POLYAMINES IN CELL PROLIFERATION AND DIFFERENTIATION

Organizers: Laurence J. Marton, Anthony E. Pegg
February 9-14, 1990

This meeting is dedicated to the memory of Diane H. Russell

Plenary Sessions Page

Poster Sessions

Polyamines in Cell Proliferation and Differentiation

Keynote Address

CH 001 THE GROWTH AND DEREGULATION OF THE POLYAMINE INDUSTRY, Seymour S. Cohen, Marine Biological Laboratory, Woods Hole, MA 02543

The paper begins with a discussion of the contributions of the recently deceased Diane Russell, who helped to initiate the considerable growth of polyamine studies in the period of 1968 through 1989. She was among the first to describe the marked increase and decline of ornithine decarboxylase in the growth of mammalian tissue. Russell was also active in building an "invisible college" and in developing symposia, including the Gordon Conference on Polyamines. She also developed and began the test of the hypothesis that analysis of the appearance of polyamines in body fluids might be helpful in the diagnosis and treatment of cancer. These contributions are considered in the context of the recent history of polyamine research.

Currently work on the polyamines results in the publication internationally of about a thousand papers per year. Some problems of keeping up with the expansion of experimentation in the diverse areas concerned with polyamine structure, roles and metabolism are discussed.

CH 002 THE END IS WHERE WE START FROM, H.G. Williams-Ashman, Ben May Institute, University of Chicago, Chicago, IL 60637.

"What we call the beginning is often the end/And to make a new end is to make a beginning. The end is where we start from."....."We shall not cease exploration/And the end of all our exploring/Will be to arrive where we started/And know the place for the first time." (T.S. Eliot, Little Gidding, Stanza V) We now know a lot about the metabolism of polyamines and its enzymatic and genetic basis; changes in intracellular polyamine levels that accompany the growth or differentiation of may sorts of cells; and effects of drugs that specifically inhibit various polyamine-transforming enzymes on cellular dynamics. Numerous interactions of spermidine and spermine with biological mini- and macro-molecules have also been delineated in cell-free systems. Yet our comprehension of which of these actions of polyamines are of prime significance for any type of living cell is meager. This presentation will briefly discuss three specialized topics in mammalian polyamine physiology that relate to the foresaid general issue. (1) The possible functional importance of the large amounts of spermidine and/or spermine found in the semens of a restricted number of species. (2) Relationships of intracellular polyamines to the amplification of hormonal signals that evoke the growth or differentiation of certain reproductive tissues. (3) Mechanisms of polyamine secretion by, and/or influx of extracellular polyamines into, various mammalian cells.

Regulation of Polyamine Biosynthesis by Signal Transduction Pathways

CH 003 CHARACTERISTICS AND GENETICS OF POLYAMINE TRANSPORT IN ESCHERICHIA COLI, Takemitsu Furuchi, Keiko Kashiwagi, Nobuo Hosokawa, Hiroshi Kobayashi and Kazuei Igarashi, Faculty of Pharmaceutical Sciences, Chiba University, Chiba 260, Japan

Polyamine transport was studied with both intact cells and membrane vesicles of an Escherichia coli polyamine-requiring mutant, MA261. The results show that the uptake is dependent on proton motive force and that the putrescine transport system differs from the spermidine and spermine transport system. We have tried to isolate the polyamine transport deficient mutant by treatment of E. coli MA261 with nitrosoguanidine. Cells which could not grow well in the presence of putrescine and spermidine were selected, and their transport activity was examined. We could isolate E. coli KK313, which is deficient in spermidine transport. E. coli NH1596, which is deficient in both putrescine and spermidine transport, was obtained by treatment of KK313 with nitrosoguanidine. We next tried to isolate the clone for the gene of polyamine transport system using pACYC184 as a vector and E. coli NH1596 as a host strain. We were able to isolate two clones for the putrescine transport system and one clone for the spermidine transport system by measuring the transport activity of transformed E. coli NH1596. By comparison of the restriction map of the clones and Kohara's physical map, and by pulsed field gel electrophoresis of E. coli DNA, two putrescine transport genes were mapped at 16 and 19 min, respectively, and the spermidine transport gene was mapped at 40 min. The nucleotide sequence of the spermidine transport gene was determined. It contained four open reading frames encoding A, B, C, and D proteins. A protein (Mr 45K) was a membrane-associated, ATP-binding subunit. B (Mr 32K) and C (Mr 29K) proteins consisted of six putative transmembrane spanning segments linked by hydrophilic segments of variable length. B protein had a leucine zipper structure, which was found in the potassium channel and in the glucose transport protein. D protein (Mr 42K) was inferred to be a spermidine binding protein existing in a periplasmic fraction. Putrescine transport was also observed in E. coli NH1596 carrying the spermidine transport gene, but it was strongly inhibited by spermidine.

CH 004 ANDROGEN REGULATION OF ORNITHINE DECARBOXYLASE AND S-ADENOSYLMETHIONINE DECARBOXYLASE GENE EXPRESSION, Olli A. Jänne, Anne Crozat, Mervi Julkunen, Leonard Eisenberg, and Li-Xin Shan, The Population Council and The Rockefeller University, New York, NY 10021

Ornithine decarboxylase (ODC) and S-adenosylmethionine decarboxylase (AdoMetDC) are two key enzymes in polyamine biosynthesis. Previous studies from this and other laboratories have indicated that regulation of ODC activity by androgens in murine kidney occurs at multiple levels, including the rate of ODC gene transcription, stabilization of ODC mRNA species, and prolongation of the enzyme protein's half-life. Comparison of androgenic responses in murine and rat kidneys revealed significant differences: the induction of ODC activity and ODC mRNA accumulation was transient in the rat but sustained in the murine renal cells. In addition, in situ hybridization experiments showed that expression of the ODC gene occurs in different subpopulations of epithelial cells of the proximal tubules in mice and rats. Detailed studies on the promoter region of the murine ODC gene are being carried out, in order to delineate the nucleotide sequences involved in the basal and androgen-regulated expression of this gene. Initial studies have also suggested that androgen administration increases the concentration or activity of nuclear proteins interacting with the ODC gene promoter sequences. ODC and AdoMetDC genes are coordinately regulated by androgens in rodent accessory sex organs, whereas only the ODC gene is androgen-responsive in rodent kidney. ODC and AdoMetDC mRNAs are expressed and androgen-regulated in the same epithelial cell types of accessory sex organs, as judged by hybridization histochemistry. Isolation and sequencing of the promoter region of the AdoMetDC gene has been accomplished and it will now permit detailed studies of factors regulating the expression of this gene.

CH 005 **RESPONSES OF THE ORNITHINE DECARBOXYLASE GENE TO ACTIVATION OF SECOND MESSENGER PATHWAYS,** David R. Morris and Mitchell Abrahamsen, Department of Biochemistry, University of Washington, Seattle, WA 98195

The ornithine decarboxylase (ODC) gene shows an immediate-early response to mitogenic activation. In both T-lymphocytes, activated through the antigen receptor, and Swiss 3T3 fibroblasts, activated by serum mitogens, ODC mRNA begins to be elevated above the resting level by 30 min and is maximally induced 10- to 20-fold by several hours after activation. Activation of protein kinase C is necessary and sufficient for elevation of ODC expression in both cell systems. Transcription of the ODC gene is undetectable in runon assays with nuclei from resting fibroblasts and is induced upon mitogenic activation of these cells. In contrast, the ODC gene is strongly transcribed in nuclei from resting T-cells and there is no change in transcription rate after stimulation by mitogen. In neither cell type is there evidence for involvement of transcriptional pausing or stabilization of mature ODC mRNA. Thus, regulation of the ODC gene in T-cells may involve intranuclear stabilization of pre-mRNA molecules.

Response of ODC expression to elevated cAMP is also quite different in the two cell types. In lymphoid cells, elevation of intracellular cAMP results in down-regulation of ODC expression. On the other hand, activation of protein kinase A in fibroblasts (and in an adrenal cell line) stimulates ODC transcription ca. 10-fold. DNA sequences 265 bp upstream of the ODC cap site are sufficient to confer cAMP-inducibility to chimeric expression constructs. Within this region, there are 2 potential CREs, both of which footprint with cell extracts.

Translational and Post-Translational Control of Polyamine Biosynthesis-I

CH 006 DOMAINS OF EUKARYOTIC ORNITHINE DECARBOXYLASE THAT DETERMINE STABILITY AND POLYAMINE-DEPENDENT REGULATION

P. Coffino, & L. Ghoda, Department of Microbiology, Univ. of California, San Francisco, CA 94143

Mouse ODC is a labile, cytosolic protein whose intracellular activity falls when polyamine levels rise. Previously, we have shown that a mutation deleting 37 amino acids from the carboxy-terminus of ODC results in a stable protein when expressed in ODC$^-$ CHO cells. This protein is, nonetheless, regulated by polyamines, much like the wild-type ODC. Smaller truncations of the mouse protein resulted in similarly stable ODCs, all of them responsive to polyamines.

To further extend these observations, we determined (in collaboration with C.C.Wang, UCSF) the stability and polyamine-dependent regulation of *Trypanosoma brucei brucei* ODC expressed in CHO cells. This ODC is largely homologous to the mouse ODC except in two regions; those corresponding to the internal PEST sequence in mouse ODC, and the carboxy-terminal region, previously found in mouse to be responsible for rapid degradation of the protein. The protozoan ODC expresssed in CHO was found to be stable in the presence of cycloheximide, thus behaving like the mutant mouse protein lacking the carboxy-terminus. Unlike both the wild-type and truncated mouse ODC, however, the trypanosome protein was found to be refractory to polyamine regulation, i.e., activity was unchanged in cells exposed to putrescine.

A hybrid protein containing the carboxy-terminus of the mouse protein and the amino-terminus of the trypanosome was constructed. This hybrid, when expressed in CHO, had a half-life similar to the mouse protein in the presence of cycloheximide, but was not regulated by polyamine treatment. These results (see table) support the hypothesis that two domains, one regulating constitutive degradation, and polyamine-dependent regulation are separable.

Amino Terminus	Carboxy Terminus	Constitutive Degradation	Polyamine Regulation
M1-461	(M)	+	+
M1-424	(none)	-	+
T1-445	(none)	-	-
T1-389	M370-461	+	-

Supported by NIH grants CA 47721 & CA 29048 and NSF grant DCB 8707375.

CH 007 ROLE OF ANTIZYME IN REGULATION OF ORNITHINE DECARBOXYLASE, Shin-ichi Hayashi, Yasuko Murakami and Senya Matsufuji, Department of Nutrition, Jikei University School of Medicine, Minato-ku, Tokyo 105, Japan

Antizyme is a unique regulatory protein involved in the negative feedback regulation of ornithine decarboxylase (ODC). Induced by polyamines, it binds specifically to ODC and inhibits its activity. Two lines of evidence from this and other laboratories suggest the possibility that binding with antizyme accelerates ODC degradation. First, ODC decay rate is accelerated by exogenous polyamines after a short lag period and this acceleration is blocked by cycloheximide but not by actinomycin D, indicating requirement of new protein synthesis but not of new RNA synthesis. It is known that antizyme induction by polyamines needs protein synthesis but not RNA synthesis. We recently confirmed that induction of hepatic antizyme upon putrescine administration was not accompanied by any increase in the amount of hybridizable antizyme mRNA. Second, ODC decay rate changes markedly during and after its induction period and there is a good correlation between fractional decay rate of ODC and cellular antizyme/ODC ratio in cultured HTC cells. Such a correlation was also observed in normal and DFMO-treated HTC cells and in male and female mouse kidneys. A hypothesis has been proposed that antizyme stimulates ODC degradation in a recycling manner, based on the fact that cellular amount of antizyme is usually much less than that of ODC. We recently compared the effects of various protein synthesis inhibitors on acceleration of ODC decay and indution of antizyme in putrescine-treated CHO cells. Both pactamycin and emetine, like cycloheximide, inhibited the two processes nearly completely. On the contrary, amino acid analogs ethionine and 5-fluorotryptophan did not inhibit either process at all, although they inhibited ODC induction in normal cells nearly completely. Puromycin moderately inhibited antizyme induction and blocked the acceleration of ODC decay to variable extents, probably depending on cellular antizyme/ODC ratio. These results supported the proposed role of antizyme in ODC degradation. Transfection study with antizyme cDNA is under way in our laboratory in order to obtain more direct evidence for the role of antizyme.

CH 008 PEST SEQUENCES ARE TRANSPLANTABLE PROTEOLYTIC SIGNALS, Pius Loetscher, Spencer Watt and Martin Rechsteiner, Department of Biochemistry, University of Utah School of Medicine, Salt Lake City, UT 84132.

Several years ago we proposed that polypeptide regions rich in proline (P), glutamic acid (E), serine (S) and threonine (T) target proteins for destruction. The original PEST hypothesis was based on literature data for 10 proteins plus experimental data for 2 proteins, a- and ß-casein, after their injection into HeLa cells. Results generated over the past 3 years have greatly strengthened the hypothesis. Follow up surveys show that 26 of 28 rapidly degraded proteins contain PEST sequences whereas such sequences are only found in 1 of 20 randomly selected proteins. Moreover, the PEST hypothesis has received direct experimental support from the studies by Coffino and his colleagues on ornithine decarboxylase and by recent studies in our laboratory. We have cloned the C-terminal PEST region from ornithine decarboxylase onto the C- and N-terminus of mouse dihydrofolate reductase (DHFR). DHFR and DHFR fusion proteins were overexpressed in E. coli and purified to apparent homogeneity. The stabilities of the radioiodinated proteins were assayed in the presence of ATP in rabbit reticulocyte lysate and frog egg extract. The proteins were stable in reticulocyte lysate. In frog egg extract, however, the degradation rates of DHFR carrying the PEST region at the C- or N-terminus was increased 9- and 5-fold, respectively. Since all proteins were isolated by affinity chromatography and since all of the purified enzymes exhibit similar specific activities, the attachment of the PEST region to either terminus does not seem to poison the proper folding of DHFR. These results demonstrate that frog egg extract contains all the components needed for the degradation of PEST proteins and that PEST sequences are transplantable proteolytic signals.

Translational and Post-Translational Control of Polyamine Biosynthesis-II

CH 009 REGULATION OF TRANSLATION IN EUKARYOTES BY MESSENGER RNA STRUCTURE, Marilyn Kozak, Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260

According to the scanning model for initiation of translation (1), selection of the correct AUG codon is determined by the *position* of the AUG codon relative to the 5' end of the mRNA and by *sequences flanking the AUG codon*. Evidence in support of this hypothesis has previously been obtained in vivo (2); the results of in vitro translation experiments (3) will now be reported. The importance of context and position in identifying the functional initiator codon is supported by sequence data from a wide variety of cellular and viral mRNAs (4); results from the most recent survey of published mRNA sequences will be described.

Other features that affect translational efficiency will be discussed, including the effects of leader length, two AUG codons in close proximity, and secondary structure near the 5' end of the mRNA.

The effects of local secondary structure were found to depend in a straightforward way on the strength of the hairpin structure and its position relative to the 5' end of the mRNA. Stem-loop structures of -30 kcal/mol can be melted by the scanning 40S ribosome/factor complex; consequently, such structures did not impair translation unless the hairpin was so close to the 5' end that the 40S ribosomal subunit could not bind (3). In contrast, hairpin structures of -50 kcal/mol cannot be disrupted by the scanning 40S ribosome. In wheat germ extracts, a hairpin of this sort did not prevent the 40S ribosomal subunit from binding but caused the 40S subunit to stall on the 5' side of the hairpin, exactly as the scanning model predicts. Efforts are underway to study these abortive initiation complexes by electron microscopy. Whereas secondary structure located *between the cap and the AUG codon* inhibited translation, a stem-loop structure was found to enhance initiation when the hairpin occurred *downstream* from the AUG codon, especially when the AUG triplet was in an unfavorable primary sequence context. The realization that mRNA secondary structure can have positive as well as the more usual negative effects on translation, along with the fact that the translatability of structure-prone mRNAs may vary with changes in the cytoplasmic environment (5), underscores the complexity of the problem in eukaryotic cells. Notwithstanding such complications, the circumstances and mechanisms of regulation by mRNA secondary structure are slowly coming into view.

(1) Kozak, J. Cell. Biol. (1989) 108:229.
(2) Kozak, Cell (1986) 44:283.
(3) Kozak, Mol. Cell. Biol. (1989) in press.
(4) Kozak, Nucleic Acids Res. (1987) 15:8125.
(5) Kozak, Mol. Cell. Biol. (1988) 8:2737.

