STRESS-INDUCED PROTEINS

Organizers: Mary Lou Pardue, James Feramisco and Susan Lindquist
April 10-16, 1988

Page

Plenary Sessions

Poster Sessions

Regulation of the Response in Eukaryotes

P 001 REGULATION OF THE EXPRESSION OF HEAT SHOCK GENE FAMILIES OF SOYBEAN, Janice Kimpel*, Ronald T. Nagao and Joe L. Key, Department of Botany, The University of Georgia, Athens, Georgia 30602 and *Agrigenetics Advanced Science Company, Madison, Wisconsin 53716. The heat shock (HS) response of soybeans is characterized by the synthesis of a complex set of low molecular HS proteins (25 to 30 in the 15 to 27 kD range) and a set of high molecular weight HS proteins similar in pattern and complexity to those of many other eukaryotes. HS mRNAs for many of these HS proteins increase to their maximum levels by 1 to 2 hr of continuous HS; after about 2 hr the levels gradually decline at HS temperatures. In contrast, at control temperatures the steady state level of HS mRNAs is reduced to very low levels within 4 hr indicating that HS mRNAs are more stable at HS temperatures than at control temperatures. HS protein synthesis continues for 6 to 8 hr during continuous HS, but slows after about 4 hr. After a shift from HS to control temperatures, HS protein synthesis slows in parallel with the decay of HS mRNAs (not detectable after 4 hr). Those HS proteins synthesized during a HS treatment are generally stable for many hours. Yet, successive HS regimes following a 3-to-4 hr recovery at control temperatures result in a new round of HS mRNA and protein synthesis, although at somewhat diminished levels during the successive cycles. Studies using run-off transcription assays address the issue of the role of induction and turn-off of transcription in the level of HS mRNAs which accumulates during different HS regimes. We have isolated representative cDNA and genomic clones for most of the families of HS proteins in soybean. The sequence analysis of several of these genes and the proteins they encode as well as some aspects of the regulation of HS gene expression will be presented.

P 002 HSR93D: A DIFFERENT SORT OF DROSOPHILA HEAT SHOCK LOCUS. W.G. Bendena, M.E. Fini, J.C. Garbe, G.M. Kidder. S.C. Lakhotia, and M.L. Pardue. Dept. of Biology, Massachusetts Institute of Technology, Cambridge, MA.

The 93D heat shock locus of D. melanogaster differs from the loci at the other major heat shock puffs in not encoding a major heat shock protein and having a sequence that diverges significantly in other species. Each Drosophila species has one heat shock puff with phenotypic characteristics of 93D. The 93D-equivalent puffs in other species yield transcripts resembling hsr93D transcripts in structure, in spite of the many differences in sequence. The transcripts do have several short regions of conserved sequence that may give clues to the function of the locus. The locus is transcribed in most cell types and the level of transcription is modulated by heat shock and by developmental controls. In addition hsr93D is induced independently of other heat shock loci by several environmental agents, suggesting that this locus is especially responsive to cellular conditions. We suggest this locus has a regulatory role in cell metabolism generally. Although the 3 major hsr93D transcripts have the same transcription start site, we can find no evidence that the largest transcript is a precursor to the other transcripts. Instead it seems to be a product of altered termination. The abundance and several conserved features of the largest nuclear transcript suggest that this RNA molecule has a role in the nucleus. The smaller nuclear transcript does appear to be a precursor to the cytoplasmic RNA. The cytoplasmic transcript is 1.2 kb in size yet the longest possible open reading frame (ORF) would encode only 34 amino acids. The only reading frame that shows any sequence conservation would encode 24-27 amino acids, depending on the species. We have not been able to detect any of the polypeptides that might be encoded by these reading frames yet several kinds of experiments show that the conserved ORF is translated, possibly to perform a regulatory function.

P 003 MICROINJECTION OF UBIQUITIN: CHANGES IN PROTEIN DEGRADATION IN HeLa CELLS SUBJECTED TO HEAT-SHOCK, Martin Rechsteiner, University of Utah, Salt Lake City, UT 84132

Abstract. Ubiquitin was radiolabeled by reaction with ^{125}I-Bolton-Hunter reagent and introduced into HeLa cells using erythrocyte-mediated microinjection. The injected cells were then incubated at 45°C for 5 min (reversible heat-shock) or for 30 min (lethal heat-shock). After either treatment, there were dramatic changes in the levels of ubiquitin conjugates. Under normal culture conditions, ~10% of the injected ubiquitin is linked to histones, 40% is found in conjugates with molecular weights greater than 25,000, and the rest is unconjugated. After heat-shock, the free ubiquitin pool and the level of histone-ubiquitin conjugates decreased rapidly, and high molecular weight conjugates predominated. Formation of large conjugates did not require protein synthesis; when analyzed by two-dimensional electrophoresis, the major conjugates did not co-migrate with heat-shock proteins before or after thermal stress.

Concomitant with the loss of free ubiquitin, the degradation of endogenous proteins, injected hemoglobin, BSA, and ubiquitin was reduced in heat-shocked HeLa cells. After reversible heat-shock, the decrease in proteolysis was small, and both the rate of proteolysis and the size of the free ubiquitin pool returned to control levels upon incubation at 37°C. In contrast, neither proteolysis nor free ubiquitin pools returned to control levels after lethal heat-shock. However, lethally heat-shocked cells degraded denatured hemoglobin more rapidly than native hemoglobin and ubiquitin-globin conjugates formed within them. Therefore, stabilization of proteins after heat-shock cannot be due to the loss of ubiquitin conjugation or inability to degrade proteins that form conjugates with ubiquitin.

P 004 ANION TRANSPORT IS LINKED TO HEAT SHOCK INDUCTION, M. M. Sanders, K. John-Alder and A. C. Sherwood, Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

The heat shock response in Drosophila is characterized by profound changes in the regulation of gene expression and by a collapse of the intermediate filament cytoskeleton, decreased anion uptake, decreased cell volume and slowed growth. In Drosophila Kc cells, we have observed that a signal generated at the cell membrane by impermeant inhibitors of anion transport typified by 4,4'-diisocyanostilbenedisulfonate (DIDS) induce all of these characteristics of the heat shock response at the normal growth temperature.

We have taken two approaches to investigating the relationship between anion transport and heat shock induction in Kc cells. First, three ion transport systems capable of $^{36}Cl^-$ uptake have been identified and partially characterized. They differ in affinity for Cl^-, in transport capacity, in temperature sensitivity and in sensitivity to DIDS, NO_3^- and vanadate inhibition. The high affinity Cl^- uptake system has some properties typical of Cl^-/HCO_3^- exchange involved in intracellular pH regulation in other systems. The relationship of the high affinity Cl^- uptake system to intracellular pH regulation and heat shock induction in Drosophila is under investigation.

A second approach has been to select Kc cell variants which are resistant to the growth inhibiting properties of the anion transport blockers. The variants are able to grow in the presence of anion transport inhibitors and they show lower level heat shock protein induction than the parent cells. They also have increased capacity for $^{36}Cl^-$ uptake and concentration via a DIDS-sensitive transport system and they have amplified three abundant immunologically related proteins of 46, 62 and 116 kD MW. The 46 and 62 kD proteins are more than 10-fold amplified and are components of the intermediate filament cytoskeleton which collapses in heat shocked cells. The 116 kD protein is much more highly amplified and is an exterior membrane protein which is anchored in the cytoskeleton. Revertants of the variants lose their altered anion transport, heat shock and protein amplification phenotypes simultaneously suggesting the phenotypes are interrelated.

These observations are all consistent with the possibility that anion transport function is linked to heat shock induction in Drosophila. The data collected up to the present time suggest signalling roles for intracellular pH and/or the cytoskeleton.

P 005 THE DEGRADATION SIGNAL IN A SHORT-LIVED PROTEIN, Alexander Varshavsky, Department of Biology, M.I.T., Cambridge, Massachusetts 02139.
I shall discuss recent results from this laboratory that address the mechanism of substrate recognition in selective protein turnover and the functions of genes for ubiquitin and ubiquitin-specific enzymes.

Regulation of the Response in Prokaryotes

P 006 THE ROLE OF THE ESCHERICHIA COLI HEAT SHOCK PROTEINS IN BACTERIOPHAGE LAMBDA GROWTH, C. Georgopoulos[1], D. Ang[1], A. Cegielska[1], O. Fayet[1], C. Johnson[1], B. Lipinska[1],A. Maddock[1], S. Sell[1], J. Spence[1], T. Zeigelhoffer[1], K. Liberek[2], and M. Zylicz[2]. [1]Department of Cellular, Viral, and Molecular Biology, University of Utah Medical Center, Salt Lake City, Utah 84132, [2]Division of Biophysics, University of Gdansk, Kladki 24, Gdansk 80-822, Poland.

The heat shock response of E. coli is essential for both bacteriophage λ early development (DNA replication) and late development (head morphogenesis). In λ DNA replication two distinct states can be demonstrated in vitro. The first is the assembly of a protein complex at the origin of λ DNA replication, oriλ. The assembly of this complex does not require any heat shock proteins. The complex consists of the O^{λ} protein , which binds specifically to oriλ, and of the P^{λ} protein which binds specifically to both O^{λ} and the host-coded DnaB protein. This DNA-protein complex is very stable and can be shown to be capable of in vitro DNA replication provided that it is supplemented with other host-coded components (see below). The second demonstrable stage is the addition of the host-coded DnaK, DnaJ and GrpE heat shock proteins to the complex at oriλ, resulting in the formation of a larger and stable complex. The heat shock proteins are atrracted to the oriλ complex through very specific DNA-protein and protein-protein interactions. The addition of ATP releases the P^{λ} protein from the complex, simultaneously liberating the DnaB helicase protein. The DnaB protein is thusly positioned on nearby single-stranded DNA and unwinds λ DNA using ribosotriphosphate hydrolysis as an energy source. The host DnaG primase protein locates the single-stranded DNA-DnaB complex and makes an RNA primer at apparently random sites. The E. coli DNA polymerase III holoenzyme extends efficiently this RNA primer into DNA. In summary, the overall role of the heat shock proteins in λ DNA replication is the disassembly of the O^{λ}-P^{λ}-DnaB complex, thus allowing DnaB to gain access to single-stranded DNA nearby oriλ.

The E. coli heat shock proteins GroEL (a decatetramer of 60,000-Mr subunits) and GroES (an oligomer of 11,000-Mr) are absolutely essential for the assembly of the bacteriophage λ head-tail connector (itself a dodecamer of 50,000-Mr subunits). In addition to these host proteins the phage-coded Nu3 scaffold protein is also essential for this process in vivo. The two GroE proteins interact as judged by both genetic and biochemical criteria. It is believed that the GroE proteins provide a surface onto which the oligomerization of B^{λ} subunits into a dodecameric structure can take place. Subsequently to the assembly of the λ connector, the correct assembly of the λ prohead is initiated. Following this, DNA is packaged inside the head, and a tail is attached to give rise to an infectious virus.

P 007 REGULATION OF THE HEAT SHOCK RESPONSE IN *E. COLI* , Carol A. Gross, David B. Straus, James W. Erickson, Yan-ning Zhou and Laura Heisler

When *E. coli* cells are shifted to high growth temperature, the synthesis of about 20 heat shock proteins is transiently induced as a result of changes in transcription initiation by $E\sigma^{32}$ at heat shock promoters. After a temperature downshift, the synthesis of the heat shock proteins is transiently decreased as a result of changes in transcription from heat shock promoters. Upon shift to lethal temperature, protein synthesis is devoted almost entirely to heat shock proteins. We have analyzed these responses and have foundthat: (1) The amount of σ^{32} increases transiently after temperature upshift due to changes in the synthesis and stability of σ^{32}. These changes in σ^{32} level directly regulate expression of the heat shock genes. Several heat shock proteins negatively regulate both the synthesis and stability of σ^{32}. (2) The response to downshift may be mediated by inactivation, but not degradation, of σ^{32}. (3) At lethal temperatures σ^{32} levels are maintained because *rpoH* (encoding σ^{32}) is transcribed by RNA polymerase containing a new 24 kd σ factor.

P 008 HEAT-SHOCK RESPONSE IS INDUCED IN ESCHERICHIA COLI CELLS DEFICIENT IN 4.5S RNA. David B. Bourgaize*, Teresa A. Phillips+, Pamela G. Jones+, Ruth A. VanBogelen+, Maurille J. Fournier*, and Frederick C. Neidhardt+, Department of Biochemistry*, University of Massachusetts, Amherst, MA 01003, and Department of Microbiology and Immunology+, University of Michigan Medical School, Ann Arbor, MI 48109-0620.

The Escherichia coli version of the universal cellular heat-shock response consists of the induction of 19 proteins upon a shift-up in temperature. Their genes are subject to transcriptional control by sigma-32, the product of htpR (rpoH, hin). Artificial elevation of the cellular level of sigma-32 by overproduction from a plasmid-carried htpR driven by the ptac promoter induces nearly all the heat-shock proteins in a manner closely similar to that of temperature shift. Of the various toxic chemicals that induce subsets of the heat-shock regulon, only ethanol (4%) closely mimics the response brought on by a shift from 28 to 42°C.

The 4.5S RNA of E. coli is a small, stable RNA that is essential for cell growth. A strain has been constructed in which the synthesis of 4.5S RNA can be turned off by removing an inducer of the lac operon. As previously reported, depriving the cell of 4.5S RNA inhibits cell growth, and leads to the accumulation of translationally defective ribosomes (1). Analysis of the pattern of protein synthesis reveals that one of the earliest observable consequences of 4.5S RNA deprivation is induction of the heat-shock response. The response is virtually indistinguishable from that produced by shift to high temperature, both with respect to the number of proteins induced and to the magnitude of their induction. The induction appears to be the consequence of a greatly elevated cellular level of sigma-32. We shall discuss the implications of this result for the cellular role of 4.5S RNA and for the function and mechanism of induction of the heat-shock response.

1. Bourgaize, D. B., and M. Fournier. Initiation of translation is impaired in E. coli cells deficient in 4.5S RNA. Nature 325: 281-284.

Regulation of Heat Shock and Other Stress Responses

P 009 Genetics of the Heat Shock Response in Drosophila. J. Jose Bonner, Janice Parker-Thornburg Mark Hallett, and Monica McAndrews. Department of Biology, Indiana University, Bloomington, IN 47405

In order to investigate the regulatory mechanism involved in heat shock gene transcription in Drosophila, we have sought mutations which affect the heat shock response. Using an hsp70-Adh gene fusion to provide a selectable marker, we have isolated a large number of constitutive mutations. These all activate the hsp70-Adh fusion, and thus confer ethanol-tolerance in the absence of heat shock. The majority of these mutations appear to be in genes unrelated to the heat shock system, but which confer a cell type-specific "biochemical stress" which triggers the heat shock response at normal growth temperatures in a tissue-specific manner. Using similar screens to select for mutations which result in the inability to respond to heat shock, we have found remarkably few. We attribute this difficulty, in part, to the possibility that genes required for the heat shock response may also be involved in other processess essential for normal cellular metabolism. As a second approach to defining genes required for the heat shock response, we have performed a systematic analysis of deletions. From this analysis, we have identified three autosomal loci which are required for a normal heat shock response.

P 010 THE SELECTIVE DEGRADATION OF ABNORMAL PROTEINS AND THE HEAT-SHOCK RESPONSE, Alfred L. Goldberg, Department of Physiology and Biophysics, Harvard Medical School, Boston, MA 02115)

Animal and bacterial cells rapidly destroy cellular proteins with highly abnormal structures, as may arise by mutation, synthetic errors, denaturation, or damage by free radicals. Such proteins are hydrolyzed by soluble ATP-dependent pathways. In E. coli, the rate-limiting enzyme in the degradation of many abnormal proteins is the ATP-dependent protease La, the lon gene product. In addition to its active site, protease La contains a regulatory domain, whose interaction with unfolded proteins induces proteolytic activity. For each peptide bond cleaved in proteins, two ATPs are utilized. The ADP generated remains tightly associated with the enzyme and prevents proteolysis in vivo until the enzyme binds to another unfolded protein. The complete base sequence of the lon gene has been determined. It contains an unusual heat-shock promoter and an ATP-binding domain, but is not homologous to other proteases.

When E. coli generate large amounts of unfolded proteins (e.g. at high temperatures or upon expression of cloned proteins), transcription of lon increases 2- to 4-fold as part of the heat-shock response. This induction is sufficient to enhance protein degradation, but it also reduces cell growth. The heat-shock response appears to be induced when the cell's capacity to degrade abnormal proteins is exceeded. No such increase in protease La is seen in htpR mutants, which show a reduced capacity to degrade abnormal polypeptides. A build-up of unfolded proteins may be the common signal triggering the heat-shock response. In frog oocytes, the injection of an unfolded protein activates heat-shock genes. This response appears to enhance the cell's capacity to eliminate abnormal proteins. One hsp is ubiquitin, a component of the ATP-dependent pathway, but there also exist other proteolytic pathways in eukaryotic cells. The major intracellular proteolytic activity is a 700KDa enzyme complex, which we call the "proteosome". It corresponds to the 19S "prosome" particle. Their precise function and mode of regulation are unknown.

P 011 CELLULAR RESPONSES TO HEAVY METAL IONS, PHORBOL ESTER TUMOR PROMOTERS AND DNA DAMAGE. Michael Karin, Department of Pharmacology, UCSD School of Medicine, La Jolla, Ca 92093. The human metallothionein II_A (hMT-II_A) gene makes a convenient system for studying alterations in transcription patterns induced by exposure to various environmental stresses. Transcription of the hMT-II_A gene, which is ubiquitously expressed in all human cell types, is rapidly induced by heavy metal ions and various stress hormones including glucocorticoids, IL-1 and interferons. In addition, hMT-II_A transcription is induced by tumor promoting phorbol esters and various DNA damaging agents such as mitomycin C and UV. Genetic and biochemical analysis of the hMT-II_A promoter has revealed the presence of at least six different control elements that serve as binding sites for distinct transcription factors. Basal expression is determined by the TATA and GC boxes and two basal level enhancer elements (BLEs). Interspersed between these basal elements are four metal responsive elements (MREs) that have no effect on basal expression but are responsible for induction of hMT-II_A by heavy metal ions. Another element that has no effect on basal expression but is responsible for induction by glucocorticoid hormones is the glucocorticoid responsive element (GRE), which is located upstream to the distal BLE. To understand the molecular mechanisms which control expression of the hMT-II_A gene we have characterized and purified most of the transcription factors that interact with its promoter. Using purified proteins we find that the GC box serves as a recognition site for Sp1, while the two BLEs contain binding sites for the enhancer binding factors AP-1 and AP-2 and the GRE is recognized by the glucocorticoid receptor. Sp1, AP-1 and AP-2 are capable of stimulating transcription from the hMT-II_A promoter in vitro. To our surprise we found that in addition to their role in establishing basal activity of the hMT-II_A promoter, AP-1 and AP-2 mediate its induction by phorbol esters and cyclic AMP. Recent experiments suggest that AP-1 is the product of the c-jun proto-oncogene, which we have recently isolated from a HeLa cDNA library. The availability of cDNA probes and anti-sera for these factors should facilitate studies directed toward understanding the mechanisms by which activation of cellular protein kinases by second messengers generated in response to stress leads to an increase in the activity of trans-acting factors such as AP-1 and AP-2.

P 012 CHARACTERIZATION OF A BACTERIAL RESPONSE TO OXIDATIVE STRESS,
Gisela Storz, Louis A. Tartaglia, Michael F. Christman, and Bruce N. Ames,
Department of Biochemistry, University of California, Berkeley CA 94720.

Treatment of *S. typhimurium* or *E. coli* with 60 μM H_2O_2 results in resistance to killing by higher doses of H_2O_2 and the induction of at least 30 proteins (1). Several of the H_2O_2-inducible proteins overlap with heat shock and other stress proteins (2). The expression of 9 of the H_2O_2-inducible proteins is controlled by the positive regulatory element *oxyR*. Constitutive mutants, *oxyR1* and *oxyR2*, are resistant to oxidants and overexpress the 9 proteins, which include catalase, glutathione reductase, and a novel alkyl hydroperoxide reductase.

We have begun to study the molecular details of the regulation by OxyR. The *oxyR* gene encodes a 34 kD protein which shows homology to NodD and LysR, other bacterial regulatory proteins. Studies of *oxyR* and *katG* (catalase) expression in *oxyR* deletion strains show that OxyR is a negative regulator of itself and a positive regulator of the other H_2O_2-inducible genes. A 50-fold higher level of *katG* mRNA in the constitutive mutant compared to wild type suggests that OxyR regulates transcription, and mobility shift gels indicate the OxyR protein binds to the *katG* promoter. The site of OxyR action has been defined by deletion analysis of the *oxyR*, *katG*, and *ahp* (alkyl hydroperoxide reductase) promoters. We are now overexpressing and purifying OxyR and have shown that the constitutive phenotype of the *oxyR2* mutant is due to an Ala to Val missense mutation in the C-terminal portion of the protein.

(1) Christman, M.F., Morgan, R.W., Jacobson, F.S., and Ames, B.N. (1985) *Cell 41*: 753-762.
(2) Morgan, R.W., Christman, M.F., Jacobson, F.S., Storz, G., and Ames, B.N. (1986) *Proc. Natl. Acad. Sci. USA 83*: 8059-8063.

Genetic Analysis of the Function of Heat Shock Proteins and their Cognates (during Heat Shock and in Normal Development)

P 013 TRANSCRIPTIONAL REGULATION OF THE HEAT SHOCK GENES BY CYCLIC AMP IN YEAST, Kazuma Tanaka, Kunihiro Matsumoto*, and Akio Tohe, Depart. Ferment. Technol. Hiroshima Univ. Higashihiroshima, 724, Japan. and *DNAX Res. Inst., Palo Alto, CA 94304-1104.

The fact that the mutants in which the cAMP-pathway is permanently activated, such as the bcy1, RAS2-val19, and ppd1 mutants, are hypersensitive to acute heat shock indicates the important role of the cAMP-pathway in the heat response in S. cerevisiae. We examined the expression of one of the heat shock genes, UBI4, which encodes poly-ubiquitin (1), in the various mutants with a defect in the cAMP-pathway.

When cAMP was depleted from the culture medium of the cyr1 mutant, the UBI4 gene was induced several fold. This effect was also seen in the presence of cycloheximide. Because the expression of the UBI4 gene of the cyr1 bcy1 double mutant did not respond to a change of the cAMP level, we concluded that the UBI4 gene is regulated by cAMP-dependent protein kinase. Our results suggest that the induction of the UBI4 gene in stationary phase cells (1) is caused by the reduction of the intercellular cAMP level. Next we examined the response of the UBI4 gene to heat in the cyr1, bcy1, and ppd1 mutants. The UBI4 gene was clearly induced by heat shock in all of these mutants. The result indicates that heat response is not mediated by cAMP.

The data of the response of other heat shock genes to cAMP and analyses of upstream sequences of the UBI4 gene responding to cAMP will also be presented.

(1) Finley et al., (1987). Cell 48, 1035-1046.

P 014 REGULATION AND FUNCTION OF STRESS-INDUCED PROTEINS OF Escherichia coli, Bernd Bukau, Caroline E. Donnelly, Christine C. Dykstra, and Graham C. Walker, Biology Department, Massachusetts Institute of Technology, Cambridge MA 02139. We have been engaged in a series of studies designed to probe the regulation and function of stress-induced proteins in Escherichia coli. Continued analysis of ΔdnaK mutants (Paek and Walker, 1987, J. Bacteriol. 169:283) has revealed new phenotypes observable at lower temperatures. Introduction of the ΔdnaK allele into the chromosome of strain MC4100 by P1 transduction at 30°C resulted in translucent colonies containing strongly filamenting cells. In each of these colonies, papillae appeared that contained cells with a reduced or non-filamentation phenotype. Since cells carrying these secondary mutations were the only transductants capable of further growth at 30°C it appears that ΔdnaK mutants must acquire at least one suppressor mutation in order to grow at 30°C. Additional studies have indicated that at least one other class of suppressor mutation is required in order for these ΔdnaK strains to grow at 16°C or 43°C. We also found that the lethal filamentation of ΔdnaK transductants could be suppressed by the presence of a plasmid carrying the ftsZAQ genes, implying that DnaK might interact directly or indirectly with the normal FtsZAQ-dependent cell division machinery.

Introduction of a multicopy plasmid carrying the $umuD^{+}C^{+}$ genes into a lexA51(Def) strain results in cold-sensitivity for growth (Marsh and Walker, 1985, J. Bacteriol. 162:155). This cold sensitivity can be suppressed by mutations in certain heat shock genes such as lon or groE or by Tn5-generated css (cold sensitivity suppression) mutations that map to at least six different locations in the E. coli chromosome. Mutations that suppress this cold sensitivity can be separated into at least two different classes - those such as lon that act, at least in part, by stabilizing the LexA51 protein, and those such as groE that act by a different mechanism. In addition, we have identified four different restriction fragments of E. coli DNA that suppress this cold sensitivity when they are present on multicopy plasmids.

P 015 YEAST HSP 70 STIMULATES POST-TRANSLATIONAL TRANSLOCATION OF PREPRO-α-FACTOR INTO YEAST MICROSOMES, M. Gerard Waters, William J. Chirico, Rubén Henríquez and Günter Blobel. Laboratory of Cell Biology. Howard Hughes Medical Institute. The Rockefeller University. New York, NY 10021.

We are studying the process by which proteins are translocated into or through the membrane of the endoplasmic reticulum. Using a subfractionated extract of *Saccharomyces cerevisiae* and mRNA for a yeast secretory protein precursor, prepro-α-factor, we have developed an *in vitro* system capable of translocation and core glycosylation of this and other proteins. In contrast to the requirement in higher eukaryotic cells that secretory protein translocation must occur co-translationally, prepro-α-factor can be translocated into yeast microsomes post-translationally. This process requires ATP and both soluble and membrane associated components. Recently we have developed an assay for the soluble activity and have used it to purify the component to near homogeneity. A post-ribosomal supernatant was prepared and applied to a DEAE anion exchange column. The activity was eluted quantitatively and applied to a GTP-agarose column, through which the activity flows but several proteins which would be contaminants in the next step are retained. This flow-through was applied to an ATP-agarose column, onto which the activity binds and was eluted specifically with ATP. By one dimensional SDS-PAGE the preparation is a virtually homogeneous 70 kD protein which can be photolabeled with [α-^{32}P]-ATP or [α-^{32}P]-8-azido-ATP. This purification scheme, molecular weight and ATP binding capacity suggested that the protein might be related to HSP 70. Indeed, the preparation cross-reacts with a monoclonal antibody (from Susan Lindquist and Hugh Pelham) prepared against Drosophila HSP 70. Yeast constitutively express two HSP 70 related proteins termed SSA1p and SSA2p, which are 97% homologous. Two dimensional electrophoresis of our preparation reveals two 70 kD proteins with isoelectric points consistent with SSA1p and SSA2p. We believe that both the proteins are active because genetic data from Elizabeth Craig's group indicates they are not functionally unique. Several groups have speculated that post-translational translocation may require unfolding of the precursor prior to or during transit across the membrane. Furthermore, it has been proposed that HSPs might serve this role. To try to correlate unfolding with the stimulatory effect of our preparation on post-translational translocation we have used prepro-α-factor that was denatured in 6M urea prior to addition to the microsomes. We find that denatured prepro-α-factor is translocated rapidly, even in the absence of the SSA1p/SSA2p preparation. Our data therefore support the idea that unfolding is necessary for translocation and that constitutively expressed heat shock proteins may catalyze this process by a mechanism which remains to be elucidated.

Molecular Analysis of the Function of Heat Shock Proteins and their Cognates

P 016 REGULATION OF HEAT SHOCK TRANSCRIPTION IN YEAST, Hugh Pelham and Peter Sorger, MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England.

S. cerevisiae, like other eucaryotes, responds to heat shock by increasing the rate of transcription of heat shock genes. This activation is mediated by a specific regulatory DNA sequence, the heat shock element (HSE). A synthetic version of the consensus HSE sequence, which was originally shown to confer heat-inducibility on a promoter in mammalian cells, is also functional in yeast. Extracts from both heat-shocked yeast and HeLa cells contain a binding activity which specifically footprints the synthetic DNA sequence. Yeast differs from mammalian and Drosophila cells, however, in that binding activity is also detectable in control, unstressed cells. We have purified the protein responsible for this activity from both unshocked and shocked yeast; its electrophoretic mobility in the presence of SDS indicates a molecular weight of approximately 150 kd in control cells, but it appears larger after heat shock. This size change can be detected in crude extracts using antibodies raised against the purified protein, and it is abolished by treatment of the extract or purified protein with phosphatases. Careful examination of the effects of heat shock suggests that the protein is phosphorylated at multiple sites in response to stress, with more extreme stress resulting in more extensive phosphorylation. This modification does not affect the DNA binding properties of the protein, nor its ability to form multimeric aggregates. Presumably, phosphorylation allows it to interact with other components of the transcriptional machinery and thus activate the expression of heat shock genes; multiple phosphates may provide an acidic motif analogous to the activating domains of other yeast transcription factors such as GCN4 and GAL4.

P 017 STRUCTURE AND FUNCTION OF THE MAMMALIAN STRESS PROTEINS, W.J. Welch, L.A. Mizzen, A.-P. Arrigo, and K. Riabowol, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

Work in our laboratory has continued to focus on identifying, purifying, and characterizing all of the major stress-induced proteins of mammalian cells. The stress proteins are comprised of two major families: (i) the heat shock proteins (HSPs) and (ii) the glucose regulated proteins (GRPs). Extensive "cross-over" exists between the two families including their reciprocal induction in the cell and the fact that the two groups of proteins are related to one another as determined by sequence homology, immunological cross-reactivity, and in some cases biochemical properties. We have succeeded in purifying the individual stress proteins, have raised specific antibodies to the proteins, and have determined a number of properties of the stress proteins in both the normal cell as well as in the cell experiencing stress. These results will be summarized and discussed in relation to the possible functions these proteins serve in the cell. In an attempt to further delineate the in vivo role of the stress proteins, and in particular the 70kDa family of proteins, we have recently begun studies in which the purified 70kDa stress proteins or their corresponding antibodies are introduced into the cell via glass needle microinjection. Using this approach, questions relating to the possible protective role of 70kDa, its autoregulation, and finally the effects of inhibiting 70kDa protein function are being addressed.

