STEROID HORMONE ACTION

Gordon Ringold, Organizer
January 17 - January 23, 1987

Plenary Sessions

Poster Sessions

Steroid Receptor Gene Structure and Function

STEROID HORMONE RECEPTORS : STRUCTURE-FUNCTION RELATIONSHIPS.

B 001 P. Chambon, M. Berry, M.T. Bocquel, M. Devic, M.V. Govindan, S. Green, H. Gronemeyer, J.M. Jeltsch, Z. Krosowski, A. Krust, V. Kumar, M. Meyer, G. Stack, C. Stricker, U. Stropp and B. Turcotte, LGME/CNRS and U.184/INSERM, Faculté de Médecine, 67085-Strasbourg Cédex, France.

cDNAs of the human oestrogen (hER) (1,2), glucocorticoid (hGR) (3) and progestin (hPR) receptors and of the chicken oestrogen (cER) (4) and progestin (cPR) (5) receptors have been cloned and sequenced. Cytosolic and nuclear extracts of HeLa cells, transiently expressing vectors containing the different cDNAs, contain the corresponding steroid binding protein with the expected molecular weight and affinity for the hormone. A sequence comparison of the hER (595 amino acids) and cER (589 amino acids) has revealed three highly conserved regions A (AA 1 to 38, 87% homology), C (AA 180 to 263, 100% homology) and E (AA 302 to 553, 94% homology), and three less conserved regions B (AA 39 to 179, 56% homology), D (AA 264 to 301, 38% homology) and F (AA 554 to 595, 41% homology). All receptors and the v-erbA protein contain sequences homologous to ER regions C and E (4,5). Thus the difference in length between the various receptors lies mainly in their amino-half (regions A and B). Transient expression in HeLa cells of vectors containing the wild type and in vitro mutated cDNAs has been used to define the the protein domains responsible for the various functions of the hER, i.e. oestrogen-binding (6), tight nuclear binding (6) and transcription activation. Transcription activation was analyzed in HeLa cells by cotransfection of wild-type or mutated hER vectors and recombinants in which the E.coli CAT sequence is under the control of either a chimeric promoter region containing the HSV tk promoter and the Xenopus vitellogenin estrogen responsive element (ERE) (7) or the promoter of the human oestrogen-responsive gene pS2 (8). Region E is the oestrogen-binding domain and region C, which contains potential DNA-binding "fingers" (4,5), is indispensable for tight nuclear binding and therefore most probably for DNA binding. Both regions C and E are required for efficient activation of EREs of vitellogenin and pS2 genes, whereas domains A and B appear to be dispensable using these assays. A chimeric receptor protein, in which region C of the hER has been replaced by that of the hGR, activates the glucucorticoid responsive element (GRE) of the MMTV LTR. Thus, the steroid hormone and target gene specificities reside in domains E and C, respectively. Possible models accounting for steroid receptor functions will be discussed.

1) P. Walter et al., PNAS (1985) 82, 7889; 2) S. Green et al., Nature (1986) 320, 134; 3) M.V. Govindan et al. NAR (1985) 13, 8293; 4) A. Krust et al. EMBO J. (1986) 5, 891; 5) J.M. Jeltsch et al. PNAS (1986) 83, 5424; 6) V. Kumar et al. EMBO J. (1986) 5, 2231; 7) P.M. Druege et al. NAR (1986), in press; 8) A.M.C. Brown et al. PNAS (1984) 81, 6344.

B 002 STEROID RECEPTOR GENE STRUCTURE AND FUNCTION, R.M. Evans, C. Weinberger, V. Giguere, S. Hollenberg, C. Thompson, J. Arriza and N. Yang. Howard Hughes Medical Institute, Gene Expression Laboratory, The Salk Institute, San Diego, California 92138.

Steroid hormones exert potent effects on development and differentiation, and their actions are mediated as a consequence of their interaction with specific, high affinity binding proteins referred to as receptors. On the basis of amino acid sequence of hGR induced from the cloned cDNA (1), the locations of functionally and immunologically important regions of the protein have been proposed (2). To confirm the proposed sites of functional domains we developed an expression system in which the synthesis of functional hGR is directed by the transcription of transfected cDNA present in an expression vector. Characterization of 38 insertional mutants and receptor deletions led to the identification of at least four functional domains, two of which correspond to the predicted DNA- and steroid-binding domains (3). The other two domains are referred to as tau ( ) for their potent effects on transcription. Based on these studies a model for the receptor is shown below.