CH 010 STRUCTURE AND REGULATION OF MAMMALIAN S-ADENOSYLMETHIONINE DECARBOXYLASE, Anthony E. Pegg, Ingvar Holm and Bruce Stanley, Departments of Cellular and Molecular Physiology and of Pharmacology,The Milton S. Hershey Medical Center, Pennsylvania State University College of Medicine, P.O. Box 850, Hershey, PA 17033.

The activity of S-adenosylmethionine decarboxylase (AdoMetDC) is the limiting step in the conversion of putrescine into the polyamines spermidine and spermine in the biosynthesis of polyamines. The activity of this enzyme is highly regulated by polyamines. This regulation has been studied using a specific antiserum and cDNA for the proenzyme and by expressing the human proenzyme in E. coli. The enzyme is synthesized as a proenzyme of 38,000 M. W. which is then cleaved in a reaction which generates the pyruvate prosthetic group from the serine residue at position 68 and two sub-units containing 67 and 266 amino acids respectively. The larger subunit contains the pyruvate prosthetic group at its amino terminus. Putrescine stimulates the activity of the enzyme and also accelerates the rate of processing of the proenzyme to the active form but does not influence the synthesis of the proenzyme. The cellular content of AdoMetDC protein is inversely related to the cellular spermidine and spermine level. This relationship is due to an increased rate of degradation of the protein and a decreased rate of synthesis when the cellular polyamine levels are increased. Two forms of mRNA are present in mammalian cells having sizes of about 2.1 and 3.5 kb. These forms differ in the 3' non-translated sequence and probably represent the use of alternate polyadenylation signals. The cellular content of mRNA and the relative proportions of the two forms are changed in response to the stimulation of cell growth with fresh serum and in response to changes in the cellular spermidine level. Depletion of spermidine leads to an increase in the cellular content of mRNA for AdoMetDC. Spermine is strongly inhibitory to the translation of AdoMetDC mRNA. These factors act to maintain the cellular polyamine content by changing the level of AdoMetDC to meet the requirement for de novo synthesis of polyamines. This research was supported by grants CA-18138 and HL-07223 from the National Institutes of Health.

CH 011 INHIBITION OF TRANSLATION BY THE 5' UNTRANSLATED REGION OF ORNITHINE DECARBOXYLASE mRNA, Ann Grens and Immo E. Scheffler, Department of Biology, B-022, University of California, San Diego, La Jolla, California 92093.

We describe the cloning and characterization of the 5' untranslated region of the ornithine decarboxylase (ODC) cDNA from Chinese hamster ovary cells, and an examination of its role in post-transcriptional regulation of the enzyme. A series of expression vectors were constructed in which portions of the ODC 5' UTR were placed flanking a reporter gene coding sequence, either firefly luciferase or chloramphenicol acetyl transferase, so as to generate a hybrid transcript. Translation of these chimeric genes in transient expression assays in wild-type and ODC-deficient hamster cells was examined in the presence of normal or depleted polyamine pools. The ODC 5' UTR suppresses translation of the coding sequence it precedes irrespective of polyamine levels, and this effect is shown to be due to the GC-rich 5' segment of the UTR. The GC-rich region has the potential to form a very stable hairpin structure, and inhibits translation in a position dependent but orientation independent manner. Insertion of the 3' UTR of ODC downstream of the translation termination codon but prior to the polyadenylation signal, in addition to the 5' UTR upstream of the coding sequence, has two effects: the overall translatability of the message improves dramatically, and the amount of translation increases when polyamine pools are depleted.

CH 012 SPERMIDINE BIOSYNTHESIS AND FUNCTION IN *ESCHERICHIA COLI* AND IN *SACCHAROMYCES CEREVISIAE*, Herbert Tabor, Celia W. Tabor, and Qiao-Wen Xie, National Institutes of Health, Bethesda, MD 20892

A key enzyme for the biosynthesis of spermidine is *S*-adenosylmethionine decarboxylase. This enzyme has been purified from a variety of sources, and has been shown to contain a novel cofactor, namely, covalently bound pyruvoyl groups that are essential for activity. We will summarize our studies on the structure of *S*-adenosylmethionine decarboxylase from both *E. coli* and *S. cerevisiae*[1] with special emphasis on the gene structure and on the formation of a pyruvoyl moiety during the post-translational conversion of the proenzyme to the active form.

In *E. coli*, *speD* (the structural gene for *S*-adenosylmethionine decarboxylase) is present as part of an operon (2.5 Kb), containing a promoter, an upstream open reading frame of unknown function, *speE* (the gene for spermidine synthase), and *speD*. With E. W. Hafner we had previously obtained deletion mutants for *speE* and *speD* in *E. coli*, and, with C. M. Cashel, we are now preparing null mutants by insertion-deletion techniques in all three parts of the *speED* operon.

SpeD mutants of *E. coli* contain no spermidine. In the past we have not been able to demonstrate any phenotypic effect of this deficiency except for a 15% decrease in the growth rate. However, with K. W. Minton[2], we have very recently found that such mutants have a striking increase in the toxicity of paraquat, a known source of superoxide; this increase in toxicity is prevented by the addition of spermidine exogenously or endogenously. We have previously shown (with M. S. Cohn) that spermidine-deficient mutants of *S. cerevisiae* have a more striking growth defect; in addition they do not sporulate and do not maintain the dsRNA killer factor.

[1] Kashiwagi, K., Tabor, C. W., Tabor, H., Liu, D. T., and Nguyen, N. Y., manuscript in preparation.
[2] Minton, K. W., Tabor, C. W., and Tabor, H., manuscript in preparation.

Polyamines in Macromolecular Synthesis and Growth

CH 013 INTRACELLULAR PHOSPHORYLATION OF ORNITHINE DECARBOXYLASE (ODC) IN RAW 264 CELLS: ITS REQUIREMENT FOR ODC ACTIVITY. Mari K. Haddox, Kathleen M. Rose, and Laura L. Worth. Department of Pharmacology, University of Texas Medical School, Houston, Texas 77030. Ornithine decarboxylase (ODC), the initial rate-limiting enzyme in polyamine biosynthesis, has been well characterized in terms of the regulation of enzyme protein synthesis and turnover. The role of phosphorylation in regulating ODC activity has been explored, but has remained unresolved. Purified ODC preparations from RAW 264 cells, a transformed macrophage cell line, contain two major and one minor ionic form of ODC, as demonstrated by two dimensional electrophoresis. All three forms have a molecular weight of 55,000. Fractionation by isoelectric focusing of immunoprecipitates isolated from RAW 264 cell extracts with an antiserum to ODC, demonstrated that the two major forms of ODC were always present within the cell, and that the minor form could occasionally be detected. The two major forms of the protein have pI values of 5.2 and 5.1 and the minor form is more acidic. Analysis of immunoprecipitates from ^{32}P-metabolically radiolabeled cells indicated that the 5.1 form of the enzyme is the major ODC phosphoprotein in the cell, while the pI 5.2 form of ODC is not phosphorylated intracellularly. Amino acid analysis of hydrolyzed metabolically radiolabeled protein showed that threonine was the predominant phosphoamino acid although some phosphoserine was present. Reverse phase HPLC demonstrated that the radiolabeled threonine is present in a highly hydrophilic tryptic fragment. Dephosphorylation of ODC with alkaline phosphatase resulted in a loss of the immunodetectable pI 5.1 form of the protein and an increase in the pI 5.2 form. The loss of the pI 5.1 form of the protein correlated linearly with the loss in ODC activity.

CH 014 THE ROLE OF INITIATION FACTOR eIF-4D AND ITS HYPUSINE MODIFICATION IN TRANSLATION. John W. B. Hershey, Zeljka Smit-McBride and Joachim Schnier, Dept. Biological Chemistry, School of Medicine, University of Californa, Davis, CA 95616.

Initiation of protein synthesis in mammalian cells is promoted by proteins called initiation factors. The initiation factors are detected by their stimulation of *in vitro* assays for protein synthesis or for partial reactions of the initiation pathway, and 10 initiation factors have been purified to homogeneity. One of these, eIF-4D, stimulates the synthesis of methionyl-puromycin, a reaction which mimics formation of the first peptide bond. Interest in eIF-4D was stimulated by the discovery by M.H. Park and colleagues (NIH) that one of its lysine residues is uniquely modified to hypusine (N^{ϵ}-(2-hydroxy-4-aminobutyl)-lysine; abbrev: Hpu). To better characterize the factor and the role of Hpu in translation, we cloned a human cDNA encoding eIF-4D based on amino acid sequences provided by W.C. Merrick and colleagues (Case Western Res. U.). Three independent approaches to the role of Hpu were undertaken. 1) The cDNA was expressed in *E. coli* which lacks the machinery for synthesizing Hpu, and ec-eIF-4D(Hpu^-) was purified. The factor lacked activity in the methionyl puromycin synthesis assay, nor did it compete with active, human eIF-4D. However, after *in vitro* modification to ec-eIF-4D(deoxy-Hpu) carried out by M.H. Park and E. Wolffe, the protein was active. We conclude that the Hpu modification is essential for the *in vitro* activity of the factor. 2) The human cDNA and a mutant form (where the Lys residue normally converted to Hpu was changed to Arg) were individually transfected into COS-1 cells. No change in the efficiency of protein synthesis was detected with either cDNA, suggesting only that eIF-4D may be in excess in cells. 3) A gene homologous to human eIF-4D was cloned and sequenced from the yeast, *Saccharomyces cerevisiae*. The deduced eIF-4D protein sequence shares with the human factor 61% identical and another 18% similar amino acids, confirming that eIF-4D is a very conserved protein. There is one eIF-4D gene (*TIF44*) per haploid, although a second hybridizing signal suggests that a related sequence may be present in the genome. When *TIF44* is inactivated by deletion, the null mutant is viable, sporulates, but grows 1.7-fold more slowly in rich medium. The null strain lacks two 18 kDa proteins detected by [^{14}C]spermidine labeling and 2D gel electrophoresis, but still exhibits a 20 kDa protein that is more weakly labeled. The results indicate that eIF-4D and its Hpu modification are not essential for cell viability in yeast. To make this conclusion more rigorous, work is in progress to determine whether or not there is another yeast protein with eIF-4D-like properties. We propose a mechanism of action for eIF-4D in translation. *Supported by grant GM22135 from the NIH.*

CH 015 NOVEL METABOLIC AND MOLECULAR PERTURBATIONS ELICITED BY POLYAMINE ANALOGS.
C.W. Porter, Roswell Park Memorial Institute, 666 Elm St., Buffalo, N.Y. 14263
As an alternative approach to the use of enzyme inhibitors to interfere with polyamine biosynthesis and ultimately cell growth, we (in collaboration with R. Bergeron, U. Florida, Gainesville) have been developing polyamine analogs which restrict polyamine biosynthesis by suppressing ornithine and S-adenosylmethionine (AdoMet) decarboxylase synthesis. This later is accomplished in part by post-transcriptional mechanisms which are selective for the decarboxylase proteins. In addition to these intended activities, certain of these analogs have been found to have other significant cellular effects. Specifically, bis(ethyl) derivatives of spermine or its homologs produce extraordinary increases in the polyamine catabolic enzyme, spermidine/spermine-N-acetyltransferase (SSAT) in certain but not all cell lines. In MALME-3 human melanoma cells, the massive enzyme increase is due initially to stabilization of the SSAT protein and may be followed by sustained shifts in the rate of enzyme accumulation. The effect is directly linked to analog structure and does not appear to be causally related to natural polyamine function. In other cell types, these same analogs produce a rapid and significant loss in mitochondrial (mt) DNA. By agarose gel analysis, the loss was found to be selective for mtDNA and to occur in the absence of apparent direct DNA damage. It is separable from analog-induced polyamine depletion but may be related to analog substitution at natural polyamine binding sites involving mtDNA or the organelle itself. The magnitude of these novel analog effects suggests that, in cell types where they are most apparent, inhibition of cell growth may be directly related to them. Thus, in addition to providing possible insight into polyamine function and dynamics, these analog effects may also serve as biochemical determinants of drug action in their further development as anticancer agents (CA-37606, CA-22153; Drs. P. Libby and P. Vertino).

Compartmentalization and Interactions with Cellular Components

CH 016 POLYAMINE TRANSPORT AND SEQUESTRATION IN *NEUROSPORA*, Rowland H. Davis, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.
Polyamines distribute themselves non-randomly in *Neurospora*. The metabolism of isotopically labelled putrescine and spermidine, as they are converted to spermine in living cells, demonstrates a sequestration of over 90% of the resident polyamines (1). Only the small remaining pools participate in the biosynthetic pathway as intermediates or as regulators of the first enzyme of the pathway, ornithine decarboxylase. While some (ca. 25%) of the sequestered polyamine is in vacuoles, bound to polyphosphate (2), the remainder probably binds ionically to ribosomes, DNA and membranes. The small size of the diffusible pools assure a rapid regulatory response to polyamine deprivation or excess (3). A study of polyamine transport by cells growing in normal growth medium reveals that entry of polyamines in this condition is entirely diffusional, and that the extent of uptake is determined largely by the fixed titratable anions of the cell interior (4). Accordingly, diffusional exit through the cell membrane is the major mechanism of disposing of excess intracellular polyamine. A new mutation of *Neurospora*, *puu-1*, causes cells to take up polyamines to toxic levels, indicating that the control of uptake is an important regulatory feature of polyamine metabolism in this organism. Studies of mutations blocking the biosynthetic pathway between putrescine and spermidine (*spe-2* and *spe-3*) show that the spermidine requirement is greatly spared if putrescine accumulates in the cell. This indicates either that putrescine can satisfy the spermidine requirement, or that high levels of putrescine can displace tightly bound spermidine and make it more effective in its indispensable function(s).

(1) Paulus, T. J., Cramer, C. L., and Davis, R. H. (1983) *J. Biol. Chem.* **255**, 8608-8612.
(2) Cramer, C. L., and Davis, R. H. (1984) *J. Biol. Chem.* **259**, 5152-5157.
(3) Davis, R. H., Krasner, G. N., DiGangi, J. J., and Ristow, J. L. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 4105-4109.
(4) Davis, R. H., and Ristow, J. L. (1989) *Arch. Biochem. Biophys.* **271**, 315-322.

CH 017 A ^{13}C NMR STUDY OF THE BINDING OF (^{13}C)-SPERMIDINE TO tRNA, Benjamín Frydman, Carlos de los Santos and Rosalía B. Frydman, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, Buenos Aires, Argentina.