Transcriptional Regulation in Eukaryotes

P 018 MULTIPLE REGULATORY PATHWAYS IN TRANSCRIPTION OF HUMAN HEAT SHOCK GENES, Richard I. Morimoto, Gregg Williams, Terrill McClanahan and Richard Mosser, Department of Biochemistry, Molecular and Cell Biology, Northwestern University, Evanston IL 60208.

Transcription of the major heat shock gene HSP70 in human cells is induced by exposure to heat shock and heavy metal stress, in response to serum stimulation, by adenovirus E1A trans-activation and during early embryonic development. These forms of regulation are mediated through multiple distinct cis-acting promoter elements which lie within 188 bp of the transcription initiation site. We have constructed promoter fusions to the bacterial chloramphenicol acetyl transferase gene and assessed the effects of deletions and insertions in both transient assays and in pooled stable human cell lines. Heat shock and cadmium sulfate, two representative forms of stress-inducers, require upstream sequences between -107 to -68. Between -107 and -80 are two overlapping heat shock elements (HSE), the distal HSE is a perfect fit with consensus (8/8) while the proximal overlapping HSE has a 4/8 fit. The sequences required for serum responsiveness and adenovirus E1A activation both map to the -69 region. From the analysis of closely spaced 5' deletion mutants and linker-scanner mutants it appears that both E1A and serum mediate their transcriptional effects primarily through the CCAAT element positioned at -68 to -64. Fusion of a 47 bp sequence containing the HSP70 CCAAT element to the TATA box of an inactive HSV-TK gene confers both forms of transcriptional control to the TK gene. Nuclear proteins from HeLa cells which interact with these cis-acting sequences have been identified by gel shift assays and the boundaries mapped by footprinting methods. The role of these trans-acting factors in providing specific transcriptional activation through the HSP70 cis-acting promoter elements will be discussed.

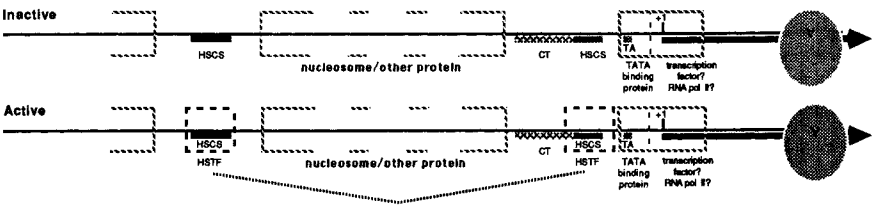
P 019 REGULATION OF HEAT SHOCK GENE TRANSCRIPTION, Carl Wu, Allan Hansell, Kris Lambert, Barbara Walker, Susan Wilson and Vincenzo Zimarino, Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892.
Heat Shock Activator Protein (recently commonly renamed Heat Shock Factor, HSF, was initially identified by in vivo studies of protein-DNA interactions at heat shock gene promoters in Drosophila; HSF is bound to the heat shock control element, HSE, after, but not before heat shock stimulation. We have purified HSF to homogeneity from shocked Drosophila cells by affinity chromatography. Drosophila HSF has a M_r of 110 kilodaltons, binds to the HSE with great affinity and specificity, and strongly stimulates transcription of the Drosophila hsp70 gene when microinjected into Xenopus oocyte nuclei. Polyclonal antibodies raised against purified HSF react with the native and denatured protein, and have been used to identify several positive plaques in a screen of a cDNA library. In normal Drosophila cells, HSF pre-exists in an inactive, non-binding form that is converted to the specific DNA-binding form within minutes of heat or chemical shock, most likely by a post-translational modification. HSF has also been identified in yeast, chicken, frog, mouse and human cells, and the properties of the factor in these different species will be discussed.

Chromatin Organization and Structure Around Heat Shock Genes

P 020 THE CHROMATIN STRUCTURE OF hsp 26, S.C.R. Elgin, T.J. Dietz, D.S. Gilmour, E. Siegfried, and G.H. Thomas, Dept. Biology, Washington University, St. Louis, MO 63130.

Mapping of the chromatin of the Drosophila melanogaster hsp 26 gene has shown that it exists in an ordered structure both prior to and after heat shock induction. There are two DNase I hypersensitive sites 5′ to the gene; the heat shock consensus sequences (HSCS) (8/10 match) lie within these open regions. An ordered nucleosome array is seen downstream across the gene. On activation, the region of transcription becomes accessible to DNase I, and the nucleosome pattern becomes smeared (1). Studies using specific antibodies and others using camptothecin (a specific inhibitor) demonstrate high levels of topoisomerase I associated with the region of transcription; this might facilitate an "unfolding" of the chromatin fiber. High resolution mapping of the 5′ region using the indirect end-labelling technique demonstrates the presence of the TATA box binding protein prior to heat shock. A large region of protection (150 bp) characterized by 10 bp periodic DNase I cleavage sites is observed between the two 8/10 HSCS's, suggesting the presence of a nucleosome. Following heat shock, additional footprints are detected over these HSCS's. In vitro binding studies utilizing an exonuclease III protection assay have demonstrated the presence of a general TATA-box binding factor in nuclear extracts from control and heat-shocked embryos; only the latter contain significant amounts of protein binding to the HSCS. We suggest that the binding of the TATA-box factor and of a specifically positioned nucleosome (see below) generates the upstream DNase I hypersensitive sites, leaving the HSCS's accessible; the folding of the DNA by the nucleosome brings these sites into proximity, perhaps facilitating gene activation.

(1) Cartwright and Elgin, 1986, Molec. Cell Biol. 6, 779-791.



P 021 DYNAMICS OF TRANSCRIPTIONAL ACTIVATION AT HEAT SHOCK LOCI,

John T. Lis, Cornell University, Ithaca, NY 14853.

A gene's transit from a transcriptionally dormant to a highly active state requires a large battery of specific molecular interactions and reactions. The heat shock genes can be induced several hundred-fold within minutes of a stress stimulus. The initiation of these events is dependent on the interaction of a protein factor with short stretches of DNA sequence, the heat shock regulatory elements. We are using the heat shock response as a model in Drosophila and yeast to investigate the mechanism of gene activation and the accompanying changes in chromatin structure. Our efforts have focused on interacting experimental approaches of reverse genetics (1), photo-crosslinking (2,3), and biochemical purification. Recent results obtained using these approaches will be presented and the implications on transcription initiation and elongation, and on the establishment of a domain of active chromatin will be discussed .

1. Simon J.A. and J.T. Lis (1987) A germline transformation analysis reveals flexibility in the organization of heat shock consensus elements. Nucleic Acids Res. 7, 2971-2988.
2. Gilmour, D.S., Pflugfelder, G., Wang, J.C., and Lis, J.T. (1986) Topoisomerase I interacts with transcribed regions in Drosophila cells. Cell 44, 401-407.
3. Gilmour D.S. and J.T. Lis (1986) RNA polymerase interacts with the promoter region of noninduced hsp70 gene in D. melanogaster cells. Molec. Cell Biol. 6, 3984-3989.

Post-transcriptional Control

P 022 A HOMOLOG OF THE VERTEBRATE 7SL RNA IS HEAT SHOCK-INDUCED IN TETRAHYMENA THERMOPHILA.

R. Hallberg, Department of Zoology, Iowa State University, Ames, Iowa.

In response to a continuous non-lethal heat shock, T. thermophila cells rapidly accumulate a 307 nucleotide long, RNA polymerase III-transcribed RNA. This RNA, which we call G-8 RNA, is undetectable in normal growing cells (<160 copies per cell), but reaches a level of about 100,000 copies within 30 min of the administration of heat shock. It accumulates in the cytoplasm of these cells where it is found quantitatively associated with the small ribosomal subunit. Initially, G-8 is found only on monosomic ribosomes but later it becomes polysome associated. The timing of this change in localization correlates with a shift in the array of proteins being synthesized in these cells. At this time the level of G-8 RNA in the cell is such that there is approximately one molecule of G-8 per polysome.

G-8 RNA is not dissociable from the ribosome by salt alone (up to 0.8 M KCl) but is dissociable (as an RNP particle) by 1.5 molar urea in 0.5 M KCl. By transcribing the sense strand of a cloned cDNA copy of this RNA we found that it will hybridize to both the 17S and 26S rRNAs. Computer searches of possible sequences in the rRNAs with which G-8 might form duplexes revealed a number of likely possibilities, several of which fell at the 3' end of the 17S rRNA.

A sequence comparison of G-8 RNA with a number of known polymerase III-transcribed RNAs showed regions of homology with known or presumed sequences involved in RNA polymerase III transcription factor binding. A more extensive region of homology was found when G-8 RNA was compared with Xenopus laevis 7SL RNA. The majority of this homology occurs in the internal 50% of the two, approximately identical sized molecules. This roughly coincides with that portion of the 7SL RNA which occupies the signal recognition and ribosome binding portion of the SRP.

Starvation, another physiological stress which, like heat shock, induces a change in the translational properties of the cell, also induces the accumulation of G-8 RNA. The behavior of G-8 RNA in these cells is currently being investigated.

P 023 THE SMALL HSPS OF DROSOPHILA MELANOGASTER FORM GLOBULAR CYTOPLASMIC 16S RNP-PARTICLES, Peter M. Kloetzel and Christian Haass, ZMBH/Molekulare Genetik, Im Neuenheimer Feld 282, 6900 Heidelberg, FRG

The synthesis of the small hsps of D. melanogaster can either be induced during normal development by ecdyson in Schneider S3 tissue culture cells and by stress and their synthesis has been correlated with the gain of increased thermotolerance. Using ecdyson induced S3 tissue culture cells, we have identified and characterized a cytoplasmic 16s complex which contains the small hsps as major constituents. The 16s complexes possess a buoyant density of $\rho = 1.34 g/cm^3$ in Cs_2SO_4 which is typical of RNP-complexes. Electronmicroscopy shows that the 16s RNPs possess an average diameter of 12nm and a globular morphology which is distinct from that of the 19s cylinder type scRNPs. Analysis of embryos, early pupae and heat-shocked ^{35}S-labelled culture cells shows that the small hsps form 16s RNPs independent of their mode of induction. As revealed by non-denaturing gel-electrophoresis only a subset of hsps appears to be required for the formation of the RNP-complex. Furthermore, both the development and stress induced 16s RNPs possess the ability to shuttle between different cytoplasmic fractions upon stress induction and recovery. Our results suggest that the small hsps of Drosophila can unfold their so far unknown biological function during development and after heat-shock via the formation of small cytoplasmic RNP-complexes which possess the potential to interact with "free" mRNP-complexes.

P 024 STUDIES ON THE FUNCTION AND REGULATION OF HSPs IN YEAST AND FRUIT FLIES. S. Lindquist, K. Borkovich, M. Fortin, K. Golic, R. Petersen, J. Rossi, R. Susek, J. Yost. Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL 60637.

Our laboratory has two major research interests. First, we are using the heat shock response as a model system to study mechanisms of eukaryotic gene regulation. Second, we are trying to determine the individual molecular functions of the heat shock proteins. Recently, we have found that heat shock messenger RNAs in Drosophila cells are preferentially translated during heat shock by virtue of sequences in their 5' untranslated leaders and preferentially repressed during recovery by virtue of sequences at their 3' ends. We have also determined that high-temperature heat shocks disrupt the processing of messenger RNA precursors while mild pre-heat treatments, which induce the synthesis of heat shock proteins, protect it from disruption. We have created a series of mutations in yeast and Drosophila cells to determine which of the proteins is important in rescuing RNA processing, in regulating the repression of heat shock synthesis, and in helping cells to recover from the toxic effects of high temperatures. The results of these investigations will be discussed.

P 025 PHENOCOPIES AND THERMOTOLERENCE, Nancy S. Petersen,* Patricia Young,* and Herschel K. Mitchell** ; * Molecular Biology Department, Univ. of Wyo. Laramie, WYO 82071, **Biology Division, Caltech, Pasadena, CA 91125.

Short high temperature heat shocks at defined times in Drosophila pupal development cause stage specific developmental defects in adult flies. The developmental defects can be prevented by a mild heat shock just prior to the high temperature heat shock. The conditions which induce thermotolerence with respect to phenocopy induction are the same as those which induce survival thermotolerence, suggesting that the molecular mechanism may be the same in some cases.

The prevention of heat induced developmental defects is a more narrowly defined problem than survival thermotolerence. The developing wing system we are using is a particularily simple one because no cell division is occuring, only terminal differentiation. Furthermore, in some cases the gene involved in causing the developmental defect has been identified. These are the cases where a mutant phenotype can be induced in pupae which are heterozygous for a recessive gene causing abnormal wing hair development.

The heat induced developmental defects appear to be the result of failure to synthesize a normal developmentally expressed gene product either in sufficient quantity or at the correct time in development. The effect of the thermotolerence inducing treatment is therefore expected to be on gene expression at some level. When we have compared recovery of the program of gene expression under conditions where thermotolerence either is, or is not induced, we find that all aspects of gene expression which we have measured recover much faster in thermotolerent wings. Synthesis of heat shock proteins, heat shock mRNA decay, recovery of normal protein synthesis, and normal mRNA synthesis and decay, all occur much faster in thermotolerent wings. From data of others we know that several other processes including recovery of cytoskeletal organization, mRNA processing, and nucleolar morphology, also occur sooner in thermotolerent cells. Either the conditions which induce thermotolerence affect each of these processes separately, or as we prefer to think, one or a few processes may be the primary ones affected and the others may be regulated in concert with these.

Medical Relevance of Stress Responses (joint)

P 026 BIOCHEMICAL AND CELLULAR RESPONSES TO HYPERTHERMIA IN CANCER THERAPY, Eugene W. Gerner, David J.M. Fuller, James R. Glass, Margaret E. Tome, Monique MacKrell and John J. Duffy, University of Arizona, Department of Radiation Oncology and Cancer Center, Tucson, Arizona 85724

Hyperthermia is cytotoxic to human tumor cells at temperatures above 41°C, with killing dependent on exposure time. Hyperthermia can also enhance the effectiveness of other cytotoxic cancer therapies, such as radiotherapy and chemotherapy, and this enhancement can occur at temperatures between 37 and 41°C for certain agents. Non-lethal hyperthermic exposures induce a transient, non-heritable resistance to subsequent hyperthermic stresses, a phenomenon(a) termed thermotolerance. Depletion of intracellular pools of the polyamines putrescine and spermidine sensitizes cells to the cytotoxic effects of heat shock. Thermotolerance development is unaffected in polyamine depleted cells, but this resistance decays more rapidly in these cells than in cells with normal putrescine and spermidine contents. Ornithine decarboxylase (ODC), the enzyme which catalyzes the formation of putrescine from ornithine and is generally rate-limiting in polyamine biosynthesis, is regulated at normal temperatures, in part, by its rapid rate of degradation ($t_{1/2}$≃30 min). Since heat shock induces the expression of a number of enzymes and proteins involved in protein degradation, we wondered whether this enzyme was selectively inactivated at elevated temperatures. In cell lysates, ODC activity was heat stable at temperatures up to 49°C for 2 hours. ODC degradation required spermidine; in cells depleted of spermidine by nutritional means, the enzyme protein was stable for greater than 6 hours, but was degraded with a half-time of 30 min when spermidine pools were restored. The spermidine-mediated degradation of ODC could be blocked by inhibitors of protein synthesis, but not by inhibitors of lysosomal proteases. Spermidine-dependent ODC degradation occurred in ts85 cells at both the permissive and nonpermissive temperature for ubiquitin-dependent protein degradation. Heat shock, and certain chemicals which sensitize cells to the toxic effects of hyperthermia, cause polyamine oxidation to occur by inducing the enzyme spermidine N^1-acetyltransferase (SAT), the first enzyme in polyamine catabolism. SAT induction occurs by a unique translational mechanism, also requiring spermidine. Thus, thermotolerance decay, which appears to be dependent on putrescine and spermidine contents, may itself be dependent on ODC degradation and SAT induction after heat stress.

P 027 DISTINCT MEDIATORS AND MECHANISMS REGULATE HUMAN ACUTE PHASE GENE EXPRESSION DURING INFLAMMATION. David H. Perlmutter, Departments of Pediatrics, Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110

During the host response to inflammation/tissue injury, there is fever and profound changes in multiple metabolic pathways. This 'acute phase response' is thought to result from activation of local tissue and circulating blood mononuclear cells with consequent release of specific cytokines. Accordingly we examined the effect of these several cytokines on expression of acute phase plasma proteins in human hepatoma cells (HepG2, Hep3B) and in primary cultures of human monocytes and macrophages. Recombinant human IL-1β or TNFα increased steady state levels of mRNA for and rate of synthesis of complement proteins C3, factor B, α-1-antichymotrypsin and decreased steady state levels of mRNA for and rate of synthesis of albumin and transferrin. Recombinant human IFN-γ increased steady state levels of mRNA for and rate of synthesis of IL-1- and TNF- unresponsive complement protein C4. Recombinant human IL-2 also elicited hepatic acute phase gene expression but through an indirect pathway involving the induction of monocyte IL-1 release. The effect of these cytokines on hepatic acute phase genes (factor B, C4) was also evident in mouse fibroblasts transfected with cloned human factor B or C4 genes suggesting the presence of regulatory elements within the gene or its flanking regions. The expression of α-1-antitrypsin was not affected by any of these cytokines. Tissue-specific regulation of α-1-antitrypsin in human macrophages was elicited by serine elastase and bacterial lipopolysaccharide. These results indicate that human hepatic acute phase gene expression is likely to involve several different cellular sites of synthesis, several different mediators, acting through several pathways.

P 028 STRESS-INDUCED PROTEINS AS ANTIGENS IN INFECTIOUS DISEASES. Douglas B Young, MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, London W12 OHS, UK.

Current strategies for development of novel diagnostic reagents and "subunit" vaccines against bacterial and parasitic diseases entail molecular characterisation of components of the pathogen which are involved in immune recognition. Mycobacterial proteins which are recognised by antibodies and by T lymphocytes during infection with leprosy and tuberculosis have been characterised by epitope mapping and by sequence determination. Antigens of molecular weight 71kD and 70kD (from M.tuberculosis and M.leprae respectively) are members of the hsp70 gene family and 65kD antigens isolated from both organisms show strong sequence homology with the GroEL heat shock protein of E.coli. A further 18kD antigen from M.leprae has some conserved sequence features characteristic of low molecular weight heat shock proteins. In combination with recent results derived by analysis of antigens from a variety of parasite and bacterial pathogens, these results indicate that stress proteins are a common target of the immune response to infection.

It is likely that stress proteins of pathogens will be induced during the process of infection - for example, as a consequence of parasite differentiation, or by exposure of bacteria to oxidative metabolites following engulfment by host macrophages - and this may contribute to their prominent role as antigens. Since the stress proteins of the pathogen share extensive sequence homology with the corresponding host proteins, their recognition must be restricted by the self tolerance mechanisms of the immune system. Immune recognition of a conserved region of such proteins would constitute an autoimmune response which could have important pathological consequences. Detailed examination of stress protein determinants and immune response mechanisms will be important in order to understand the relationships between stress proteins, infection and immunity.

P 029 STRESS RESPONSES AND TREATMENT OF CANCER, George M. Hahn, Robin L. Anderson and George A. Fisher, Department of Therapeutic Radiology, Stanford University, Stanford, CA 94305.
The exposure of target cells to hyperthermic temperatures (42-50°C) is rapidly becoming an important tool in the treatment of localized cancers. It may also become useful in dealing with other cell-proliferative diseases such as psoriasis. Hyperthermic treatments are usually administered in multiple "doses". Therefore, modification by heat of the cells' subsequent heat sensitivity ("thermotolerance") becomes an important clinical consideration. In the treatment of cancer, hyperthermia is almost invariably combined with the more traditional approaches of radiation or chemotherapy. Hence, the possible modification by the heat-induced stress response of the cells' sensitivity to X-rays or to drugs needs to be considered.
The converse (i.e., the modification of hyperthermic sensitivity by a drug-induced stress response) may also be of importance. For example, steroids are frequently administered to cancer patients, and it is known that these can induce thermotolerance. Curiously, no studies have examined the very distinct possibility that the steroid-induced stress response can also induce resistance to drug therapy.
Clearly, the possible unintentional induction of stress responses during chemotherapy (or by chemotherapy itself) is an area of investigation of considerable importance, but one that has received very little attention by oncologists and cell biologists.

Supported in part by NIH grant CA-19386.

P 030 EVENTS IN HEAT-SHOCKED AVIAN FIBROBLASTS. Milton J. Schlesinger, Nancy Collier, Ursula Bond and Neus Agell., Dept. Microbiol. & Immunol. Washington University Medical School, St. Louis, MO 63110. In addition to the induction of heat shock proteins, primary cultures of chicken embryo fibroblasts that have been stressed by a temperature (45°C, 1hr) or chemical agent (100μM arsenite) show a variety of changes in their metabolic activity and their morphology. Under our conditions of stress, protein synthetic capacity decreases very little (<20%) whereas DNA synthesis virtually stops and total RNA synthesis decreases by >50%. Processing of rRNA and splicing of mRNA are inhibited with prolonged stress. Neither the intracellular pools of ATP and phosphocreatine nor the intracellular pH and calcium levels change significantly. The free ubiquitin pool decreases slightly with a concomittant increase in the level of ubiquitin conjugates; however, one specific ubiquitin conjugate, U-H2A, loses its ubiquitin almost immediately after the stress begins. Both ubiquitin pools rise during prolonged stress. Turnover of protein does not change during the stress period but increases 2-fold immediately upon removing cells from stress. The intermediate filament(IF) network collapses shortly after stress is imposed but the microfilaments and microtublin system are intact. IF returns to normal after removal of stress but not if actinomycin D is present during stress, thus implicating a need for heat shock protein(s) in recovery of IF. Upon prolonged stress or during a restress, the small heat shock protein, HSP 24, aggregates into huge perinuclear granules that appear to consist almost entirely of HSP 24.

Induction of the Heat Shock Response; Chromatin Structure and Transcription of Heat Shock Change; Genetic Regulation of Heat Shock Response

P 100 A FRESH LOOK AT THE HEAT SHOCK REGULATORY SEQUENCE, Jahanshah Amin, Jayakumar Ananthan and Richard Voellmy, Dept. of Biochemistry and Molecular Biology, University of Miami School of Medicine, Miami, Florida 33101

One of two regions (between -45 and -65) containing essential heat shock regulatory elements (each region includes a heat shock consensus sequence) in a Drosophila hsp70 - -galactosidase hybrid gene with a 90 bp-long hsp70 promoter segment was mutated. The activity of genes with mutant promoters was assessed by measuring -galactosidase levels produced by these genes in transfected, heat-treated Drosophila cells. Some mutations were found to render the hsp70 promoter inactive even though they were found to render the shp70 promoter inactive even though they were outside of the consensus sequence element, CNNGAANNTTCNNG. Additional experiments strongly suggested that a functional heat shock regulatory element consisted of at least three GAA blocks that were arranged at two-nucleotide intervals and in alternating orientations. Arrangements with a one block gap were functional provided that flanking GAA blocks were in phase and appropriately oriented. The influence of nucleotides at positions between GAA blocks on the competence of the regulatory sequence will also be discussed.

Supported in part by State of Florida High Technology Council

P 101 HEAT SHOCK TRANSCRIPTION FACTOR PREFERENTIALLY BINDS TO SUPERCOILED DNA IN VIVO, Jayakumar Ananthan, Jahanshah Amin, Duri Rungger and Richard Voellmy, University of Miami School of Medicine, Miami, Florida 33101.

DNA topological requirements for the various events leading to transcription initiation of eukaryotic genes are being widely discussed. Binding of heat shock transcription factor to heat shock regulatory sequences constitutes a distinct step in transcription initiation of heat shock genes. We have singled out and investigated this critical step using a tagged Drosophila hsp70 (heat shock) gene microinjected into Xenopus oocytes. Expression of this gene was totally inhibited when a plasmid containing isolated multiple heat shock regulatory elements devoid of other heat shock gene sequence was co-injected with the tagged test gene in a supercoiled but not in linear form. This suggests that heat shock transcriptional factor binds preferentially to supercoiled templates in vivo.

P 102 THE YEAST PRT1 GENE AFFECTS HEAT SHOCK PROTEIN PRODUCTION. C. A. Barnes, G. C. Johnston and R. A. Singer, Faculty of Medicine, Dalhousie University, Halifax, N.S., Canada B3H 4H7.

Mutations in the PRT1 gene of Saccharomyces cerevisiae affect both the regulation of cell proliferation (1) and protein synthesis (2,3). At appropriate non-permissive temperatures prt1 mutant cells show regulated arrest of cell proliferation with only moderate inhibition of global protein synthesis (1). Under these same conditions synthesis of two polypeptides was greatly decreased. The two polypeptides differentially underproduced in prt1 mutant cells have mobilities similar to those of the two major yeast heat shock proteins HSP90 and HSP70. Quantification of rates of synthesis by pulse labelling and immunoprecipitation of lacZ gene-fusion polypeptides showed that one of these proteins underproduced in prt1 mutant cells is related to a yeast HSP70 protein.

(1) Hanic-Joyce, Johnston and Singer (1987) Exp. Cell Res. 172: 134-145.
(2) Hartwell and McLaughlin (1968) J. Bacteriol. 96: 1664-1667.
(3) Feinberg, McLaughlin and Moldave (1982) J. Biol. Chem. 257: 10846-10851.

P 103 CONTRIBUTION OF CONSERVED AND NONCONSERVED BASES IN THE TATA-PROXIMAL HSE OF THE PROMOTER OF SOYBEAN HEAT SHOCK GENE *Gmhsp17.5E*. Dulce Barros, E. Czarnecka and W. B. Gurley. Microbiology and Cell Science, Univ. of Florida, Gainesville, FL 32611. The TATA-proximal heat shock element (HSE) of the soybean heat shock gene *Gmhsp17.5E* matches the *Drosophila* consensus (5'-CT-GAA--TTC-AG-3') in nine of the specified bases. Oligonucleotide site-directed mutagenesis was applied to introduce single base changes in the HSE in order to assess the contribution of each nucleotide to the overall activity of the HSE and to identify the optimum HSE configuration for higher plants. Twenty two mutations have been made and the mutated HSEs were inserted into the promoter of *Gmhsp17.5E* and were cloned into a T-DNA-based double reference vector. The contribution of the mutated bases was assessed in sunflower tumors. We have observed that mutations in both conserved and nonconserved positions of the *Drosophila* consensus have a significant effect (positive and negative) on the thermoinducibility of the promoter. These results suggest that each position in the fourteen bp that comprise the consensus may contribute to the activity of the HSE.

P 104 REGULATION AND CHROMATIN STRUCTURE OF DROSOPHILA HSP70 SEQUENCES IN AN SV40-BASED MINICHROMOSOME. P. Beard[1], W. de Bernardin[2] and H. Bruggmann[1]. [1]Swiss Institute for Experimental Cancer Research, 1066 Epalinges/Lausanne, Switzerland and [2]Institute for Cell Biology, ETH, 8093 Zürich, Switzerland.

We constructed a substituted SV40 (SVHS6) in which the early region is replaced by a DNA segment containing the drosophila hsp70 promoter with some coding sequence. This virus grows in COS cells and allows us to isolate the hsp70 in the form of chromatin. The hsp70 promoter in SVHS6 is induced by shifting infected cells to 43°C.

The superhelical density of extracted SVHS6 DNA is similar to that of wild-type SV40 implying that the corresponding chromosomes have a similar number of nucleosomes per length of DNA. Crosslinking SVHS6 chromosomes with psoralen and observing the purified DNA under denaturing conditions in the electron microscope leads to the same conclusion.

SVHS6 DNA extracted from infected cells shifted to 43°C had about one extra negative superhelical turn than control DNA from cells at 37°C. This result implies that, for the bulk of SVHS6 chromosomes, there is no great overall change in the nucleosomal structure on shifting to 43°C, at least as reflected in DNA superhelicity. To look specifically at the transcribed SVHS6 chromosomes, electron microscopy after psoralen crosslinking, in which both nucleosome positions and nascent RNA can be visualised, will be used.

P 105 PRODUCTS OF OXYGEN REDUCTION AS HEAT SHOCK PROTEIN INDUCERS Anne-Marie Courgeon, Michèle Ropp, Emmanuelle Rollet, Jacqueline Becker, Claude Maisonhaute and Martin Best-Belpomme, UA CNRS 1135 et Université Paris VI, F-75005 Paris.

Reoxygenation of Drosophila cells following a 24h period of anaerobiosis was shown to induce a twofold increase of oxygen consumption and the synthesis of heat shock proteins (hsps). As this combination of oxygen deprivation followed by an excess generates an over-production of products of oxygen reduction (superoxide ion, hydrogen peroxide ...) we suggested that these highly reactive products, related to many diseases, could play a role in the mechanism of hsp induction (1).

In order to test this hypothesis, we recently treated Drosophila cells with exogenous hydrogen peroxide (H_2O_2, 1 mM). H_2O_2 was shown to induce the synthesis of some hsps (hsps 70-68 and hsp 23) and an important increase of the synthesis of actin. The corresponding genes were transcriptionally activated within ten minutes of treatment.

(1) Ropp, M., Courgeon, A.M., Calvayrac, R. and Best-Belpomme, M. (1983) Can. J. Biochem. Cell. Biol. 61, 456-461.

P 106 STRESS INDUCED PROTEINS IN RICE, Chumpol Borkird and Marc van Montagu, Laboratorium voor Genetika, Rijksuniversiteit Gent, Ledeganckstraat 35, Gent 9000, Belgium.