(5,8-$^{13}C_2$)-Spermidine ((^{13}C)-spd) was added to a suspension of E.coli cells and its binding to macromolecules was monitored using ^{13}C NMR spectroscopy at 20 MHz. It was found that spd preferentially binds to membranes and to the tRNA present in the soluble fraction. The binding to tRNA was of a non-covalent type, and the (^{13}C)-spd could be displaced by Mg^{2+} and by (^{12}C)-spd. No binding took place in the presence of a strong ionic force (500 mM KCl). The binding of (^{13}C)-spd to tRNA was easily detected by the broadening of its ^{13}C-resonances (47.8 ppm; d, J=5.8 Hz; C_5, and 39.6 ppm; d, J=5.8 Hz; C_8). The electrostatic nature of the binding was evident from the fact that bound (^{13}C)-spd was released by treatment with perchloric acid or with (^{12}C)-spd. No displacement could be achieved by addition of either putrescine or cadaverine. In order to obtain a further insight into the details of the binding of spd to tRNA, the line widths of (^{13}C)-spd were measured as a function of temperature. It was found that the width of the signals decreased with increasing temperatures. Although it is possible to ascribe this decrease of the relaxation rates solely to a decrease of the correlation time of bound spd, it was assumed that this also reflected the fact that the equilibrium is fast on the NMR timescale. Therefore, the half linewidth of the observed resonances are:

$$\nu_{1/2}^{obs} = 1/\pi \left(X_f/T_{2f} + X_b/T_{2b} \right)$$

where T_{2f}, T_{2b} are the spin-spin relaxation times of free and bound spd and Xf, Xb are their relative concentrations. To simplify the system, subsequent studies were carried out using mixtures of (5-^{13}C)- and (8-^{13}C)-spd, where the ^{13}C - ^{13}C coupling was avoided. An equation was obtained which allowed an estimation of Kd (the dissociation constant) and of n (the number of equivalent independent binding sites per tRNA molecule) by monitoring $\nu_{1/2}^{obs}$ vs C_n^* (concentration of tRNA) at constant C_s^* (concentration of spd) and temperature. Thus a Kd= 3.10^{-3} M and n=12 was obtained from the C_5 data, as well as a Kd=2.10^{-3} M and n=14 from the C_8 data. The Table summarizes the obtained results:

δ	T_1^f(sec)	T_1^b(sec)	τ_c^f(sec)	τ_c^b(sec)	$\nu_{1/2}^b$ (calc)	$\nu_{1/2}^b$ (found)
47.8	1.4	0.09	$1.8.10^{-11}$	$3.7.10^{-10}$	4.6 Hz	4.7 Hz
39.6	1.8	0.14	$1.3.10^{-11}$	$2.1.10^{-10}$	2.7 Hz	2.7 Hz

CH 018 EFFECTS OF POLYAMINES ON MEMBRANE FUSION,

Demetrios Papahadjopoulos, Francis Schuber, Paul Meers and Keelung Hong, Cancer Research Institute, University of California, San Francisco, CA 94143

We have studied the effect of the polyamines (spermine, spermidine, and putrescine) on the aggregation and fusion of large (approximately 100 nm in diameter) unilaemellar liposomes in the presence of 100 mM NaCl, pH 7.4. Spermine and spermidine at physiological concentrations aggregated liposomes composed of pure phosphatidylserine (PS) or phosphatidate (PA) and mixtures of PA with phosphatidylcholine (PC) but did not induce any fusion. However, liposomes composed of mixtures of acidic phospholipids, cholesterol, and a high mole fraction of phosphatidylethanolamine could be induced to fuse by spermine and spermidine in the absence of divalent cations. Putrescine alone in the phsyiological concentration range was ineffective for both aggregation and fusion of these liposomes. Liposomes made of pure PC did not aggregate in the presence of polyamines. Addition of aggregating concentrations of spermine caused a drastic increase in the rate of Ca^{2+}-induced fusion of PA liposomes and a large decrease in the threshold Ca^{2+} concentration required for fusion. This effect was less pronounced in the case of PS or PA/PC vesicles. Preincubation of PA vesicles with spermine before the addition of Ca^{2+} resulted in a 30-fold increase in the initial rate of fusion. Equilibrium dialysis was used to measure the binding of spermine and calcium to large unilamellar vesicles (liposomes) of phosphatidate (PA) or phosphatidylserine (PS). Spermine bound to isolated PA and PS liposomes with intrinsic association constants of approximately 2 and 0.2 M^{-1}, respectively. Spermine enhanced calcium binding to PA, while it inhibited calcium binding to PS, under the same conditions. This difference explained the small effect of spermine on the overall rate of calcium-induced fusion of PS liposomes as opposed to the large effect on PA liposomes. The preference for binding of spermine to PA over PS suggested a preference for accessible monoesterified phosphate groups by spermine. This preference was confirmed by the large effects of spermine on aggregation and overall fusion rates of liposomes containing phosphatidylinositol 4,5-diphosphate. Arachidonic acid was found to act synergistically with promoters of liposomal aggregation, such as Mg^{2+}, spermine, and synexin, to enhance the overall rate of liposome fusion, as would be expected from action at separate kinetic steps.

CH 019 *IN SITU* NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY TO STUDY INTRACELLULAR ENVIRONMENTS AND MOLECULAR INTERACTIONS, Richard L. Weiss, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90024-1569

The compartmentation of metabolites in eukaryotic cells can have profound effects on metabolic and regulatory processes by separating enzymes from their substrates and regulatory effectors. Determination of enzyme localization is now routinely performed by a variety of biochemical and cell biological techniques. Techniques have recently been developed to examine the distribution of small molecules between intracellular compartments and their association with macromolecules. One such technique is nuclear magnetic resonance spectroscopy, a non-invasive technique which can provide information about the environment of molecules in intact cells. The utility of this technique will be illustrated for arginine compartmentation in *Neurospora crassa* (1). Little or no catabolism of arginine is observed in *N. crassa* despite a large intramycelial arginine pool and a high basal level of its catabolic enzyme, arginase, in mycelia growing in minimal medium; addition of arginine to the growth medium results in immediate initiation of catabolism even in the absence of protein synthesis (2). These observations suggest that arginine is not randomly distributed in mycelia. Suspensions of intact mycelia labeled with $^{15}NH_4Cl$ give well-resolved ^{15}N nuclear magnetic resonance spectra for metabolites of nitrogen metabolism. Pulse-chase experiments indicate the existence of a metabolically stable pool of arginine (3). The half-height line widths of the δ- and ω,ω'-nitrogens of [^{15}N]arginine are consistent with a localized intramycelial pH of 6.1-6.5. The half-height line widths for intramycelial alanine and proline are consistent with a localized intramycelial pH of 7.1-7.2. These observations are consistent with a vacuolar localization of intramycelial arginine and a cytosolic localization of alanine and proline (4). The nitrogen-15 spin-lattice relaxation time, T_1, for the $^{15}N_\gamma$ of glutamine (cytosol) is only slightly shorter than that in the culture medium. This indicates that the microenvironment of the cytosol surrounding the glutamine molecules is not much higher than 1.3 cP. By contrast, for $^{15}N_{\omega,\omega'}$ of arginine, the intramycelial T_1 is only one-fourth of that in the medium. These results suggest either that the vacuolar viscosity is substantially above 2.8 cP or that the ω,ω'-nitrogens of vacuolar arginine are associated with a polyanion, possibly polyphosphate (5). These results illustrate the effectiveness of NMR as a tool for identifying and characterizing metabolite compartmentation in eukaryotic cells.

(1) Davis, R. H. and Weiss, R. L. (1988) *Trends Biochem. Sci.* **13**, 101-104.
(2) Weiss, R. L. and Davis, R. H. (1977) *J. Bacteriol.* **129**, 866-873.
(3) Legerton, T. L., Kanamori, K., Weiss, R. L., and Roberts, J. D. (1981) *Proc. Natl. Acad. Sci. USA* **78**, 1495-1498.
(4) Legerton, T. L., Kanamori, K., Weiss, R. L., and Roberts, J. D. (1983) *Biochemistry* **22**, 899-903.
(5) Kanamori, K., Legerton, T. L., Weiss, R. L., and Roberts, J. D. (1982) *Biochemistry* **21**, 4916-4920.

Polyamine-Nucleic Acid Interactions

CH 020 MULTIVALENT CATION EFFECTS ON DNA BEHAVIOR. Victor A. Bloomfield, Department of Biochemistry, University of Minnesota, St. Paul MN 55108.

DNA is condensed into toroidal or rodlike particles by multivalent cations such as spermidine^{3+}, spermine^{4+}, and $Co(NH_3)_6^{3+}$. These condensates have attracted much attention because of their structural similarity to DNA from the capsids of gently lysed bacteriophage[1,2]. We are investigating a number of physical chemical issues, including determinants of size and shape of the condensed particles, the equilibrium and kinetics of condensation, and the coupling of condensation with helix-helix transitions.

Condensed particles have approximately the same volume, regardless of their shape or the length of the DNA or the shape. We have determined the distribution in number n of whole- and half-length molecules of the plasmid pUC12 (2700 base pairs) incorporated in condensates, and are attempting to fit this distribution to the form proposed by Tanford[3] for micelles:

$$\ln X_n = -n\Delta\mu_n^\circ/kT + n \ln X_1 + n \ln f + \ln n$$

using the Post-Zimm[4] coil-globule transition free energy function in the form

$$\frac{\Delta\mu_n^\circ}{kT} = \ln v_2 - (N-1)(1-v_2) + \chi N(1-v_2)^2 + N[(\chi - 1) + \frac{B_2 v_2}{2^{3/2}} + \frac{B_3 v_2^2}{2 \cdot 3^{5/2}}] + \frac{\Delta\mu_{bend}}{kT}$$

Light scattering study of condensation kinetics suggests that the initial aggregate is a loose assembly of uncondensed coils, which becomes more compact as more molecules are added.

Among the forces that have been suggested to stabilize the condensed state are crosslinking by condensing ligands, intrinsic curvature of charge-neutralized DNA, coupled charge fluctuations in the ion atmospheres surrounding the DNA helices, and attractive hydration structure. We suggest an additional factor that may be important: coupling of condensation with transition from B-DNA to some other helical form. This has been observed in Z-forming synthetic DNA[5] and in P-DNA formed at high alcohol concentrations[6]; and is inferred from studies of aggregation with divalent metal cations at somewhat elevated temperatures[7]. Bases which become unpaired and unstacked during such a transition could bind to similarly perturbed bases in adjacent helices.

[1]Klimenko, S.M., Tikkchonenko, T.I. & Andreev, V.M. (1967) *J. Mol. Biol.* **23**, 523-533.
[2]Chattoraj, D.K., Gosule, L.C. & Schellman, J.A. (1978) *J. Mol. Biol.* **121**, 327-337.
[3]Tanford, C. (1974) *J. Chem Phys.* **78**, 2469-2479.
[4]Post, C.B. & Zimm, B.H. (1982) *Biopolymers* **21**, 2123-2137.
[5]Thomas, T.J. & Bloomfield, V.A. (1985) *Biochemistry* **24**, 713-719.
[6]Zehfus, M.H.&J., W. C., Jr. (1984) *Biopolymers* **23**, 1269-1281.
[7]Knoll, D.A., Fried, M.G. & Bloomfield, V.A.(1988) in *Structure & Expression. DNA and Its Drug Complexes* (eds. Sarma, R.H. & Sarma, M.H.) 123-145 (Adenine Press, New York).

CH 021 RELATIONSHIPS BETWEEN POLYAMINE STRUCTURE AND NUCLEIC ACID CONFORMATION. **Burt G. Feuerstein[1,2], Hirak S. Basu[2], Jean-Guy Delcros[2], Peter Csokan[2], Janos Szöllösi[2], and Laurence J. Marton[1,2], [1] Brain Tumor Research Center of the Department of Neurosurgery and [2] Department of Laboratory Medicine, University of California, San Francisco, CA 94143.** Flexibility of DNA appears to be an important characteristic of its structure and function. Polyamines both associate with DNA and alter its conformation in vitro. We hypothesize that polyamines play an important role in secondary and tertiary DNA structure in the cell. In order to explore what imbues polyamines with their abilities to bind and alter DNA structure, we have studied spermine and a variety of polyamine analogs for their abilities to promote conformational transitions in vitro, and have used molecular modeling as a tool to help visualize DNA/polyamine interactions. We find that ability to promote conformational transition is not a simple function of polyamine charge, structure, or binding activity. In particular, both the central carbon chain and the terminal amino groups of spermine appear to be important for its effects on DNA. Both modeling and experimental results will be discussed.

Potential Mechanisms for Therapeutic Intervention

CH 023 POLYAMINE/DNA INTERACTIONS AND THEIR POTENTIAL RELATIONSHIP TO CANCER THERAPY, *Laurence J. Marton, Hirak S. Basu, Burt G. Feuerstein and Dennis F. Deen, Departments of Laboratory Medicine, Pediatrics, and Radiation Oncology, and the Brain Tumor Research Center of the Department of Neurological Surgery, University of California, School of Medicine, San Francisco, CA 94143.*

Strong interactions of polyamines with nucleic acids are described in historic and modern day literature. Our studies of the molecular mechanics and dynamics of polyamine/nucleic acid interactions and of the physico-chemical interaction of polyamines with nucleic acids, along with the studies of others, are defining a basic understanding of the chemistry of these interactions. Although these studies are either theoretical or usually based on data obtained using "naked" nucleic acids, and although our knowledge in this sphere continues to evolve as new techniques are applied and as additional crystallographic data become available, it appears that chemical alterations of polyamines which cause modification of specific physico-chemical properties of polyamine/DNA interactions are likely to yield compounds capable of interfering with cell growth and/or killing cells. The evolution of polyamine analogues, such as those produced by Bergeron and his co-workers (1), has been exceedingly helpful in providing defined compounds with clear effects which could be used to corroborate our findings. For instance, compounds which combine affinity for DNA with a decreased potential for aggregating DNA, appear to inhibit cell growth. Predicated on these and other data, several compounds were selected or designed by us as potential agents against tumor cells in culture. These agents have been tested and found to be growth-inhibitory and in many instances cytotoxic. Further elaboration on this "rational" approach to the development of polyamine analogues will be presented.

1 Bergeron, R.J., Hawthorne, T.R., Vinson, J.R.T., Beck Jr., D.E. and Ingeno, M.J. Cancer Res. 49:2959-2964,1989.

CH 024 Ornithine Decarboxylase in Trypanosomes. MARGARET PHILLIPS, LUCY GHODA, PHILIP COFFINO and C.C. WANG* (University of California, San Francisco).

Ornithine decarboxylase (ODC) from the African trypanosomes is an important target for antitrypanosomal chemotherapy. DL-difluoromethylornithine (DFMO), a catalytic, irreversible inhibitor of ODC, can effect the cure of African trypanosomiasis without any toxic effect on the mammalian host. We have recently purified the ODC from one of the African trypanosomes *Trypanosoma brucei brucei*, and found it somewhat less susceptible to DFMO inhibition than the mammalian ODC, thus failed to explain the observed therapeutic value of this drug. The gene encoding *T. brucei* ODC has since been cloned and sequenced, and the deduced amino acid sequence bears significant homology with that of mouse ODC with the exception that the C-terminal 36 amino acids in mouse ODC are missing from the *T. brucei* enzyme.

Since the C-terminal peptide in mouse ODC has been postulated to be responsible for its rapid turn-over *in vivo* (Rogers et al., Science *234*, 364, 1986), we examined the half-life of ODC in *T. brucei* and found no sign of turning over of the enzyme. This discrepancy between the mammalian and *T. brucei* ODC can adequately explain the selective *in vivo* toxicity of DFMO on *T. brucei*. Further investigations using Chinese hamster ovary (CHO) ODC-mutant cells transfected with various DNA constructs indicated that; 1). mouse ODC has a very short half-life in CHO cells; 2). mouse ODC truncated at the C-terminus has a very long half life; 3). *T. brucei* ODC has a very long half-life; 4). *T. brucei* ODC with the mouse ODC C-terminals 36 amino acids added to its C-terminus has a shortened half-life. Thus, the C-terminal peptide in mouse ODC is the deciding factor of its rapid turnover in a variety of eukarytic cells.

Physiological Mechanisms for Regulating Polyamine Content

CH 100 EFFECT OF N^1,N^{14}-BE-4-4-4 AND N^1,N^{19}-BE-4-4-4-4 ON THE GROWTH AND SURVIVAL OF HUMAN BRAIN TUMOR CELLS, Hirak S. Basu[1], Malgorzata Pellarin[1], Burt G. Feuerstein[2,3], Dennis F. Deen[1], and Laurence J. Marton[1,2], [1]Brain Tumor Research Center of the Department of Neurosurgery, [2]Department of Laboratory Medicine, and [3]Department of Pediatrics, School of Medicine, University of California, San Francisco, CA, 94143.

A spermine analog, N^1,N^{14}-(bis)-ethyl-homospermine (BE-4-4-4), was tested on U-87 MG, SF-126, U-251 MG, and SF-188 human brain tumor cells, and a pentamine N^1,N^{19}-bis(ethylamino)-5,10,15-triazanondecane (BE-4-4-4-4) was tested on U-87 MG and U-251 MG cells for their effects on growth, polyamine levels, and survival of the tumor cells in tissue culture. Both analogs, at a concentration between 5-10 μM, were growth inhibitory, caused a significant decrease of putrescine, spermidine and spermine levels, and were cytotoxic to all cell lines after 72 hours of treatment. The affinities of the analogs for calf-thymus DNA (determined by T_m measurement studies) were higher than those of spermine, but the analogs were poorer than spermine in aggregating DNA. The growth inhibitory and cytotoxic effect of these analogs support our hypothesis, that polyamine analogs which can enter cells, deplete intracellular polyamine levels and replace the natural polyamines from their binding sites on DNA should act as an antiproliferative agent.

CH 101 TRANSGLUTAMINASE ACTIVITY AND POLYAMINE INCORPORATION INTO PROTEIN OF MURINE MELANOMA CELLS WITH DIFFERENT METASTATIC POTENTIAL. Simone Beninati* and Massimo Cardinali‡,+ *Department of Biology, 2nd University of Rome, "Tor Vergata", Italy and +National Institutes of Health, NIDR, Bethesda, MD.

There is increasing evidence to suggest that polyamines play an important role in mammalian tissues through covalent interaction with macromolecules. In this regard the post-translational modification of proteins by incorporation of polyamines through γ-glutamyl linkages is catalyzed by transglutaminase, as is the production of ε-(γ-glutamyl)lysine bridges. Several reports describe reduction in both transglutaminase activity and ε-(γ-glutamyl)lysine in neoplastic tissue. We examined metastasizing and non-metastasizing cells in order to determine whether there are differences in transglutaminase-catalyzed polyamine incorporation in these cells. An inverse correlation was found between polyamine incorporation in proteins and metastatic potential in two melanoma cloned cell lines originating from a spontaneous tumor in C57Bl/6 mouse. Although substantial amounts of ε-(γ-glutamyl)lysine and γ-glutamylpolyamines were found in protein from B16-F1^{LPR}, which have low metastatic potential, significantly less of these transglutaminase products were present in B16-F10 cells, which yield a high incidence of pulmonary metastases. These findings are consistent with a role of polyamine-protein conjugates in neoplastic events.