Plants in natural environments daily encounter many different forms of stress including high and low temperatures, acidity and basicity of soils, anaerobic conditions from flooding, changes in metal compositions in the environments etc.. Plants have to endure these stresses by physiological adaptation governed by changes in gene expression. We are investigation these changes in gene expression of rice cells challenged with different stresses including high and low temperatures (42 and 4 degree C), high and low pH of growth medium (pH 9 and pH 4), low water availability (20% polyethyleneglycol) and toxic levels of iron. We found that these stresses induced characteristic sets of proteins revealed on one and two-dimensional gels. Three heat-shocked proteins are induced by other stresses such as high levels of iron or high pH environments. Stress adapted rice cell lines capable of growth under stress conditions have been isolated and their protein profiles on two-dimensional gels are being analyzed. Our laboratory has developed protein blotting and amino acid sequencing systems (Bauw et al.,1987. Proc.Natl.Acad.Sci.USA. 84:4806-4810) which yield the N-terminal amino acid sequence of proteins detectable on two-dimensional gels. These techniques will be used to identify the N-terminal amino acid sequences of stress-induced proteins in rice. The corresponding genes will be isolated using oligonucleotide probes deduced from the amino acid sequences. The functional significance of these genes will be assessed in transgenic plants.

P 107 A SET OF *ESCHERICHIA COLI* GENES INDUCIBLE BY HEAT, ETHANOL, AND BACTERIOPHAGE f1. Janice L. Brissette, Tracy L. Ripmaster, and Peter Model. The Rockefeller University, New York, NY 10021.

Morphogenesis of the filamentous phage f1 requires the participation of phage gene products I and IV, as well as certain bacterial gene products such as thioredoxin. Although these two phage-encoded proteins are essential for f1 assembly, they are not found in the mature virus particle. We have been studying gene IV protein (pIV) in an effort to define its role in phage morphogenesis. We have cloned gene IV and localized its product to the membrane and periplasm. During the course of our experiments with the cloned gene, we noticed that gene IV expression induced the synthesis of a previously unidentified bacterial cellular protein (phage shock protein, Psp25; Mw = 25 Kdal). We then prepared antibodies to Psp25 and further characterized the induction process. Psp25 is a soluble protein and its induction by pIV, a membrane protein, requires a functional gene IV protein. Psp25 is also induced by heat and ethanol, but it is not a conventional heat shock protein. More extreme conditions are required, and this induction is independent of the heat shock sigma factor (σ^{32}). Recently, we cloned the gene encoding Psp25 from an *E. coli* library using a degenerate oligonucleotide (17mer) probe deduced from the protein sequence. This clone expresses Psp25, as well as two other proteins (16K and 12K) upon induction. The induction is at the level of transcription. Mapping, sequencing and deletion analysis are currently in progress. These studies may provide insight into the mechanism of induction of these stress-induced proteins.

P 108 REGIONAL DIFFERENCES IN HEAT SHOCK GENE EXPRESSION IN THE RABBIT BRAIN Ian R. Brown and Geoffrey.K. Sprang, Dept of Zoology, Univ of Toronto, Scarborough Campus, West Hill, Ontario, Canada M1C 1A4

Elevation of the body temperature of adult rabbits to levels similar to that attained during fever induces the rapid synthesis of a 74K hsp in brain tissue. Northern blot analysis of brain RNA isolated 1 hr after a 2°C increase in body temperature reveals the massive induction of a 2.7 kb heat shock mRNA. In control animals the presence of a 2.5 kb constitutively expressed mRNA is apparent. In situ hybridization was employed to determine whether regional differences exist in the expression of members of the 70K gene family. If the ability of cells to survive heat shock and other stresses is related to their capacity to induce hsps, an analysis of the pattern of expression of heat shock genes in the brain may further understanding of the selective vulnerability of certain brain cells to various forms of trauma and neurological diseases. Striking regional differences in the expression of constitutive and inducible heat shock genes are apparent in the rabbit brain. Constitutive expression was observed in neuronal enriched layers of the hippocampus and cerebellum. One hr following hyperthermia, fibre tracts throughout the forebrain show a dramatic induction of heat shock mRNA, a pattern consistent with a strong glial response to heat shock. In the cerebellum induction of heat shock mRNA was observed in large Purkinje neurons, the granule cell layer and in glial cells in the deep white matter. Induction of hsps in glial cells of fibre tracts and rapid export of these proteins into adjacent axons could provide a 'fast response' mechanism to deliver hsps to distal regions of neurons in the mammalian brain. (Supported by MRC, Canada)

P 109 NEW PHENOTYPES OF A ΔdnaK-MUTANT OF Escherichia coli. Bernd Bukau and Graham C. Walker, M.I.T., Cambridge, MA 02139.

The DnaK protein is one of the major heat shock proteins in E. coli. Previously reported phenotypes of dnaK-mutants suggested an essential role for DnaK in several cellular processes after heat shock, including DNA- and RNA-synthesis, cell division, and adaptation to heat shock. Here we report new phenotypes of a ΔdnaK-mutant (Paek and Walker, 1987, J. Bacteriol. 169:283) at low temperatures, and the isolation of secondary mutations which suppress certain phenotypes of the ΔdnaK-mutation. Upon introduction of the ΔdnaK-allele into the chromosome of strain MC4100 by P1-transduction at 30°C, transductants formed pale colonies containing strongly filamenting cells. In each ΔdnaK-transductant colony papillae appeared that contain cells with a reduced filamentation phenotype. These cells contained a secondary mutation which is unlinked to dnaK. Filamentation of the ΔdnaK-transductants could also be suppressed by the presence of a multicopy plasmid that encodes the ftsZAQ-proteins, implying that DnaK might interact directly or indirectly with the regular, FtsZAQ-dependent cell division machinery. Filamentation of cells was also observed when the dnaJ259 mutation was crossed into the chromosome or when DnaK and DnaJ were strongly overproduced in wild-type cells. We observed that ΔdnaK-mutants isolated at 30°C were unable to grow at 16°C and at 43°C. In the permissive temperature range (20°C to 37°C), fresh ΔdnaK-transductants were always strongly filamenting, until a secondary mutation reduced filamentation, as described above. Thus, the DnaK protein broadens the temperature range for growth of E. coli and is involved in cell division at all temperatures.

P 110 HEAT SHOCK CAUSES TURNOVER OF POLYPHOSPHOINOSITIDES AND INCREASES INTRACELLULAR FREE CALCIUM IN MAMMALIAN CELLS.

S.K. Calderwood, M.A. Stevenson, E.K. Farnum, L. Mannheim, G. Jansen and C.R. Hunt, Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115.

Heat shock causes a rapid increase in intracellular free calcium in eucaryotic cells. The increase is correlated with rapid (within 30 sec) turnover of phosphoinositides (PI) and production of inositol trisphosphate (IP_3) and diacylglycerol. The response is observed in human, rat, mouse and hamster cells and resembles effects induced by growth factors such as serum, thrombin and PDGF. In digitonin-permeablized cells, heat shock causes Ca^{++} release from a non-mitochondrial internal pool through production of Ca^{++} mobilizing compound IP_3. Heat shock-induced release of IP_3 is dependant on GTP, suggesting a role of G proteins in the response. We are currently assessing the role of these changes in transmembrane signalling pathways in expression of the heat shock response.

P 111 STRESS INDUCED POLYAMINE OXIDATION, Steven W. Carper, David J.M. Fuller, Paul M. Harari, Janet Matuszek and Eugene W. Gerner, University of Arizona, Department of Radiation Oncology and Cancer Center, Tucson, Arizona 85724

In mammalian cells, heat shock induces the enzyme spermidine N^1-acetyltransferase (SAT), the first enzyme in polyamine catabolism. This induction is inhibited by cycloheximide, but not actinomycin D. Inducibility of SAT by heat stress or certain chemicals which sensitize cells to the cytotoxic effects of hyperthermia, such as diethyldithiocarbamate, is dependent on intracellular spermidine content as SAT is not induced in spermidine depleted cells. Since SAT is not expressed in unstressed cells which have high spermidine levels but is induced in polyamine depleted cells when endogenous pools are restored by exogenous putrescine, the induction of this enzyme apparently occurs when stresses alter normal cellular polyamine compartmentalization. In prokaryotes, cold shock can also induce this enzyme. When E. coli are placed on ice, a significant amount of their spermidine pool is acetylated by SAT. Since polyamines occur at high intracellular concentrations (near millimolar) in proliferating cells, spermidine acetylation utilizes large amounts of acetyl CoA, as the acetate donor, and produces significant quantities of hydrogen peroxide, since N^1-acetylspermidine is efficiently oxidized by the constituitively expressed enzyme, polyamine oxidase. Thus, polyamine oxidation occurs in both pro- and eukaryotes in response to a variety of physical and environmental stresses, and can affect several metabolic processes in ways which are potentially deleterious to the cell.

P 112 IDENTIFICATION OF NUCLEAR FACTORS THAT INTERACT WITH REGULATORY SEQUENCES WITHIN THE SOYBEAN HEAT SHOCK GENE *Gmhsp17.5E* PROMOTER. Eva Czarnecka, P. C. Fox and W. B. Gurley, Univ. of Florida, Gainesville, FL 32611. We have previously localized regulatory domains of the *Gmhsp17.5E* heat shock promoter of soybean by 5' and internal deletion analysis. Factors were found in crude nuclear extracts of soybean plumules that bind *in vitro* specifically to the *cis*-regulatory elements of *Gmhsp17.5E* promoter. Gel retardation assays and DNase I footprinting revealed a large number of interactions with the *Gmhsp17.5E* promoter proximal and distal domains consistent with observations that 179 nucleotides of the proximal domain contributed 50% of the promoter activity and the remaining 50% was due to the distal domain (-1175 to -180). The analysis was simplified by employing oligomer probes as well as by using partially fractionated extracts. We have identified seven to nine sites of DNA:protein interactions which correspond to regions previously shown by mutational analysis to be critical for activity. We have evidence for at least two discrete factors, heat shock transcription factor (HSTF) and AT binding factor (ATBF). The total binding activities for each of these factors increased in crude extracts prepared from heat shocked soybean plumules as compared to activity in control (28°C) extracts. Competition binding assays demonstrated that HSTF binding to the heat shock element consensus (HSE) was strongly dependent on the degree of homology to the *Drosophila* HSE and was distinct from the ATBF binding site.

P 113 MUTATIONS WHICH SUPPRESS THE COLD SENSITIVE PHENOTYPE DUE TO OVEREXPRESSION OF THE E. coli umuDC OPERON, Caroline E. Donnelly, Christine C. Dykstra and Graham C. Walker, M.I.T., Cambridge, MA 02139.
Overexpression of the SOS inducible umuDC operon of E. coli results in the inability of these cells to grow at 30°C (Marsh and Walker, 1985; J. Bacteriol. 162:155-161). Mutations in seven heat shock genes (htpR, dnaK, lon, dnaJ, grpE, groEL and groES) suppress cold sensitivity. In addition a series of Tn5 generated insertion mutations also suppress this phenotype. One of these suppressors (lon) appears to lengthen the half life of a defective LexA repressor. This results in repression of the umuDC operon at 30°C. Other suppressor mutations (htpR, groEL and groES) do not appear to act at this level because these mutations repress cold sensitive growth in a lexA null background. All of the Tn5 generated cold sensitive suppressor mutations (css::Tn5) produce a 25,000 dalton protein which is not seen in wild type cells or the heat shock mutants. We feel that the overexpression of this protein may be responsible for suppression of cold sensitive growth. We have cloned sequences of the E. coli genome which when present on a high copy plasmid allow the umuDC overexpressing strain to grow at 30°C. One of these clones produces a 25,000 dalton protein which migrates on a 2 D gel at a position similar to that of the 25,000 dalton protein produced by the css::Tn5 mutants.

P 114 DIBROMOPROPAMIDINE INDUCTION OF THE STRESS RESPONSE IN E. COLI, Christine C. Dykstra, National Institute of Environmental Health Sciences, Research Triangle Park, NC 27709.
Dibromopropamidine (DBP) is an aromatic diamidine used as an anti-bacterial and anti-protozoal drug. It is also an inhibitor of trypsin-like proteases. We have found that DBP induces the stress response in E. coli in an rpoH-dependent manner. In addition, DBP causes filamentation in a lexA-independent, sulA-dependent manner. Mutations in lon affect the extent of filamentation. These results support the connection between the stress response and the regulation of protein turnover. A more specific protease inhibitor, BABIM, also induces the stress response and causes filamentation, but the filamentation is not affected by mutations in lon. Other diamidines with different specificities are now being tested in order to separate different pathways that regulate the stress response via protein stability mechanisms.

P 115 IN VIVO POINTS OF PROTEIN-DNA CONTACT WITHIN THE PROMOTER REGION OF THE YEAST HSP82 GENE. David S. Gross,[+] Kerry W. Collins,[+] and William T. Garrard,[*] [+]Dept. Biochemistry & Molecular Biology, Louisiana State University Medical Center, Shreveport, LA 71130, and [*]Dept. Biochemistry, University of Texas Health Science Center, Dallas, TX 75235.

We report here the identification of regulatory protein contacts within the promoter of a heat-shock inducible gene in *Saccharomyces cerevisiae*, *HSP82*. We have subjected yeast cells to the *in vivo* dimethyl sulfate (DMS) footprinting procedure of Church and Gilbert (1984), and have assessed the extent to which the N-7 moiety of guanines, which resides in the major groove of DNA, is accessible to methylation. In both basal and transcriptionally induced states, we find evidence for either partial or complete methylation protection at seven guanine residues within the primary heat shock element (HSE), spanning -161 to -174 relative to the transcription start site. The spacing of these protected sites, which occur on both upper and lower strands, suggest that the yeast heat shock transcription factor (HSTF) engages in major groove interactions largely on one face of the double helix. The constitutive binding of HSTF to its cognate sequence element is in agreement with a recent report from Pelham and co-workers (1987) that HSE binding activity is present in nuclear extracts from both control and heat-shocked yeast cells. Notable by its absence is any evidence for binding of the TATA box (at position -80) by its transcriptional factor using the DMS *in vivo* methylation technique, suggesting, perhaps, that the TATA factor interacts with DNA within the minor groove and/or along the phosphate backbone. Current experiments using a newly described footprinting reagent, Iron(II)·EDTA, should help clarify this.

P 116 PREFERENTIALLY ENHANCED SYNTHESIS OF HSP90 AND A MEMBER OF THE HSP70 FAMILY IN MITOGEN ACTIVATED HUMAN T CELLS. Linda K. Hansen, Robert N. Haire and James J. O'Leary, University of Minnesota, Minneapolis, MN 55455.

Mitogen activation of quiescent peripheral blood T cells preferentially enhances the synthesis of HSP90 and a constituitively synthesized member of the HSP70 stress protein family. The 73kD protein, P73, appears identical to P72 (Morimoto) or HSC70 (Pelham) and the constituitively expressed clathrin uncoating ATPase. In addition, we find evidence on 2-D gel electrophoresis of two distinct HSP90 proteins of identical pI's and slightly different M_r that are equally induced by either PHA or heat shock. Maximal synthesis of HSP90 and P73 is achieved relatively late in G0/G1 (12-18 hours) and is sustained as the cells enter S phase. Mild heat shock (42° for 20') induces HSP90, P73, and HSP70 in human T cells, but has no effect on subsequent proliferative response. At the higher heat shock temperature the proliferative response is inhibited, and HSP90 synthesis is suppressed relative to HSP70, consistent with observations that HSP90 has introns. Intriguingly, the synthesis of HSP90 may also be suppressed relative to HSP70 after the 42° heat shock in lymphocytes from older human donors (greater than 75 years), suggestive of a defect in mRNA processing with aging. PHA activation in the G0/G1 interval may make T cells more tolerant to a stronger heat stress (45° for 15'). Preliminary studies of expression of the mRNA for these proteins after mitogen activation appear consistent with mitogen induced enhancement in gene activity for HSP90. Supported by NIH grants AG02338 and CA39692.

P 117 INDUCIBLE EXPRESSION OF CDNAS IN A VECTOR BASED UPON THE MOUSE HSP70 HEAT-SHOCK PROMOTER, Clayton R. Hunt and Stuart K. Calderwood, Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115.

Mouse cells heat-shocked at 43 C. induce transcription of at least two HSP70 RNAs 3.1 and 2.7 kb in size. In N.I.H. 3T3 cells and most other mouse cell lines there is no basal HSP70 synthesis, therefore the relative magnitude of induction is quite large. The DNA sequence controling the transcriptional activation of the 3.1 kb mRNA has been isolated, characterized, and used to construct a cDNA expression vector. The vector promoter consists of 800 bp from the mouse HSP70 gene cloned into the Eco R1 site of pUC18. This fragment contains all the transcription control sequences as well as an untranslated leader sequence encoded by the HSP70 gene. A 3' noncoding segment from a human HSP70 gene has been cloned downstream of the mouse promoter. This fragment contains polyadenylation and transcription termination signals. Between the 5' and 3' sequences are unique cloning sites for Sac 1,Xma 1, and BamH 1. When a cDNA encoding the polyoma middle T antigen was cloned into the BamH 1 site, efficient production of mRNA was obtained by heat-shock while no synthesis was observed in control cells. Western blot analysis indicated the mRNA was effectively translated into cellular protein. A DNA cassete containing the 5' signals-cloning sites-3' signals has been transferred to a retrovirus and is currently being tested as a vector for gene transfer into cultured cells or animals.

P 118 STRESS ACTIVATES G PROTEINS. G.J. Jansen, M.A. Stevenson, L. Mannheim, C.R. Hunt and S.K. Calderwood, Joint Center for Radiation Therapy, Harvard Medical School, Boston, MA 02115.

Heat shock has been shown to activate a number of signal transduction pathways known to be regulated by guanine-nucleotide binding (G) proteins. These include the adenylate cyclase and phospholipase C and A2 pathways. The usual primary step in these pathways is the binding of a ligand to its membrane receptor, which induces a conformational change in associated G proteins. Previous work in our lab (Calderwood et al 1986) has shown that the presence of added GTP, chemical (e.g. vanadate or arsenate) or physical stress (hyperthermia) can activate the PI cycle, bypassing receptor interaction. To determine if proteins can be activated by chemical or physical stress, we have synthesized two photoaffinity probes, ^{32}P-GDP-azidoanilide and ^{35}S-GTP-azidoanilide. Our data indicates both physical and chemical stress activate G proteins and furthermore that activation does not appear to be uniform, but is dependent on both the quantity and type of chemical inducer. In both heat-shocked and chemically-stressed cells proteins of molecular weight 69, 55 and 46 kDa became labelled.

P 119 CONTROL OF HSP_{70} GENE EXPRESSION BY AMPLIFICATION OF ITS PROMOTER SEQUENCE, R.N. Johnston, University of Calgary, Calgary, AB, Canada T2N 1N4

We have tested the concept that varying the availability of *trans*-acting regulatory proteins may regulate the expression of endogenous cellular genes. To vary the availability of these proteins, we have used an indirect method whereby highly amplified promoter sequences, to which the regulatory proteins bind, are used to titrate out the proteins. The test system we have used in these studies is that of the major heat shock protein, HSP_{70}. The heat shock regulatory element for HSP_{70}, with four binding sites for the heat shock transcription factor (HSF), was cloned into a vector that includes the gene for dihydrofolate reductase. The hybrid vector was used to transfect Chinese hamster cells that had previously been rendered *dhfr-*, thereby restoring them to a *dhfr+* phenotype. The chemotherapeutic drug methotrexate was then added to the cells, resulting in new populations that had coamplified the *dhfr* gene (thereby providing methotrexate resistance) along with the heat shock regulatory element. Several transfected lines have now been obtained with varying degrees of amplification of these sequences. In one line, designated CHO DH(f), the degree of amplification is approximately 10,000 fold. When cells of this line are heat shocked, the normal induction of synthesis of HSP_{70} is prevented and greater than 99.9% of the cells die, whereas control unamplified cells survive the heat exposure. These observations are consistent with a reduction in availability of the HSF, and a consequent reduction in inducibility of the endogenous HSP_{70} gene. Amplification of gene control sequences provides a novel method for elucidating genetic control pathways, and for modulating the expression of eukaryotic genes and testing their function. In this instance, we have demonstrated a functional relationship between the acquisition of thermotolerance in a higher eukaryote and the synthesis of the HSP_{70} protein.

P 120 HEAT SHOCK STIMULATES THE EXPRESSION OF THROMBOSPONDIN BY ENDOTHELIAL CELLS IN CULTURE, N.V. Ketis, J. Lawler* and M. J. Karnovsky. Department of Pathology, Harvard Medical School, Boston, MA 02115; and *St. Elizabeth's Hospital, Department of Biomedical Research and Medicine, Division of Hematology/Oncology, Boston, MA 02135.

Heat-shock proteins of endothelial cells from different origins were analyzed by SDS polyacrylamide gels and were found to include 28,000, 71,000, 73,000, 89,000, 90,000 and 100,000 D polypeptides. In addition to the increased synthesis of the classical heat-shock proteins, there is an increase of a 180,000 D polypeptide in the growth media of the heat-shocked cells. Immunoprecipitation with specific antiserum indicates that the 180,000 D polypeptide is thrombospondin. Assay of mRNA levels coding for thrombospondin after brief hyperthemic treatment (45°C. ten minutes), followed by a recovery of two hours at 37°C resulted in about a 2-fold increase in mRNA abundance. In contrast, there was a 50% decrease in mRNA abundance for fibronectin and actin mRNA levels remained unaltered. Analysis of the 71,000 D heat-shock protein (HSP71) gene revealed that the abundance of the mRNA is greatly increased (i.e. many fold). However, the activation level of the HSP71 mRNA occurred at an earlier time than that of the thrombospondin mRNA. In addition, marked differences in intracellular and extracellular distribution of thrombospondin was noted in endothelial cells exposed to hyperthermia. No gross morphological changes in extracellular matrix staining of fibronectin was noted. However, the vimentin cytoskeleton collapsed around the nucleus after heat shock. We therefore conclude that the expression of thrombospondin is heat-shock stimulated.

P 121 TRANSMISSION OF BASAL AND REGULATORY SIGNALS IN A HUMAN hsp70 PROMOTER. John M. Greene and Robert E. Kingston. Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114 and Department of Genetics, Harvard Medical School. We have used a genetic approach to explore interactions between five promoter elements that are responsible for basal and heat regulated transcription of a human hsp70 gene. Four of these elements lie within 110 bases of the hsp70 transcription start site: a CCAATC box in inverted orientation at -67, a GC-rich element strongly resembling an SP1 binding site at -49, a TATA box at -28, and a heat shock element (HSE) at -106. Analysis of mutant promoters containing various combinations of these four elements demonstrates that they function independently in a multiplicative manner to stimulate transcription: the strength of any promoter containing more than one mutation can be accurately predicted by multiplying the effects of each individual mutation. Our data imply that these four elements all alter the same rate-limiting step of transcription, and show that proper levels of heat-induced transcripts from this promoter require not only the HSE, but the three proximal basal elements as well. A distal element, consisting of sequences between -1250 and -120, stimulates basal expression of this promoter five to ten-fold in human cell lines. We demonstrate that, in contrast to the HSE, this distal element functions only when the TATA element is intact. Thus, while the proximal elements can each directly and independently stimulate expression from this promoter, the distal element requires a TATA element to transmit its effect. We suggest that a general function of the mammalian TATA element is to mediate action of distal signals. Work is in progress to determine if function of the HSE can be made dependent upon a proximal element(s) by translocation to a distal location.

P 122 GroE: KEY HEAT SHOCK PROTEINS REQUIRED FOR CELL GROWTH OF ESCHERICHIA COLI, Noriko Kusukawa, Takashi Yura, Institute for Virus Reseach, Kyoto University, Kyoto 606, Japan.
The rpoH null mutant that lacks sigma 32 protein is capable of growing only at extremely low temperatures, i.e. bellow 20C, and produces trace amounts of heat shock proteins at steady-state growth and during heat shock (Zhou et al in prep). This indicates that a substantial amount of certain heat shock protein(s) is required for cellular growth at normal and high temperatures. To examine the relation between growth temperatures and the requirements of specific heat shock proteins, we isolated a series of temperature resistant revertants from ΔrpoH cells. Most of the revertants produced GroE proteins in higher quantities than their ΔrpoH parent; the level of production was well correlated to the upper limit of growth temperature for each strain (30-40C). In agreement, ΔrpoH strains that carry groE plasmids also were capable of growing at 37C. Thus, GroE seems to be the most required heat shock protein for supporting cellular growth at a wide range of temperatures (>20-40C). Analysis of two-step revertants, and of ΔrpoH strains carrying dnaK and/or groE plasmids, suggested that the other major heat shock protein DnaK, is active primarily at much higher temperatures (>37C).

P 123 HEAT-INDUCIBLE BINDING OF HUMAN HSF TO THE HEAT SHOCK ELEMENT IN VITRO AND PURIFICATION OF THE FACTOR FROM HEAT SHOCKED HELA CELLS. Jeffrey S. Larson, Thomas J. Schuetz, and Robert E. Kingston. Department of Molecular Biology, Massachusetts General Hospital, Boston MA 02114 and Department of Genetics, Harvard Medical School. A protein that binds the HSE, the regulatory sequence element sufficient for heat shock induction, has been identified in several species, and in drosophila has been shown to function as a transcription factor. The ability of this protein to bind to the HSE is heat inducible in drosophila and human cells by a mechanism that involves post-translational modification. We show here that we can activate the HSE-binding capabilities of a human factor by heating a HeLa cytoplasmic extract. Denatured protein, previously suggested to be the trigger for the heat shock response, does not alter the rate or extent of activation of the HSE binding activity in vitro. Instead, the data suggest that activation of the human HSE-binding factor requires at least two components, one of which directly senses an increase in temperature. This in vitro system should allow dissection of the precise mechanism by which human cells respond to heat and provide a model for understanding the biochemical changes that result in activation of human transcription factors. We are studying this mechanism by another approach; we have purified the activated factor from heat shocked HeLa cells by sequence specific affinity chromatography. The major band identified by SDS-PAGE has an apparent molecular weight of approximately 85 kilodaltons. We are in the process of characterizing this factor.

P 125 DEFICIENT ACTIVATION OF HEAT SHOCK GENE TRANSCRIPTION IN EMBRYONAL CARCINOMA CELLS, Valérie Mezger, Olivier Bensaude and Michel Morange, Institut Pasteur, 75724 Paris Cedex 15, France.

Heat shock protein (HSP) synthesis cannot be induced by stress in the cleavage stage embryos of many different species. For instance, no HSP synthesis can be induced in the mouse embryo before the formation of the blastocyst. Similarly, HSP synthesis is not stress inducible in some embryonal carcinoma (EC) cell lines such as PCC4 and PCC7-S-1009 (1009). We show that RNAs coding for the major stress inducible murine heat shock protein, HSP68, do not accumulate in PCC4 and 1009 EC cells in response to a stress. Using an in vitro nuclear transcription assay, we demonstrate that the transcription of the corresponding genes is not activated after a stress. A specific gene switch-off due to DNA methylation or chromatin conformation is unlikely to account for this result. Indeed, stress does not promote the activation of the heterologous Drosophila HSP 70 heat shock promoter in transfection assays of these cells. In contrast, the same promoter, like endogenous HSP synthesis, becomes stress-inducible in 1009 cells after in vitro differentiation. This suggests that, in contrast to differentiated cells, these EC cells, and maybe the very early mouse embryonic cells, could lack a trans-acting activating transcription factor or contain a repressor. DNA-protein complexes of Drosophila HSP70 or mouse HSP68 promoter with crude nuclear or total cellular extracts are presently analysed by gel shift assays.

P 126 HEAT SHOCK GENE EXPRESSION IN RICE EMBRYOS UNDER ANOXIC CONDITIONS, Bernard Mocquot, Bérénice Ricard and Alain Pradet, Station de Physiologie Végétale, INRA, BP 131, 33140 Pont de la Maye, France.

Heat shock proteins (hsps) are induced by a number of oxidative stresses. It has been suggested that the reduction products of oxygen could initiate hsp induction. We have tested this proposal in rice embryos, capable of coleoptile growth under oxygen-free conditions.
In such embryos, hsps have been detected by both *in vivo* labeling, *in vitro* translation of RNA using the reticulocyte lysate system, and by DNA-RNA hybridization.
Our results show for the first time that the hsps can be induced in plant tissues in the lack of oxygen. For rice, at least, the mechanism for hsp induction does not involve oxygen.

P 127 TRANSCRIPTION FACTOR SP1 BINDS TO AND ACTIVATES A HUMAN HSP70 GENE PROMOTER. William D. Morgan, McGill University, Montreal, Canada H3A 1B1.

Transcription of a human gene encoding the major 70 kilodalton heat shock protein hsp70 has been shown to respond to processes such as serum stimulation, activation by nuclear oncogenes, and cell cycle control, as well as heat shock. We investigated binding of transcription factor Sp1, purified to homogeneity from HeLa cells, to this hsp70 promoter by DNAase I footprinting. Three binding sites were detected within the upstream promoter region, including one located between the TATA-box and CCAAT-box elements. In vitro transcription demonstrated that the proximal site, centered at -46, is capable of responding to stimulation by purified Sp1. A linker-scanning mutant in the GC-box did not bind Sp1 and had reduced transcription activity in crude nuclear extract. Footprinting of the proximal promoter domain with both Sp1 and CTF showed that these factors can bind simultaneously, although the recognition sites are closely spaced and the protected regions overlap substantially. Although Sp1 alone has some transcriptional effect, CTF seems to be required for maximal activation. These results suggest that Sp1 can contribute to constitutive expression in vivo, and might also be involved in heat shock and the various other regulatory responses that affect this gene. Since HSTF (heat shock transcription factor), CTF (CCAAT-box-binding transcription factor), and AP-2 (enhancer-binding activator protein) also interact with multiple sites on this promoter, further work will be necessary to analyze the relationship among these different control elements. Supported by a grant from NSERC.