CH 102 STABILIZATION OF LOOP STRUCTURE IN ETHIDIUM BROMIDE -POLY(dA-dT) COMPLEX BY SPERMINE, Jean-Guy Delcros[1], Hirak S. Basu[1], Richard H. Shafer[4], Janos Szalosi[3], Burt G. Feuerstein[2,4], and Laurence J. Marton[1,2], [1]Brain Tumor Research Center of the Department of Neurosurgery, [2]Department of Laboratory Medicine, [3]Department of Pediatirics, School of Medicine, and [4]Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, CA, 94143.

Spermine (Spm) like other DNA binding agents is able to displace ethidium bromide (EB) from its complex with DNA. Circular dichroic, spectrophotometric, fluorescence intensity and steady-state fluorescence polarization measurements have been carried out to investigate the interaction of Spm with DNA and synthetic polynucleotides complexed with EB. Spm is able to displace EB in a cooperative manner from poly(dG-dC), CT DNA, and poly(dA).poly(dT), and in a non-cooperative manner from poly(dA-dT). Spm induced release of EB from DNA and polynucleotides was interpreted in terms of Spm-induced stabilization of DNA in a conformation less favorable for the binding of EB. A remarkable conformational change is observed for poly(dA-dT)-EB complex in the presence of molar ratios of Spm/nucleotide > 0.2. This change is characterized by changes in the CD spectra, a sharp decrease in fluorescence polarization of the complex and the appearance of a low temperature melting region in fluorescence detected T_m measurements. These unusual effects have been interpreted as the stabilization of loop structure in poly(dA-dT) as a result of a local untwisting and bending generated by EB and Spm respectively. The stabilization of such structures in a specific base sequence of DNA *in vivo* may have some role in the biological function(s) of Spm.

CH 103 COMPARISON OF BINDING SITES FOR TWO PHOTOAFFINITY DERIVATIVES OF SPERMINE AND ONE DERIVATIVE OF SPERMIDINE ON NUCLEOSOME CORE PARTICLES, Harry R. Matthews, Elizabeth M. Clark, Richard Swank and James E. Morgan, Department of Biological Chemistry, University of California, Davis, CA 95616. Polyamines bind to DNA in nucleosome core particles and stabilize the core particles (Morgan J.E., Blankenship J.W. & Matthews H.R., 1987, Biochem. 26, 3643-3649). A photoaffinity derivative of spermine, (azidonitrobenzoyl)spermine (ANB-spermine), was used to photoaffinity label nucleosome core particles and 7 discrete labeling sites were observed superimposed on a "background" of uniform labeling (Morgan J.E., Calkins C.C. & Matthews H.R., 1989, Biochemistry, 28, 5095-5106). A new derivative has now been synthesized, (azidobenzyl-amidino)spermine (ABA-spermine), and the analogous spermidine derivative, ABA-spermidine. These compounds also photoaffinity label the DNA in nucleosome core particles. They show a "background" of uniform labeling, like the ANB-spermine sites, but do not show enhanced labeling at the same discrete sites as were seen with ANB-spermine. A different set of discrete sites is seen with ABA-spermidine, but additional experiments are being carried out to test the possibility that they may be due to an artefact caused by irradiation. ABA-spermidine, but not ANB-spermine, can be taken up by growing yeast cells. ABA-spermidine partly inhibits the growth of these cells while ANB-spermine is without effect on growth. The absence of physiological activity of ANB-spermine suggests that the ABA- compounds may be better models for natural polyamines. Supported by National Science Foundation Grant DCB 8705378.

Role of Polyamines in Cellular Physiology

CH 200 DIFFERENTIAL SENSITIVITY TO -DIFLUOROMETHYLORNITHINE BY CLINICAL ISOLATES OF TRYPANOSOMA BRUCEI RHODESIENSE, Cyrus J. Bacchi, Nigel Yarlett and Henry Nathan, Haskins Laboratory and Biology Department, Pace University, New York, NY 10038

2-Difluoromethylornithine (DFMO) is an enzyme activated irreversible inhibitor of ornithine decarboxylase (ODC), the first enzyme of polyamine biosynthesis. DFMO is effective in the treatment of West African sleeping sickness caused by Trypanosoma brucei gambiense, but has had mixed results against the East African parasite, T.B. rhodesiense. Seven of 14 isolates of East African origin Kenya Trypanosomiasis Research Institute (KETRI) strain bank to be moderately to highly refractory to DFMO treatment at dose levels of 2% or 4% in drinking water for 3 or 6 days. The specific activity of ODC was found to be similar in the amount of drug required to cause 50% inhibition of ODC activity, was unchanged for all the strains tested. DFMO uptake was reduced by approximately 50% in one isolate, KETRI 2285, but unchanged in all the other strains examined. Uptake of [3H] putrescine and [3H] spermidine in blood forms in vitro was low (< 10 pmoles/mg protein/h), however in 3 refractory strains, spermine uptake was 4 to 10 times the levels in sensitive isolates. There was no evidence of interconversion of spermine to spermidine or putrecine in sensitive or DFMO refractory isolates. These studies are continuing and will also focus on the levels of polyamines and precursor amino acids in these isolates and the activity of other polyamine synthetic enzymes. (Support: NIH AI 17340, WHO 890064)

CH 201 CATALYTIC IRREVERSIBLE INHIBITION OF TRYPANOSOMAL S-ADENOSYL-L- METHIONINE DECARBOXYLASE BY A SUBSTRATE ANALOG AND ITS EFFECTS ON MURINE TRYPANOSOMIASIS, Alan J. Bitonti, Timothy L. Byers, Cyrus J. Bacchi, Allen B. Clarkson, Jr., Peter P. McCann and Albert Sjoerdsma, Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215.

A substrate analog of S-adenosyl-L-methionine (AdoMet), MDL 73811, was found to irreversibly inhibit Trypanosoma brucei brucei AdoMet decarboxylase (AdoMet DC). The inhibition was time-dependent ($t_{1/2}$ = 0.6 min), exhibited pseudo-first order kinetics (K_i = 1 μM) and was apparently irreversible. AdoMet protected the enzyme from inactivation suggesting the MDL 73811 was directed at the enzyme's active site and was probably catalytically activated. MDL 73811 administration to rats infected with T.b. brucei resulted in a depletion of spermidine and an increase in putrescine in the trypanosomes isolated from the treated rats. AdoMet DC activity in the trypanosomes was also found to be inhibited. Treatment of T.b. brucei-infected mice with MDL 73811 (20 mg/kg i.p., 3 times/day for 4 days) resulted in cures of the trypanosome infection. MDL 73811 also increased the survival of mice infected with T.b. rhodesiense, a species causing human trypanosomiasis, and induced cures in about 40-50% of these mice. In combination with α-difluoromethylornithine (DFMO), MDL 73811 cured all mice infected with a multidrug-resistant strain of T.b. rhodesiense. The two drugs appeared to be synergistic against T.b. rhodesiense and thus may offer a new chemotherapeutic strategy for the treatment of drug-resistant trypanosome infections in man.

CH 202 INCREASED IMP AND ATP CONTENT BY POLYAMINES IN CULTURED MOUSE LEUKEMIC LYMPHOID CELLS SC-1 TREATED WITH ADENOSINE AND 6-THIOINOSINE, S.-C. Cho Department of Biology, School of Hygiene, Fujita-Gakuen Health University, Toyoake, Aichi 470-11,Japan. To study the relationship between polyamines and cellular nucleotide content, mononucleotide content and adenine nucleotide content of mouse leukemic lymphoid cells SC-1 were measured by high-performance liquid chromatography, after the cells were treated with 6-thioinosine 0.5 mM, adenosine 0.3 mM, and polyamines (0.05 or 0.5 mM) for ca. 6 h.

Mononucleotides were analyzed by a 250 x 4.6 mm I.D. column prepacked with a MCI CDR-10 anion-exchange resin. 1 M ammonium acetate buffer (pH 3.3) was eluted at a flow-rate of 1 ml/min. The column temperature was maintained at 60°C. Adenine nucleotides was analyzed using ion-pair reversed-phase HPLC (Sellevold et al. '86). Acid soluble nucleotides were extracted from cells with 0.5 M perchloric acid, and neutralized with alamine/Freon solution by the method of Khym ('75). The aqueous solution was concentrated to dryness and then dissolved in 0.5 ml of water.

The IMP and ATP content increased markedly in the presence of 6-thioinosine, adenosine, and polyamines: spermine was the most effective, followed by putrescine, spermidine, cadaverine, and N^1-acetylspermine. The accumulation of ATP by polyamines was very low, though the IMP content increased, in tne case of adenine and 2'-deoxyadenosine in place of adenosine. The result suggests that polyamines may affect the adenosine salvage pathway, AMP deaminase activity, or cellular energy charge system.

CH 203 THE EFFECT OF DEPLETION OF INTRACELLULAR POLYAMINES ON THE REPLICATION OF HUMAN CYTOMEGALOVIRUS IN VITRO,J.R. Clarke,Division of Virology, Dept of Med Microbiology,St. Mary's Medical School, London. Human cytomegalovirus (HCMV) is an important human pathogen,which can cause serious disease in the newborne and in immunocompromised patients. Congenital infection is characterised by chronic excretion of virus in the urine of affected babies for prolonged periods. Although the majority of infected babies are asymptomatic at birth some develop sequelae most commonly sensori-neural deafness.CMV complications of the immunocompromised and AIDS patients include pneumonia and retinitis.There is an urgent need for an effective chemotherapeutic agent to combat the effects of CMV. The effect of ODC inhibitors on HCMV replication in vitro was assessed. The inhibitors used were DFMO, monofluoromethyl-dehydro-ornithine methylester (Δ MFMO-CH_3) and RR methlacetyl-enicputrescine (RRMAP). It was discovered that the replication of 9 out of 9 strains of CMV were inhibited by Δ MFMO-CH_3. The Colburn strain of CMV was not inhibited in cells treated with Δ MFMO-CH_3 This was the first indication that the requirements of the variours strains of HCMV for polyamines may differ. Furthermore, most of HCMV strains were only partially inhibited in the presence of RRMAP.Molecular studies have shown that ΔMFMO-CH_3 and DFMO partially inhibit viral DNA replication.

CH 204 INHIBITION OF RADIATION RECOVERY, Dennis F. Deen, Laura E. Kendall and Laurence J. Marton, Brain Tumor Research Center, University of California, San Francisco, California 94143-0520

In 1980 we reported that treatment of 9L rat brain tumor cells with the polyamine inhibitor alpha difluoromethylornithine (DFMO) had no effect on subsequent radiosensitivity of the cells. Since that time, there have been several studies on other cell lines in which DFMO has either increased radiosensitivity or inhibited recovery from radiation damage. We have investigated the ability of DFMO to alter the radiation response of 5 human brain tumor cell lines, SF-126, U-87 MG, U-138 MG, U-251 MG and U-343 MGA. Cells (1×10^5) were seeded into 25 cm^2 tissue culture flasks and allowed to incubate at 37°C for 48 hr. DFMO (1 mM) was added and the cells were incubated for 96 hr, at which time putrescine and spermidine levels were minimal, and the cells were growth-inhibited. At this time cells were either irradiated and assayed immediately for cell survival, irradiated and allowed time to recover from potentially lethal damage (PLD), or irradiated with a split dose protocol in order to measure recovery from sublethal damage (SLD). Radiosensitization did not occur in any of the cell lines. However inhibition of PLD occurred in all 5 cell lines, and inhibition of SLD occurred in 3 of 3 cell lines studied. Thus inhibition of radiation recovery by DFMO appears to be a general phenomenon in human brain tumor cells. Because DFMO produces only minor and reversible toxicity in humans, it should be possible to integrate DFMO treatment into the radiation therapy protocols for patients with brain tumors. *Supported by NIH Grants CA 13525 and CA 42779.*

CH 205 MODIFICATION OF RADIATION BRAIN DAMAGE BY a-DIFLUOROMETHYLORNITHINE (DFMO), John R. Fike, Glenn T. Gobbel, Laurence J. Marton, Brain Tumor Research Center, University of California, San Francisco, California 94143-0520

Recent laboratory studies suggest that polyamines mediate the response of normal brain to focal injury and the formation of vasogenic edema. Because edema and its associated mass effect is a common finding in radiation brain injury, we hypothesized that polyamines are involved in the disruption of the normal blood brain barrier and the resultant edema, and that depletion of polyamines using α-difluoromethylornithine (DFMO) may modify the extent and/or development of focal radiation injury. Removable high activity I-125 sources were implanted into the frontal white matter of normal beagle dogs until a 20 Gy dose was delivered 0.75 cm away. Animals received a constant i.v. infusion of DFMO (150 mg/kg/day) 2 days before implantation and continuing for 14 days. The extent and evolution of tissue necrosis and edema were quantified weekly to 6 wks using computed tomography, and blood-to-brain transfer constants and regional cerebral blood flow were quantified using cine-CT. Tissue polyamine levels were determined using HPLC. Putrescine levels in non-irradiated brain were less than 8 pmoles/mg, whereas in irradiated tissues they averaged 150 ± 50 pmoles/mg. DFMO treatment reduced putrescine levels in irradiated brain to approximately 30 pmoles/mg. Volume of edema averaged over the 6 wk follow-up was reduced 25-40% relative to radiation alone. Permeability of irradiated vasculature paralleled wk to wk changes in edema volume; DFMO appeared to affect radiation-induced permeability. Such studies enable us to determine if permeability and/or blood flow are involved in the observed DFMO effects, and perhaps elucidate the role of polyamines in the development and expression of radiation brain injury. *Supported by NIH Grant CA 13525.*

CH 206 REDUCTION OF DIFLUOROMETHYLORNITHINE (DFMO)-INDUCED THROMBOCYTOPENIA WITH ORNITHINE Bruce Grossie, David Ota, Jaffer Ajani, and Kenji Nishioka. Department of General Surgery, UT MD Anderson Cancer Center, Houston, Texas 77030.

The dose-limiting host toxicity of DFMO is thrombocytopenia. Thrombocytopenia induced by a continuous infusion of 2.0 g/kg/d was ameliorated by concomitant ornithine (Orn) administration (Cancer Res. 49:4159, 1989). The purpose of this study was to determine if Orn can ameliorate thrombocytopenia at higher doses of DFMO. This might allow an increase in dose and tumor growth inhibition. Fischer 344 rats with a transplantable sarcoma (sc) were treated with continuous infusion of 2.0 and 3.5 g/kg/d DFMO alone or with Orn added to the infusate at a molar ratio to DFMO of 0.4. After 8 days tumors were measured and rats were exsanguinated. Platelet counts and tumor weight change (ratio of the final-to-initial tumor weight) were determined. Polyamines were determined by HPLC. Tumor putrescine and spermidine levels were decreased and spermine and S-adenosylmethionine decarboxylase activity were increased with DFMO treatment; Orn+DFMO did not alter tumor polyamine levels. DFMO-induced platelet suppression (Table) can be ameliorated by concomitant Orn treatment without affecting its antitumor activity. Our data suggest that this is independent of DFMO dose when an appropriate Orn:DFMO molar ratio is maintained. Further success in increasing antitumor activity may depend upon additional reduction in tumor levels of spermidine and spermine.

Dose DFMO (g/kg/d)	Orn:DFMO	n	tumor wt. change	Platelets (x1000)
0	0	5	1.63±0.48	332±145
2.0	0	5	0.59±0.18*	79± 44*
2.0	0.4	5	0.60±0.02*	489±190
3.5	0	5	0.46±0.13*	31± 10*
3.5	0.4	5	0.56±.018*	359±131

*Mean differs (P<0.05) vs controls.

CH 207 POLYAMINE ACCUMULATION AND VASOGENIC EDEMA IN THE GENESIS OF LATE DELAYED RADIATION INJURY OF THE CENTRAL NERVOUS SYSTEM(CNS). Philip H. Gutin, Michael W. McDermott, Pak Chan, Sylvia Chen, Kenneth A. Levin, Oktar Babuna, Laurence J. Marton. Brain Tumor Research Center, University of California, San Francisco, CA 94143. Endothelial injury with abnormal blood-brain barrier (BBB) permeability and vasogenic edema may be an etiologic factor in the demyelination and white matter necrosis of CNS radiation injury. Polyamine accumulation has been previously associated with BBB breakdown in the rat brain after cold injury, both being reversed by DFMO. In a model of cervical spinal cord delayed radiation injury in F344 rats, putrescine (Pu) and spermine levels were significantly elevated at 4 months post-irradiation as compared to unirradiated controls ($P<0.001$). The water content of cervical spinal cord rose transiently within 60 days of irradiation (71.6+/-1.2%) and then abruptly at paralysis(74.5+/-2.1%). Tissue Na^+ and K^+ measurements confirmed vasogenic edema. DFMO given at a dose of 500 mg/kg I.P., Q12H X 2, to paralyzed animals reduced the levels of Pu significantly as compared to irradiated paralyzed controls ($P=0.005$). Spinal cord Pu levels were significantly reduced in unirradiated rats on 0.5% and 1.0% DFMO in drinking water. The blockade of polyamine synthesis might be a reasonable therapeutic alternative to corticosteroid drugs in treating the vasogenic edema of radiation injury and may improve CNS radiation tolerance. Results of such experiments will be reported.

CH 208 POLYAMINES,DIETARY ARGININE, AND HYPOXIA-INDUCED PULMONARY HYPERTENSION. Allen Hacker and Carl Christensen, Department of Medicine, Medical College of Wisconsin, Milwaukee, WI 53226
Increases in lung polyamine levels are required for the development of monocrotaline (MCT)-induced pulmonary hypertension (PH) in the rat (A. Hacker and C. Byus Circulation 76:IV-471, 1987). Arginine (ARG) is a semi-essential amino acid. It is converted to ornithine by arginase and is the dietary source of ornithine which is the precursor for polyamine biosynthesis. We have previously reported that reduction of arginine intake protects against the development of MCT-induced PH in the rat and is associated with an inhibition of increased levels of lung polyamines. To test the hypothesis that a diet deficient in arginine might protect against the development of hypoxia -induced PH, we exposed rats to 10% oxygen or air and divided them into 3 groups of 5 each (Control+ARG, HYPOXIA+ARG, or HYPOXIA-ARG). The diets used were isocaloric and animals were pair-fed. Twenty-one days after hypoxia animals in the HYPOXIA-ARG group had decreased lung polyamine lung levels and right ventricular hypertrophy compared to those in the HYPOXIA+ARG group. These results are consistent with our working hypothesis that elevated lung polyamine levels are required for the development of pulmonary hypertension. (HL 36123)

CH 209 POLYAMINE LEVELS IN ERYTHROCYTE OF LEUKEMIC PATIENTS
Haruhisa Nagoshi, Yoshimasa Nakamura, Teruhisa Ayabe, Susumu Shimizu, Kazuhiko Someya, The Third Division of Internal Medicine, St. Marianna University School of Medicine, Sugao, Miyamae-ku, Kawasaki, Kanagawa 213, JAPAN
The polyamine content in erythrocytes was determined in an attempt to establish a staging marker of cell growth of leukemia. Erythrocytes from patients with acute (AL) and chronic leukemia and myelodysplastic syndrom (MDS) were subjected to determine polyamine content, using high-performance liquid chromatography. The spermidine (SPD) and spermine (SPM) levels in 40 healthy controls were 15.0±6.4 and 9.0±4.8 u mole/1 ml of packed red cells, and SPD/SPM ratio was 1.98±1.04. SPD and SPM levels in that of AL were higher than normal and in MDS, those were similar levels. SPD/SPM ratio, however, was under 1 in both AL and MDS. After chemotherapy and at the recovery state of leukemia, SPD and SPM levels were increased compairing with healthy controls, but SPD/SPM ratio was over 1. Serial studies in AL indicate that the polyamine levels of erythrocyte did not correlate with clinical stage, but SPD/SPM ratio, correlated well with the state of leukemic or normal cell growth. From this study, the determination of polyamine in erythrocytes and calibration of SPD/SPM ratio will be useful for determine the clinical stage of leukemia.

CH 210 INHIBITION OF POLYAMINE SYNTHESIS IN THE BROWN-ROT FUNGUS POSTIA PLACENTA. Barbara Illman, Forest Products Laboratory, FS/University of Wisconsin, One Gifford Pinchot Drive, Madison, Wisconsin 53705.
As part of the effort to find alternatives to the current use of metabolic poisons to control wood decay fungi, methods for regulating polyamine biosynthetic enzymes were tested. Enzyme-activated irreversible inhibitors of polyamine biosynthesis were used to study polyamine metabolism in the brown-rot fungus, Postia placenta. The enzyme-specific inhibitors for ornithine decarboxylase (ODC) and arginine decarboxylase (ADC) were difluoromethylornithine (DFMO) and difluoromethylarginine (DFMA), respectively. The inhibitors suppressed in vitro mycelial growth indicating that polyamines are essential for the fungus. Growth was retarded more by DFMO than by DFMA. Addition of the ODC pathway products, putrescine and spermidine, reversed the DFMO-induced inhibition. Growth in wood samples was also inhibited by DFMO in ASTM standard wood-block tests, suggesting that polyamine inhibitors should be further studied for their use as wood preservatives.

CH 211 CONSEQUENCES OF CONCOMITANT INHIBITION OF S-ADENOSYLMETHIONINE (ADOMET) BIOSYNTHESIS AND POLYAMINE BIOSYNTHESIS OR CELLULAR METHYLATION, Debora L. Kramer, Janice R. Sufrin, Ronald T. Borchardt, and Carl W. Porter, Roswell Park Cancer Institute, Buffalo, NY 14263.
AdoMet serves two metabolic processes, cellular transmethylation and polyamine biosynthesis. Following treatment of L1210 cells with L-2-amino-4-methoxy-cis-but-3-enoic acid (L-cisAMB), a competitive methionine-analog inhibitor of AdoMet synthetase, AdoMet pools are rapidly depleted. Unexpectedly, methylation of DNA and RNA are markedly decreased while polyamine pools remain unaffected, indicating that the latter is spared at the expense of methylation. When L-cisAMB was combined with inhibitors of ornithine or AdoMet decarboxylase, growth inhibition was no greater than either inhibitor alone. In fact, the polyamine inhibitors increased AdoMet pools and abrogated depletion of AdoMet by L-cisAMB. L-cisAMB was also combined with inhibitors of S-adenosylhomocysteine (AdoHcy) hydrolase which inhibit methylation by raising levels of AdoHcy, a potent product inhibitor of methyltransferases. This latter drug combination raised AdoHcy and concomitantly lowered AdoMet, resulting in AdoHcy/AdoMet ratios 4-fold greater than with either hydrolase inhibitor alone. This was accompanied by a marked and sustained interference with DNA and RNA methylation and a near-total inhibition of cell growth. Overall, the data indicate that while the combined inhibition of AdoMet synthetase and polyamine biosynthesis is not advantageous, inhibition of AdoMet synthetase and AdoHcy hydrolase may have therapeutic implications in antiproliferative or antiviral strategies targeting methylation. (CA-13038; CA-24538).

CH 212 RELATIONSHIP BETWEEN POLYAMINE LEVELS IN BRAIN TUMORS AND OUTCOME OF THE PATIENTS, H. Kurihara[1], S. Matsuzaki[2], H. Yamazaki[1], T. Tsukahara[1], M. Tamura[1] and C. Ohye[1], Department of Neurosurgery, School of Medicine(1), and Department of Physiology, Institute of Endcrinology(2), Gunma University, Maebashi 371, Japan. We studied the relationship between tissue polyamines levels and outcome in 112 cases of primary brain tumors. The patients whose tumors contained high tissue levels of N^1-acetylspermidine (N^1-AcSpd)($\geq$10 nmol/g) showed far worse prognoses, significantly lower recurrence-free survival rate and survival rate, compared with the patients whose tumors contained lower N^1-AcSpd levels (<10 nmol/g). Furthermore, tissue N^1-AcSpd levels showed a significant positive correlation with bromodeoxyuridine labeling index (BUdR LI). Other polyamines such as putrescine, spermidine (SPD) and spermine (SPM), and SPD/SPM ratio showed no significant correlation with recurrence-free survival rate, survival rate and BUdR LI. These results suggested that elevation of tissue N^1-AcSpd levels is closely associated in the patients' outcome, recurrence-free survival rate and survival rate. Determination of tissue polyamine levels, especially N^1-AcSpd, may predict the biological malignancy of human brain tumors and the prognosis of individual patients, and thereby supplemrnt the histopathological diagnosis and cell kinetic studies such as BUdR LI. In the meanwhile, hemangiopericytic meningioma and medulloblastoma often indicated low N^1-AcSpd levels in spite of their poor outcome, and neurinoma often indicated high N^1-AcSpd levels in spite of their good outcome.

CH 213 POLYAMINE TITRE CORRELATES WITH SEED LIFE SPAN IN CORN
Jose L. Lozano, Scott H. Wettlaufer and A. Carl Leopold, Agronomy Department and Boyce Thompson Institute, Cornell University, Ithaca, NY 14853.

The correlation of embryo putrescine (PUT) and spermidine (SPD) titre with maize seed performance during storage was investigated in polymorphic crosses and in random samples from a seed germplasm bank. We have found a correlation between high PUT and SPD titre, but not for other polyamines, with good storage characteristics. Mean generation analysis indicates that high PUT and SPD titre are determined by nuclear dominant genes.

CH 214 *IN VITRO* AND *IN VIVO* STUDY OF THE SPERMIDINE UPTAKE BY ERYTHROCYTES FROM NORMAL AND LEWIS LUNG CARCINOMA (3LL) GRAFTED MICE,

Moulinoux J-Ph., Quemener V., Khan N.A., Delcros J-G., Department of Cell Biology, University School of Medicine, 35043 Rennes Cedex, France.

During Lewis lung carcinoma growth, red blood cells (RBC) spermidine (Spd) level evolution was proportional to the increase in tumor volume and inversely correlated with the decrease in tumor Spd concentration. Similarly, in cancer mice i.p. injected with [14C] Spd, the increasing amount of RBC [14C] Spd was in proportion with tumor volume enhancement and linked to decrease in tumor tissue [14C] Spd concentration. After i.p. injection of [14C] Spd, putrescine or spermine, contrary to normal mice, cancer mice provided [14C] Spd in their erythrocytes. When injected with [14C] methionine, [14C] polyamines were never found in normal or cancer mice erythrocytes. As noted *invitro*, increasing amounts of polyamines produced by tumor tissue and excreted into blood would modify erythrocyte stroma proteins involved in the [14C] Spd uptake process (presence of a compact band 3). These data first demonstrate the tumor origin of polyamines carried by erythrocytes. Since RBC polyamine levels are directly correlated with tumor progression, these experimental results reinforce the clinical use of this index of cell proliferation in malignant diseases with a short doubling time.

CH 215 DFMO RESISTANT AND REVERTANT LINES OF A HUMAN COLON CARCINOMA CELL LINE,Kenji Nishioka,Joyti R. Wagle and Domitilia Patenia,Dept. of General Surgery/Surgical Res. Lab.,The University of Texas M. D. Anderson Cancer Center,Houston,TX 77030

Human colon carcinoma cell line (DLD-1,clone A) was exposed to stepwise increases in DFMO concentrations (0.05 to 10 mM) over a period of months to select cells which continued to grow in presence of high DFMO concentrations. DFMO (0.05 to 1 mM) was cytostatic to the parental cells while 5 to 10 mM DFMO were cytotoxic. Revertant lines were obtained by growing the resistant cells in absence of DFMO for several weeks. Determination of intracellular polyamines in resistant cells revealed low but measurable amounts of putrescine and spermidine, and above parental level of spermine. Polyamine levels were restored in revertant lines with higher putrescine level, which corresponded to elevated ODC activity in revertant cells. DFMO transport study revealed that the resistant cells accumulated less DFMO and effluxed more DFMO as compared to the parenteral and revertant cells. There were increases in ornithine and arginine levels in resistant cells. ID_{50} to 5-fluorouracil, the most commonly used chemotherapeutic agent against human colon carcinoma, were 0.005, 0.01 and 0.01 mM for parental, resistant (10 mM DFMO) and revertant (10 mM DFMO) cells, respectively. This study may help explain the clinical situation where different levels of resistance of colorectal cancers to continuous infusion of DFMO have been observed.

CH 216 NUCLEAR COMPARTMENTALIZATION OF BIS BENZYL POLYAMINES, Ronald D. Snyder, Gregory F. Davis and Prasad S. Sunkara, Department of Cell Biology, Merrell Dow Research Institute, Cincinnati, OH 45215. Rapid cell lysis techniques have been used to determine the intracellular localization of the naturally occurring polyamines and exogenously added synthetic polyamine analogs. We have determined that bis benzylated polyamines regardless of their overall charge or charge distribution accumulate exclusively in the nuclei of HeLa cells. Within 5 minutes after treatment, at least 50% of total cellular bis benzyl polyamine is nuclear, this increasing to 80% after 1 hour. This nuclear accumulation is not observed with the naturally occurring polyamines or other polyamine derivatives not containing at least one benzyl group. The process by which nuclear compartmentalization occurs is not clear but studies with isolated nuclei have shown no preferential retention of benzylated polyamines upon washing. Moreover, no obvious relationship exists between the ability of benzylated polyamines to bind DNA and their ability to accumulate in nuclei. Studies examining the mechanism of this phenomenon and possible alterations in chromatin structure in these nuclei are currently underway.

CH 217 5-METHYLTHIORIBOSE KINASE: A TARGET FOR ANTIMICROBIAL DRUG DESIGN. M.K. Riscoe, P.A. Tower, A.J. Gianotti, P.A. Conte, J.H. Sheley, L.L. Johnson, A.J. Ferro, and J.H. Fitchen, Medical Research Service, VAMC, Portland, OR 97201 and Epitope, Inc., Beaverton, OR 97006. 5'-Deoxy-5'-methylthioadenosine (MTA), a product in the synthesis of polyamines, is recycled into methionine by one of two mechanisms. In mammalian cells, MTA is degraded directly to 5-methylthioribose-1-phosphate (MTR-1-P) and adenine by MTA phosphorylase. However, in certain microbes, MTA is hydrolyzed by MTA nucleosidase to MTR and adenine, with subsequent conversion of MTR to MTR-1-P by MTR kinase. In both cases, MTR-1-P is recycled to methionine. Because MTR kinase is "unique" to microbes (it is also found in plant tissue) and since it is essential to microbial methionine recycling, we hypothesized that MTR kinase is a promising target for drug design. Indeed, we have shown that the ethyl analog of MTR (ethylthioribose) selectively inhibits MTR kinase-containing protozoa (Riscoe et al., Antimicrob Agents Chemother 32:1904,1988). We have now synthesized additional MTR analogs (substituted at the 5-position) and tested them against MTR kinase-containing *(Klebsiella pneumoniae)* and MTR kinase-deficient *(Escherichia coli)* bacteria. Of the 15 analogs, 5-trifluoromethylthioribose (TFMTR) showed the greatest potency against *K. pneumoniae* (IC_{50}~40nM). At 1000-fold greater concentrations, TFMTR was without effect against *E. coli* or cultured mammalian cells. We purified MTR kinase from *K. pneumoniae* 4,400-fold and used this preparation for kinetic studies. TFMTR competitively inhibited MTR kinase activity (Ki~7μM). Furthermore, TFMTR was shown to be a substrate for MTR kinase (Km~1.7μM), suggesting that the drug could be converted to toxic products (e.g., trifluoromethionine or carbonothionic difluoride) in enzyme-containing organisms. Structural analogs of MTR represent a new class of compounds with the potential for treating diseases caused by MTR kinase-containing microorganisms.

CH 218 INVOLVEMENT OF POLYAMINE BIOSYNTHESIS AT EARLY AND LATE STAGES IN HCMV REPLICATION, A Stanley Tyms, Antiviral Research Unit, MRC Collaborative Centre, Mill Hill, London, NW7 1AD, England. Inhibition of polyamine biosynthesis by treatment with the putrescine analogue RR-MAP failed to block viral DNA synthesis or progeny virus growth. In contrast, suppression of polyamine biosynthesis by the ornithine analogue Δ MFMO methyl ester corresponded to major inhibition in virus replication. This early temporal nature of virus-induced synthesis of polyamines was verified by the lack of response to inhibition using the selective inhibitor of viral DNA synthesis, ganciclovir. Suppression of virus-induced cellular DNA synthesis by thymidilate synthetase however effectively blocked virus-induced polyamine biosynthesis and virus growth. Thus, the early induction of polyamine biosynthesis corresponded to virus-induced cellular events. At metabolically less effective concentrations of Δ MFMO methyl ester, viral DNA was synthesised but virus growth was still inhibited. This appeared due to an alteration in the regulation of abundant production of 'late' structural proteins.