P 129 Isolation of a Yeast Gene Potentially Involved in Regulation of the Stress Response. Charles M. Nicolet and Elizabeth A. Craig, Univ. of Wisconsin-Madison, Madison, WI 53706.

We have cloned a gene from Saccharomyces cerevisiae based on its ability to increase expression of a heat shock gene promoter-DHFR gene fusion. The cloned gene is denoted SRL for Stress Related Locus. If present on a multicopy plasmid, SRL results in increased expression of two different reporter genes which are fused to the SSA4 promoter. The SRL gene encodes an approximately 2.0 kb mRNA, sufficient to code for a 70-80 kDa protein. Interestingly, the SRL mRNA is induced 5-10 fold by heat shock. SRL, however, bears no homology to the SSA subfamily of heat shock proteins (as measured by low stringency DNA-DNA hybridization). Expression of the SRL gene also increases in cells concomitant with increasing OD. Cells that carry disruptions at the SRL locus are competent for mitotic growth at 30°C, but are temperature- and cold-sensitive. We are currently investigating the exact nature of the modulation of heat shock gene expression by SRL, and determining which other heat shock genes in yeast are affected by this gene product.

P 131 ANALYSIS OF STRESS-INDUCED PROTEINS FROM BACILLUS SUBTILIS, Uldis N. Streips, M. Walid Qoronfleh, Brian Miller, Robin Staples, Peter Khoury and Ralph A. Slepecky, University of Louisville, Louisville, KY 40292 and Syracuse University, Syracuse, NY 13210.

Stress shock in Bacillus subtilis features at least 16 polypeptides, which are accelerated in synthesis following exposure to heat or several other stress-inducing agents. A mutant has been isolated, following transposon mutagenesis, which is missing a subset of stress shock proteins and exhibits pleiotropic phenotypic alterations such as different secretion capabilities and markedly diminished heat resistance in vegetative cells and spores. Fractionation of heat shocked cells reveals that the absence of these proteins alters subcellular location of the major (66kDa) heat shock protein- in the mutant the 66kDa protein is primarily cytosolic, while in wild type cells this protein is membrane associated. All of these traits can be concommitantly transfered to recipient cells by transduction. Also, bacteriophage ϕ105clz infection induces a subset of stress proteins. Further mutants in the stress response are being isolated from mutagenized cells which are phage resistant. The 23kDa heat shock protein has been identified to be a 48C-specific protease.

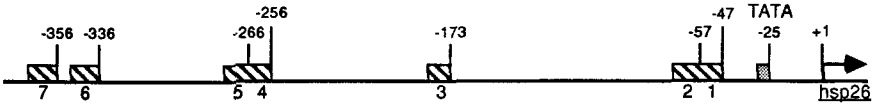
P 132 METHYLATION OF CORE HISTONES AND REGULATION OF TRANSCRIPTION DURING HEAT SHOCK, Robert M. Tanguay and Richard Desrosiers, Ontogénèse et Génétique Moléculaires, Centre Hospitalier Université Laval, Ste-Foy, Québec, Canada G1V 4G2

In Drosophila, the rapid heat-induced activation of the heat shock genes is accompanied by the inactivation of many of the genes active prior to the heat shock treatment. While the changes in the structure of heat shock gene chromatin are well documented, very little is known about the molecular mechanisms responsible for the silencing of genes active prior to heat shock. Changes in the post-translational methylation patterns of histones are rapidly induced in response to heat: there is a increase in the methylation level of histone H2B and a concomitant decrease in that of histone H3. No changes in the methylation patterns are observed when stresses (ethanol, suboptimal temperatures) known to induce heat shock genes without impairing the expression of normal genes are used suggesting a relationship with gene repression. This is further substantiated by a study of the kinetics of recovery of the normal pattern of histone methylation during cellular recovery and by the use of transcriptional inhibitors acting at the chromatin level.

In order to understand the functional significance of these changes, we have determined the nature of the methylated amino acids in the core histones. At 23°C, H3 and H4 are methylated on lysine residues. Under heat shock conditions, there is a decrease in the extent of methylation of lysine and the appearance of new methylation on arginine residues in H3. In H2B, the methylated amino acid has been identified as N-methylproline located at the N-terminal end. These quantitative (H2B) and site-specific (H3) changes in the methylation of certain core histones could be involved in the restructuration of chromatin accompanying the repression of normal genes during heat shock. The hypermethylation of H2B may also be involved in its protection from increased ubiquitin-mediated proteolysis activity observed under these stress conditions.

Supported by the MRC of Canada and the FCAR.

P 133 MAPPING OF PROTEIN FOOTPRINTS 5' OF THE DROSOPHILA HSP26 GENE IN INTACT NUCLEI, Graham H. Thomas and Sarah C.R. Elgin, Washington University, St.Louis, MO 63130. The active and inactive states of the Drosophila melanogaster hsp26 promoter have distinct, ordered chromatin structures. These two structures are different (I.L. Cartwright and S.C.R. Elgin, 1986. Mol. Cell. Biol. 6;779-791). The aim of this study is to define, to the base pair, the location of the protein/DNA interactions within these structures in isolated nuclei, using the high-resolution indirect-end-labelling technique of G.M. Church and W. Gilbert (1984, Proc. Natl. Acad. Sci. 81;1991-1995). The hsp26 promoter contains seven copies of the heat shock consensus sequence, a 14bp sequence found 5' of all heat shock genes (H.R.B. Pelham, 1982. Cell 30;517-528: see figure). On the inactive promoter we have found a footprint at the TATA box and a region of periodic DNase I cleavage which begins at around -150bp and encompasses some 140-150bp of DNA 5' of this location. The periodicity is 10-11 base pairs indicative that the DNA in this region is bound to a surface. We believe that the extent and nature of this cleavage indicates the existence of a phased nucleosome in this region. On the active promoter, in addition to the above features we see footprints on four of the heat shock consensus sequences (numbers 1, 2, 6, and 7 located at -47 to -60, -57 to -70, -336 to -349 and -356 to -369bp respectively) 5' of hsp26. These footprints presumably represent binding of the heat shock factor. Heat shock consensus sequences within the putative nucleosomal region (numbers 3, 4 and 5) are not footprinted on heat shock, suggesting that they are inaccessible. The observed chromatin configuration places the critical heat shock consensus sequences in accessible sites, perhaps defined by the TATA binding protein and the phased nucleosome. (see Figure in Elgin et al, this volume).



P 134 EXPRESSION OF HEAT SHOCK PROTEIN BY INSULIN, Ling-Pai Ting, Chia-Ling Tu and Chen-Kung Chou, National Yang-Ming Medical College, Taipei, Taiwan, Republic of China.

Heat shock protein with molecular weight 70 KD was greatly induced by heat shock in human hepatoma cell line. When these cells were arrested by serum starvation, the expression of hsp70 gene was induced by insulin. The effect of induction correlates very well with the dissociation constant of insulin receptor of these cells, suggesting that the insulin effect is indeed via the insulin receptor. The kinetic change of hsp70 transcript in this hepatoma cell line induced by insulin is different from the S phase-specific expression of hsp70 gene induced by serum in HeLa cells. The hsp70 transcript in this cell line starts to increase and reaches the peak at 6 hrs after the addition of insulin, which does not correlate with the DNA synthesis.

P 135 STRUCTURE OF THE CHICKEN HSP 90 PROMOTER. Claire Vourc'h, Nadine Binart, Beatrice Chambraud, Etienne-Emile Baulieu and Maria-Grazia Catelli. INSERM U 33, Lab Hormones, 94275 Bicêtre Cedex, France.

In most cells although hsp 90 is abundant at normal temperatures, its expression is induced by heat. Both the basal level and the induced level vary as a function of development. As a first step in understanding the mechanism regulating hsp 90 expression we studied the chicken hsp 90 promoter region. Using a cDNA (1) containing the coding region for the first 329 AA of the chick hsp 90 and some of the 5' non translated leader, we have screened a chicken genomic library constructed in λ 47.1 (2). Four different clones have been isolated and one has been studied in detail on the basis of Southern blots analysis. This clone is ~ 11 Kb and contains the 5' region of the cDNA with ~ 10 kb upstream from the translational start codon. There are at least two introns in the 5' non coding region one of which is ~ 1.3 Kb and is located just before the translational start codon. Although the transcription start site has not yet been accuretely defined, sequence analysis shows, within 120 bp, two overlapping heat shock responsive elements (HSE), a CAAT box and a TATA box; the latest is 70 bp upstream of the 5' end of the previously clone cDNA.

1) Catelli, MG, Binart, N., Feramisco, J.R. and Helfman, D. Cloninig of the chick hsp 90 cDNA in expression vector. Nucleic Acids Research, 13, 6035-6047, 1985. 2) Ballivet, M., Nef, P., Stalder, R. and Fulpius, B. Genomic sequences encoding the α-subunit of acetylcholine receptor are conserved in evolution. Cold Spring Harbor Symposia on Quantitative Biology, Vol. XLVIII, 83-87, 1983.

P 136 REGULATION OF HUMAN HSP70 GENE EXPRESSION BY SERUM STIMULATION AND E1A TRANSACTIVATION. Gregg T. Williams, Terrill K. McClanahan, Dick D. Mosser and Richard I. Morimoto. Northwestern University, Evanston, IL. 60208.

Expression of the human HSP70 gene is controlled through multiple regulatory pathways which utilize transcriptional and post-transcriptional mechanisms. Transcription of the HSP70 gene is induced by conditions of stress, by serum stimulation and by infection with adenovirus. To define essential promoter elements responsible for the growth-regulated expression of the HSP70 gene, three approaches have been utilized. First, analysis of a series of 5' deletions and linker-scanning mutations in HeLa cells revealed that promoter sequences to -74, containing a CCAAT element at -68, are required for both serum stimulated expression and E1A activation. Linker-scanning mutants containing sequences to -100 and abolishing the CCAAT element significantly reduce basal expression and E1A response. Second, fusion of the promoter sequences from -100 to -44 of the HSP70 gene to the TATA box of an inactive HSV-thymidine kinase gene confers E1A- and serum responsiveness to this test gene. Constructs linking various promoter elements of the HSP70 gene to the truncated thymidine kinase promoter have been constructed to elucidate minimal sequence element requirements of serum and E1A-responsiveness of the HSP70 promoter. Third, the interactions of protein factors with this promoter have been studied by gel mobility shift assays. Oligonucleotides containing sequences encompassing the CCAAT element and the TATA element bind protein factors in a sequence-specific manner. These studies reveal that a CCAAT-containing region between -68 and -44 of the HSP70 promoter is necessary to confer regulatory function during serum stimulation and adenovirus E1A transactivation.

P 137 *IN VITRO* STUDIES ON THE REGULATION OF THE HEAT SHOCK RESPONSE IN *ESCHERICHIA COLI*. Susan Skelly, Timothy Coleman, Chee-Fook Fu, Barbara Dalie, Diane Skaleris, Nathan Brot and Herbert Weissbach, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

It is now well documented that when *Escherichia coli* cells growing at 30°C are shifted to 42° the rates of synthesis of at least 17 proteins are increased. This response requires the presence of the *htpR* gene product, the 32 Kd heat shock sigma factor (σ^{32}). We have employed *in vitro* DNA-directed protein synthesizing systems to study the expression of heat shock (HS) and non-heat shock genes (non-HS). It was found that S-30 extracts prepared from cells that were grown at 33° and shifted to 45° express HS genes *in vitro* about 8 times better than extracts prepared from cells maintained at 33°. In contrast, the expression of non-HS genes in S-30 extracts from the heat induced cells was only about 40% of that found in extracts from cells at 33°. These results were in close agreement with the levels of σ^{32} and σ^{70} found bound to RNA polymerase. Thus, there was about an 8 fold increase in σ^{32} and a 60% decrease in σ^{70} associated with RNA polymerase obtained from cells shifted to 45°. Part of the increase in the level of σ^{32} could be accounted for by a 3-fold increase in the amount of σ^{32} mRNA during the induction.

In addition, in other studies, we have reinvestigated the question of the specificity of $E\sigma^{70}$ and $E\sigma^{32}$ towards transcribing HS and non-HS genes. The present results confirm previous *in vitro* data that showed that $E\sigma^{32}$ could efficiently transcribe HS genes such as DnaK but could not transcribe a non-HS gene. In contrast, however, purified $E\sigma^{70}$ preparations could transcribe HS genes. We have now shown that antibodies against σ^{32}, specifically inhibit the ability of these preparations to transcribe HS genes. Thus, these data indicate that $E\sigma^{70}$ is inactive in transcribing HS genes and that the observed activity is due to a small contamination of $E\sigma^{32}$ in the $E\sigma^{70}$ preparations. These results are in agreement with a previous study (Grossman *et al. Cell* **38**, 383-390, 1984) in which it was shown in a run off transcription assay that $E\sigma^{70}$ could not transcribe HS genes. It is also of interest that in the *in vitro* system the optimal amount of $E\sigma^{32}$ required for transcription of HS genes is about 5-10 fold less than the optimal concentration of $E\sigma^{70}$ needed for a non-HS gene. This greater activity of $E\sigma^{32}$ may account for the significant levels of the heat shock proteins, DnaK and GroEL, that accumulate in *E. coli* grown at 30°.

Developmental Regulation of Heat Shock Genes; Post Transcriptional Regulation in the Heat Shock Response

P 200 DIFFERENTIAL EFFECTS OF STRESS ON PRE-mRNA PROCESSING IN HeLa CELLS. Ursula Bond and Joan A. Steitz. Yale University Medical School, New Haven, CT 06510.

Cells have devised many stratagies to cope with the onslaught of thermal and chemical stresses: alterations in mRNA transcription, processing and translation allow the cell to recover and survive from the interruption in homeostasis. We are studying the changes occurring in RNA processing in stressed cells. Unspliced mRNA of the small heat shock gene, hsp27, and the heat inducible ubiquitin gene accumulate in heat shocked and sodium arsenite treated HeLa cells. To analyse this inhibition further nuclear extracts were prepared from heat or sodium arsenite treated HeLa cells and assayed for their ability to splice an exogenously supplied substrate. Extracts from sodium arsenite treated cells behaved identically to untreated cells while extracts from heat treated cells were incapable of splicing the pre-mRNA, suggesting that subtle differences may exist between these two responses. In the latter case the degree of inhibition of splicing was dependent on the severity of the stress and splicing could be restored if the cells were allowed to recover for three hours. Analysis of small nuclear ribonucleoprotein (SnRNP) particles by gel electrophoresis indicates a correlation of the inhibition of splicing in the heat treated cells with changes in two SnRNPs, U2 and U5. We are currently trying to determine the mechanism whereby two apparently identical stresses, indistinguishable in their stress response at the molecular level in vivo, behave differently in their ability to process pre-mRNA in vitro.

P 201 AUTOPROTEOLYSIS OF DROSOPHILA AND HUMAN HEAT SHOCK PROTEIN 70 MADE IN VITRO, John J. Duffy and Joseph E. Rabinowitz, Department of Radiation Oncology, University of Arizona, AZ 85724.

Autoproteolytic activity of heat shock protein 70 (hsp70) in Drosophila and CHO cells has been observed during acrylamide gel electrophoresis in the presence of SDS (H.K. Mitchell, N.S. Petersen, and C.B. Buzin, 1985, Proc. Natl. Acad. Sci. USA 82:4969-4973). We have inserted the entire transcribed sequence of the Drosophila gene from the 87C1 locus coding for hsp70 and the human sequence coding for hsp70 along with 5' and 3' non-translated sequences into in vitro transcription vectors. RNA synthesized from the vectors codes for a 70 KDal protein made in vitro in a rabbit reticulocyte system in the presence of ^{35}S-methionine. The hsp70 proteins exhibit proteolytic activity subsequent to SDS/PAGE as obeserved on a second dimensional SDS/PAGE to give as the major products of 41 to 47 KDal and 22 to 27 KDal peptides. Mouse ornithine decarboxylase synthesized and electrophorysed under the same conditions showed no evidence of proteolytic activity. The proteolytic activity is not induced by SDS alone nor by high temperature but is surmised to function during PAGE when the protein is focused to a high concentration. The in vitro system will allow analysis of the proteolytic activity when deletion mutants are used as templates for RNA synthesis.

This work is supported in part by grants from the National Cancer Institute, CA-30052, and the Arizona Disease Control Commission.

P 202 **Novel Behaviors of the Transcripts of Hsr93D of D. melanogaster.**
M. Elizabeth Fini and Mary Lou Pardue, Dept. of Biol. MIT, Cambridge, Mass.

The gene at locus, 93D, in D. melanogaster, a major heat-induced puff is also expressed constitutively. Three major RNA species are transcribed from the 93D gene, each of which displays unusual behaviors. The largest species of 10 kb, which contains the sequences of the single intron, is confined to the nucleus, outside of the nucleolus. It accumulates in this organelle to unusually high levels suggesting that it may play an important role as an RNA at this location. The 1.9 kb RNA is also confined to the nucleus where it apparently acts as a splicing precursor to a 1.2 kb cytoplasmic RNA. This smaller nuclear species is more abundant in the nucleus than a typical splicing precursor. In addition, we find an unusual accumulation of splicing intermediates derived from this RNA, suggesting that its processing, to yield the cytoplasmic RNA, is under some type of unusual control. The levels of 1.2 kb cytoplasmic RNA are further controlled by a specific mechanism to regulate the rate of RNA turnover. Such controls may be important in regulating the ultimate levels of a short-lived peptide of 3 kd, which appears to be the translation product of the cytoplasmic RNA. We report here our investigation into the sequences important in nuclear restriction of the 10 kb RNA, controlled splicing of the 1.9 kb RNA, and half-life regulation of the 1.2 kb RNA. We further have examined possible roles for the translation product of the cytoplasmic RNA.

P 203 EFFECT OF HEAT SHOCK ON NONHEATSHOCK PROTEINS OF THE INSECT INTEGUMENT.
Catherine M. Fittinghoff and Lynn M. Riddiford. University of Washington, Seattle, WA 98195.

The heat shock response of insect integument is of interest because the integument, composed of epidermis and overlying cuticle, provides the insect's first barrier to heat and dehydration. The tobacco hornworm, Manduca sexta, is nearly ideal for examination of the integumental heat shock response because its integument can be cleanly dissected, cultured in vitro, and is sufficiently large that pooled samples are not required. Studies of protein synthesis during heat shock demonstrate that larval Manduca epidermis produces at least seven heat shock proteins similar in molecular weight to those of Drosophila. Unlike Drosophila and other Diptera, Manduca, a lepidopteran, does not eliminate synthesis of nonheatshock proteins during heat shock, although a moderate decline may occur. Furthermore, synthesis of certain nonheatshock proteins is apparently enhanced during heat shock. These enhanced proteins cross-react with an antibody to larval cuticle and are presumably cuticular proteins. Preliminary evidence suggests that the apparent increase in synthesis may actually reflect a failure to transport cuticular proteins out of the epidermis and into the cuticle.

P 204 CHARACTERIZATION OF HSP 70, HSP 30 AND UBIQUITIN GENE EXPRESSION DURING XENOPUS LAEVIS DEVELOPMENT, John J. Heikkila, P. Krone and N. Ovsenek, Univ. of Waterloo, Waterloo, Ontario., Canada N2L 3G1.
Exposure of Xenopus laevis neurula embryos to heat shock (33°C) induced the accumulation of ubiquitin as well as hsp 70 mRNA. The ubiquitin transcript sizes ranged from 1.7 to 3.1 kb. Heat shock induced accumulation of both ubiquitin and hsp 70 mRNA were not detectable until after the mid-blastula stage. Unlike hsp 70 mRNA, constitutive levels of ubiquitin mRNA were detectable throughout development. In contrast to the aforementioned hsp mRNAs, heat-induced hsp 30 mRNA (1.1 kb) accumulation was first detectable at the tail bud stage. While these genes are regulated differently during early development, we have found that hsp 30, hsp 70 and ubiquitin genes are expressed coordinately at the tadpole stage. For example, continuous exposure of tadpoles to heat shock induced a coordinate, transient accumulation of hsp 30, hsp 70 and ubiquitin mRNA. A coordinate temporal pattern was also observed for the decay of all three hsp mRNAs during recovery from heat shock. Cardiac actin mRNA levels were not affected in these experiments. In order to gain more insight into the regulation of these hsp mRNAs during early development, we are currently analyzing the expression of microinjected hsp 70 and 30 fusion genes in Xenopus embryos. Preliminary experiments have shown that microinjected hsp 70 genes are not heat-inducible prior to the mid-blastula stage. (Supported by NSERC)

P 205 CHANGES IN hsp 70 SYNTHESIS AND CELLULAR CONTENT DURING THE COMMITMENT OF LEUKEMIC CELLS TO TERMINAL DIFFERENTIATION. John A Hickman, Frances Richards and Andrea Watson, CRC Experimental Chemotherapy Group, Pharmaceutical Sciences Institute, Aston University, Birmingham B4 7ET, UK.

HL-60 Myelomonocytic leukemia cells undergo terminal cell differentiation to metamyelocytes when incubated with marginally cytotoxic concentrations of a variety of polar solvents, including ethanol and N-methylformamide (NMF) (Langdon and Hickman, Cancer Res., **47**: 140, 1987). A heat shock of 43.5° for 1 h induced up to 34% of HL-60 cells to differentiate to metamyelocytes, and continuous treatment with sodium arsenite and procaine induced >60% differentiation. Analysis of protein synthesis by SDS-PAGE of equal cell numbers incubated with [^{14}C]-leucine after a heat shock or 1 M NMF (a toxic concentration) showed an induction of the synthesis of proteins of ~70, 90 and 110 kD, corresponding to the major hsps. In the untreated cells there was a significant synthesis of a 70 kD protein. When cells were incubated with the optimum concentration of NMF (170 mM) to promote >70% differentiation, while maintaining >80% viability, no induction of the synthesis of hsps was observed: instead a significant and early (~2h) fall in the constitutive synthesis of the 70 kD protein took place. Immunoblotting with an antiserum to human hsp 70 showed insignificant amounts of the protein in untreated cells but levels began to rise after 24 h of incubation with 170 mM NMF. This rise corresponded to the period in which NMF irreversibly commits the cells to terminal differentiation. The relationship between the rise in cellular levels of hsp 70 and changes in gene expression are under investigation.

P 206 ALTERATIONS IN UBIQUITIN AND UBIQUITIN-PROTEIN CONJUGATES IN D. DISCOIDEUM WHEN EXPOSED TO HEAT SHOCK AND DURING DEVELOPMENT, Edwin Jahngen and Helen Czarniewicz University of Lowell, Department of Chemistry, Lowell, MA 01854.

The exposure of cells to sublethal temperatures that impair growth, results in a Heat Shock Response (HSP). The HSP is characterized by the synthesis of a variety of proteins, the function of which is still the focus of investigation. Among these HSP proteins is the 8Kd Ubiquitin. The HSP in D. discoideum has been well documented by Loomis and co-workers (Dev. Biol. (1980) 79: 399; ibid., (1982) 90: 412). More recently, Gerish (FEBS Letts. (1986), 209: 92) and Enna (Mol. Cell. Biol. (1987) 6: 2097) have reported the isolation and characterization of developmentally regulated genes for Ubiquitin from D. discoideum. In our laboratory using antibodies prepared against human erythrocyte Ubiquitin, we demonstrated the presence of Ubiquitin as well as Ubiquitin-Protein conjugates in D. discoideum. In response to a shift of D. discoideum from growth at 22°C to 30°C we observed a transient increase in the synthesis of free endogenous Ubiquitin followed by an increase in higher molecular weight conjugates. In cell free lysates we demonstrated that the formation of the higher molecular weight conjugated from exogenous Ubiquitin requited ATP. Very similar results were noted when cells grown at normal temperatures were placed in non-nutrient medium in order to initiate morphodifferentiation. Subcellular fractionation indicated that the majority of the Ubiquitin was located in the cytosolic fraction with major concentrations also found in the nucleus and mitochondrial fractions. Our experiments indicate that a rapid turnover of Ubiquitin and Ubiquitin conjugates occur when cells are stressed by Heat Shock and during differentiation.

P 207 UBIQUITIN CONJUGATES INCREASE WITH AGE IN HUMAN LENSES. J.H. JAHNGEN AND A. TAYLOR, USDA HNRC AT TUFTS UNIVERSITY, BOSTON, MA 02111

During aging the lens is subject to a variety of stresses such as exposure to active oxygen species and ultraviolet light. Simultaneously, antioxidant defenses are diminished, particularly in the oldest tissue or core which may last the entire life span of the individual. Thus, by comparing lens epithelial tissue, which is the most recently elaborated tissue of the lens, with core, it is possible to determine changes in structure and function of biomolecules in the lens upon stress and aging.

We have examined human lenses of ages (45 min-68 yr) for protein content, ubiquitin changes and for the presence of endogenous ubiquitin-lens protein conjugates. There is a trend towards generation of (1) very low, and (2) very high-molecular mass proteins, as well as (3) a heterogeneous group of proteins which leads to the the loss of resolution of the major lens proteins with increasing age. The extent of recognition of free ubiquitin increases with age, such that more free ubiquitin is found in older lens tissue. The distribution of ubiquitin lens protein conjugates also changes with age. More conjugates of very high-molecular mass are observed in epithelial tissue, while in core tissue the majority of conjugates are in the 17-45 KD range. Furthermore, it appears that more conjugates are recognized by antisera to ubiquitin in lens tissue obtained from older individuals.

Supported by grants from USDA ARS, the Daniel and Florence Guggenheim Foundation, and the Massachusetts Lions Eye Research Fund, Inc.

P 208 INDUCTION OF A TISSUE SPECIFIC SET OF HEAT SHOCK PROTEINS IN THE FLESH FLY SARCOPHAGA CRASSIPALPIS, Karl H. Joplin, Cheng-ping Chen, George D. Yocum and David L. Denlinger, The Ohio State University, Department of Entomology, Columbus, OH 43210.

The flesh fly Sarcophaga crassipalpis has a heat shock protein (hsp) response similar to the heat shock response seen in Drosophila. The hsp are first induced in vivo at 40°C and maximal labeling is seen at 43°C. All protein production, including hsp, is repressed at 45°C. Although the induction temperatures are higher than in Drosophila, the hsp profiles of the two species are very similar: major labeling occurs in proteins with molecular weights of 87,74,70 and in a set of four small proteins of <30 kd. The heat shock induced proteins from the integument and brain, labeled in vitro, are similar to the in vivo labeled proteins. We expected the same proteins to occur in other tissues labeled in vitro. However, the accessory glands of three day old male adult flies synthesize a complex set of heat shock induced proteins that are not only very different from the protein pattern observed in accessory glands labeled at 25°C but also differ from the heat shock proteins observed with in vivo labeling of the whole organism or the in vitro labeling of the integument and brain. We can resolve 28 proteins that range in size from 104 to 33 kd with a major band appearing at 63 kd. The 63 kd band can also be seen in flight muscle, testis and fat body. This difference may reflect a differential hsp expression in mesodermal and ectodermal tissues.

P 209 GENE REGULATION OF A *DICTYOSTELIUM* STRESS-PROTEIN: DIFFERENTIAL USE OF TWO MRNAS FROM A SINGLE COPY GENE, Markus Maniak and Wolfgang Nellen, Dept. of Cell Biology, Max-Planck-Institute for Biochemistry, Klopferspitz, D-8033 Martinsried, West Germany.

We have characterized a cDNA clone, P8A7, from *Dictyostelium discoideum*, encoding a potential membrane-protein as implied from the derived amino acid sequence. The corresponding single copy gene is transcribed into two translated mRNAs which differ in the length of their 5'regions, but share at least 100 amino acids of the protein coding region.

The characterization of regulation of the two mRNAs makes it possible to assign them to different classes of genes:

While the P8A7S (small, 700 nts) message has characteristics of a cell-type-non-specific gene, the large message is also induced by the formation of cell-substrate contacts in vegetative cells, and cell-cell contacts in development. This suggests an involvement of the P8A7 gene product in cell adhesion.

Surprisingly, P8A7L is not only induced by cell-contact formation. Heat-shock, cold-shock and Cadmium also induce accumulation of the large message, whereas the small transcript is destabilized in heat-shock. The response of other stress genes is different upon cold shock: *Dictyostelium* ubiquitin is also induced, but hsp 70 remains unaffected. Under heat- and cold-shock conditions splicing is impaired as demonstrated by the accumulation of an unprocessed precursor, P8A7P in the nucleus.

The possible mechanisms for generating two differentially regulated mRNAs from a single copy gene will be discussed. Further investigations will cover the function of the gene product(s).

P 210 REGULATION OF THE HEAT-SHOCK RESPONSE BY INTERFERON IN MOUSE L CELLS, Michel Morange, Marie-Françoise Dubois, Chantal Ferrieux, Pierre Lebon and Olivier Bensaude, Institut Pasteur, 75724 Paris Cedex 15 and INSERM U 43, Hôpital St. Vincent de Paul, 75014 Paris, France.

Interferon (IFN) pretreatment of mouse L cells has already been shown (J. Cell. Physiol., **127**: 417-422) to enhance the decrease of overall protein synthesis which follows a heat shock, and to stimulate the accumulation of heat-shock proteins (HSPs) by increasing the amount of mRNAs coding for these proteins.

We show here that the synthesis of a protein (the Hepatitis B virus surface Antigen) under the control of a Drosophila HSP 70 promoter is also stimulated by pretreatment with IFN of cells having incorporated this gene. In addition the synthesis of endogenous HSPs is prolonged after pretreatment with IFN. Experiments performed in the presence of actinomycin D demonstrate that this effect is, at least partially, due to a stabilization of mRNAs coding for HSPs by IFN pretreatment. Experiments are in progress to know whether mRNA stabilization after a heat-shock by IFN pretreatment occurs for all mRNAs or is limited to a special class of mRNAs.

P 211 PHENOTYPIC PROFILES OF MYXOCOCCUS XANTHUS Tn5 HEAT-SHOCK MUTANTS, Kevin P. Killeen and David R. Nelson, The University of Rhode Island, Kingston, RI 02881.
Myxococcus xanthus, a Gram-negative, social bacterium which inhabits soils is always found in multicellular masses. Cells interact to promote growth, motility, and development. Starvation or the addition of glycerol induces vegetative rods to differentiate to ovoid, environmentally-resistant myxospores. We have previously characterized the heat-shock response of both vegetative and developmental M. xanthus and have shown that heat-shock of vegetative cells causes premature expression of developmental proteins and accelerates myxospore formation. We hypothesized that some heat-shock proteins possess developmental functions and their induction by heat-shock can accelerate development. To test this hypothesis, Tn5 mutants of M. xanthus were constructed and screened for vegetative and developmental aberrations. Initial screens included heat sensitivity at 35°C and ability to grow in casitone yeast extract broth plus 3% ethanol. Over 1500 mutants were screened and 12 were classified as heat-shock mutants. Subsequent screens included ability to develop at 30°C and 35°C. Pleiotropic mutants could be placed in one of three general categories. One group was heat and ethanol sensitive during vegetative growth yet was developmentally functional during myxospore formation. A second subset was heat and ethanol sensitive while developmentally defective only at 35°C. A third group was heat and ethanol sensitive with developmental defects at both 30°C and 35°C. All of these mutants failed to accelerate development when heat-shocked prior to the development inducing signal. Cloning of these putative heat-shock/developmental mutant genes is in progress to determine their roles in the heat-shock response and in development.

P 212 EFFECT OF AGE ON THE INDUCTION OF HEAT SHOCK PROTEINS IN RAT HEPATOCYTES, Ahmad Heydari, Bo Wu, Mahmood Ghassemi and Arlan Richardson, Illinois State University, Normal, IL 61761.
A characteristic feature of senescence is the progressive decline in the ability of an organism to respond to environmental and cellular stimuli. Therefore, the ability of hepatocytes isolated from rats of various ages to synthesize heat shock proteins in response to an increase in temperature was studied. Hepatocytes were isolated from 5- and 30-month-old male Fischer F344 rats by in situ collagenase perfusion. The hepatocytes were incubated at either 37 or 42°C for 10 minutes. [^{35}S]-L-Methionine was added to the hepatocyte suspension, and the suspensions were incubated at 37°C for 2 hours. The hepatocytes were lysed, and the lysates were subjected to two-dimensional gel electrophoresis. Exposure of the hepatocytes to 42°C resulted in the induction of a protein with a molecular weight of 70 Kd. Autoradiographs of the two-dimensional gels showed that the induction of the heat shock protein was much lower in hepatocytes isolated from 30-month-old rats compared to hepatocytes isolated from 5-month-old rats. To quantify the age-related changes in the induction of the 70 Kd protein, the section of the gel containing this protein was removed, and the radioactivity present in the gel was quantified using a scintillation counter. In hepatocytes isolated from 5-month-old rats, the heat shock resulted in a 9-fold increase in the synthesis of the 70 Kd protein. However, only a 3.8-fold increase in the synthesis of the 70 Kd protein was observed in hepatocytes isolated from 30-month-old rats. Thus, aging appears to result in a decrease in the ability of hepatocytes to express at least on heat shock protein.

P 213 DEVELOPMENTAL REGULATION AND BIOCHEMICAL ANALYSIS OF A 21Kd HEAT SHOCK PROTEIN IN SEA URCHINS. J. Rimland, O. Akhayat, D. Infante and A. A. Infante. Dept of Mol. Biol. & Biochem., Wesleyan Univ., Middletown, Conn. 06457

A 21,000 dalton heat shock protein has been found in *S. purpuratus* and *L. pictus*. Synthesis of this protein normally begins 12hr after fertilization. Its rate of synthesis increases from 18hr to 24hr and remains constant thereafter. Western blot analysis using a polyclonal antibody, showed that the constitutive hsp 21 is present at all stages examined, but its level fluctuates, decreasing from eggs to 18hr embryos and increasing afterwards. Before 18hr, heat shock induces hsp 70, but not hsp 21; at 18hr and after, both are coordinately induced. Both the constitutive and heat inducible hsp 21 exist in a particle with at least two other proteins of 24Kd and 20Kd of which the 24Kd protein is not inducible by heat. The particle sediments at 15S in high salt and SDS sucrose gradients and can be purified on DEAE-cellulose. The heat inducible 21Kd protein has the same isoelectric point, sedimentation characteristics and buoyant density as the constitutively expressed 21Kd protein. Both the constitutive and heat inducible hsp 21 are resistant to digestion by proteinase K. Phenol extraction of the semi-purified particle from a sucrose gradient and 3' end labeling have revealed many low MW RNA species. Electron microscopy indicates the particle is spherical with a diameter of about 38nm. Using differential centrifugation, the constitutive and heat inducible hsp 21 are found predominately in the cytoplasmic post-ribosomal "free RNP" fraction of cells at all developmental stages examined. The hsp 21 is differentially expressed in endoderm and ectoderm of late stage embryos. During non-heat shock conditions, the ectoderm constitutively synthesizes hsp 21 at a higher rate, but during heat shock the synthesis of hsp 21 is greater in the endoderm.
(Supported by NSF grant DCB-8616077)

P 214 ESTROGENIC REGULATION OF MURINE UTERINE 90 KD HEAT SHOCK PROTEIN, G. Shyamala, C. Ramachandran, M.G. Catelli and W. Schneider, Lady Davis Institute for Medical Research - The Jewish General Hospital, Montreal, Quebec and Lab Hormones, Bicêtre, France.

Recently two lines of evidence have implicated that cellular heat shock proteins (hsp) may play a role in steroid hormonal regulation of target tissues. One is the demonstration that cellular hsp90 can complex with steroid receptors *in vitro* and inhibit their ability to interact with DNA and second, the demonstration that in avian oviduct sex steroids can regulate the synthesis of hsp108. As yet, there is no report that these steroids can regulate hsp90 synthesis. In these studies we have examined the estrogenic regulation of murine uterine hsp90. We find (a) ovariectomy reduces the cellular concentration of hsp90 by approximately 4-fold, (b) estradiol causes a time-dependent increase in uterine hsp90 level as early as 4 hrs after steroid administration reaching a maximum between 18-24 hrs, (c) the effect is specific to estrogens and not elicited by other steroid hormones, (d) the estrogenic effect on hsp90 is specific to uterus and does not occur in non-target tissue for estradiol such as spleen and (e) the magnitude of estrogenic regulation of hsp90 is comparable to that seen with progesterone receptor, a well characterized and a specific estrogenic response in target tissue for estradiol. Based on these findings we propose that hsp90 may play a critical role in the estrogenic regulation of uterine function. (These studies were supported by the National Cancer Institute of Canada.)

P 215 ACCUMULATION OF mRNA FOR ACHLYA Hsp85, A COMPONENT OF THE SCHLYA STEROID RECEPTOR, CAN BE INDUCED BY EITHER HEAT SHOCK OR STEROID HORMONE. Julie C. Silver[1], Robert Riehl[2], and Shelley Brunt[1]. [1]University of Toronto, Scarborough, Ontario, Canada and [2]University of Texas, San Antonio, Texas.

The steroid hormone antheridiol regulates sexual development in the fungus Achlya. Analyses of in vivo labeled proteins revealed that one of the major Achlya heat shock proteins,hsp85, appeared identical to an Achlya 85 kDa cytoplasmic and nuclear protein which is induced by the steroid hormone antheridiol. Analysis of in vitro translation products of RNA isolated from control, heat-shock or hormone-treated cells demonstrated an increased accumulation of mRNA encoding a similar 85 kDa protein in both the heat-shocked and hormone-treated cells. A monoclonal antibody, AC88, specific for an 85-88 kDa protein component of the Achlya steroid hormone receptor has been isolated. This antibody recognizes also the 90 kDa non-hormone binding component of steroid receptors in chick and mouse. Immunoaffinity isolation using the antibody AC88, has shown that Achlya hsp85 and the Achlya 85 kDa steroid hormone-induced protein are both antigenically related to the non-hormone binding component of the Achlya steroid receptor. These data suggest that an identical 85 kDa protein is independently regulated by the steroid hormone antheridiol and by heat shock and that this protein is part of the Achlya steroid hormone-receptor complex. Southern hybridization analysis suggests that there are at least two putative hsp85 genes in the Achlya genome. These show roughly 70% homology to a Drosophila hsp83 clone. Whether these putative Achlya hsp85 genes are each regulated by both stress and steroid hormone remains to be determined. (Supported by NSERC Canada).

P 216 POST-TRANSCRIPTIONAL REGULATION OF HUMAN HSP70 EXPRESSION. Nicholas G. Theodorakis and Richard I. Morimoto, Dept. of Biochem., Mol. Biol., and Cell Biol., Northwestern Univ., Evanston, IL 60208

HSP70 expression is regulated at a variety of post-transcriptional levels. Whereas the translational efficiency of HSP70 mRNA is not affected by heat shock, the stability of HSP70 mRNA increases at least ten-fold upon heat shock or in the presence of protein synthesis inhibitors. The increase in mRNA stability upon heat shock or the inhibition of protein synthesis is a feature that HSP70 mRNA shares with other growth regulated mRNAs, suggesting a common regulatory pathway for short-lived mRNAs. An increase in mRNA stability may be necessary to preserve the levels of short-lived mRNAs when RNA Polymerase II transcription is inhibited by heat shock. Post-transcriptional regulation of HSP70 expression is likely to play an important role in normal cell growth as well. When HeLa cells are stimulated to enter the cell cycle by the addition of serum, the transcriptional activation of the HSP70 gene precedes the accumulation of HSP70 mRNA by several hours. Other post-transcriptional effects on HSP70 expression occur during adenovirus infection. When cell line 293 cells are infected with adenovirus-5, the levels of HSP70 mRNA increase approximately two-fold early in the infection and fall to undetectable levels late in infection, while HSP70 gene transcription remains constant during the course of infection. We are currently attempting to understand the mechanisms by which HSP70 expression is regulated at the level of post-transcriptional control.

P 217 EXPRESSION OF THE 84 AND 86 kDa HEAT SHOCK PROTEIN ISOFORMS DURING RECOVERY FROM HEAT SHOCK: TRANSLATIONAL AND TRANSCRIPTIONAL ANALYSIS, S. J. Ullrich, S. K. Moore and E. Appella, National Cancer Institute, Bethesda, MD 20892

Among the proteins induced upon stress are the 84 and 86 kDa heat shock protein (hsp) isoforms. Northern blot analysis was performed on total RNA isolated during recovery from heat shock using two non-crosshybridizing murine cDNA probes specific for each isoform. Analysis of both NIH 3T3 cells and Meth A tumor cells indicated that transcripts for both isoforms are rapidly induced and peak around 5 h post heat shock; the levels of hsp 84 and hsp 86 transcripts increased 1.5- and 2.0-fold, respectively, then decreased to control levels 24 h post heat shock. Analysis of protein synthesis after heat shock indicated that hsp 86 isoform synthesis increased 2- and 3-fold in NIH 3T3 and Meth A cultures, respectively, and that hsp 84 synthesis increased 1.5-fold in NIH 3T3 cells but no increase was found in Meth A cells. Elevated synthesis of these hsps continued for up to 9 h post heat shock in contrast to that of hsp 70 whose synthesis is elevated only during the first 3 h post heat shock. Basal levels of each isoform at 37°C were identical in both cell lines. Thus, the induction of hsp 84 and 86 transcripts and synthesis continues for a relatively longer period than that of hsp 70 and suggests that continued synthesis of these isoforms is necessary for recovery from heat shock. Lack of heat shock response in the synthesis of hsp 84 isoform in Meth A suggests different translational and/or post-translational regulation of these isoforms in transformed cells.

P 218 THE HALF-LIFE OF HSP70 MRNA IS REGULATED DURING HEAT SHOCK, Lee A. Weber, David Lloyd, Susan Hughes, and Eileen Hickey, University of South Florida, Tampa, FL 33620.

In HeLa cells subjected to sustained heat shock at 42^{0}, synthesis of each class of heat shock protein shows a characteristic pattern of induction and repression. Hsp70 synthesis is maximal between 2 to 4 hr at 42^{0}, and then rapidly declines. Synthesis of hsp27 and hsp89 remains at high levels for more than 12 hours. The abundance of hsp27, 70, and 89 mRNA parallels the rate of synthesis observed for each protein. Nuclear run-on experiments show that the pattern of transcription of the heat shock genes is similar, with maximum transcription taking place during the first hr at 42^{0}. Therefore, temporal differences in synthesis of the different hsps is largely the consequence of differences in mRNA stability. We measured the half-life of hsp70 mRNA at different times during heat shock. If actinomycin D is added after 3 hrs of heat shock, when hsp70 mRNA levels are declining, the remaining mRNA decays with a 1-2 hr half-life. If the drug is added after 30 or 60 min of heat shock, when hsp70 mRNA is still increasing in abundance, the half-life of hsp 70 mRNA is in excess of 4 hr and decay shows a complex kinetic pattern. These results suggest that hsp70 mRNA is specifically destabilized late in the heat shock response or, conversely, becomes stabilized early in the response. Inhibition of protein synthesis with cycloheximide of emetine have little effect on hsp70 mRNA turnover. Thus, turnover of hsp70 mRNA may be mechanistically different from many other mRNAs, which are stabilized by inhibition of protein synthesis.

P 219 THE EFFECTS OF HEAT SHOCK INDUCERS ON PROTEIN DEGRADATION, Tim Westwood and Rick Steinhardt, Univ. of California, Berkeley, 94720

It was suggested several years ago that heat and other inducers of the stress response might result in the generation of "abnormal" proteins in cells. Recently, it has been proposed that the increased protein degradation resulting from the generation of such proteins might indirectly be responsible for the induction of the heat shock (hs) genes. We examined protein degradation rates in Chinese Hamster Ovary and Drosophila Kc cells by pulse labeling the cells with ^{3}H-leucine, cold chasing, then monitoring the liberation of TCA soluble counts as a function of time. After chasing, cells were subjected to different heat treatments or to different hs inducers such as amino acid analogues (5 mM canavanine added during labeling), arsenite (0.1 mM), or Cd^{++} (0.1 mM) to determine what erfects they had on the protein degradation rate. Mild hs temperatures initially increased degradation but prolonged hyperthermia lowered the degradation rate. Severe hs temperatures produced a quick burst in degradation followed by a drastic inhibition of degradation. Canavanine stimulated degradation tremendously but took much longer than heat to induce hsp (hs protein) synthesis. Neither Cd^{++} nor arsenite appeared to have much effect on degradation even though both induce hsp synthesis. These results suggest that increased protein degradation may not be correlated with the induction of hs genes.

P 220 MOLECULAR CLONING OF A HEAT-SHOCK 70 PROTEIN GENE FROM RAINBOW TROUT AND ITS EXPRESSION IN THE ABSENCE OF HEAT SHOCK IN FISH TISSUES AND CELL LINES.

M. Zafarullah, S. Misra & L. Gedamu. Dept. of Biological Sciences, University of Calgary, Calgary, Alberta, Canada T2N 1N4

In order to study the molecular mechanisms involved in induction of stress (heat shock, heavy metals) proteins and to investigate the possible function of these proteins in fish, we have screened a trout genomic library with a Drosophila hsp70 probe. Restriction enzyme mapping of several clones and partial DNA sequence analysis of a 5.3 Kb HindIII fragment from one of these clones revealed strong amino acid homology with the heat shock 70 proteins from other organisms such as man, rat and xenopus. The gene was found to be interrupted by at least 4 introns within a 3 Kb DNA region sequenced so far. The position of introns is also conserved when compared with the rat and human heat shock 71 cognate genes. By using the probes from different regions of the coding part of the gene we have been able to study the expression of both the constitutive and heat inducible genes. In the rainbow trout hepatoma (RTH) and chinook salmon embryonic (CHSE) cell lines the mRNA corresponding to this gene was present at high levels even in the absence of heat shock. The mRNA level increased with higher growth density of cells. The mRNA specific to this gene was also detected in various fish tissues such as brain, liver, kidney, gills, spleen and different stages of testis development. From the high level synthesis of mRNA in somatic and germline tissues as well as cell lines we suggest that the gene codes for a heat shock 70 cognate polypeptide and that this protein is required for the normal growth and development of fish cells. (Supported by NSERC and AHFMR).

P 221 Expression and Inducibility of Heat Shock Proteins During Mammalian Development.

Z.F. Zakeri[1]., W. J. Welch[2]., and D.J. Wolgemuth[1].[1]Center for Reprod.Sci. & Dept. of Genetics & Development, Coll. of P&S, Columbia University, 630 West 168th St., New York, NY 10032.[2]Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

We have recently reported the expression of different members of the HSP 70 gene family in the mammalian testis. These transcripts are developmentally regulated during mouse spermatogenesis, one being most abundant in cells at meiotic prophase (pachytene spermatocytes) and the other appearing in abundance in post-meiotic germ cells. Our observations suggested that the two members are regulated differently and that Hsp 70 genes play a role in normal mammalian male germ cell development. To understand the a possible function for Hsp 70 genes in the male germ line we have examined the expression and the inducibility of the hsps at the protein level, by *in vitro* heat shocking and ^{35}S-methionine labeling of proteins synthesized after heat shock. The pattern of newly synthesized protein was analyzed by one and two dimensional gel electrophoresis. In the adult testis, proteins of 73 kDa and a 72 kDa protein were detected, with only a slight increase in the level of the 72 kDa protein after heat shock. However when testes of immature day 7-8 neonates, which contain the full range of somatic cells but only pre-meiotic germ cells, were subjected to heat shock, a substantial level of induction of the 72 kDa was detected. This suggested that the 72 kDa is induced most abundanthly in the pre-meiotic germ cells and/or the somatic cells of the testis. Further support for this observation was obtained in experiments demonstrating the induction of 72 kDa after heat shock in germ cell free mutant mice testes. In addition to the 73 kDa and 72 kDa protein present in both the adult and immature testis there appears to be a specific form of 73 kDa protein present in the adult testis which is absent in the immature testis.

Molecular Analysis of Heat Shock Proteins; Related Responses

P 300 CHARACTERIZATION OF THE MAMMALIAN LOW MOLECULAR WEIGHT HEAT SHOCK PROTEIN AND PROTEASOME PARTICLES, A.-P. Arrigo[1], J. Suhan[1], A.L. Goldberg[2], and W.J. Welch[1], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 and [2]Harvard Medical School, Boston, Massachusetts 02115.

In mammalian cells there appears to be a single low MW HSP, which we refer to as 28kDa. Phosphorylation of 28kDa (but not its synthesis) increases within minutes following exposure of cells to either tumor promoters (e.g., PMA), calcium ionophores (e.g., A23187) or simply the addition of fresh serum or defined growth factors. In the case of heat shock treatment both the synthesis and phosphorylation of 28kDa are observed to increase. Similar to the situation in Drosophila (where there are four low MW HSPs), mammalian 28kDa exhibits differential solubility characteristics as a function of the state of the cell (i.e., normal or stress). We have succeeded in purifying the protein by classical methods and found it to exist as a multimer with an average molecular mass of about 500,000 daltons after the heat stress. Analysis of the purified protein by electron microscopy reveals it to have a distinct and defined morphology. Using an antibody raised against the purified protein, we will present data describing the different intracellular distributions of the protein as a function of stress. A number of studies during the past fifteen years have described a ring-shaped 19-20S particle present in a variety of eukaryotic cells. These structures, called "prosomes," are composed of several polypeptide subunits which range in size between 20 and 30kDa and have a very characteristic morphology in the electron microscope. Using specific antibodies, we demonstrate that the mammalian prosome does not contain the low molecular weight HSP 28kDa. Interestingly, we have found that the prosome is identical to a large multifunctional protease complex and have renamed this particle "proteasome." We will present data describing the behavior of this protease as a function of stress.

P 301 CHARACTERIZATION OF cDNA SEQUENCES ENCODING THE HUMAN HEAT SHOCK PROTEIN HSP 89. Bernd-Joachim Benecke,Birgit Drabent, Thomas Walter and Heike Krebs. Dept.of Biochemistry, Ruhr-University, D-463 Bochum, Fed.Rep.of Germany.

Several hsp 89 cDNA clones were isolated from a cDNA library obtained with mRNA from heat-shocked HeLa cells. The specificity of these clones was verified by hybridization selection and in-vitro translation of HeLa cell mRNA. In northern blots, these clones identified a mRNA of about 3000 nucleotides in length.This mRNA, though synthesized in significant amounts in control cells, was induced 4-5 fold upon heat shock. Sequence analysis of the clones revealed that at least two different hsp 89 genes with considerable differences in nucleotide sequence but high homology in amino acid sequence are expressed in human cells.Interestingly, these cDNAs reveal an insertion of 5 amino acids in the 5'coding region of one hsp 89 gene. This insertion is also found in two otherwise identical 84-kDa mouse hsp genes (Ullrich et al.,1986, PNAS 83,3121). In Southern blots of whole genomic DNA, the cDNA containing the five additional amino acids identified three genomic fragments homologous to this cloned sequence.

P 302 CLONING AND NUCLEOTIDE SEQUENCE OF THE CHICK 90 kDa HEAT SHOCK PROTEIN, Nadine Binart, Beatrice Chambraud, Bruno Dumas, Jean Garnier* and Etienne E. Baulieu, INSERM U33, 94275 Kremlin Bicêtre, France. * Laboratoire de Biochimie Physique, Universite Paris-Sud, 91405 Orsay Cedex, France.

Steroid hormone receptors found in the cytosol of target cells have an approximate 8S sedimentation coefficient corresponding to an oligomeric structure with a molecular mass of ~ 250-300 kDa. This complex contains a non hormone binding 90 kDa component identical to the 90 kDa heat shock protein (hsp 90) or stress protein. The complete nucleotide sequence of the chick cDNA 90 kDa heat shock protein (2917 bp) and the deduced amino acid sequence are presented. A single long open reading frame encodes a protein of 84 kDa. The sequence analysis of this gene reveals extensive similarity with the human 83 kDa mouse, fly, yeast, trypanosoma and E. Coli 90 kDa heat shock proteins. A comparison of the translated product of the chick cDNA to the other published heat shock proteins reveals more than 60% similarity at both the nucleotide and amino acid levels. Several regions of 50 aa or more show greater than 90% identity. Analysis of this 90 kDA protein sequence shows a particularly charged domain (predicted in α-helix) which could interact with other proteins such as steroid hormone receptors.

P 303 DEMONSTRATION THAT THE 90-kDa HEAT SHOCK PROTEIN IS ASSOCIATED WITH TUBULIN IN L CELL CYTOSOL AND IN INTACT PtK CELLS, Emery H. Bresnick, Timothy Redmond, Edwin R. Sanchez, William B. Pratt, Michael J. Welsh, University of Michigan, Ann Arbor, MI 48109.

The distribution of the 90-kDa murine heat shock protein (hsp90) in cultured PtK (Potorous tridactylis kidney) cells was examined by indirect immunofluorescence using the AC88 monoclonal as primary antibody. In interphase cells, hsp90 was distributed like tubulin in a fibrillar array located in the cytoplasm and around the periphery of the nucleus. In cells undergoing mitosis, hsp90 was located primarily in the mitotic spindle. Incubation of L cell cytosol with Affi-Gel-coupled monoclonal antibodies directed against α or β tubulin results in the immune-specific adsorption of hsp90, identified by Western blotting with the AC88 monoclonal antibody. Similarly, the AC88 antibody, which is specific for hsp90, causes the immune-specific isolation of both α and β tubulin from hypotonic cytosol. These observations demonstrate that a significant portion of hsp90 is associated with a tubulin-containing complex in intact marsupial kidney epithelial cells and in a hypotonic cytosol preparation from mouse fibroblasts. Supported from Grant CA28010 (W.B.P.) and Grant GM33980 (M.J.W.).

P 304 DEFINITION OF DOMAIN OF HSP 90 INTERACTING WITH STEROID RECEPTORS, MG Catelli, C Radanyi, JM Renoir, N Binart and EE Baulieu, INSERM U33, Bicêtre, France.

Steroid hormone receptors are found in target tissue extracts in supramolecular complexes associated with hsp90. This receptor form sediments at ~ 8S and does not bind DNA. High ionic strength and/or heat dissociate the hsp90 from the steroid binding subunit which is then able to bind to DNA and sediments as a 4S form. The primary structure of hsp90 has two stretches of polar amino acids (defined as the A and B regions, A being richer in negative charges) that are present in all species from yeast to human. In order to verify our hypothesis that region A may cap the DNA binding site of steroid receptors, we prepared an antiserum (hsp90 A1) to a synthetic peptide from the chicken A region. The reactivity of hsp90 A1 was compared to that of other anti hsp 90 mAbs (Ac 88 and BF4). Hsp90 A1 cross-reacted with hsp 90 from all tested species between human and chicken and in mammalian species, it interacted with a double band in the 90 kDa MW region. In the V8 digest of immunopurified chicken hsp90 the three antibodies recognized fragments of different size. Similarly to Ac88 and BF4, hsp90 A1 antiserum immunoprecipitated almost all the cytosoluble hsp90. However, it was not able to interact with the 8S form of steroid receptors (unlike BF4). These results indicate that hsp90 A1 epitopes are accessible to antibodies only when the hsp90 is not complexed with the binding subunit of receptors. It is conceivable therefore that the negatively charged A region represents at least one of the domains of the hsp90 interacting with the steroid binding molecules. By masking the DNA binding site of steroid receptors this polyanion stretch of hsp90 may play a role in maintaining the receptors in inactive forms.

P 305 TRANSIENT INDUCTION OF PHOTOLYASE ACTIVITY IN CULTURED FROG CELLS BY ULTRAVIOLET LIGHT. Chuck C.-K. Chao[1] and Sue Lin-Chao[2], [1]Dept of Medicine (Oncology) and [2]Dept of Genetics, Stanford University School of Medicine, Stanford, CA 94305

ICR 2A (Rana pipiens) frog cells were established at growing or arrested state and the regulation of photolyase activity was studied. Using clonogenic assays, we show that the enzyme activity is inducible by sublethal doses of 254nm-ultraviolet (UV) light in arrested cells with an eight-fold increase 2-3 days after induction. In contrast, the induction of photolyase activity in growing cells was not detected. Nonetheless, constitutive photolyase activity in both arrested and growing cells remained the same. The inducible function of photolyase was not detected in a mutant cell line hypersensitive to solar-UV wavelengths (310-330nm), neither in cells of high population doublings (HPD). Both mutant and HPD cells demonstrated a global change in DNA function. This includes i) a reduced induction of sister-chromatid exchanges by a predose of r-rays, and ii) a slow formation of repair-associated, DNA single-stranded breaks, and iii) an inefficiency in replicon reinitiation from DNA arrested by UV light. Furthermore, the inducible photolyase activity was undetected in cells by solar-UV wavelengths (310-330nm). We conclude that in addition to exogenous inducer(s) regulation of photolyase activity in ICR 2A cells can be partially determined by endogenous DNA or chromosome conformation.

P 306 Reduction of Endogenous GRP78 Levels in CHO cells and its Effect on Secretion. Andrew J. Dorner, Maryann G. Krane, and Randal J. Kaufman. Genetics Institute, Cambridge, MA 02140.

GRP78, a member of a stress-inducible family of proteins, is localized in the endoplasmic reticulum where it may associate with improperly folded or underglycosylated proteins which fail to be efficiently secreted. We have constructed a mammalian cell expression vector containing the cDNA sequence for hamster GRP78 and used this vector to generate antisense RNA transcripts in Chinese hamster ovary (CHO) cells. The levels of GRP78 mRNA and protein have been reduced 5-10 fold in CHO cells as measured by Northern and Western analysis. We examined the effect of reducing GRP78 levels on the secretion of a heterologous protein in CHO cells. A mutant of tissue plasminogen activator in which the N-linked glycosylation site sequences have been altered to produce an unglycosylated molecule (tPA3x) was used. Wild type CHO cells expressing tPA3x display elevated levels of GRP78. tPA3x was detected in an intracellular complex with GRP78 and inefficiently secreted. Fusion of tPA3x producing cells with antisense GRP78 cells resulted in 2-5 fold reduction in GRP78 levels compared to the original tPA3x line as measured by Western and Northern analysis. The level of tPA mRNA was unchanged. The antisense GRP78/tPA3x line produced 2-3 fold higher levels of secreted tPA activity and the secreted protein had a similar specific activity compared to the original cell line. Analysis of the intacellular processing of tPA3x showed that in the antisense GRP78/tPA3x line tPA3x displayed reduced association with GRP78 and a higher proportion of the intracellular protein was processed to the mature form and efficiently secreted. These results suggest that alterations of GRP78 levels are possible and can influence the secretion efficiency of proteins associated with GRP78.

P 307 SUPEROXIDE INDUCIBLE GENES, Spencer Farr[1,2], Don Natvig[1], Bruce Ames[2] and Tokio Kogoma[1], [1]Depts. of Cell Biology and Biology, University of New Mexico, Albuquerque, NM 87131, [2]Dept. of Biochemistry, University of California, Berkely, CA 94720.

We have screened a MudX1 insertion library of E. coli for cells carrying lacZ, Ap^R operon fusions that produce Beta-galactosidase when exposed to superoxide radical (O_2-) generating conditions. Three such fusions were found and and two of the soi::lacZ, ApR have been cloned (soi = superoxide inducible). Hfr mapping, kinetics of induction, sensitivity to paraquat and restriction analysis show that the fusions map in different locations. The inserts do not map in sodA, katG or nfo. Each of the soi::lacZ, Ap^R fusions is induced by paraquat, plumbagin, HpO_2 and in mutants lacking superoxide dismutase activity. They are not induced by H_2O_2 or other peroxidizing agents. Preliminary evidence suggests that soi genes may be commonly regulated by a trans-acting regulatory element, but their regulation is oxyR, rpoH and recA independent. The Soi response may be a novel network response to an increase in the intracellular flux of superoxide radicals. We are currently trying to clone the wild type soi alleles in order to characterize their regulation and products.