CH 219 EFFECT OF BIS(BENZYL)POLYAMINES ON POLYAMINE METABOLISM OF FILARIAL PARASITES, Rolf D. Walter[1], Sylke Müller[1], Alan J. Bitonti[2] and Peter P. McCann[2], [1] Bernhard Nocht Institute, Hamburg, FRG, [2] Merrell Dow Research Institute, Cincinnati, USA Cytotoxic and cytostatic effects of bis(benzyl)polyamines on plasmodia and hepatoma cells have been reported (Bitonti et al., 1989a; Bitonti et al. 1989b). The bis(benzyl)polyamine derivative MDL 27695 also shows a potent filaricidal effect; adult *Brugia malayi* maintained *in vitro* were killed by 1 μM of this compound (Müller et al., 1989). MDL 27695 was found to inhibit the polyamine transport system as well as the polyamine oxidase activity of filarial worms. These results indicate that the polyamine metabolism of these organisms may be a target for the chemotherapeutic attack of MDL 27695. Here, we report on uptake of bis(benzyl)polyamines into *Brugia pahangi* and *Onchocerca volvulus* and their effects on polyamine levels. Surprisingly the uptake rate of ^{14}C-MDL 27391 (4μM) was not affected by putrescine and spermine, when concentrations were increased 100 fold, indicating an independent uptake mechanism for the drug. The K_m-value for MDL 27391 transport was determined to be 2 μM, a value comparable to those for putrescine and spermine uptake. No evidence for degradation of ^{14}C-MDL 27391 by filarial worms was found, when *Brugia pahangi* were maintained in medium supplemented with the drug for 3 days. This result corresponds with enzymatic studies which demonstrated that bis(benzyl)polyamines act as inhibitors but not as substrates of the polyamine oxidase reaction of nematodes.

CH 220 Endogenous Polyamine Titre Modification in Zea mays (L.) Seeds. S.H. Wettlaufer, P.N. Myers, A.C. Leopold and J.L. Lozano. Boyce Thompson Institute and Agronomy Department, Cornell University, Ithaca, N.Y. 14853.
An in vitro system that allows endogenous polyamine (PA) titre modification in maize seeds is described. Addition of putrescine (PUT) or spermine (SPM) to the culture medium of developing caryopses of maize selectively altered endogenous PA levels. Five days after pollination (DAP), single maize kernels (Pioneer 3925) were placed in medium supplemented with 100 nM PUT or SPM. Kernels were cultured at 27° C in a dark, humidified chamber for 30 days. Kernels were harvested at 35 DAP, when physiological maturity was reached. PUT treatment decreased PA in the endosperm and had no effect on embryo PA titre. SPM treatment did not change PA titre in the endosperm but in the embryo all PA titres were increased following culture in medium containing SPM.

Interaction of Polyamines with Macromolecules and Cellular Structures

CH 300 CELL TYPE-SPECIFIC MECHANISMS OF REGULATING EXPRESSION OF THE ODC GENE AFTER GROWTH STIMULATION, Mitchell Abrahamsen and David R. Morris, Department of Biochemistry, University of Washington, Seattle, WA 98195.

Ornithine decarboxylase (ODC) mRNA is strongly and rapidly induced by mitogenic activation. In quiescent Swiss 3T3 fibroblasts, activated by serum, TPA or elevated cAMP, and resting T-lymphocytes, activated with conconavalin A (Con A), ODC mRNA levels begin to rise within 30 min and reach a maximal induction of 10- to 20- fold within several hours . Nuclear runon analysis reveals a low level of nascent transcripts in resting fibroblasts which is elevated upon activation. In contrast, there is a high level of transcription across the entire ODC gene in resting T-cells, which remains unchanged upon activation. In neither cell type is there any evidence for transcriptional pausing being involved in the regulation of message production. The stability of the mature ODC message was also examined and found to be unaffected by mitogenic stimulation. These results indicate that ODC mRNA levels are regulated transcriptionally in Swiss 3T3 cells and post-transcriptionally within the nucleus of T-lymphocytes in response to mitogenic stimuli.

We have initiated studies to determine the mechanism by which elevated cAMP increases transcription of the ODC gene. The chloramphenicol acetyl-transferase (CAT) gene preceded by 620 bp of ODC 5' flanking sequences, including exon 1 and part of intron 1, is regulated by cAMP in transiently transfected cells. Within this region, there are 2 potential CREs, both of which footprint with whole-cell extracts and with a bacterial CREB fusion protein.

CH 301 PROLACTIN MEDIATED CHANGES IN POLYAMINE CONCENTRATIONS IN BOVINE MAMMARY EPITHELIAL CELLS. Michael R. Bedford and David Zadworny. Department of Animal Science, Macdonald College of McGill University,21,111 Lakeshore Rd.,Ste Anne de Bellevue,Montreal,Quebec,CANADA. H9X 1C0. (514)-398-7978. Fax (514)-398-7964.
A mammary epithelial cell line established in our laboratory by Hung and Turner (in press) expresses and secretes the casein proteins within 48 hours of prolactin (PRL) exposure. The concurrent effects of PRL on the synthesis of polyamines were investigated. Cells grown on plastic and collagen (seeded at 1 x 10^6 per flask) were allowed to grow for 48 hours in DMEM (supplemented with 3% FBS, 5ug/ml insulin, 1ug/ml hydrocortisone) before rinsing in PBS (2 x) and replacing with treatment media (alpha-methylornithine ,AMO (30mM) and PRL (5ug/ml) in factorial combination) for 24 hours. Cells were harvested and analysed for polyamine content and ODC activity. PRL did not affect ODC activity at 24 hr (although a time series experiment indicated that ODC activity was markedly induced in the first 6 hours of exposure). Control cell putrescine (PUT) concentrations were high (8.6 nM/10^6 cells) relative to spermidine (SDN) and spermine (SMN). PRL significantly increased spermidine (SDN) concentrations as has been reported previously, but much of this increase was offset by a decrease in putrescine concentration. Concurrent treatment with AMO abolished this effect and the synthesis of B-casein in collagen plated cells. Results suggest that the PRL induced increase in SDN and subsequent casein gene expression is not neccessarily dependent upon increased ODC activity due to a large putrescine pool.

CH 302 A POTENTIAL ROLE FOR ORNITHINE IN THE REGULATION OF ODC ACTIVITY AND POLYAMINE ACCUMULATION, Craig V. Byus and Jennifer L. Harrington, Division of Biomedical Sciences & Department of Biochemistry, University of California, Riverside, CA 92521

The activity of ornithine decarboxylase (ODC) is believed to be regulated in a negative manner by the polyamines; at the level of translation and turnover of the ODC protein. A number of studies have implicated spermidine rather than putrescine or spermine as the polyamine responsible for the observed decrease in ODC activity. We have observed marked decreases in ODC activity in cultured cells treated with exogenous ornithine. This decrease in ODC has been correlated with increased intracellular putrescine with no significant change in intracellular spermidine. In studies where cells were incubated with ^{3}H-ornithine, a pool of newly synthesized spermidine has been identified as the possible regulator of ODC activity (similar to reports in non-mammalian cells). Furthermore, the export of putrescine and not spermidine from the cells to the culture medium appears to play a major role in the regulation of intracellular polyamine levels. Intracellular ornithine appears to exist in two separate pools and can markedly reduce the export of putrescine as the level of ornithine increases. The roles of newly synthesized spermidine, putrescine export, and ornithine uptake will be discussed in relation to ODC and polyamine accumulation.

CH 303 STRESS-INDUCED POLYAMINE ACETYLATION IN *E. coli*, Stephen W. Carper, Derall Willis, Kristan Manning and Eugene W. Gerner, University of Arizona Health Sciences Center, Departments of Radiation Oncology and Biochemistry, Tucson, Arizona 85724.

Exposure of mammalian cells to heat shock, and certain other stresses, stimulates polyamine catabolism by inducing the enzyme spermidine/spermine N^1-acetyltransferase (N^1-SAT). Stress-induced polyamine acetylation is a conserved response in prokaryotes. Heat shock, cold shock, ethanol and alkaline shift, but not H_2O_2, induce spermidine acetylation in these organisms. However, acetylation occurs at both the N^1 and N^8 position of spermidine with nearly equal frequency. Cold shock induced spermidine acetylation, as first described by C.W. Tabor (BBRC 30:339-342, 1968), does occur in *E. coli* strains deficient in their ability to express gene products of the htpR and oxyR regulons. Thus, genes involved in this stress response are not part of these two stress regulons. Cold shock induced spermidine acetylation does not occur in *E. coli* strains CAG 2242 or CAG 2243. The lac operon in these strains is disrupted (they contain a stop codon in the lactose permease structural gene). In strains capable of expressing the lac operon, lactose and IPTG also induce polyamine acetylation. These and other data suggest that either the lac Y (permease) or A (transacetylase) gene products, or both, but not the lac Z gene product (β-galactosidase), are involved in cold shock induced spermidine acetylation.

CH 304 HIGH SPECIFIC INDUCTION OF SPERMIDINE/SPERMINE-N^1-ACETYLTRANSFERASE IN A HUMAN LUNG CARCINOMA LINE, Robert A. Casero[1], Paul Celano[1] and Anthony E. Pegg[2], [1]The Johns Hopkins Oncology Center Laboratories, Baltimore, MD 21231 and [2]The Pennsylvania State University Hershey Medical Center, Hershey, PA 17033.

The cytotoxic response of the human large cell lung carcinoma line NCI H157 to exposure to the polyamine analogue N^1,N^{12}-bis(ethyl)spermine (BESpm) is preceded by an extremely high induction of spermidine/spermine N^1-acetyltransferase (SSAT). The human enzyme has been purified >300-fold to apparent homogeneity and cross-reacts with antisera raised against rat liver SSAT. Although, other acetylases are capable of acetylating polyamines using acetyl CoA as the acetyl donor, the >1,000-fold induction was found to be specifically SSAT since essentially all activity was precipitable by the specific anti-SSAT antisera. The human enzyme appears to be similar to the rat enzyme in subunit size (18,000 daltons), substrate specificity and kinetic parameters. Preliminary results using actinomycin D and cycloheximide are consistent with the hypothesis that the unusually high induction of SSAT in the human lung cancer line is resultant from new mRNA and protein synthesis. This hypothesis is further substantiated by *in vitro* translation experiments using poly A+ mRNA from control and BESpm treated cells.

The large cell lung carcinoma line NCI H157 represents a useful system to produce large amounts of the SSAT protein and to study the molecular events responsible for the induction and control of this important polyamine metabolic enzyme. Further, this system should be useful in determining whether there is an association between the unusually high induction of SSAT and the observed cytoxicity in the NCI H157 cells.

CH 305 POLYAMINE OXIDATION IN HUMAN AND RODENT CELLS, David J.M. Fuller, Jung-Ren Chen, Stephen W. Carper and Eugene W. Gerner, Department of Radiation Oncology, The University of Arizona Health Sciences Center, Departments of Radiation Oncology and Biochemistry, Tucson AZ 85724.
Acetylation by spermidine N^1-acetyltransferase (N^1-SAT) initiates the catabolism of the polyamines. The acetylated polyamine is then oxidized by the flavin-dependent peroxisomal enzyme polyamine oxidase (PAO) with the formation of the parent homolog, acetamidopropanal and hydrogen peroxide. We have assayed the activity of PAO by three methods, two measuring the formation of spermidine (Spd) in vivo and in vitro by high performance liquid chromatography (HPLC) and the other the production of H_2O_2 by means of a peroxidase-coupled colorimetric technique. Strict 1:1 stoichiometry was demonstrated by assaying in the presence or absence of N^1-(buta-2,3-dienyl)-N^2-methylbutane-1,4-diamine (MDL 72,521), a polyamine oxidase inhibitor (PAOI). CHO cells display high constitutive expression of PAO (0.4nm/min/10^6 cells). Unlike N^1-SAT, PAO is a very stable enzyme with no apparent turnover after 6 hours in 20 μg/ml cycloheximide and no change in activity after a 45°C x 20 min heat shock. PAO is also expressed in human tumor cell lines, including those derived from brain tumors (D54, D247, and SF126), lung carcinoma (A549) and breast cancer (MCF-7), but at very low levels compared to rodent cells (4% of the rodent lines). Unlike N^1-SAT, PAO is not inducible by stresses such as heat shock in either rodent or human tumor cells, but does appear to undergo growth phase-dependent activity changes (increasing in plateau compared to log phase of growth). We thank Merrell Dow Pharmaceuticals for the gift of MDL 72.521.

CH 306 INTRACELLULAR PHOSPHORYLATION OF ORNITHINE DECARBOXYLASE (ODC) IN RAW264 CELLS: REQUIREMENT FOR ODC ACTIVITY. Mari K. Haddox, Kathleen M. Rose, and Laura L. Worth. Department of Pharmacology, University of Texas Medical School, Houston, Texas 77225. Ornithine Decarboxylase (ODC), the initial rate-limiting enzyme in polyamine biosynthesis, has been well characterized in terms of the regulation of protein synthesis and turnover. The role of phosphorylation in regulating ODC activity has been explored, but has remained unsolved. Purified ODC preparations from RAW264 cells, a transformed macrophage cell line, contain two major and one minor ionic form of ODC, as demonstrated by two dimensional electrophoresis. All three forms have a molecular weight of 55,000. Fractionation by isoelectricfocusing of immunoprecipitates isolated from RAW264 cells extracts incubated with an antiserum to ODC, demonstrate that all three forms of the enzyme are present in the intact cell. The two major forms of the protein have pI values of 5.2 and 5.1. Analysis of immunoprecipitates from P^{32}-metabolically radiolabeled cells indicated that only the 5.1 form of the protein is a phosphoprotein. Amino acid analysis of hydrolyzed metabolically radiolabeled protein showed that threonine was the predominant phosphoamino acid, although some phosphoserine was present. Reverse phase HPLC demonstrated that the radiolabeled threonine is present in a highly hydrophilic tryptic fragment. Dephosphorylation of ODC with alkaline phosphatase resulted in a loss of the immunodetectable pI 5.1 form of the protein that positively correlated in a linear manner with a loss in enzyme activity. Therefore, intracellular phosphorylation of ODC appears to be an essential requirement for the expression of enzyme activity.

CH 307 **CELL TYPE SPECIFIC DISTRIBUTION OF S-ADENOSYLMETHIONINE DECARBOXYLASE mRNA IN POLYSOMES.** John R. Hill, Michael W. White*, and David R. Morris, Dept. of Biochemistry, University of Washington, Seattle WA 98195; (* present address; Veterinary Research Laboratory, Montana State University Bozeman, MT 59717

In normal T-lymphocytes and lymphoid cell lines, S-adenosylmethionine decarboxylase (SDC) mRNA shows an unusual distribution in polysomes. In experiments where cytoplasmic extracts from quiescent T-lymphocytes were fractionated on sucrose gradients, the majority of SDC mRNAs have been found to be associated with small polysomes containing only one to two ribosomes [Mach, M. et.al JBC 261, pp.11697-11703 (1986)]. This observation suggests a model where SDC mRNA is inefficiently initiated by ribosomes. This model predicts that at any given time a percentage of SDC mRNAs would not be loaded with any ribosomes and would be associated with the ribonucleoprotein pool. However, little if any of the SDC mRNA was detected in fractions containing particles that sedimented slower than the 80S monosome. This suggests the distribution of SDC mRNA in polysomes in T-lymphocytes is a specific regulatory phenomenon. In sharp contrast is the distribution of SDC mRNA in polysomes from fibroid cell lines such as murine swiss 3T3 and baby hamster kidney. In these cell lines, SDC mRNA is much more broadly distributed in polysomes that range in size containing from 2 to 12 ribosomes. In T-lymphocytes, SDC mRNA distribution in polysomes is modulated by addition of mitogenic factors. In the swiss 3T3 cell line, SDC mRNA distribution in polysomes can be modulated by the depletion of endogenous polyamines. These results suggest that SDC mRNA distribution in polysomes can be regulated both by mitogenic activation and by endogenous polyamine concentrations.

CH 308 PURIFICATION AND PROPERTIES OF HUMAN SSAT, Paul R. Libby, Raymond J. Bergeron, and Carl W. Porter, Department of Experimental Therapeutics, Roswell Park Cancer Institute, Carlton and Elm Streets, New York, NY 14263 (PRL and CWP) and University of Florida, Gainesville, FL 32610 (RJB)

Human spermidine/spermine acetyltransferase (SSAT) has been purified to homogeneity from human melanoma cells after induction with bis-ethyl norspermine (BENSPM). Treatment with BENSPM (48 hrs, 10 μM) causes a 1,000-to 4,000-fold increase in cellular SSAT levels. Purification consists of two affinity chromatography steps: 1) binding on immobilized spermine and elution with spermine; and 2) binding on Matrex Blue A and elution with coenzyme A. The enzyme shows one band on SDS PAGE, with a subunit MW of 20,300. Native enzyme has MW of 80,000 by gel chromatography. K_m's were: acetylcoenzyme A, 5.9 μM, spermidine, 55 μM, spermine, 5 μM, N^1-acetylspermine, 36 μM, norspermidine, 1.6 μM, and norspermine, 4 μM. Bis-ethylated polyamine derivatives are competitive inhibitors of spermidine acetylation, with K_i values of 7.9, 0.8, 1.9 and 17 μM for BESPD, BENSPM, BESPM, and BEHSPM, respectively. The inhibition of SSAT by spermine analogs correlates with their relative abilities to increase SSAT in MALME-3M cells and with analog-induced increases in SSAT half life, suggesting that stabilization of SSAT by analogs may explain, in part, the massive increases in enzyme levels observed in certain tumor cell types.