P 308 CHARACTERIZATION OF CONSTITUTIVE DNA-DAMAGE-INDUCIBLE (DIN) PROMOTER MUTATIONS IN BACILLUS SUBTILIS. B. M. Friedman, M. W. Tibbetts, D. H. Flaherty & R. E. Yasbin, University of Rochester, Department of Microbiology & Immunology, Rochester, NY 14642.

All living organisms possess specific mechanisms for their handling of environmental stress(es). In the gram positive developmentally regulated bacterium Bacillus subtilis, constitutive and inducible systems exist that respond not only to DNA damage but also to the stress induced during differentiation. In order to investigate the molecular mechanisms of this system, Din operon fusions were generated by the use of Tn917 transpositional mutagenesis. The regulatory region of one such fusion, din-17, has been subcloned onto plasmid pPL603b which contains a promoterless chloramphenicol acetyltransferase gene. In the presence of the DNA damaging agent mitomycin C, the recombinant plasmid confers resistance to 75ug/ml chloramphenicol in contrast to 10ug/ml in the absence of DNA damage. Following mutagenesis with ethyl methane sulfonate, we were able to isolate a mutation(s) in the din-17 regulatory region which presumably causes its constitutive transcription in the absence of mitomycin C. In addition, a chromosomal mutation(s) was isolated which allows constitutive transcription of the non-mutated din-17 promoter. This mutation(s) is presumably in a cellular regulator that normally represses the din-17 locus or a mutation in the recE gene, the major affector molecule for the B. subtilis DNA-damage-inducible regulatory network. The characterization of the chromosomal mutation(s) along with the DNA sequence of the din-17 regulatory region will be presented and their significance discussed.

P 309 MUTATIONS THAT SUPPRESS CELL KILLING AND MUTAGENESIS IN ESCHERICHIA COLI OXYR⁻ MUTANTS, Jean Toby Greenberg and Bruce Demple, Harvard University, Cambridge. MA 02138.

The oxyR gene of Escherichia coli positively regulates a set of at least 9 polypeptides in response to H_2O_2 and other redox agents. Bacteria that harbor a deletion of oxyR have several phenotypes. These include high sensitivity to killing by compounds that cause oxidative stress, and an elevated spontaneous mutation rate. We have mutagenized ΔoxyR bacteria and selected for cells that are resistant to H_2O_2. Most of these mutants overproduce catalase (HPII) or alkyl hydroperoxide reductase, both of which are scavenging enzymes in the oxyR regulon. Elevated levels of either activity confer resistance to paraquat, menadione and N-ethylmaleimide, implicating peroxides in the pathway of cell killing by these agents. In addition, both types of mutations suppress the elevated spontaneous mutation rate in aerobically grown ΔoxyR E. coli.

P 310 The dissociation of clathrin from coated vesicles by the Uncoating ATPase. Lois Greene and Evan Eisenberg, Laboratory of Cell Biology, NHLBI, NIH.
The 70 kDa-heat shock protein has been shown to be highly homologous to the uncoating ATPase, an enzyme which is able to remove clathrin from coated vesicles in an ATP-dependent reaction. This enzyme was discovered and has been extensively studied in the laboratory of Dr. J. Rothman (Stanford). Using synthetic clathrin baskets as a substrate in most of their studies, they found that only enzymatic amounts of uncoating ATPase were needed to obtain complete removal of clathrin from the baskets. In our experiments we used the more physiological substrate, coated vesicles, which we isolated from bovine brain. In contrast to the results obtained by Rothman and his collaborators with baskets, we find that the enzyme is not able to remove unlimited amounts of clathrin from the coated vesicles. Although we could obtain up to 80% uncoating if we added a 3-fold molar excess of enzyme over clathrin, at lower concentrations of enzyme, we obtained proportionately less uncoating. No matter how long the enzyme was left with the vesicles, further net uncoating did not occur and this was not due to ADP inhibition or denaturation of the enzyme. These results could be caused by inhibition of the uncoating ATPase activity by free clathrin. Alternatively, the enzyme may be inducing a steady state level of free clathrin which remains constant as the uncoating activity of the enzyme is balanced by the rebinding of clathrin to the vesicles. This latter explanation might account for the difference observed between the activity of the uncoating ATPase with coated vesicles and with synthetic clathrin baskets.

P 311 SYNTHESIS OF CALMODULIN-BINDING PROTEINS DURING HEAT SHOCK IN CULTURED PLANT CELLS, H. M. Harrington, A. H. Albert, J. I. Stiles, University of Hawaii, Honolulu, HI 96822.
Heat shock treatments lead to the development of thermotolerance in cultured tobacco (cv. Wisconsin-38) cells. Analysis of cell extracts by ^{125}I-calmodulin (CaM) overlay indicated the presence of several HSPs which bind to calmodulin in a calcium-dependent manner. Similar results were obtained with ^{35}S-methionine-labeled extracts from heat-shocked cells analyzed by calmodulin sepharose affinity chromatography. The apparent molecular weights of these HSPs ranges from 14 to 65 kD on SDS gels. A c-DNA library has been constructed from poly(A) RNA isolated from heat shock polysomes. Research is currently in progress to isolate clones containing CaM-binding HSP by direct expression screening using the above methods and by analysis of hybrid select <u>in vitro</u> translation products.

P 312 THE GENE ENCODING HUMAN HSP89α CONTAINS MULTIPLE INTERVENING SEQUENCES, Eileen Hickey, Georgeann Smale, David Lloyd, and Lee A. Weber, University of South Florida, Tampa, FL 33620.
Vertebrate cells synthesize two different forms of the 83-90 Kd heat shock protein. We have isolated cDNAs representing both forms of human hsp 89 mRNA (hsp89α and hsp89β). Both mRNAs are heat inducible, but only hsp89α is induced by the adenovirus E1A gene product. In order to investigate the molecular basis of hsp89α gene regulation by different effectors, we have isolated and sequenced a human hsp89α gene that shows complete sequence identity with hsp89α cDNA. The gene contains 10 intervening sequences as indicated by S1 analysis and comparison with hsp89α cDNA sequences. The 5′ flanking region contains a single TATA box and overlapping and inverted consensus heat shock control elements. The spliced mRNA sequence contains a single open reading frame encoding an 84,564 Da protein. The deduced hsp89α protein sequence differs from the human hsp89 sequence of Rebbe et al (which is identical to our hsp89β cDNA) in at least 99 out of the 732 amino acids. The first 30 amino acids of human hsp89α are identical to the N-terminal polypeptide of mouse hsp86. Human hsp89β is more similar to mouse hsp84, differing at only 6 amino acid positions. The evolutionary conservation of 2 types of hsp89 genes in higher eukaryotes suggests that there may be functional differences between the proteins.

P 313 MUTATIONS WHICH ACTIVATE P53 FOR TRANSFORMATION RESULT IN COMPLEX FORMATION BETWEEN P53 AND HSC70. Philip W. Hinds, Cathy A. Finlay, Catherine F. Clarke, Alan B Frey and Arnold J. Levine, Princeton University, Princeton, NJ 08544.
A non-transforming, wild-type p53 cDNA clone can be activated for transformation of primary rat fibroblasts with T24 HA-ras by linker insertion mutagenesis. Unlike the wild-type p53, these mutant p53s bind to hsc70, the constitutively produced member of the mammalian 70Kd heat shock protein family, and are not recognized by the monoclonal antibody PAb246. Rat cells transformed by a mutant p53 genomic clone and ras express two populations of p53 proteins, one of which can be recognized by PAb246 ($PAb246^+$) and one of which cannot ($PAb246^-$). $PAb246^-$ p53 is found associated with hsc70 and has a half-life 4-20 fold extended over that of free ($PAb246^+$) p53. p53 genes derived from methylcholanthrene transformed cells also direct the synthesis of a $PAb246^-$, hsc70 binding, stable p53 that is capable of cooperating with activated ras to transform primary rat cells. All p53s which bind to hsc70 and transform differ from wild-type p53 in at least one amino acid residue, suggesting that mutations activate p53 for hsc70 binding, which may be functionally required for the transformation process. Evidence for a role of hsc70/p53 complexes in transformation is also provided by the observation that bacterially synthesized wild-type and mutant p53s bind to dnaK, the bacterial hsc70 cognate. Since hsc70 and dnaK share a remarkable 50% amino acid homology as well as the ability to bind to p53, the functional role of dnaK in replication, cell division and transcriptional control may be conserved in the mammalian homologue, hsc70.

P 314 84 AND 86KD HEAT SHOCK GENE SEQUENCES OF MOUSE AND MAN: TWO RELATED GENE COPIES ENCODE NH_2-TERMINAL SEQUENCES FORMERLY IDENTIFIED AS TUMOR SPECIFIC TRANSPLANTATION ANTIGENS, Thomas Hoffmann and Bernd Hovemann, Zentrum für Molekulare Biologie (ZMBH), 6900 Heidelberg, F. R. of Germany
Mouse hsp84 gene cDNA clones have been isolated. A long open reading frame deduced from their nucleotide sequence encoded a polypeptide of 83kd molecular weight. The aminoterminal half of this cDNA crosshybridized to a second class of mouse cDNA clones. Northern blot hybridization experiments revealed a 2.6kb poly $(A)^+$ RNA when probed with the hsp84 clone and a 2.85kb signal with the hsp84 related cDNA that were both increased after heat shock. The aminoacid sequences deduced from the first contiguous open reading frame of the hsp84 and the hsp84 related cDNA appeared to coincide with the aminoterminal end sequence of formerly identified 84 and 86kd tumor associated antigens (Ullrich et al., PNAS USA 83, pp. 3121-3125, 1986). In addition, the aminoacid composition of the putative 84kd mouse heat shock protein described here matched that of the 84kd tumorantigen of Ullrich et al. very closely. Both notions support the assumption that these heat shock proteins are identical with the 84 and 86kd tumorantigens. Using these mouse hsp84 and hsp86 gene clones as hybridization probes the corresponding pair of human cDNA clones was isolated. Partially characterizing their sequence and RNA expression pattern we obtained indications for a strong evolutionary constraint concerning these two genes in mouse and man.

P 315 INDUCED EXPRESSION OF THE c-*myc* and c-*ras* ONCOGENES FROM THE HSP-70 PROMOTER. **Päivi Koskinen**, Lea Sistonen, Erkki Hölttä, Gerard Evan* and Kari Alitalo. Departments of Virology and Pathology, University of Helsinki and the *Ludwig Institute for Cancer Research, M.R.C. Centre, Cambridge
We have cloned the c-*myc* and c-Ha-*ras* genes under the heat- and serum-/cell cycle-inducible HSP 70 promoter (a gift from Dr. Morimoto), and studied the proteins, as well as the dose-effects of induced oncogene expression in both transient expression assays in COS and 293 cells and in transfected NIH/3T3 cell lines. After an about 30-fold heat shock-induced p64 (c-*myc*) expression, we find an unexpected distribution of *myc*-immunofluorescence in nuclei: in several c-*myc* positive cells fluorescence is distributed in large nuclear structures, and **in some of the cells *myc* fluorescence colocalizes with the HSP 70 immunostain associated with enlarged nucleoli.** The variation in the distribution of the induced c-*myc* protein suggests, that its localization is controlled by cell cycle parameters after heat shock. A fraction of the HSP 70 protein is immunoprecipitated with anti-c-*myc* antibodies from the induced cells. The c-*myc* protein constitutively expressed from the SV 40 early promoter is localized within COS cell nuclei exluding nucleoli, as has been described in previous studies. In cells where p64 (c-*myc*) is heat-induced from an amplified HSP-70-SV 40 vector, the levels are apparently cytotoxic, as shown by lysis of the cells, a result earlier reported by Wurm et al. (PNAS 83, 5414, 1986).

We have also derived stable lines of NIH/3T3 cells expressing the HSP 70 promoter-c-Ha-*ras* proto-oncogene or oncogene constructs. Induction of p21 (c-Ha-*ras*) oncoprotein expression is associated with a gradual appearance of transformation parameters, such as increased fluid-phase pinocytosis and rounded cell morphology in video time-lapse recordings. Using this model, we have studied the effect of p21 (c-Ha-*ras*) on the expression of ornithine decarboxylase.

P 316 MAMMALIAN 90 KD HEAT SHOCK PROTEIN : mRNA EXPRESSION IN MURINE EMBRYONAL CARCINOMA CELL LINES AND PHOSPHORYLATION IN HUMAN CELLS, Vincent Legagneux, Carole Quelard, Michel Morange and Olivier Bensaude. Departement de Biologie Moléculaire, Institut Pasteur, 75724 PARIS CEDEX 15.

A partial cDNA clone has been isolated from a murine embryonal carcinoma cell line expression library, using an anti-HSP90 antibody prepared in our laboratory. The primary structure deduced from the nucleotidic sequence of this clone shows that it corresponds to the "heavy" (thermo-inducible) form of HSP90. The level of corresponding mRNAs is greatly enhanced in fibroblasts after heat shock and spontaneously elevated in embryonal carcinoma cell lines PCC4 and F9.
In vitro phosphorylation of cellular proteins has been studied in HeLa cell line. After stringent heat shock, in vitro phosphorylation of HSP90 is observed, which is hardly detectable in extracts from non heat-shocked cells. This phenomenon could be explained by in vivo dephosphorylation of HSP90 at high temperature, providing free sites for subsequent phosphorylation.

P 317 ASSOCIATION OF P72 AND HSP70 WITH hnRNP COMPLEXES K.L. Milarski, W.J. Welch* and R.I. Morimoto. Dept. Biochem., Molec. Biol., Cell Biol., Northwestern University, Evanston, IL 60208. *Cold Spring Harbor Labs, Cold Spring Harbor, NY.
The major heat-inducible protein in human cells, HSP70, is expressed following a variety of physiological stresses. In addition, synthesis of the protein is growth regulated in unstressed cells. HSP70 is expressed at the G_1/S boundary of the cell cycle and the protein becomes concentrated in the nucleus in early S phase. In order to study the function of HSP70 we have begun to identify proteins associated with HSP70 in the nuclei of control or heat shocked cells by non-denaturing immunoprecipitation with anti-HSP70 antibodies. When monoclonal antibodies which recognize only HSP70 on Western blots and in denaturing precipitations are utilized, P72 the constitutive member of the HSP70 family, is immunoprecipitated along with HSP70; suggesting that P72 and HSP70 may be associated in the cell. In addition, a number of other nuclear proteins are immunoprecipitated under stringent conditions (1% TX-100, 1% DOC, .1% SDS). We have identified some of these proteins as those which are associated with heterogeneous nuclear RNA (hnRNPs). When antibodies to hnRNP proteins are used to isolate hnRNP complexes from control cells we find that P72 is associated with these complexes. Following heat shock both P72 and HSP70 are associated with complexes immunoprecipitated by antibodies to hnRNP proteins. We are currently using methods other than immunoprecipitation to isolate hnRNP complexes from control and heat shocked cells to extend these results.

P 318 STRESS INDUCED GENE EXPRESSION IN FISH CELL LINES, S. Misra, M. Zafarullah, J. Price Haughey and L. Gedamu, Dept. of Biological Sciences, University of Calgary and Biotechnica Canada, #170, 6815 - 8th Street NE, Calgary, Alberta.
We have examined the effect of heavy metals on the expression of two major groups of stress induced proteins; the hsp 70 and metallothioneins (MTs), in fish cell lines. The rainbow trout hepatoma (RTH) and Chinook Salmon embryonic (CHSE) cell lines synthesized the hsp 70 protein in response to cadmium, zinc as well as heat shock. The synthesis of this 70 Kda protein was correlated with the accumulation of hsp 70-mRNA as measured by hybridization to a trout hsp-70 gene probe. Heavy metals also induced the synthesis of MTs in RTH cells. However, heat shock did not result in induction of this heavy metal binding protein. Unlike RTH cells, CHSE cells did not synthesize MT in response to heavy metal exposure. When these cells were treated with 5-azacytidine prior to heavy metal treatment, the synthesis of MT was induced suggesting that DNA methylation may play a role in MT gene expression in this embryonic cell line. Northern blot analysis of total RNA from such treated cells using coding region of TMT-B cDNA as RNA-probe indicated that the time course of induction and the maximal level of MT-mRNA accumulation in response to cadmium and zinc parallels that observed in RTH cells. Copper and dexamethasone were ineffective in inducing MT-mRNA. In conclusion, our results indicate that the MT is specifically induced in response to heavy metal treatment, whereas, synthesis of hsp70 appears to be a rather general stress response. Furthermore, the induction of MT is differentially regulated by the heavy metals and dexamethasone and shows cell-type specific expression. (Supported by NSERC and AHFMR).

P 319 A SPECIFIC COMPLEX BETWEEN THE p53 ONCOPROTEIN AND hsp70. Moshe Oren, Dan Michalovitz, Daniel Eliyahu and Orit Pinhasi-Kimhi Department. of Chemical Immunology, The Weizmann Institute of Science Israel,
p53 is a cellular protein whose aberrant expression can lead to neoplastic transformation and can therefore be regarded as an oncogene product. In a variety cells overproducing p53, this protein is found in a specific, tight physical complex with members of the hsp70 family of heat-shock proteins. Formation of this complex appears to be correlated with an increased protein half-life of p53. The ability to form a complex depends on the sequence of p53, and there appears to be a correlation between the transforming activity of various p53 mutants and their ability to bind hsp70. Finally, animals bearing tumors which contain this complex produce antibodies against hsp70 (and also p53). These findings suggest that hsp70 may play some role in p53-dependent transformation. However, exposing cells to elevated temperatures actually decreased the efficiency of p53-mediated transformation.

P 320 CHARACTERIZATION OF DROSOPHILA HEAT SHOCK COGNATE GENE HSC3; A GENE SIMILAR TO MAMMALIAN GRP78. K. Palter[+*], J. Doctor[+], J. Natzle[**], and E. Craig[+], [*]Dept. of Biology, Temple Univ., Philadelphia, PA 19122, [**]Dept. of Zoology, UC Davis, Davis, CA 95616, [+]Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison, WI 53706. We have isolated four previously unidentified constitutively expressed Drosophila genes related to Hsp70. The chromosomal map positions for three of the genes have been determined and are as follows: Hsc3:10E3,4; Hsc5:50E4-7; and Hsc6:5C5,6. Hsc3 is believed to encode the abundantly expressed 72 kda cognate protein, hsc72. Hybridization data has shown that Hsc3 is extremely homologous to the nematode Hsp70C gene (obtained from T. Snutch, M. Heschl and D. Baillee, Simon Fraser Univ., Canada), the nematode equivalent of mammalian Grp78. Both Grp78 and Hsp70C contain signal sequences and Grp78 encodes a protein found in the lumen of the endoplasmic reticulum. The DNA sequence of Hsc3 is currently being determined to establish if it also possesses a signal sequence. Consistent with the presence of a signal sequence, we have shown that Hsc3 mRNA is found on membrane-bound polysomes. We have also shown that Hsc3 mRNA levels are increased 10 fold in response to 20-hydroxyecdysone in imaginal discs. The induction of Hsc3 mRNA in imaginal discs occurs when the discs are actively secreting the pupal cuticle, suggesting that Hsc3 may play a role in this process.

P 321 OXYGEN REGULATION OF GENE EXPRESSION IN STREPTOCOCCUS SANGUIS. Robert G. Quivey, Jr., William H. Bowen and Ronald E. Yasbin, University of Rochester,
Rochester, NY 14642. It is known that oxygen plays a role in the gene expression of a number of the species of the Enterobacteriacae. Futhermore, oxygen tensions vary greatly in the microenvironments of the oral cavity which could affect the pathogenic potential of plaque. Therefore, to explore the role of oxygen in the regulation of gene expression in the gram-positive oral bacteria, we have decided to study S. sanguis NCTC 10904 because of its ecology, physiology, and genetic manipulability. Cultures of S. sanguis were grown to late exponential phase either aerobically or anaerobically in medium containing 1% glucose. Extracts prepared from harvested culture samples were examined by 2-D gel electrophoresis for comparison of their protein profiles. Electropheretograms of these extracts revealed a number of differences between the two growth conditions that were seen throughout a wide range of molecular weights. To begin dissection of the regulation of these and other proteins, we have isolated a number of mutant strains following Tn 916 transposon mutagenesis. These strains were selected on the basis of their tetracycline resistance and differential growth on solid media in the presence or absence of oxygen. Three classes of strains bearing insertions were isolated: i) no effect on growth ii) long lag phase, poor growth aerobically, long lag phase anaerobically; and iii) long lag phase and poor growth aerobically(>3 hr. generation time), no lag and normal growth anaerobically. The presence of Tn916 in these strains will facilitate the identification of their altered gene products via genetic cloning and comparative 2-D electrophoresis.

This study was supported by NIDR Cariology Training Grant T32DE07165

P 322 MOLECULAR EVENTS OCCURRING IN *Tetrahymena pyriformis* SUBJECTED TO STRESS CONDITIONS. C.Rodrigues-Pousada, L. Galego, I.Barahona, R.Coias and M.Amaral, Instituto Gulbenkian de Ciência, Dept. Microbiol., Apartado 14, 2781 OEIRAS CODEX, Portugal.
Molecular and cellular events associated with the response of *Tetrahymena pyriformis* to stress induced by heat-shock (HS) and sodium metaarsenite (As) have been examined by pulse-labelling experiments. Comparison of the results obtained by both types of stress shows: induction of two main group of proteins, 70-75 kDa and 25-29 kDa as well as 92 kDa and 35 kDa displaying identical peptide maps and of that which seem to be either HS-specific (58 kDa and 46 kDa) or As-specific (83 kDa, 46 kDa presenting different pI, and two forms of 42 kDa). Results obtained by Northern blotting followed by hybridization show that the mRNA coding for the 70 kDa stress protein is present only in stressed cells whereas 27 kDa coding mRNA is present both in stress and in unstressed cells. It seems therefore that the 70 kDa is controlled at transcriptional level whereas 27 kDa is subjected to transcriptional/translational control. Tubulin is one of the most abundant proteins of this microorganism and like most of the constitutive proteins is sharply repressed during HS response. We have investigated the mechanisms by which the modulation of tubulin synthesis takes place during stress conditions. The results revealed that the mRNAs coding for α-and β-tubulin are specifically degraded in contrast to most other mRNAs which seem to be stored in the cytoplasm. We tested the effects of inhibitors of protein and RNA synthesis on the stability of tubulin mRNAs. These results indicate that the process of specific mRNA destabilization requires the synthesis of a heat-shock induced factor.

P 323 SIMILARITY BETWEEN THE 90 KDA PEPTIDE OF THE HEME-REGULATED eIF-2α KINASE AND THE hsp 83-90 FAMILY OF PROTEINS. D.W. Rose, G. Kramer and B. Hardesty, Dept. of Chem. and Clayton Foundation Biochemical Inst., University of Texas, Austin 78712

Highly purified preparations of the heme-regulated eIF-2α kinase that effects translational control in reticulocytes contain a series of peptides that are immunologically cross-reactive with HeLa cell hsp 90 and both subunits of erythroid spectrin. The most abundant of the kinase-associated peptides is a highly elongated 90 kDa species that appears to be structurally related or identical to the 83-90 kDa family of heat shock proteins. The sequence of a 14 amino acid tryptic phosphopeptide derived from the reticulocyte 90 kDa protein that had been phosphorylated by casein kinase II is identical to a sequence of the Drosophila, mouse and human hsp. A very high degree of similarity exists in the amino terminal sequences as well (Rose et al., Biochemistry 26, 6583, 1987). No sequence similarity is evident between the hsp 90 peptides and the known portion of the spectrin subunits that might explain the immunological crossreactivity. The 90 kDa reticulocyte peptide and the isolated spectrin subunits are similar in their ability to increase the enzymatic activity of the eIF-2α kinase and to inhibit protein synthesis in reticulocyte lysates. A possible physiological role for the association of an eIF-2α kinase with a heat shock protein may be implied by data from several laboratories suggesting a contributory role of eIF-2α phosphorylation in the inhibition of normal cellular translation which occurs in stressed tissue culture cells.
(Supported by grants CA 16608 and CA 09182 from NIH.)

P 324 HEAT SHOCK RESPONSE OF NEUROSPORA CRASSA: INDUCTION, PURIFICATION AND CHARACTERIZATION OF HSP80, H.S. Roychowdhury and M. Kapoor, Cellular, Molecular and Microbial Biology Division, Department of Biological Sciences, The University of Calgary, Canada T2N 1N4.
HSP80 is one of the 11 distinct heat shock proteins of Neurospora crassa, resolved by one-dimensional SDS-polyacrylamide gel electrophoresis. This protein was not induced by toxic metal ion treatment whereas sodium arsenite induced it at normal growth temperatures. The induction of HSP80 was investigated during growth on carbon-sufficient medium as well as under carbon starvation conditions. It was synthesized in carbon-deficient medium even under non-heat shock conditions, when the medium was supplemented with 0.02% sucrose, 0.3% acetate, 0.2% lactate or ethanol. The expression of HSP80 appears to be under dual control of heat stress and carbon catabolite repression. HSP80 has been purified to near homogeneity using gel filtration, ion exchange and affinity chromatography. The purified protein migrates as a single band on SDS gels but on 2-D gels it resolves into 4 polypeptide spots with isoelectric points in the acidic range. HSP80 has been shown to be a glycoprotein with an approximate size of 610 kDa in the native state.

P 325 PRIMARY EFFECTS OF ALLELOCHEMICALS: SOS RESPONSE MECHANISMS, Matthew Ryuntyu, Department of Agronomy and Soil Science, University of New England, Armidale. N.S.W. 2351.

The presence of scopolamine in the seed coat of thornapple points to an elegant physiological mechanism which had the following inhibition of early growth of sunflower. The latter interaction could be retarded by activity of the metabolism of food reserves. That interference with membrane function at the level of the cell can explain some secondary allelopathic phenomena, e.g. inhibition of mineral uptake. The allelopathic effects are associated with impaired mitochondrial function, increased vacuolar activity and phagocytosis (Lovett & Ryuntyu, 1987. Proc. 8 Aust. Weeds Conf., Sydney:179-183). TEM shows arrested development of metaphase and anaphase by sunflower root tips. Lipid bodies are abundant in the cell and amyloplasts may also be present. The latter features suggest that metabolism of food reserves has been disrupted. It is possible to confirm the hypothesis that the detoxification effect of allelochemicals is to interfere with SOS response mechanism through the metabolism of energy sources in the cells of germinating seedlings.

P 326 DEMONSTRATION THAT THE 90-kDa HEAT SHOCK PROTEIN IS ASSOCIATED WITH TUBULIN IN L CELL CYTOSOL AND IN INTACT PtK CELLS, Edwin R. Sanchez, Timothy Redmond, Emery H. Bresnick, William B. Pratt, Michael J. Welsh, University of Michigan, Ann Arbor, MI 48109.

The distribution of the 90-kDa murine heat shock protein (hsp90) in cultured PtK (Potorous tridactylis kidney) cells was examined by indirect immunofluorescence using the AC88 monoclonal as primary antibody. In interphase cells, hsp90 was distributed like tubulin in a fibrillar array located in the cytoplasm and around the periphery of the nucleus. In cells undergoing mitosis, hsp90 was located primarily in the mitotic spindle. Incubation of L cell cytosol with Affi-Gel-coupled monoclonal antibodies directed against α or β tubulin results in the immune-specific adsorption of hsp90, identified by Western blotting with the AC88 monoclonal antibody. Similarly, the AC88 antibody, which is specific for hsp90, causes the immune-specific isolation of both α and β tubulin from hypotonic cytosol. These observations demonstrate that a significant portion of hsp90 is associated with a tubulin-containing complex in intact marsupial kidney epithelial cells and in a hypotonic cytosol preparation from mouse fibroblasts. Supported from Grant CA28010 (W.B.P.) and Grant GM33980 (M.J.W.).

P 327 cDNA CLONING OF A HUMAN MITOMYCIN C INDUCIBLE GENE, N.A. Stevens and M. Buchwald, The University of Toronto and the Hospital for Sick Children, Toronto, Canada.

Mitomycin C (MMC) is a bifunctional alkylating agent that produces DNA interstrand crosslinks as a primary lesion. In E. coli, exposure to MMC induces the expression of 17 genes whose gene products play roles in DNA repair, mutagenesis, and recombination. We have documented that exposure of primary human fibroblasts to 1 ug/ml MMC for eight hours results in an increased abundance of a set of seven abundant proteins detected by resolving ^{35}S-methionine labelled total proteins by 2D PAGE. Furthermore, we have begun the isolation of human fibroblast cDNAs corresponding to MMC induced mRNAs in order to characterize the MMC response in human cells at the molecular level and to investigate the expression of the corresponding genes in Fanconi Anemia (FA) cells. Cultured cells from patients with FA; an autosomal recessive disorder of unknown molecular basis, exhibit approximately a 10 fold greater sensitivity to MMC compared to normal as assayed by survival curves. We screened about 10,000 clones of a λgt10 cDNA library as well as about 1,000 clones of a pBR322 cDNA library; both libraries constructed from normal human fibroblasts treated with 1 ug/ml MMC for eight hours. The clones were screened differentially using ^{32}P labelled cDNA synthesized from total poly A+ mRNA from induced or uninduced cells. In total, 18 clones were isolated that reproducibly corresponded to to mRNAs of increased abundance in the induced population. Cross hybridization analysis revealed that all were representatives of the same mRNA. According to Northern analysis, the normalized levels of the mRNA increase in abundance over time of exposure to MMC up to about 13 times the basal level at 19 hours. Induction starts at about 4 hours after the initiation of treatment and increases for the next 15 hours. Preliminary Northern analysis of one SV40 transformed FA fibroblast line suggests that the mRNA correponding to this cDNA exists at a lower basal level compared to normal and does not undergo induction upon exposure to MMC. Genomic Southern analysis strongly suggests the existence of a single corresponding gene. Sequencing of an apparently full length cDNA is currently well in progress. To date, no significant homology to human sequences in the GENBANK data base have been found. *Supported by MRC, Canada.*

P 328 INDUCERS OF GLUCOSE REGULATED PROTEINS DEPLETE TOPOISOMERASE II. J.W. Shen, W.E. Ross* and J.R. Subjeck. Roswell Park Memorial Institute, Buffalo, NY 14263 and *University of Florida, Gainesville, FL.

We have previously reported that several inducers of glucose regulated proteins (grps) also produce resistance to the drugs adriamycin and VP-16. One prominent target for these two drugs is the nuclear enzyme topoisomerase II. We report here that 2-deoxyglucose, anoxia, glucose starvation, A23187, and EGTA, all inducers of the grp system, lead to a rapid and selective depletion of topoisomerase II from the nuclear fraction. No visible change in other nuclear proteins was detected (i.e., the depletion in topoisomerase II was not a reflection of a general loss in nuclear protein content). This depletion appears to precede the induction of grps. This change is also not a general response to stress as indicated by the fact that a 10 min., 45°C heat shock does not alter topoisomerase II levels. We suggest that this grp induced depletion in topoisomerase II is the basis for the drug resistance phenomenon which we have previously described. This information additionally indicates that several "glucose regulated stresses" result in rapid changes in nuclear composition and function.

These studies were supported by PHS grant 40330.

P 329 TWO-DIMENSIONAL GEL ANALYSIS OF PROTEINS INDUCED BY SUPEROXIDE RADICAL LACKING SUPEROXIDE DISMUTASE, Linda K. Walkup, Spencer B. Farr, Donald O. Natvig and Tokio Kogoma, University of New Mexico, Albuquerque, NM 87131

All aerobic organisms have mechanisms to protect themselves from reactive oxygen species which arise even during normal respiration and metabolism. Protective enzymes include superoxide dismutases (SOD's) which catalyze the conversion of the superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2). E. coli SOD^- mutants lack all superoxide dismutase activity, and thus are constitutively stressed by their own endogenously generated superoxide radicals. Lack of SOD activity also makes them especially sensitive to exogenous O_2^- generators such as plumbagin and paraquat. Earlier studies indicated that a protective stress response induced by O_2^- flux is separable from that induced by H_2O_2 treatment (Farr, Natvig and Kogoma, J. Bact. 164 : 1309, 1985). To examine the proteins induced in this postulated O_2^- response, we ran two-dimensional gels of proteins extracted from wild-type and SOD^- cells that were either growing aerobically or were stressed with plumbagin and paraquat treatments. Proteins from H_2O_2 treated and heat shocked wild type cells were used for correlation with other stress induced proteins. Nine proteins are constitutively induced above wild type levels in the aerobically growing mutant, only one of which is also induced due to H_2O_2 treatment. Three of these proteins are also induced in wild type cells treated with plumbagin or paraquat, as well as two additional proteins seen only in the treated wild type cells. Six additional proteins are induced in the mutant after treatment with O_2^- generators, four of which are also seen in treated wild type cells. Three of these four proteins are also induced by heat shock, and the fourth is induced by H_2O_2 treatment.

P 330 ORGANIZATION AND EXPRESSION OF THE HUMAN GRP78 GENES S.S. Watowich and R.I. Morimoto. Dept. Biochem., Molec. Biol., Cell Biol., Northwestern University, Evanston, IL 60208. Human cells express a 78,000 dalton protein under normal growth conditions which is structurally and antigenically related to the major heat inducible protein, HSP70. This protein has been previously identified as the major glucose regulated protein, GRP78. We are interested in studying the expression and regulation of GRP78 to determine its relationship within the human HSP70 gene family. Multiple cDNA clones as well as two distinct genomic clones have been isolated from human libraries screened for GRP78 sequences. Southern blot analysis indicates at least two loci for GRP78 sequences in the human genome; each locus appears to contain a single copy of the GRP78 gene. One of the human GRP78 genes, unlike the rat GRP78 gene, does not appear to contain introns.

The cloned GRP78 sequences and the human HSP70 gene, previously isolated in this laboratory, have allowed us to study the expression of GRP78 and HSP70 in human cells treated with a variety of metabolic inhibitors. We find that the inhibitors tested fall into three classes; the first class activates the expression of GRP78 alone, the second activates the expression of both genes, and the third activates GRP78 expression and represses HSP70 expression (Watowich, S.S. and R.I. Morimoto, MCB, in press). The increase in GRP78 expression is due to an increase in the transcription rate of the gene whereas the repression of HSP70 is regulated post-transcriptionally. Our results suggest that the specificity of physiological damage may independently regulate the expression of both stress genes and that this regulation can occur at different levels of gene expression.

P 331 CHARACTERIZATION OF THE MAMMALIAN LOW MOLECULAR WEIGHT HEAT SHOCK PROTEIN AND PROTEASOME PARTICLES, A.-P. Arrigo[1], J. Suhan[1], A.L. Goldberg[2], and W.J. Welch[1], [1]Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724 and [2]Harvard Medical School, Boston, Massachusetts 02115.

In mammalian cells there appears to be a single low MW HSP, which we refer to as 28kDa. Phosphorylation of 28kDa (but not its synthesis) increases within minutes following exposure of cells to either tumor promoters (e.g., PMA), calcium ionophores (e.g., A23187) or simply the addition of fresh serum or defined growth factors. In the case of heat shock treatment both the synthesis and phosphorylation of 28kDa are observed to increase. Similar to the situation in Drosophila (where there are four low MW HSPs), mammalian 28kDa exhibits differential solubility characteristics as a function of the state of the cell (i.e., normal or stress). We have succeeded in purifying the protein by classical methods and found it to exist as a multimer with an average molecular mass of about 500,000 daltons after the heat stress. Analysis of the purified protein by electron microscopy reveals it to have a distinct and defined morphology. Using an antibody raised against the purified protein, we will present data describing the different intracellular distributions of the protein as a function of stress. A number of studies during the past fifteen years have described a ring-shaped 19-20S particle present in a variety of eukaryotic cells. These structures, called "prosomes," are composed of several polypeptide subunits which range in size between 20 and 30kDa and have a very characteristic morphology in the electron microscope. Using specific antibodies, we demonstrate that the mammalian prosome does not contain the low molecular weight HSP 28kDa. Interestingly, we have found that the prosome is identical to a large multifunctional protease complex and have renamed this particle "proteasome." We will present data describing the behavior of this protease as a function of stress.

P 332 HSP70 PROTEINS FROM YEAST: THEIR ROLE IN MITOCHONDRIAL FUNCTION. M. Werner-Washburne, J. Kramer, J. Kosic-Smithers, and E. A. Craig. University of Wisconsin-Madison, Madison, Wisconsin 53706

Saccharomyces cerevisiae contains a large family of genes related to the heat-inducible Hsp70 gene of Drosophila. These genes have been grouped into 4 subfamilies, based on genetic evidence and DNA sequence comparisons. Two of these subfamilies, SSA and SSC, are essential for vegetative growth.

The SSA proteins are involved in mitochondrial transport. Analysis of a conditional SSA mutant under nonpermissive conditions revealed that this mutant is defective in the in vivo import of the mitochondrial F_1ATPase ß-subunit. We are currently studying this mutant to determine whether the import of other mitochondrial proteins is affected and whether the import defect can be demonstrated in vitro.

SSC1 is a mitochondrial protein. The predicted amino acid sequence of the SSC1 protein contains an amino terminal extension that resembles a mitochondrial transit peptide. In vitro translation and import experiments were used to confirm that the SSC1 protein is synthesized as a precursor and that this precursor can be imported into isolated mitochondria. We are now determining the intramitochondrial localization of the SSC1 protein and are examining the effect of an SSC1 mutation on the mitochondrial protein import and assembly.

P 333 CHARACTERIZATION OF AN M_r~90,000 HEAT SHOCK PROTEIN ASSOCIATED WITH THE RAT GLUCOCORTICOID RECEPTOR . Ann-Charlotte Wikström, Marc Denis and Jan-Åke Gustafsson. Dept. of Medical Nutrition, Karolinska Institute, Huddinge University Hospital F-69, S-141 86 Huddinge, Sweden

Employing immunoaffinity chromatography of rat liver glucocorticoid receptor, a non-hormone binding M_r~90,000 protein has been shown to be associated with GR in low salt buffers or in the presence of molybdate. Following immunoaffinity chromatography this M_r~90,000 protein has been further purified to homogeneity by high-performance ion-exchange chromatography. By a two-step purification procedure using high-performance anion-exchange and gel permeation column chromatography mg amounts of pure protein is obtained. Twenty-five N-terminal amino acids of this protein have been identified. Comparisons with other heat shock proteins show extensive N-terminal amino acid sequence homology to heat shock proteins from drosophila (DHS83) and yeast (YHS 90). The M_r~90,000 protein exists as a dimer in the molybdate-stabilized GR complex as demonstrated by sodiumdodecylsulphate-polyacrylamide gel electrophoresis and gel permeation chromatography. After ligand-binding to GR the dissociation of the M_r~90,000 dimer from GR is a prerequisite for GR to convert to its activated DNA-binding form. Using monospecific rabbit antibodies to the M_r~90,000 protein in immunocytochemical studies of the rat Rueber hepatoma cell line H-4-II-E, specific staining in both the cell cytoplasm and nucleus is demonstrated. Furthermore, the capacity of the purified M_r~90,000 to bind the lectins Concanavalin A and Dolichos Biflorus Agglutinin indicates that it is a glycoprotein.

P 334 THE RAT GENE ENCODING THE 78,000 DALTON GLUCOSE-REGULATED PROTEIN GRP78: ITS STRUCTURAL ORGANIZATION, SIGNAL SEQUENCE, AND CIS-REGULATORY CONTROL ELEMENTS, Scott K. Wooden, Raj P. Kapur, Jerry Ting, Elpidio Resendez Jr., and Amy S. Lee. Department of Biochemistry, University of Southern California School of Medicine, Los Angeles, CA 90033.

GRP78 exists as a single copy in the rat genome and comprises of seven exons. The protein profile is highly hydrophilic with the notable exception of several highly conserved hydrophobic domains among members of the 70-kilodalton stress-inducible protein family. The GRP78 signal sequence within the first exon targets the protein into the endoplasmic reticulum (ER). We have fused this leader sequence and the GRP78 promoter in frame with the chloramphenicol acetyltransferase gene (CAT) and transfected the plasmid into a hamster fibroblast cell line. In these stable transfectants, the CAT protein, like GRP78 itself, is localized in the ER and is inducible under several stress conditions, notably those that block glycosylation and disrupt sequestered Ca^{2+}. We have further examined the transcriptional regulation of the GRP78 gene and found the cis-acting element responsible for Ca^{2+} ionophore and glycosylation block induction lies within a 50nt region unpstream of the transcriptional initiation site. This site also binds with high affinity to nuclear protein factors.

Thermal Tolerance and Thermal Regulation; Heat Shock Response and Pathogens

P 400 RAT VL30 INDUCTION BY ANOXIA: STRESS ACTIVATION OF A PUTATIVE MAMMALIAN TRANSPOSABLE ELEMENT. Garth R. Anderson, Daniel L. Stoler, Lisa A. Scarcello and Kenneth F. Manly, Department of Molecular and Cellular Biology, Roswell Park Memorial Institute, Buffalo, NY 14263.

Anoxic stress induces normal rat fibroblasts to transcribe very large amounts of RNA related to VL30 elements. Induction ranges up to more than 500-fold. The induced RNA is of 5.4 kb and has both VL30 internal and termini sequences, indicating complete VL30 elements are induced. Optimal induction occurs under complete anoxia, although a lesser response is seen under atmospheres up to two per cent oxygen. Phorbol esters and diacylglycerol, which induce mouse VL30 by around eight fold, show no effect on the rat VL30 system. VL30 RNA is induced within one hour after onset of anoxia, considerably before the appearance of a set of at least seven major anoxic stress proteins. The anoxic conditions optimal for induction of rat VL30 appear to represent a relatively mild stress, being associated with no reduction in cell viability.

VL30 elements have structural and biological properties closely resembling known transposable elements. Sequence analysis reveals similarities to discrete regions of feline and murine retroviruses. Particularly extensive sequence similarity is seen to the endonuclease domain of a retroviral pol gene. In lower organisms, transposable elements are known which are stress inducible. The rat VL30 response appears to represent an analogous mammalian system.

P 401 A NEW 70 kD PROTEIN IN HEAT RESISTANT MAMMALIAN CELLS. Robin L. Anderson, Ine van Kersen, Vatsala Basrur, Manohar Adwankar and George M. Hahn, Stanford University, Dept. of Therapeutic Radiology, Stanford, CA 94305, USA.

One proposed function of the heat shock proteins (HSP) is to protect cells from heat damage. The way in which these proteins may protect is not clear, but could involve stabilization of critical heat sensitive organelles or macromolecules.

We have isolated heat resistant mutants of a mouse tumor cell line (RIF-1) to explore the possible role of HSP in conferring the increased resistance to heat. The cells were selected by multiple cycles of 45°C heating followed by regrowth of survivors at 37°C. The mutants display a markedly increased and stable resistance to hyperthermia.

Two dimensional gel electrophoresis of total cell proteins from three different heat resistant lines reveals a new protein in the 70kD region, with a slightly lower pI than the constitutive HSP 70. In addition, there is constitutive expression of the normally heat-inducible 68kD and 89kD proteins, although the expression is much less than that observed after heat shock. After heating, the mutant lines display a similar pattern of heat-inducible proteins as the parent line. The new protein is not markedly heat inducible. Preliminary evidence suggests that the new protein is a member of the HSP 70 family.

Another series of cells selected for resistance to amphotericin B displayed as much heat resistance as the heat selected lines. Selection for amphotericin B resistance from one line previously selected by heat revealed no further increase in heat resistance, suggesting a common pathway for development of the heat resistance. The amphotericin B resistant cells also have the same changes in their protein profile, as revealed by 2D gel electrophoresis.

P 402 SYNTHESIS OF HEAT SHOCK PROTEINS IN MESOPHILIC AND THERMOPHILIC CLOSTRIDIUM SPECIES, Andreas Pich and Hubert Bahl, Institut für Mikrobiologie, Universitat Gottingen, Grisebachstrasse 8, D-3400 Gottingen, Federal Republic of Germany.

The heat shock response in the strictly anaerobic mesophilic bacteria Clostridium butyricum and Clostridium acetobutylicum and the thermophilic Clostridium thermosulfurogenes EM1 was characterized. At least 10 different heat shock proteins (hsps) in each of the mesophilic strains were induced by a temperature upshift from 30 °C to 42 °C. In the thermophilic microorganism only 3 hsps could be identified after a heat shock from 50 °C to 62 °C. More thermophilic Clostridium species will be tested to determine whether the number of hsps is reduced in these organisms.

As judged by ^{35}S- labeling and gel electrophoresis the regulation of heat shock protein synthesis in the Clostridium strains fits in general the description of the heat shock response in E. coli. The synthesis rate of the hsps was transiently increased 4 to 20 fold reaching a maximum 5 to 15 min after the temperature shift. The increase in the synthesis rate of hsps was not in all cases strictly coordinated. In C. acetobutylicum this was especially striking for a hsp with a molecular weight of 21 KDa. The synthesis rate of this protein reached a maximium after 5 min at 42 °C and then declined rapidly to the starting level by 10 min. At that time the majority of the hsps had their highest synthesis rate. It is of interest that in C. thermosulfurogenes a protein with a similar molecular weight and isoelectric point had the overall highest synthesis rate at low temperature (50 °C) which increased slightly after the shift to 62 °C and then declined to almost zero by 60 min. Whether this protein is analogous in these strains and whether it has a function in the regulation of the heat shock response in Clostridia can only be speculated upon so far.

P 403 ENZYME INACTIVATION AND THERMOPROTECTION DURING HEAT-SHOCK AND RELATED STRESS, Olivier Bensaude, Van Trung Nguyen, and Michel Morange, Biologie Moleculaire du Stress, Institut Pasteur, 75724 Paris Cedex 15, France.

Heat-shock and related stress are well known to induce disorders in the cells. The abnormal protein formation is generally accepted to be responsible for the heat-shock gene induction of transcription. However the nature and the cause of the abnormalitie remain largely a mystery. We have undertaken an approach of the toxic effects of stress on the activity of well defined enzymes commonly used as reporters of gene activity. In cells transfected with plasmids carrying the corresponding genes, the activities of B-galactosidase and of firely luciferase are rapidly inactivated during non lethal heat shock conditions. Interestingly, stress conditions which promote a thermotolerant state are the same which bring a protection of the reporter enzymes against in vivo heat-inactivation. Thus, it is suggested that the noxious disorders induced by heat shock and some of the related stress are not due to simple heat or solvent denaturation effects, and that thermotolerance results from a prevention against these effects.

P 404 INOLVEMENT OF rRNA AND PROTEIN SYNTHESIS IN THE EXPRESSION OF THERMOTOLERANCE. Adrian R. Black and John R. Subjeck. Roswell Park Mem Inst, Buffalo, NY, 14263.

Both protein and RNA synthesis recover more rapidly after heat shock in thermotolerant cells and the rate of their recovery correlates with the level of thermotolerance. The role of rRNA synthesis in thermotolerance was investigated using 0.1 ug/ml actinomycin D. The drug itself was only mildly cytotoxic to CHO cells either alone or in conjunction with a single heat treatment. Actinomycin D (0.1 ug/ml) did not block the development of thermotolerance, but dramatically reduced the survival of thermotolerant cells when left in place for 16 h after the challenge treatment. The concentration dependence of this effect was very similar to that for blockade of rRNA synthesis. At 0.1 ug/ml, actinomycin D was able to inhibit the rapid recovery of protein synthesis following a severe heat shock in thermotolerant cells, suggesting that de novo synthesis of rRNA is an important factor in this phenomenon. The effect of blocking the recovery of protein synthesis following heat shock of thermotolerant cells was studied by adding 100 ug/ml cyclohexamide for the 16 h immediately following challenge treatment. The drug was again only mildly toxic but inhibited the expression of thermotolerance. These data show that rapid recovery of both rRNA and protein synthesis following a challenge treatment are important for the greatly enhanced survival of thermotolerant cells and that rRNA synthesis is needed for the rapid recovery of protein synthesis. Therefore, protection of rRNA synthesis seems to be a key mechanism of thermotolerance both at the subcellular and cellular level. Supported by grant CA40330.

P 405 STRESS IN THE FRESHWATER POLYP HYDRA: SPECIES WHICH DO NOT SYNTHESIZE HEAT-SHOCK PROTEINS DO NOT SURVIVE STRESS.

Thomas CG Bosch, Susan M Krylow, Hans R Bode and Robert E Steele, Developmental Biology Center, University of California, Irvine, CA 92717.

The major heat shock protein of the freshwater coelenterate hydra is a 60 kD protein which is induced at temperatures as low as 24°C. When characterizing the heat shock response in different species of hydra we found the same stress response in H.attenuata and H.magnipapillata. However, several species, e.g. H.oligactis and H.utahensis, were found not to synthesize detectable levels of any new species of proteins in response to stress. The absence of heat shock proteins was always found to be directly correlated with the inability of these species to survive short-term exposure to environmental stress. These findings predict that species of hydra characterized by a strong heat shock response would be found in a wide variety of freshwater habitats. However, species of hydra which are unable to synthesize hsp60 in response to stress would be expected to be found in a more restricted range of habitats characterized by stable temperatures. All ecological data available support these predictions. In conclusion we propose that the heat shock response might not be as universal as previously thought. Its presence might well be dispensable for organisms not exposed to temperature changes in their natural habitats.

P 406 THE EXPRESSION OF HEAT SHOCK PROTEIN GENES IN TRYPANOSOMA CRUZI IS REGULATED AT DIFFERENT LEVELS, Elizeu F. de Carvalho, Jose Francisco Carvalho, E. Rondinelli, C. M. A. Soares and F. T. de Castro, Instituto de Biofisica, UFRJ, Rio de Janeiro, Brazil, 21941. The induction of four heat shock proteins (HSPs) is observed in T.cruzi when this parasite is submitted to temperatures higher than 29oC up to 40oC. In vivo experiments in the presence of actinomycin D suggested that: 1- this induction seems to be controlled at different levels since the appearence of two HSPs is still observed 2- the amount of the pre-existing mRNAs coding for these proteins seems to be sufficient to support the HSPs synthesis for a period of time of at least 3 hours . These facts led us to postulate the existence, a 29oC, of a stock of specific mRNAs coding for these proteins in a non-polysomal compartment.The analysis of the population of polysomal and non-polysomal compartments by dot-blots probed with HSP 70 homologous clone revealed : a) the existence of HSP 70 mRNA in the non-polysomal compartment of cells kept at 29oC, b) the amount of this RNA increases in both compartments in cells incubated at 37oC. The data presented strongly suggest that at the beginning of the heat treatment, T. cruzi cells utilize preformed mRNAs for HSPs synthesis. Later on the synthesis of these proteins is dependent on the "de novo" formation of the specific mRNAs. Supported by: CNPq, FINEP, CPEG (Brazil).

P 407 EFFECT OF CONTINUOUS HEAT STRESS ON CELL GROWTH AND PROTEIN SYNTHESIS IN A.albopictus. Carvalho M.G.C. and Freitas M.S., U.F.R.J., Brasil . A.albopictus cells (clone C6/36) normally grow at 28ºC. We observe that cells in lag or exponential growth phase, if maintained at 37ºC, present inhibition of cell growth withouth death, at least for five days. In the present study, we analyse the modifications occuring on the protein synthesis during short and long term exposition of cells to heat stress. We observe that at first hour of exposition of cells at 37ºC they synthesize two heat shock proteins (hsp) of 82 KDa and 70 KDa, concomitant with inhibition of normally produced proteins at control temperature. However, for incubations longer than two hours at 37ºC, a shift to the normal pattern of protein synthesis occurs. During these transitions, a decrease of the inhibition of cellular proteins is observed and two other hsps of 76 KDa and 90 KDa are synthesized. If cells were submitted for 90 minutes at 37ºC followed by incubations for 3 hours at 28ºC and submitted to a second heat treatment, they synthesized hsp of 82KDa and 70 KDa, without , however, cellular protein synthesis inhibition. These results suggest a development of thermotolerance by the cells if they were incubated under continuous stress or if they were incubated, for a short time , to a heat treatment. Pulse chase experiments show that the hsp 70 KDa is stable at least for 18 hours when the cells are returned to 28ºC after heat treatment and for 48 hours if they were maintained at 37ºC.
(Supported by CNPq and CEPEG).

P 408 HSP SYNTHESIS IN T CELLS IS CORRELATED WITH PROTECTION OF IMMUNE FUNCTION DURING STRESS. Richard P. Ciavarra, Eastern Virginia Medical School, Norfolk, VA 23501.

I have investigated the heat shock protein (hsp) response in purified T cells, T cell subsets and a cloned helper T cell line (D10) following exposure to hyperthermia. SDS-PAGE analysis of ^{35}S-methionine labeled whole cell extracts demonstrated that a short exposure (1 hr) to 39°C induces hsp in T cells with an approximate molecular weight of 110, 93 and 79 Kd; a minor hsp is also detected at 70 Kd. A maximal hsp induction occurs at 41°C whereas 42°C severely inhibites both cell protein and hsp synthesis. Lyt-1$^+$23$^-$ T cells are responsible for most of the hsp synthesized by T lymphocytes following hyperthermic stress because hsp are induced in Lyt-1$^+$23$^-$ lymphocytes and D10 cells whereas no hsp are detected in purified Lyt-1$^-$23$^+$ cells. In contrast to hyperterminia, dexamethasone at concentrations (10^{-6}M-10^{-7}M) that inhibit generation of alloreactive cytotoxic T lymphocytes (CTL) in vito does not induce hsp in purified T cells although these concentrations are detrimental to cellular protein synthesis. Functionally, hsp$^+$ state does not protect alloreactive CTL responses from dexamethasone-induced stress. However, a stress-tolerant state can be demonstrated in hsp$^+$ lymphocytes subjected to hyperthermia. Thus, comparable CTL responses are detected in lymphocytes cultured at 37°C and 40°C in only those cells that have synthesized hsp prior to culture.

P 409 ALTERATION OF THE ANTIVIRAL STATE AND SELECTIVE DEGRADATION OF THE P68 KINASE IN INTERFERON-TREATED HELA CELLS AFTER A HEAT-SHOCK. Marie-Françoise Dubois*, Julien Galabru+ and Ara.G. Hovanessian+. INSERM U43 Hopital St Vincent de Paul * 75014 Paris and Institut Pasteur + 75015 Paris France.

The antiviral state induced by interferon can be degraded during a heat-stress: following a 43°C heat-shock, multiplication of encephalomyocarditis virus is no longer inhibited in interferon-treated human HeLa cells. The fate of the interferon-induced double-stranded RNA dependent protein kinase was investigated. This protein kinase is a 68 000 daltons protein which, when autophosphorylated, catalyzes phosphorylation of the protein synthesis initiation factor eIF2, thus mediating inhibition of protein synthesis. After a heat-shock, dsRNA-dependent phosphorylation of the p68 kinase is greatly reduced whereas the phosphorylation of other cellular proteins is not affected. This reduction in the activity of the protein kinase is due to a significant degradation occuring during the heat-shock period. Actin and other cellular proteins are not affected by such a stress. Another dsRNA-dependent enzyme induced by interferon, the 100 KDa 2-5 A synthetase, is also degraded but at a lesser extent than the kinase. During the recovery period at 37°C, the p68 kinase levels are restored and the degree of this restoration is dependent on the intensity of the thermal stress.

P 410 INDUCTION AND ACCUMULATION OF HSP 70 M-RNA IN ADULT SALAMANDERS IN RESPONSE TO DIFFERENT HEAT STRESSES, INCLUDING NATURALLY OCCURRING THERMAL STRESS CONDITIONS IN THE FIELD, Douglas P. Easton, Joseph C. Near, Paul S. Rutledge, Douglas P. Dickenson* and James R. Spotila, State University College at Buffalo, Buffalo NY 14222 and *School of Dentistry, State University of New York at Buffalo, Buffalo NY 14214. We have examined the effects of three different types of heat shock on the production of hsp 70 mRNA by tissues of intact adult salamanders. Brief shocks were given by heating the animals to the critical thermal maximum (CTM), the temperature at which the animal begins to spasm. More prolonged shocks were given by heating the animals to either 2°C below the CTM or 5°C below CTM and holding the animal at that temperature for 1 hr. Hsp mRNA was measured by probing northern blots of RNA extracted from the animals' tissues at specified times after recovery at normal temperature. Measurable accumulation of hsp mRNA occurred within 20 min. of a CTM and during the 1 hr shocks. Hsp mRNA levels peaked 3-6 hours following CTM, 9-15 hours after 1 hr 5°C below CTM and 24-48 hr. after a 1 hr. 2° below CTM treatment. Levels of hsp 70 mRNA approached those of controls after 16, 30 and 96 hrs. respectively. On a hot summer afternoon we collected 4 salamanders from sites with temperatures ranging from 25-27.7°C. We extracted RNA from the animals in the field and assayed for Hsp 70 mRNA. The animal at 27.7°C contained hsp mRNA in all tissues examined. The lower temperature individuals contained little or no Hsp 70 message.

P 411 **A NOVEL HSP70 COGNATE IN TRYPANOSOMES**, David M. Engman, Louis V. Kirchhoff, Kimberly Henkle and John E. Donelson, Departments of Biochemistry and Internal Medicine, University of Iowa, Iowa City, Iowa 52242. The protozoan *Trypanosoma cruzi* is the etiologic agent of Chagas' disease, an illness that causes considerable morbidity and death among the 10 to 12 million infected individuals in the Americas. In an attempt to identify *T. cruzi* gene products that might have potential use in vaccination, we used rabbit antiserum against *T. cruzi* to isolate cDNA clones from a *T. cruzi* λgt11 expression library. Seven recombinant phage [λTC1-λTC7] were identified and found to have cross-hybridizing cDNA inserts. Determination of the TC1 DNA sequence revealed that the cDNA's encode part of a novel hsp70 cognate, which is more similar to *E. coli dnaK* [46% aa identity and 60% nt identity] than to any other member of the eukaryotic hsp70 family, including *T. cruzi* hsp70 [38% aa identity and 48% nt identity]. In addition, TC1 possesses a region of 28% aa identity and 52% nt identity to the homeobox of the *engrailed* protein of *Drosophila melanogaster*. The specificity of the rabbit antiserum for TC1 was confirmed by hybrid-arrested in vitro translation followed by immunoprecipitation. The 70 kd TC1 polypeptide is specifically precipitated by the rabbit serum used for the initial screening and by mouse serum raised against the TC1 fusion protein. It is also the major polypeptide precipitated by serum from chronically infected humans and mice. In contrast to hsp70 mRNA, TC1 mRNA is less abundant upon heat shock. TC1 homologues are found in other kinetoplastids, including African trypanosomes and leishmania. The subcellular localization of the TC1 protein awaits further immunological and biochemical characterization.

P 412 **ABSENCE OF HEAT SHOCK RESPONSE IN TWO MURINE ASCITES TUMORS**

Umberto Ferrini, Elisabetta Mattei, Anna M. Mileo and Andrea Delpino, Regina Elena Cancer Institute, Rome, Italy.

Although the induction of stress proteins(SP) is a common response in all living organisms, we found two murine tumors (an Ehrlich and MC-sarcoma ascites forms) that were unable to express SP in response to heat and to a variety of stressors like ethanol, Disulfiram and arsenite. Further investigations also demonstrated the absence of in vivo acquired thermotolerance in these tumors. By utilizing antisera directed against the murine SP 70 (both cognate and heat-induced isoforms) and also against the SP 84-86 (since they have the same aminoacid residues at their C-terminal as SP 70) we observed in the unresponsive tumors the presence of constitutive SP 72 in same amounts both in control and in heat-conditioned cells. On the contrary the heat-inducible SP 70 was barely detectable, even if it was clearly expressed in other murine tumors. No modifications were found at the levels of 84-86 KDa species. From these data it is conceivable that the inability to express the heat-inducible SP 70, even in presence of quite large amounts of the constitutive SP 72, might be correlated with the absence of acquired transient thermotolerance in these tumors.