Supported in part by grants CA-22153 and CA-37606.

CH 309 CONTROL BY HYPERTHERMIA OF ORNITHINE DECARBOXYLASE IN EHRLICH ASCITES TUMOR CELLS Isao Matsui-Yuasa[1], Shuzo Otani[1], Seiji Morisawa[1], Katsuhiro Kageyama[2], and Yasuto Onoyama[3], [1]Department of Biochemistry, [2]Radioisotope Centre, and [3]Department of Radiology, Osaka City University, Medical School, Osaka, 545 Japan

The exposure of cells in culture to temperature above 41°C results in loss of their ability to proliferate. Neoplastic and transformed cells may be more susceptible to being killed by heat than normal cells. The mechanism leading to the hyperthermic response are not completely understood. When cultured cells or whole organisms are exposed to elevated temperature, they universally respond by synthesizing a small number of conserved proteins. In most cells the heat-shock response is regulated transcriptionally. While transcription and translation of the heat-shock proteins are under way, the synthesis of other proteins slows down or even stops. Here, the effect of hyperthermia on the activity and the messenger RNA levels of ornithine decarboxylase (ODC) was studied in Ehrlich ascites tumor cells. When the cells were incubated at 42°C, the elevation of ODC activity by a change of the medium was prevented. Total RNA was isolated from cells treated at 37°C or 42°C, and the relative abundance of the ODC mRNA was measured by Northern blot analysis. These levels in heat-treated cells were comparable to those in control cells. Inhibition by hyperthermia was reversible. The recovery was suppressed by cycloheximide but not by actinomycin D. In hyperthermic-treated cells, the biological half-life of ODC was 14 min, which was the same time as for cells cultured at 37°C. These results suggest that hyperthermic treatment of Ehrlich ascites tumor cells suppressed ODC induction during translation.

CH 310 ABNORMAL TRANSLATIONAL AND POST-TRANSLATIONAL CONTROL OF ODC IN A DFMO-RESISTANT RAT HEPATOMA CELL LINE, John L.A. Mitchell and Jane A. Hoff, Department of Biological Sciences, Northern Illinois University, DeKalb, IL 60115

Very rapid alterations in ornithine decarboxylase (ODC) activity appear to result from radical changes in the rate of translation of available message combined with an extremely short half-life of the enzyme protein. We have been investigating the role of enzyme phosphorylation and other post-translational modifications in destabilizing this enzyme in mammalian cells in culture. In this investigation comparisons have been made between a rat hepatoma cell line (HTC) and a subclone of this line that exhibits abnormal ODC degradation. This clone, HD23A, was selected by growth in the presence of 10 mM DFMO yet it exhibits an enhancement in ODC synthesis somewhat lower than expected (approx. 20 times control levels). It is unique in that the ODC protein produced is extremely stable (T1/2 > 5 h). Upon complete removal of these cells from DFMO, the level of active enzyme can exceed 1.5 ug/mg protein. After 10 days in the absence of DFMO the synthesis rate falls to a value approximating controls, however the half-life of the enzyme remains unchanged. This cell line is not only defective in its ability to inactivate ODC but it also is deficient in degrading inactive ODC protein, thereby revealing a possible sequence of intermediates in normal ODC degradation.

CH 311 EVIDENCE FOR THE EXISTENCE OF MULTIPLE POLYAMINE UPTAKE SYSTEMS IN TRANSPORT DEFICIENT CHINESE HAMSTER OVARY CELL LINES EXPRESSING A HUMAN GENE FROM HT-29 CELLS, Mark E. Nuttall, Timothy L. Byers and Anthony E. Pegg, Dept Cell. Mol. Physiol. and Pharmacol., Penn. State Univ. Coll. Med. Hershey, PA 17033. A polyamine transport deficient Chinese Hamster Ovary mutant cell-line (CHOMG) was transfected with human DNA isolated from HT-29 colon carcinoma cells enabling them to take-up both polyamines and methylglyoxal bis(guanylhydrazone)(MGBG). The transfectants possessed both the ubiquitous, repetitive alu sequence found only in the human genome and the neomycin resistance gene that was co-transfected with the human DNA. There was a direct correlation between the uptake of MGBG and the degree of cytotoxicity in the six transfectant cell-lines. In the majority of the transfected cell-lines the uptake of putrescine was sodium dependent, whereas spermine uptake appeared to be independent of extracellular sodium. Gramicidin (20nM), a membrane ionophore, inhibited the uptake of putrescine almost completely and had no effect on spermine uptake. MGBG uptake exhibited partial sodium dependency suggesting uptake by both polyamine transport systems and this was confirmed by the competitive inhibition of the drug transport by both putrescine and spermine. Supported by grants GM 26290 and CA 37606.

CH 312 POTENTIAL SIGNALING PATHWAYS FOR THROMBIN INDUCTION OF ENDOTHELIAL CELL (EC) ORNITHINE DECARBOXYLASE (ODC), Jack W. Olson, Urszula Orlinska, Sarah Allen-Gebb, Bernhard Hennig and Mark N. Gillespie, University of Kentucky, College of Pharmacy, Lexington, KY 40536.
Thrombin, which is a procoagulant and potent mitogen released during tissue repair, has been incriminated in microembolization-induced lung injury wherein EC's are a primary target. Since these injury and repair processes involve EC synthesis of proteins, RNA and DNA, we hypothesized that thrombin would increase EC ODC through a membrane signal transduction pathway(s) that, like those utilized in many other thrombin elicited cellular responses, would involve G-proteins and protein kinase C (PKC). Thrombin caused time- and dose-dependent increases in ODC activity in confluent, quiescent cultures of porcine main pulmonary artery EC's, with maximum increases occurring after a 6 hour incubation with 12 U/ml (130±5 vs 890±75 pmoles CO_2/60 min/mg protein; control vs thrombin). Immunoprecipitation studies showed the increased ODC activity at 6 hours was associated with an approximate 1.6 fold increase in ODC protein content. Pertussis toxin or PKC inhibitors were used to evaluate possible signaling pathways involved in thrombin activation of EC ODC. A 3 hour pretreatment with pertussis toxin inhibited thrombin-stimulated ODC activity in a concentration-dependent fashion suggesting involvement of G proteins in the signaling process. The PKC inhibitors H-7, H-9 and staurosporine inhibited thrombin-induced ODC activity in a concentration-related manner with IC-50 values of 2.9 and 7.4 μM for H-7 and H-9 respectively, and 1.8 nM for staurosporine. Pertussis toxin or H-7 treatment prevented the thrombin-induced increases in ODC protein content. Evaluation of the effect of thrombin on relative ODC mRNA content are in progress. These findings suggest that thrombin induction of ODC in EC's involves G-protein and PKC signaling pathways. (Supported by HL-36404 and HL-38495).

CH 313 **ORNITHINE DECARBOXYLASE EXPRESSION: STUDIES USING A POLYSOMAL RUN-OFF SYSTEM**, Lo Persson[1], Riccardo Autelli[1], Ingvar Holm[2] and Olle Heby[2], [1]Department of Physiology, University of Lund, S-223 62 Lund, and [2]Department of Zoophysiology, University of Umeå, S-901 87 Umeå, Sweden. Cellular synthesis of ornithine decarboxylase (ODC) is subject to feedback regulation by the polyamines. In the present study we have used a polysomal run-off system to analyze the mechanism by which this regulation occurs. Lysates of ODC-overproducing cells were shown to be capable of synthesizing ODC. The synthesis of ODC (as well as that of total protein) exhibited a maximal rate for less than 4 min due to a low degree of initiation. Protein synthesis in the lysate was mainly a result of continued elongation of nascent peptide chains on pre-initiated polysomes. Thus, by determining the amount of ODC produced in the lysate, we obtained an estimate of the number of ribosomes that were actively translating ODC mRNA at the moment of lysis. Using this polysomal run-off assay we demonstrated that the polyamine-mediated regulation of ODC synthesis occurs without any change in the number of ribosomes associated with the message.

CH 314 POST-TRANSCRIPTIONAL REGULATION OF ORNITHINE DECARBOXYLASE EXPRESSION BY HYPOTONIC SHOCK, Richard Poulin and Anthony E. Pegg, Dept. Cell. Mol. Physiol. and Pharmacol., Penn State Univ. Coll. of Med., Hershey, PA 17033. Ornithine decarboxylase (ODC) activity is known to be strongly induced by a decrease in medium osmolality in various mammalian cell types. We have investigated the mechanisms responsible for this effect in L1210 cell variants which express ODC at ≥ 100-fold higher levels than the original strain. A reduction in osmolality from 325 to 130 mOsmol/kg brought about by reducing the NaCl concentration in serum-free RPMI 1640 medium increased ODC activity and immunoreactive protein 20- to 30-fold within 4 hours with no detectable change in ODC mRNA content. The induction was sustained for up to 48 h after hypotonic shock with maximal levels being observed at 24 h. The dramatic accumulation of enzymatically active ODC was triggered by an up to 36-fold stimulation of the rate of ODC translation occurring within 60 min after downshock, and an increased half-life of the enzyme (35 ± 10 and 212 ± 67 min under isosmotic and hypoosmotic conditions, respectively). Hypotonically induced effects on rates of ODC synthesis and degradation were reversed by a sudden shift to control osmolality. While incorporation of radiolabeled methionine in other soluble proteins decreased by 30-50% over the first 4 h of hypotonic treatment, the fraction of protein labeling represented by ODC increased from 0.35 to 25% during the same period. Thus, ODC expression is reversibly activated by a decrease in osmolality exclusively through post-transcriptional mechanisms in these L1210 cells. Supported by grants GM 26290 and CA 37606 and by a fellowship from the Medical Research Council of Canada.

CH 315 MOLECULAR AND GENETIC CHARACTERIZATION OF AN ORNITHINE DECARBOXYLASE-DEFICIENT CHINESE HAMSTER MUTANT, R. Pilz and I.E. Scheffler, Department of Biology and Center for Molecular Genetics, University of California, San Diego, CA 92093 Our laboratory has described a CHO mutant which is completely deficient in ODC activity. The cells grow normally in the presence of putrescine, and the phenotype is very stable under these conditions. They appear to have normal amounts of the two size classes of ODC mRNA, but have no significant amount of immunological cross-reacting protein. These findings suggest a point mutation (or very small deletion) in the coding sequence of the gene. However, treatment of these mutants with 5 azacytidine leads routinely to the generation of clones which grow in the absence of putrescine. We have interpreted this finding in terms of the existence of two alleles in the wild type parental CHO cells, one of which is silenced by hypermethylation, the other being expressed. A mutation in the active allele generates the ODC-deficint mutant. Reversion by 5 azacytidine involves activation of the silent allele. We present proof that the mutant phenotype is due to a single base change resulting in the substitution of aspartate for glycine at position 381 in the protein. We also present data which support the conclusion that the gene being activated by 5 azacytidine is indeed a silent allele in the wild type and not another ODC gene. When the ODC gene is amplified by selection in the presence of DFMO, the active gene becomes globally hypomethylated.

CH 316 IDENTIFICATION OF TWO ESSENTIAL AMINO ACID RESIDUES IN HUMAN S-ADENOSYLMETHIONINE DECARBOXYLASE (ADOMETDC), Bruce A. Stanley, Carol L. Ardle, and Anthony E. Pegg, Dept Cell. Mol. Physiol. and Pharmacol., Penn. State Univ. Coll. Med. Hershey, PA 17033. Human AdoMetDC is synthesized as a 38 kilodalton proenzyme which is processed to a mature enzyme containing two subunits of approximately 7 and 31 kilodaltons. The larger subunit contains a covalently bound pyruvate prosthetic group which is generated during the proenzyme cleavage. To determine the site of this proenzyme cleavage/pyruvate formation, we made a series of site-specific mutations (Ser→Ala or Ser→Ile) in the region of the enzyme where cleavage could result in subunits of the size seen on SDS-PAGE gels. When expressed in vitro, AdoMetDC proenzymes containing mutations at serines 50, 66, 69, or 73 were cleaved in vitro and retained AdoMetDC activity, while proenzymes containing either Ser 68→Ala or Ser 68→Ile mutations showed neither processing nor activity. In addition, direct amino acid sequencing of human AdoMetDC produced in E. coli showed a peptide consistent with cleavage and pyruvate formation at serine 68. Cys→Ala mutations at positions 49, 82, or 226 did not prevent processing; however, Cys 82 mutants had no activity in spite of being processed. Cys 226 mutants had AdoMetDC specific activities similar to wild type, while Cys 49 specific activity was higher. Since Cys 49 is the only cysteine residue in the small subunit of the mature enzyme, disulfide links between the two subunits are not necessary for either processing or activity. Serine 68 and cysteine 82, however, are essential for AdoMetDC activity. (Supported by CA-18138 and HL-07223)

CH 317 **POLYAMINE-MEDIATED REGULATION OF ORNITHINE DECARBOXYLASE. ANALYSIS OF ORNITHINE DECARBOXYLASE mRNA DISTRIBUTION IN POLYSOME PROFILES,** Louise Stjernborg[1], Ingvar Holm[2], Lo Persson[3] and Olle Heby[2], Departments of [1]Zoophysiology and [3]Physiology, University of Lund, S-223 62 Lund, and [2]Department of Zoophysiology, University of Umeå, S-901 87 Umeå, Sweden. Ornithine decarboxylase (ODC) catalyzes a rate-controlling step in the biosynthesis of polyamines. The polyamines exert a feedback control of ODC by affecting the efficiency of ODC mRNA translation. In the present study we have used an ODC-overproducing cell line to investigate the mechanism by which this regulation occurs. The high expression of ODC in these cells is caused by an amplification of the ODC gene, with a concomitant increase in the steady-state level of the mRNA. In spite of the 100-fold increase in ODC expression the enzyme is still subject to a feedback control by the polyamines. Cells grown in the presence of the ODC inhibitor 2-difluoromethylornithine (DFMO) have a 10-fold higher synthesis of ODC than cells grown in the absence of the inhibitor. This increase is not caused by a comparable change in the amount of ODC mRNA indicating a difference in the translational efficiency. By studying the distribution of ODC mRNA in polysome profiles we demonstrate that the polyamine-mediated change in ODC synthesis is not correlated with a change in the number of ribosomes that are actively translating the mRNA, indicating a coordinate effect on initiation and elongation.

CH 318 GENETIC ANALYSIS OF MAMMALIAN CELL GROWTH REGULATION BY POLYAMINES. Jean-Paul Thirion, Nezha Alami, Arvind Chopra and Karoly Tihanyi, Department of Microbiology, Faculty of Medicine, University of Sherbrooke, Sherbrooke, Quebec, Canada, J1H 5N4. Two classes of somatic cell mutants of adenovirus-transformed rat cells resistant to methylglyoxal bis(guanylhydrazone) MGBG, a specific inhibitor of S-adenosyl-L-methionine decarboxylase (Ado Met DC; EC 4.1.1.50) were isolated to carry out a genetic analysis of mammalian cell growth regulation by polyamines. Class I mutants showed reduced intracellular transport of MGBG and spermidine and did not form tumors in nude mice. By cell-cell hybridization, the $MGBG^R$ alleles of the class I mutants were found to be recessive to the wild-type alleles. By complementation analysis of the class I mutants it was observed the $MGBG^R$ mutation defined three groups of complementation for drug transport and tumor formation in nude mice. This strongly suggests a common membrane motif is implicated in tumor formation and the transport of MGBG by these cells. The second class of $MGBG^R$ mutants consists of cells that have normal drug transport, but have up to 5 fold higher Ado Met DC specific activity than the wild-type cells. The Km, Ki and inhibition constants of Ado Met DC of these mutants and the parental cells were not very different from one another, indicating the mutation in these cells probably did not affect the Ado Met DC structural gene. By cell-cell hybridization, $MGBG^R$ alleles of the class II mutants were found to be dominant to the wild-type allele. Molecular characterization of the genes encoding for the Ado Met DC and its mRNAs in the class II mutants will be reported using probes provided by Drs Morris and Pegg. This study is supported by a grant from la Société de Recherche sur le Cancer de Montréal Inc to J.P.T..