P 413 GENE ACTIVATION BY STRESS: A ROLE IN REACTIVATION OF LATENT CYTOMEGALOVIRUS?

J.L.M.C. Geelen, R.P. Minnaar, W.R. Boom, and J. van der Noordaa. University of Amsterdam, Amsterdam, The Netherlands.

The molecular mechanism of latency and reactivation of latent cytomegalovirus (CMV) is not well understood. Cell lines in which the immediate-early (IE) genes of human cytomegalovirus are stably integrated, but are not expressed under normal growth conditions are used as an in vitro model to analyse cellular and viral factors that may be involved in reactivation of latent virus. In these cell lines the silent CMV-genes can be activated by inhibitors of protein synthesis, which results in a transcriptional activation of the integrated IE genes. A well known cellular stress factor which has been implicated in the reactivation of latent herpesviruses is hyperthermia. Therefore the effect of a short period of heat shock on the induction of the CMV genes was analysed. It was found, that a mild heat treatment resulted in an induction of the previously repressed CMV-IE genes. Other inducers of the heat shock response, such as sodium arsenite and salicylate were also found to induce the IE genes. No viral factors are involved in the repression and induction of the integrated CMV-genes, as the same response to stress factors was observed, when the bacterial chloramphenicol acetyl transferase gene was integrated under control of the CMV IE enhancer promoter. An octameric sequence in the four 18 bp repeats in the CMV-IE enhancer promoter resembles the heat shock element core sequence and may therefore be involved in the heat shock response.

P 414 HEAT SHOCK PROTEINS IN BOVINE LYMPHOCYTES, Vince Guerriero, Jr. and Deborah H. Raynes, Dept. of Animal Sciences, University of Arizona, Tucson, AZ 85721.

Experimental evidence suggests that the increased synthesis of heat shock proteins in response to thermostress in tissue cultured cells is necessary for cell survival. The function of and conditions that stimulate heat shock protein synthesis in the whole animal are unknown. The first step in determining the function in the whole animal would be to find conditions that would elicit the synthesis of heat shock proteins. We have monitored the synthesis of the types and relative amounts of heat shock proteins in the lymphocytes of cattle. In vitro cultivation and heat shock (41°C) of these cells demonstrated the presence of nine different heat shock proteins with molecular weights ranging from 22 Kd to 116 Kd. The most abundant are proteins of 70 Kd and 95 Kd. The synthetic rates of these two proteins were considerably higher in the warm summer months when these animals would be heat stressed and dropped to undetectable levels during the cooler months. These preliminary results are consistent with a seasonal regulation of heat shock protein levels.

P 415 **SYNTHESIS OF HSP70 DURING DEVELOPMENT OF *Blastocladiella emersonii***, M. Helena Juliani, M. Christina M. Bonato, Suely L. Gomes and Aline M. da Silva, Universidade de São Paulo, São Paulo, Brazil.

In the fungus *B.emersonii* the heat shock response is developmentally regulated. Particular subsets of heat-shock proteins are induced by heat shock during sporulation, germination and growth and some heat shock-related proteins are spontaneously expressed during sporulation (Bonato et al., 1987, Eur. J. Biochem. 163,211). As our previous results suggested that HSP70 whose synthesis induced during sporulation was quite similar to the heat-inducible HSP70, we performed an extensive characterization of both, the constitutively expressed isoforms and the heat-inducible isoforms. HSP70 is expressed abundantly under normal cellular development and is a major stainable spot on two-dimensional gels of cellular proteins from zoospores. We can not distinguish the heat shock-induced HSP70 isoforms from the constitutive hsp70 isoforms by the criteria of two-dimensional gel electrophoresis, partial proteolysis mapping, Western blotting using monoclonal antibody directed against Drosophila HSP70 (a gift from Dr. Susan Lindquist), binding to ATP-agarose and phosphorylation. HSP70 is extensively phosphorylated even at normal temperature and at least two phosphorylated isoforms can be observed. During heat shock the level of phosphorylation is increased, but the same isoforms are found. Much of our data suggest that HSP70 perform primarily a structural role during development of the fungus.

We have also found that heat shock caused acceleration of protein degradation during zoospore germination. However, the constitutive levels of HSP70 remained high. These pulse-chase experiments also showed to be unlikely interconversions between the different isoforms of HSP70 during heat shock. **(Supported by FINEP, FAPESP, CNPq)**

P 416 EFFECT OF VARIOUS STRESSES ON THE EXPRESSION OF HEAT SHOCK GENES OF *NEUROSPORA CRASSA*: MESSENGER RNA LEVELS AND TRANSLATION PRODUCTS, M. Kapoor and C.A. Curle, Cellular, Molecular and Microbial Biology Division, Department of Biological Sciences, The University of Calgary, Calgary, Canada T2N 1N4.

A probe prepared by nick-translation of a DNA fragment from a *Neurospora* heat shock-inducible, hsp70-gene equivalent, was used to monitor the stress-induced mRNA levels in mycelium subjected to hyperthermal/toxic metal ion, and oxidative stress. A 2.7 kb transcript, with homology to this and the *Drosophila* hsp70 gene fragment, was detected within 15 min of HS treatment. Its levels increased up to 60 min and declined thereafter. The synthesis of the same transcript was also induced by sodium arsenite as well as treatment with some toxic metal ions. A second, constitutively-synthesized 2.4 kb transcript was detected by hybridization with the *N. crassa* probe but not with *Drosophila* hsp70 DNA. Recovery of the heat-shocked cells was accompanied by the complete disappearance of the 2.7 kb mRNA, within 60 min of transfer to normal temperature and the restoration of normal cellular messages to pre-stress levels. The heat shock mRNA was stable and readily translatable. An mRNA-dependent, *in vitro* translation system, prepared from heat-shocked *N. crassa* cells, translated homologous heat shock mRNA and normal cellular RNA, without any preference for the former.

P 417 ENHANCED EXPRESSION AND HEAT-INDUCED PHOSPHORYLATION OF HSP28 IN HEAT-RESISTANT VARIANTS OF CHINESE HAMSTER CELLS, Jacques Landry and Pierre Chrétien, Centre de recherche en oncologie de l'Université Laval, Québec, Canada G1R 2J6.

Four heat-resistant variants (HRV) of Chinese hamster 023 (WT) cells were isolated after treatment of WT cells with ethyl methane sulfonate followed by a single-step selection with a severe hyperthermic treatment of 4 hours at 44°C. Relative survival of the HRV cells to this heat treatment for up to at least 150 generations was $5x10^{-3}$ as compared to 10^{-7} for WT cells. Two-dimensional electrophoretic analyses revealed an elevated constitutive level of HSP28 in all 4 HRV clones as compared to WT cells. Dot blot and Northern analyses showed a corresponding 2-fold increase in the constitutive level of mRNA encoding HSP28 in 3 of the HRV cell lines. HSP28 is composed of 3 isoforms, 2 of which being phosphoproteins (HSP28 B and C). Heat shock induces a rapid phosphorylation (within 10 min.) of HSP28 and an immediate increase in the relative level of HSP28 B and C. HRV cells share several characteristics with thermotolerant WT cells, that is cells induced to a physiological thermotolerant state by a sublethal heat conditioning treatment administered 18 hours before. They have similar thermosensitivity, similar increased levels of phosphorylated and total HSP28 and both are competent to further heat induction of HSP28 synthesis and phosphorylation. These results, together with findings that A23187, EGTA, arsenite and CCCP induce phosphorylation of HSP28 and thermoresistance, provide physiological and genetic evidence that HSP28 and its rapid phosphorylation upon stresses, represent key elements in the regulation of cell thermoresistance. This work was supported by the Medical Research Council and the Cancer Research Society of Canada.

P 418 DIFFERENT TYPES OF THERMOTOLERANCE IN MAMMALIAN CELLS Andrei Laszlo Washington University Medical Center, St Louis, Mo. 63108

There is a good correlation between the induction of elevated levels of the heat shock proteins (HSP), and thermotolerance, a transient resistance to lethal heat treatments. However, significant levels of thermotolerance can be achieved after a mild heat shock in the absence of the increased levels of the HSP, an observation that has been interpreted to indicate that heat may induce thermotolerance via several different mechanisms (Laszlo, Int.J.Hyperthermia,in press). On the other hand, induction of thermotolerance by a brief exposure to sodium arsenite requires the subsequent synthesis of elevated levels of HSP. Heat induced cellular perturbations are being examined in cells in which thermotolerance has been triggered by either heat or arsenite and in permanently heat resistant cells expressing elevated levels of hsp70. Modifications in such perturbations are being systematically compared in order to delineate what, if any, alterations in the cellular heat response are associated with the HSP dependent and HSP independent states of thermotolerance. The effects studied include heat-induced perturbations of the nucleus, cytoskeleton and macromolecular metabolism. Results obtained so far indicate that the recovery from heat-induced increases in nuclear protein content, nuclear translocation of hsp70 and intermediate filament collapse, is more rapid in all three types of thermaly resistant cells, indicating that the ability to repair heat-induced perturbations in cellular parameters is probably associated with the HSP-dependent state of thermotolerance. Supported by NIH CA- 42591.

P 419 OVERPRODUCTION OF HSP 70 IN SACCHAROMYCES CEREVISIAE DOES NOT PROTECT CELL FROM THERMAL STRESS, Gloria C. Li, W.C. Lee, T.Y. Taso, F.M. Shen and James C. Wang. Dept. of Rad. Oncol., UCSF, CA 94143, Inst. of Mol. Biol. Acad. Sinica, ROC and Dept. of Biochem. & Mol. Biol., Harvard Univ., MA

In mammalian systems, there is a strong correlation between the concentration of hsp 70 and heat resistance. We have examined the relationship between hsp 70 and thermal resistance in yeast S. cerevisiae by using genetic manipulation to vary the intracellular concentration of hsp 70. Rather than using null mutants, we took advantage of expression vectors that produced various amounts of hsp 70. Plasmids were constructed in which the major constitutively expressed hsp 70 gene, SSA2, was placed under the control of different promoters (gal 1, gap or its own upstream sequence). Yeast strains CH335 (ura3) was transformed with these plasmids using the LiAOc method. The growth kinetics, the protein synthesis profiles and cell survival at 50°C were examined for cells bearing these multicopy plasmids. It is found: (1) Overproduction of SSA2 protein by 2 to 10 folds does not affect cells' growth at 26°C; (2) Overproduction of SSA2 protein does not protect cells against thermal stress; and (3) Overproduction of SSA2 protein does not interfere with the induction of the other members of the hsp 70 multigene family nor inhibit the development of thermotolerance. The possible role of other members of the hsp 70 family in thermal resistance needs to be investigated.
Supported in part by NIH CA 31397.

P 420 NEW CELLULAR MODELS TO ASSESS THE ROLE OF ANTIOXIDANT ENZYMES AND HSPS IN THE DEVELOPMENT OF RESISTANCE TO HEAT AND OXIDATIVE STRESSES. Marc-E. Mirault and Rémy Moret. Department of Medicine, Laval University, Québec G1V 4G2, Canada and Swiss Institute for Experimental Cancer Research, CH-1066 Epalinges, Switzerland.

We are currently investigating the regulation and role of the cellular antioxidant defence and heat shock proteins in the development of crossresistance of mammalian cells to heat and oxidative injury. Our approach is to generate cell derivatives which overproduce specific enzymes including catalase (CAT), CuZn-superoxide dismutase (CuZn-SOD), glutathione peroxidase (GSHPx) and heat shock proteins (hsp70-related) as encoded by transfected recombinant genes, and to characterize their new cellular responses and biological properties toward oxidative stresses of various origin. Using expression vectors carrying a chimeric catalase gene, we have isolated several mouse epidermal JB6 cell transfectants which overproduce catalase constitutively. We have started to investigate their responses to oxidative agents and heat shock. We find that cells overproducing catalase are more resistant to both hydrogen peroxide treatments and exposure to 45^0C than their parent cell line. The same strategy is currently applied to increase GSH-Px, CuZn-SOD and hsp70-related ATPase levels in mouse and human cells, in order to assess their individual role in the cellular defence against stresses of various origin.

P 421 PROPERTIES OF THE THERMOTOLERANT CELL, L.A. Mizzen and W.J. Welch, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 11724.

In an attempt to elucidate the possible functions of the mammalian stress proteins, we have been characterizing a number of properties inherent to the thermotolerant cell. Using protocols similar to those used in cell survival studies (mild heat shock, recovery at 37°C, subsequent severe heat shock challenge), we find that the acquisition of the tolerant state results in an ability of the cell to recover translational activity, synthesize the stress proteins, and return to normal protein synthesis patterns markedly faster than that of the nontolerant cell following a severe heat shock challenge. The acquisition of such "translational tolerance" appears dependent upon the production of the stress proteins and in particular the family of 70kDa stress proteins. Moreover, we present quantitative data indicating that the absolute levels of 70kDa produced in the cell are apparently a function of both the severity of the stress and the preexisting levels of 70kDa. We show that the acquisition of tolerance also has manifestations at the cellular level. Migration of 70kDa into and out of the nucleolus and the recovery of normal nucleolar morphology are greatly accelerated in the tolerant cell as compared to the nontolerant cell following shock. Finally, we show that other lesions which occur in the cell following heat shock can either be prevented, or at least repaired faster, if the cell is first made thermotolerant.

P 422 CHARACTERIZATION OF THE HEAT SHOCK RESPONSE IN CULTURED SUGARCANE CELLS, Stefan Moisyadi and H. Michael Harrington, University of Hawaii, Honolulu, HI 96822.

Effects of heat shock on the growth of cultured sugarcane cells were measured. A brief heat shock treatment at 36C (2 hr) caused the development of thermotolerance to otherwise nonpermissive heat stress (54C for 7 min) after a lag period at 23C. The events leading to the subsequent development of thermotolerance were initiated by heat shock treatments as short as 30 min at 36C. Maximum thermotolerance was observed eight hours after return to 23C from the elevated heat shock temperatures; however, cells maintained continuously at 36C for 10 hr exhibited immediate tolerance to nonpermissive heat stress.

A temperature upshift from 23C to 32C is sufficient to induce the synthesis of heat shock proteins (HSP). There was a positive relationship between increased HSP synthesis and increased temperature in the range 32 to 38C. Heat shock at 40 to 42C resulted in decreased HSP synthesis. Cultured sugarcane cells produce a variety of HSPs including a complex of low molecular weight peptides in the 20 kD range. The majority of the HSPs accumulated gradually over the course of heat shock, however the synthesis of the low molecular weight complex increased sharply 30 to 60 min after the initiation of heat shock. Analysis of HSP synthesis during the lag period between heat shock and the subsequent expression of thermotolerance revealed that several "late" polypeptides were synthesized during this period. None of the heat shock treatments in this study resulted in the inhibition of "normal" protein synthesis.

P 423 Beta Lactam Antibiotic Induced Stress Response in *Staphylococcus aureus.* M. MYCHAJLONKA*, P. JABLONSKI AND P. LEINARD. The University of Michigan/Dearborn, Dearborn, MI.

A clinical isolate of *Staphylococcus aureus* was found to be tolerant (MBC >> MIC) to a number of beta lactam antibiotics including Oxacillin. Biophotometric analysis showed that a number of concentrations of Oxacillin were capable of stimulating lysis, however. Cell cultures treated with an Oxacillin concentration showing maximum rate and extent of lysis were anaylsed for protein and RNA synthesis by pulse labeling techniques. RNA was found to be initially stimulated then severely inhibited. Overall protein synthesis was not inhibited initially, however, the increase in the rate of protein synthesis expected as the result of logarithmic growth was not observed. SDS-PAGE analysis of protein samples taken one and three hours after antibiotic addition showed that the shut down of protein synthesis was not coordinate but rather was suggestive of the operation of a stress regulon perhaps similar to those responsible for heat shock, SOS and oxidation stress. The Arrhenius plot for this organism was biphasic over the range of 24 to 42 C. Heat shock combined with antibiotic challenge stimulated the rate of lysis observed and significantly decreased Eagle's "paradoxic effect" observed at high antibiotic concentrations.

P 424 GENETIC ANALYSIS OF HEAT SHOCK PROTEIN SYNTHESIS IN WHEAT. H.T. Nguyen, D.R. Porter, and R.A. Vierling. Texas Tech University, Lubbock, TX 79409.

Wheat, a cool-season C_3 species, is physiologically poorly adapted to high temperatures. Improving heat tolerance in wheat using cellular and molecular technology will require a greater understanding of the genetic mechanisms of cellular and molecular responses of wheat to high temperature stress. Our current research objectives are (1) to evaluate the genetic variability in the synthesis of heat shock proteins, (2) to determine their relationships to cellular thermal tolerance, and (3) to determine the chromosomal locations and inheritance of heat shock genes in wheat. Cultivated hexaploid (2n = 42) and wild diploid (2n = 14) wheat seedlings were evaluated for cellular thermal tolerance using a triphenyl tetrazolium chloride test and heat shock protein synthesis under various temperature conditions. Green leaf tissues were labeled in vivo and the synthesis of heat shock proteins were evaluated using two-dimensional gel electrophoresis and fluorography. Results will be presented to show substantial genetic variation, both qualitatively and quantitatively, in the synthesis of heat shock proteins among several wheat genotypes. It appears that the higher level of synthesis of some heat shock proteins is correlated with cellular thermal tolerance, suggesting that genes coding for these proteins are involved in the genetic control of heat tolerance in wheat. Preliminary results on the chromosomal locations and inheritance of the HS genes will also be presented.

P 425 INTRACELLULAR LOCALIZATION OF HSP70 IN NORMAL, TRANSIENTLY THERMOTOLERANT AND PERMANENTLY HEAT RESISTANT CHINESE HAMSTER FIBROBLASTS. Kenzo Ohtsuka and Andrei Laszlo, Washington University School of Medicine, St.Louis Mo 63108

The kinetics of accumulation in, and removal of hsp70 from, the nuclei and nucleoli of chinese hamster HA-1 fibroblasts displaying different thermal responses, using a polyclonal antibody that recognized both the constitutively expressed ($hsp70_c$) and the heat-inducible form ($hsp70_i$) of hsp70, as indicated by immunoblotting. Exposure to elevated temperatures led to an immediate translocation of $hsp70_c$ into the nucleus, with the appearance of $hsp70_i$ in the nucleoli during the subsequent recovery at 37°C. The original accumulation of $hsp70_c$ in the nucleoplasm of heated cells was found to be time and temperature dependent at all temperatures tested (42-45°C), with similar kinetics in normal, thermotolerant (induced by either heat or sodium arsenite), and permanently heat resistant cells. During the recovery from elevated temperatures, the hsp70 was eventually removed from the nucleus, again in a manner that depended on the time/temperature combination of the original exposure. However, for a given heat dose, the kinetics of the removal of $hsp70_c$ from the nucleus was more rapid in thermotolerant and permanently heat resistant cells than in their normal counterparts. These results suggest that the increased levels of $hsp70_c$ and $hsp70_i$ associated with the thermotolerant or permanently heat resistant state may play a key role in restoring and/or repairing heat-induced alterations in the nuclear and nucleolar functions. We are currently testing this hypothesis by examining the effect of modifiers of the heat-response, both sensitizers and protectors, on the nuclear accumulation and removal of hsp70. Supported by NIH CA-42591.

P 426 MOLECULAR ANALYSIS OF *H.CAPSULATUM* hsp70 GENE: TRANSCRIPTIONAL REGULATION

Patriarca, E., Carratù, L. & B. Maresca Intern'l Institute of Genetics and Biophysics, CNR, Via Marconi, 10, 80125 Naples, Italy

H. capsulatum is a dimorphic fungus which undergoes a transition from a saprobic mycelium to a pathogenic yeast after temperature shift from 25° to 37°C. We have previously described three distinct stages during the transition of mycelia to yeast in the less virulent Downs strain and in several more virulent strains of*H. capsulatum* . Under conditions of "heat shock" in Downs, there is an almost immediate uncoupling of oxidative phosporylation and a rapid decline in intracellular ATP level followed by a decrease in oxygen consumption and in RNA and protein synthesis. In Stg. 2, the cells enter a "dormant" period, lasting three to six days. In Stg. 3, they return to normal metabolic activities and cells gradually transform to the yeast phase.These physiological events are similar in all strains studied but in the less virulent Downs changes are more extreme than in the more virulent strains. We have previously found that hsp70 gene from*H.capsulatum* is differentially expressed in the two classes of strains, and its regulation is correlated with the level of pathogenicity. We will show that in Downs a brief exposure to intermediate temperatures protects oxidative phosphorylation and cells do not enter Stg. 2. at higher temperatures. A mild heat shock pre-treatment induces hsp70 mRNA at high level at 34°C, and subsequent incubation at 37°C allows further transcription. In this physiological condition mycelial cells are able to produce high level of hs RNA's at 37°C as compared to the more virulent strain G222B at 37°C. The protection of mitochondrial activity can be induced in less than 1 hour by hs and lasts about 24 hours as measured by oxygen consumption in experiments in which after the shift to 34°C, cells were returned to 25°C in order to dilute the accumulation of hsp's or their effect. Hsp70 gene is also induced under condition of stress. Mycelia incubated at 25°C in a stress medium, accumulate high level of hsp70 mRNA. Further transcription is induced by shifting the cells to 37°C. The high level of transcription of the hsp70 gene appears to be under a different type of control since this gene is only transiently induced after temperature shift in conditions of heat shock, while it appears to be continuously transcribed in condition of stress for at least 3 days. The hs response elicited in condition of stress induces the protection mechanism and allows the cells to transform to the yeast phase without entering Stg.2. We are now studying the DNA-protein interaction by gel electrophoresis-DNA binding assay to analyze the mechanism of regulation of hsp70 expression.

P 427 IONIC REGULATION OF THE HEAT SHOCK RESPONSE IN GAMETES OF *Xenopus laevis*,

Michael Pollock, Leon W. Browder and Jean Marc Renaud, University of Calgary, Calgary, Alberta, Canada T2N 1N4.

We have previously demonstrated that post-ovulatory eggs of *Xenopus laevis* are capable of a complex heat shock response, including the synthesis of a variety of heat shock proteins ("hsps"; Browder *et al.*, Dev. Biol. 124:191, 1987). The ability to respond to elevated temperature in this way, however, is regulated by components of the incubation medium; in particular, the concentration of Cl^- in the external medium has a profound influence on the synthesis of these heat-inducible species. As well, the presence of the organic acids pyruvate and oxaloacetate is required for full expression of the potential for hsp synthesis on some ionic backgrounds but is inhibitory to such synthesis on others. Because mature eggs of *Xenopus* are transcriptionally inert, the inducible synthesis of hsps must be directed by specific mRNAs selected from the store of messengers laid down during oogenesis. We will present data detailing the effects of heat shock and medium composition on specific cellular parameters (membrane potential, and cytoplasmic pH and Cl^- concentration) in *Xenopus* eggs in an effort to determine the mechanisms responsible for the selected translation of a subset of oogenic messengers. (Supported by NSERC and AHFMR.)

P 428 HSP SYNTHESIS IN TRANSPLANTABLE MORRIS HEPATOMAS.

Luisa Schiaffonati, Gaetano Cairo, Emilia Rappocciolo, Lorenza Tacchini and Aldo Bernelli-Zazzera. Istituto di Patologia Generale dell'Università degli Studi; Centro di Studio sulla Patologia Cellulare del CNR Via Mangiagalli 31, Milano, Italy.

The basal level of expression of hsp 70 genes is high in many tumor cell lines of high growth rate. We have studied the constitutive and heat-shock induced hsp synthesis in liver and in a series of transplantable hepatomas and, by using probes for different members of hsp 70 gene family, we have tried to assess their relations to heat challenge and/or to the rate of growth. 2D electrophoresis of labeled liver proteins shows strong induction by heat of polypeptides of 5.8-5.9 pI in the 68-73 Kd range: the response of slow-growing 9618A hepatoma is similar. On the contrary the 3924A fast-growing hepatoma has an elevated constitutive synthesis of the more acidic, higher m.w. polypeptides and does not produce the more basic lower m.w. hsp of this group. Hybrid selection and Northern blot experiments using the cDNA for rat hsc 73 reveal that this mRNA codes for the acidic isoforms of the 68-73 Kd proteins, is barely detectable in normothermic liver and in 9618A hepatoma and is induced 3-4 folds after heat shock. The same mRNA is present in high amount in 3924A before heat shock, and is only slightly inducible. On Northern blots a human hsp 70 probe recognizes, in addition to hsc 73 mRNA, two mRNA species which, upon heat shock, are induced in liver and 9618A hepatoma at higher extent than in the 3924A tumor.

P 429 TEMPERATURE INDUCED GENE EXPRESSION THROUGHOUT LEISHMANIA LIFE CYCLE, Michal Shapira[1], Juan G. McEwen[1] and Charles L. Jaffe[2]. Depts. of Chemical Immunology[1] and Biophysics[2], The Weizmann Institute of Science Israel,

Leishmania parasites encounter a wide range of temperature fluctuations throughout their life cycle, as the flagellated promastigote in the fly, and the non-motile amastigote within mammalian macrophages. This suggests that expression of heat shock proteins may play a role in stage differentiation. In several leishmania strains, such as L. Mexicana, it is possible to mimick the transformation process by exposure of promastigotes to elevated temperatures. This results in a morphological change of promastigotes into amastigote like organisms, which survive only for several days. Recently it has been shown that promastigotes, both in the fly and in culture differentiate along their growth curve from a non-infective form in the log phase, to a virulent form in the stationary phase. We have followed the expression of genes of the Hsp family and tubulin, which are developmentally regulated, in attempt to understand the molecular events during stage transformation of this parasite. Hsp's 70 and 83 are constitutively expressed in the amastigote stage, as shown by metabolic labelling of proteins, and Northern blot analysis of RNA. mRNA's of Hsp's 70 and 83 are already induced at 26° in stationary phase promastigotes, whereas in the logarithmic phase they may be induced only by a temperature shift from 26 to 35°C. Amastigote like hybridization pattern of β-tubulin may be induced in stationary phase promastigotes, only after exposure to elevated temperature. Therefore, tubulin expression is susceptible to temperature control in L. mexicana though direct correlation to expression of Hsp's could not be demonstrated as yet.

P 430 A MULTIGENE FAMILY IN LEISHMANIA MAJOR WITH HOMOLOGY TO EUKARYO HEAT SHOCK PROTEIN 70 GENES, D.F. Smith, S.Searle, A.J.R. Campo R.M.R. Coulson and P.D. Ready, Imperial College of Science and Techno London SW7 2AZ, U.K.

We are studying the molecular events which accompany the differentiat Leishmania major promastigotes in vitro, in order to facilitate the development of prophylactics against infective-stage organisms, which human cutaneous leishmaniasis. We have identified a small family of in this organism which encode proteins of 70-75 Kd and which are rela sequence homology, to the hsp 70 genes of Drosophila and man. One of genes shows up-regulation as promastigotes differentiate into forms w are infective to man, while another shows increased transcription fol the transformation of promastigotes to amastigotes (the intracellular parasite forms). We are interested in determining both the role of t proteins and the stimuli which are responsible for their increased production, which may include heat shock, changes in extracellular gl concentration or extremes of pH. To this end, we are using antibodies raised to fusion proteins translated from chimaeric genes study the locations and functions of the native proteins in the Leis cell. In addition, we are investigating what regulatory sequences ar required for the control of transcription of this multi-gene family.

P 431 CALMODULIN ANTAGONISTS, THE HEAT STRESS PROTEIN RESPONSE AND THERMOTOLERANCE, Stephen P. Tomasovic and Douglas P. Evans, University of Texas, M.D. Anderson Hospital and Tumor Institute, Houston, TX 77030.

We are investigating the role of calmodulin (CaM)-regulated processes in the mechansims of heat-induced cellular lethality, hsp synthesis and thermotolerance in rat mammary adenocarcinoma cells in culture. In the studies reported here we used the CaM antagonists W-7 and W-13 and their less active analogs W-5 and W-12 in clonogenic survival and protein synthesis assays to determine what effect inhibition of CaM's interactions with its binding proteins had on 42° and 43°C heat killing, hsp synthesis, and thermotolerance. These antagonists potentiated acute 43°C heat killing at concentrations near the IC_{50} for antagonism of many CaM-binding proteins in vitro but were ineffective at potentiating 42°C heat killing. The potentiation is believed to be CaM-dependent since the less active analogs did not potentiate heat killing. Several hsp including hsc/hsp90, hsc70, hsp68 and hsp22 (but not hsp112) bound to a CaM-agarose affinity column. Presumably the CaM antagonists would inhibit the interaction of CaM with hsp in vivo, if it is physiologically relevant; however, W-7 at concentrations which potentiated acute 43°C heat killing did not hinder the triggering and development of thermotolerance or alter hsp synthesis. These results suggest that if hsp synthesis is involved in mechanisms of thermotolerance, then binding of these hsp to CaM is not a necessary event in those mechanisms. Supported in part by USPHS grant CA-32745 (SPT) and CA-09299 (DPE).

P 432 STRESS-RELATED PROTEINS ARE MAJOR ANTIGENS IN LEPROSY AND TUBERCULOSIS, Douglas B. Young, MRC Tuberculosis and Related Infections Unit, Hammersmith Hospital, London W12 0HS, U.K.

A set of protein antigens which play a prominent role in humoral and cellular immune responses to Mycobacterium leprae and Mycobacterium tuberculosis has been identified. As an approach to elucidating the functions of these proteins within mycobacterial cells, we have carried out a search for related proteins in other cell types based on study of immunological cross-reactivity and comparative sequence analysis. Homologues of proteins involved in the heat-shock response of E.coli (groEL and dnaK) were found amongst the major antigens of both mycobacterial species. In addition, an open reading frame predicting an M.tuberculosis protein with sequence homology to E.coli dnaJ has been identified, and a homology between an M.leprae antigen and a plant heat-shock protein was found. These findings and recent observations with other pathogens, suggest that infectious agents may respond to the host environment by producing stress proteins and that these abundant and highly conserved proteins can be important immune targets.