CH 319 **INHIBITION OF GROWTH OF HUMAN COLONIC CANCER CELLS INCREASED POLYAMINE ACETYLATION,** Heather M. Wallace and Catherine S. Coleman, Depts of Medicine, and Therapeutics and Pharmacology, University of Aberdeen, Scotland AB9 2ZD.
Intracellular polyamine concentrations are controlled at the level of the two decarboxylase enzymes and one or more acetyltransferases. These enzymes are all highly regulated and have rapid turnover rates in the cell. Spermidine/spermine acetyltransferase (SSAT), is the rate limiting enzyme in the interconversion of the higher polyamines to putrescine. The N^1-acetyl derivative maybe lost from the cell either by excretion or broken down to spermidine or putrescine depending on the growth status of the cell. SSAT activity is induced in response to a number of stimuli including certain hepatotoxins and the growth inhibitory spermidine analogue, methylglyoxal bis(guanylhydrazone) (MGBG). It may be then, that increased SSAT activity is an early response to cellular toxicity. The aim of this study was to examine the effects of 3 growth inhibitory drugs, MGBG, 5-fluorouracil (5-FU) and methotrexate (MTX) on the growth, intra- and extracellular polyamine concentrations and SSAT activity of a human colonic cancer cell line.
HT29/219 cells were grown in medium supplemented with 10%(v/v) horse serum. Treatment of these cells with growth inhibitory concentrations of the above drugs decreased significantly the intracellular concentration of all 3 polyamines. Putrescine, spermidine and N^1-acetylspermidine were excreted from both control and drug-treated cells although extracellular polyamine concentrations were lowered significantly after treatment. SSAT activity (whole cell) was increased in response to all 3 drugs with spermidine being the preferred substrate. Analysis of the products by hplc showed a 4-fold increase in the levels of N^8-acetylspermidine in response to all 3 drugs. A similar increase in the production of N^1-acetylspermidine was found after treatment with 5-FU and MTX while MGBG increased the amount of N^1-acetylspermidine formed by approx. 30-fold. In terms of polyamines, the response of cells to growth inhibition, by whatever mechanism, appears to be a decrease in intracellular polyamine content brought about by increased SSAT activity in conjunction with excretion.

CH 320 SUPERINDUCTION OF ORNITHINE DECARBOXYLASE BY ACTINOMYCIN D. ANALYSIS OF THE MECHANISM, Margaretha Wallon[1], Ewa Dahlberg[1], Lo Persson[2] and Olle Heby[3], Departments of [1]Zoophysiology and [2]Physiology, University of Lund, S-223 62 Lund, and [3]Department of Zoophysiology, University of Umeå, S-901 87 Umeå, Sweden. We have previously shown that treatment of cells with actinomycin D, in a dose that inhibits mRNA synthesis, may cause superinduction of ornithine decarboxylase (ODC) in mammalian cells (Proc. Natl. Acad. Sci. USA 73: 4022, 1976). With the use of new tools, including a cDNA encoding mouse ODC and a monospecific antibody against mouse ODC, we are in the process of further analyzing the mechanism behind this superinduction. When Ehrlich ascites tumor cells were treated with actinomycin D (10 μg/ml) at a time of rapidly increasing ODC activity (10 h after seeding in fresh medium containing 0.2 % bovine serum albumin) there was a 3-fold increase in the activity of the enzyme, as compared to the control, within 2 h. A similar increase was seen in the ODC content as determined with a radioimmunoassay. Despite the fact that transcription was blocked by actinomycin D, the ODC mRNA content (as determined by Northern blot analysis) remained almost constant for about 6 h. A subsequent decrease suggests a half-life for ODC mRNA exceeding 10 h. Experiments are under way to determine if the superinduction is due to (1) stimulation of translation of ODC mRNA and/or (2) reduction of the turnover rate of ODC.

Alterations of Intracellular Polyamine Levels: Biological Effects and Therapeutic Potential

CH 400 DEOXYHYPUSINE HYDROXYLASE FROM RAT BRAIN: STUDIES ON ITS CATALYTIC PROPERTIES. A. ABBRUZZESE* AND S. BENINATI** DEPARTMENT OF (*) BIOCHEMISTRY AND BIOPHYSICS UNIVERSITY OF NAPLES AND DEPARTMENT OF (**) BIOLOGY UNIVERSITY OF ROME ITALY.

The unusual basic amino acid hypusine [N^{ϵ}-(4-amino-2-hydroxybutyl)lysine] is a derivative of lysine present in eukaryotic cells as a component of a single cellular protein, the eukaryotic Initiation Factor 4D (eIF-4D).Its biosynthesis occurs only by a series of post-translational reactions beginning with the transfer of the butylamine moiety from spermidine to the ϵ-amino group of a specific lysyl residue, thus forming the intermediate deoxyhypusine. The final step in hypusine formation is the hydroxylation of the deoxyhypusine residue catalyzed by deoxyhypusine hydroxylase, an enzyme apparently distinct from other reported hydroxylases. In this communication we report some of catalytic properties of deoxyhypusine hydroxylase. The enzyme partially purified from rat brain showed:a) a pH optimum of 7.4 b) a strong inhibition by various chelating agents and by high salt concentration, c) a rapid inactivation by heat treatment. On the contrary 2-oxoglutarate and several structural analogs, known to act as effective inhibitors of prolyl hydroxylase,showed no effect on deoxyhypusine hydroxylase activity. This observation confirms our hypothesis that this hydroxylating reaction occurs in eIF-4D with a unique catalytic mechanism distinct from other mixed function oxygenases.

CH 401 TRANSPORT OF POLYAMINES IN MITOCHONDRIA, D. Siliprandi, N. Siliprandi and A. Toninello, Dipartimento di Chimica Biologica-Universita di Padova- and Centro per la Fisiologia Mitocondriale del CNR, Padova. It has been shown that spermine is trasported bidirectionally across the inner membrane of rat liver mitochondria (1). Spermine enters the mitochondrial matrix space electrophoretically, apparently in form of a dication, even if it is largely present at physiological pH as tetracation. The uptake of this polyamine is dependent on high transmembrane potential, negative inside, and exhibits a non-linear current voltage relationship. Spermine is released from mitochondria in a separate process, governed by the pH gradient. Spermidine and putrescine are taken up by liver motochondria under the same conditions, but at the lower rate than spermine. The latter two polyamines decrease, unlike tetramethylammonium, the accumulation rate of spermine and vice versa, indicating a reciprocal competition for the transport, possibly occurring on the same carrier. Considering that polyamines are present in mitochondria (2) and that their synthesis occurs in the cytosol, the cycling of polyamines in mitochondria might be relevant for the modulation of either the mitochondrial genoma expression or the activity of intramitochondrial enzymes, or both.

1. A. Toninello, G. Miotto, D. Siliprandi, N. Siliprandi and K.D. Garlid; J.Biol.Chem.1988, 263, 19407-19411.
2. M. Mancon, D. Siliprandi and A. Toninello, Italian J.Biochem. in the press 1990.

CH 402 EFFECTS OF RADICAL SCAVENGERS ON THE LIPOPOLYSACCHARIDE-INDUCED ELEVATION OF N^1-ACETYLSPERMIDINE IN THE LIVER IN VIVO AND IN VITRO, Hiroyuki Sugimoto, Shigeru Matsuzaki, Koei Hamana, Mitsuo Suzuki, Shoji Yamada and Setsuo Kobayashi, Department of Physiology, Institute of Endocrinology, College of Medical Care and Technology, 1st Department of Internal Medicine, School of Medicine, Gunma University, Maebashi 371, Japan

Appearance of N^1-acetylspermidine (N^1-acetyl-SPD) in mouse liver has been shown to be closely associated with radical-production. In the present study we examined the effects of radical scavengers on lipopolysaccharide (LPS)- or paraquat-induced elevation of N^1-acetyl-SPD in vivo and in primary culture of adult rat hepatocyte. α-Tocopherol, superoxide dismutase (SOD) and reduced glutathione suppressed the LPS-induced elevation of N^1-acetyl-SPD and PUT in mouse liver in vivo. LPS elevated N^1-acetyl-SPD levels in the liver of mice fed on a vitamin E-deficient diet twice higher than in control mice. Paraquat-induced elevation of N^1-acetyl-SPD and PUT in primary cultures was reduced by the addition of SOD and α-tocopherol. In the culture, LPS only slightly affected the polyamine levels. These results suggest that superoxide anion is one of the factors which elevate N^1-acetyl-SPD and PUT and that the LPS-induced elevation of N^1-acetyl-SPD in mouse liver is induced by superoxide anion derived from Kupffer cells.

CH 403 EFFECT OF POLYAMINES ON $[Ca^{2+}]_i$ SIGNALS EVOKED BY PDGF IN A172 HUMAN GLIOBLASTOMA CELLS, J.Szöllösi[1], B.G.Feuerstein[1,2], H.A.Pershadsingh[2], H.S.Basu[1], and L.J.Marton[1,2]; [1]Brain Tumor Research Center, Department of Neurological Surgery; and [2]Department of Laboratory Medicine; University of California, San Francisco, CA 94143. Changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) evoked by platelet derived growth factor (PDGF) on confluent A172 human glioblastoma cells were monitored with the fluorescent Ca^{2+} indicator, INDO-1, using an ACAS 470 Laser Cytometer. 0.5-1.0 min after treatment with PDGF, the $[Ca^{2+}]_i$ increased sharply to three times basal levels. The $[Ca^{2+}]_i$ then decreased gradually, reaching basal levels after 12-15 min. The increase of $[Ca^{2+}]_i$ was due to both an initial release of intracellular Ca^{2+} stores and a later influx of Ca^{2+} across the plasma membrane. Depletion of cellular putrescine and spermidine with 5mM difluoromethylornithine (DFMO) for 4 days did not significantly interfere with the Ca^{2+} release from intracellular stores, but significantly decreased the duration of Ca^{2+} influx across the plasma membrane from 11-14 min to 4-5 min. Addition of 1mM putrescine to DFMO-treated cells one or three days prior to addition of PDGF restored the Ca^{2+} influx to normal. The characteristics of channels responsible for Ca^{2+} influx and the role of polyamines in the regulation of these channels are under investigation.

CH 404 HYPUSINE SYNTHESIS IN RAT HEPATOMA CELLS SENSITIVE AND RESISTANT TO GROWTH INHIBITION BY α-METHYLORNITHINE, Margaret E. Tome and Eugene W. Gerner, University of Arizona Health Sciences Center, Departments of Radiation Oncology and Biochemistry, Tucson, Arizona 85724.

Hypusine [N^6-(4-amino-2-hydroxybutyl)-2,6-diaminohexanoic acid], a rare amino, acid is synthesized post-translationally in the putative protein synthesis initiation factor eIF-4D from a protein-bound lysine residue and the polyamine spermidine. The consequences of this unique modification are currently not understood. Hypusine accumulation in rat hepatoma cells (HTC) differs from that in HMO_A cells, an HTC variant selected for resistance to growth inhibition in α-methylornithine (Mamont et al, Exp. Cell Res. 115:387-393, 1978). When α-difluoromethylornithine-(DFMO) treated cells are labeled with exogenous [^{3}H]-spermidine, incorporation of label into hypusine in protein and the M_r~18,000 protein is reduced in HMO_A, compared to HTC, cells. This decrease is evidenced both as a reduction in the rate, and the final extent, of hypusine formation. The lesser accumulation of hypusine in HMO_A cells is not due to decreased levels of the polyamines putrescine or spermidine in these cells, relative to the HTC parental line. The degradation of ornithine decarboxylase (ODC) is a polyamine-dependent process and the half-life of ODC is greatly increased in HMO_A, compared to HTC, cells. To test for a possible relationship between hypusine formation and ODC stability, the half-life of ODC in HTC cells was measured in cells treated with 1,3-diaminopropane (DAP), an inhibitor of deoxyhypusine synthesis (Park et al, JBC 263:15264-15269, 1988). DAP increases the apparent half-life of ODC in these cells from ~25 to nearly 50 minutes. These data suggest that hypusine formation in eIF-4D may play a role in the polyamine-dependent degradation of ODC in these cells.

CH 405 EFFECTS OF 3-DEAZANEPLANOCIN ON FRIEND ERYTHROLEUKEMIA CELLS, June M. Whaun[1], Nesbitt D. Brown[2], Christopher K.H. Tseng[3], Victor E. Marquez[3] and Peter K. Chiang[2], Division of Pathology[1], Division of Biochemistry[2], Walter Reed Army Institute of Research, Washington, DC 20307, Laboratory of Pharmacology and Experimental Therapeutics[3], National Cancer Institute, Bethesda, MD 20892. 3-Deazaneplanocin, a carbocyclic analogue of adenosine, is a potent inhibitor of S-adenosylhomocysteine hydrolase. It inhibits transmethylation. With the current interest in factors influencing cellular differentiation, we looked at the effect of this drug on the differentiation of Friend erythroleukemia cells. This virally transformed poorly differentiated proerythrocytic line, blocked in the expression of erythroid differentiation, can be induced to terminally differentiate by a number of small molecular weight compounds. Cells of clone DS19 were cultured in the presence of 10^{-8}M deazaneplanocin or DMSO or both, for over 48 hours because we wanted to observe terminal differentiation. Cells were collected on slides for Giemsa and benzidine staining and cell extracts were prepared for polyamine analysis. Putrescine levels decreased significantly in cultures with either deazaneplanocin or DMSO or both added. Both DMSO- and deazaneplanocin-treated cells showed similar shifts in spermidine and spermine levels, slight elevations in those with shorter exposures and slight depressions in those with longer exposures (72 hours). Stained blood films of all cultures exposed to either or both drugs for over 3 days showed terminal differentiation with benzidine positive inclusions present. These findings show deazaneplanocin is a useful agent to examine terminal differentiation.

CH 406 **EFFECTS OF EXOGENOUS SPERMIDINE ON EXPRESSION OF ENZYMES ASSOCIATED WITH THE MICROVILLAR AND BASOLATERAL ENTEROCYTE MEMBRANE DOMAINS IN THE NEONATAL RAT INTESTINE**, Gary Wild, Andrew S Daly and Gary Bennett, Departments of Medicine and Anatomy, McGill University, Montreal, Canada. Ornithine decarboxylase activity appears to play a key role in the intestinal hyperplasia seen during lactation. The present work examines the effects of spermidine on intestinal differentiation in the neonate. Neonatal Sprague Dawley rats were fed 6 micromoles of spermidine for either 1 day (S1) or 3 days (S3) prior to sacrifice on day 10. Controls were sacrificed at day 10 and at day 49 (adult reference). A progressive loss of the ileal endocytic complex and supranuclear lysosomal vacuole was seen in the S1 and S3 groups. No significant ultrastructural changes were observed in the proximal small gut and large intestine in these animals. A precocious appearance of sucrase and NaK ATPase activity was noted in the small and large intestine in the S1 group; these activities attained adult levels in the S3 group. Both enzymes exhibited a proximal to distal gradient of activity in the S3 group similar to that noted in adult controls. The premature appearance of sucrase and NaK ATPase activities was associated with a progressive decline in lactase levels in the spermidine treated neonatal rats. This fall in lactase was most pronounced in the proximal small intestine. The precocious structural and functional maturation provoked by exogenous polyamine exposure may involve modulation of transcriptional and translational events. (Supported by the Medicial Research Council of Canada).

CH 407 **DEOXYHYPUSINE SYNTHESIS: CLEAVAGE OF SPERMIDINE AND ROLE OF NAD IN THE FIRST STEP,** Edith C. Wolff, Myung Hee Park and J. E. Folk, Laboratory of Cellular Development and Oncology, NIDR, NIH, Bethesda, Maryland 20892.

Spermidine contributes the 4-aminobutyl moiety for the post-translational biosynthesis of deoxyhypusine (N^{ε}-(4-aminobutyl)lysine) in an eIF-4D precursor. Deoxyhypusine synthase, the NAD-requiring enzyme responsible for this reaction, was purified ~700-fold from rat testis. The Km values for spermidine, NAD^+, and eIF-4D precursor protein were estimated as 1 μM, 30 μM, and 0.08 μM, respectively. Upon incubation of the enzyme with [1,8-^{3}H]spermidine, NAD^+, and eIF-4D precursor protein, equal amounts of radioactivity were found in free 1,3-diaminopropane and in protein-bound deoxyhypusine. However, incubation without the eIF-4D precursor resulted in an equal distribution of radioactivity between 1,3-diaminopropane and Δ^1-pyrroline and provided evidence for the cleavage of spermidine in the absence of the eIF-4D precursor. The role of NAD^+ as the hydride ion acceptor was demonstrated by the transfer of ^{3}H from [5-^{3}H]spermidine to NAD^+ to form [4*R*-^{3}H]NADH. The findings to date are consistent with an enzyme mechanism involving imine intermediate formation.