Analysis of the receptor amino acid sequence reveals that it is related to the oncogene erbA (2). Based on this homology we have proposed that the erbA proto-oncogene family may represent a set of DNA binding regulatory proteins that are involved in transcriptional activation. Accordingly, we have isolated two members of the mammalian erbA proto-oncogene family. This family may contain as many as 4-5 different members. The steroid receptor family itself contains at least six different members corresponding to the known steroid hormones. In addition to the glucocorticoid receptor the recent cloning of the human estrogen receptor has been reported. Recently we have cloned the human mineralocorticoid (aldosterone) receptor which shows striking homology to the glucocorticoid receptor. Homologies amongst the various steroid hormone receptors will be discussed.

1. S.M. Hollenberg et al (1985). Primary structure and expression of a functional human glucocorticoid receptor cDNA. Nature 318, 635-641.
2. C. Weinberger et al (1985). Domain structure of human glucocorticoid receptor and its relationship to the v-erbA oncogene product. Nature 318, 670-672.
3. V. Giguere et al (1986). Functional domains of the human glucocorticoid receptor. Cell 46, 645-652.

B 003 STRUCTURE, CLONING AND EXPRESSION OF HUMAN ESTROGEN AND PROGESTERONE RECEPTORS, Geoffrey L. Greene, Paul Gilna, Craig Wilde and Nickki Parlet, Ben May Laboratory for Cancer Research, University of Chicago, Chicago IL 60637.

To better understand structure-function relationships in the transcriptional regulation of gene expression by steroid receptors, human estrogen receptor (ER) and progesterone receptor (PR) from two breast cancer cell lines were purified and cDNAs encoding these proteins were isolated, sequenced and expressed in heterologous cells. For ER, the purified cytosol steroid-receptor complex from MCF-7 human breast cancer cells appears to exist as an activated 5-6 S homodimer of M_r 65,000 (4 S) steroid-binding subunits. In addition, purified ER has high affinity for DNA and serves as a substrate for phosphorylation by a purified rat brain kinase. Purified PR from T47D human breast cancer cells consists of two steroid-binding components which can be photoaffinity-labeled with the synthetic progestin ^{3}H-R5020 and which migrate at 120 kDa (B) and 95 kDa (A) in reducing SDS gels. The relationship between these components has not been established. However, they share at least 13 common epitopes among a series of 14 unique monoclonal antibodies; one epitope appears to exist on B only. Extensive immunocytochemical data suggest that both ER and PR are predominantly nuclear proteins, even in the absence of hormone, and that they are associated with the euchromatin rather than the marginated heterochromatin or nucleoli. The isolation of cDNA clones corresponding to human ER mRNA from MCF-7 cells and human PR mRNA from T47D cells has been achieved. A 2.1 Kb cDNA (ORS) containing the entire translated portion of mRNA for MCF-7 ER was sequenced and expressed in Chinese hamster ovary (CHO-K1) cells to give a functional protein. An open reading frame of 1785 nucleotides in the cDNA corresponded to a polypeptide of 595 amino acids and M_r 66,200. Hypotonic extracts of transfected CHO cells contained human receptor protein which bound [^{3}H]estradiol and sedimented as a 4 S complex in salt-containing sucrose gradients and as an 8-9 S complex in the absence of salt. This [^{3}H]estradiol-receptor (E*R) complex reacted with all tested ER monoclonal antibodies and could be activated with steroid to bind to CHO nuclei. When covalently labeled with [^{3}H]tamoxifen aziridine, expressed human ER appeared as a single 65-kDa band on autoradiograms of SDS gels. Amino acid sequence comparisons revealed significant regional homology among human and chicken ER, human glucocorticoid receptor (GR), human and chicken PR, and the v-erb-A oncogene product, suggesting that steroid receptor genes and the avian erythroblastosis viral oncogene are derived from a common primordial gene. The cysteine/lysine/arginine-rich homologous region appears to represent the DNA-binding domain of these proteins. This research was supported by the American Cancer Society (BC-86), Abbott Laboratories, NIH (CA-02897, HD17103) and NSF (INT-8313623).

B 004 ANALYSIS OF ENHANCER ACTIVATION BY THE GLUCOCORTICOID RECEPTOR, Roger Miesfeld, Paul Godowski and Keith R. Yamamoto, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143-0448.

The glucocorticoid receptor protein, in association with cognate hormonal ligands, binds with high affinity to specific DNA sequences termed glucocorticoid response elements (GREs) which function as transcriptional enhancers. The receptor is absolutely required for GRE responsiveness and is therefore functioning as a hormone-dependent enhancer-activating protein. Our isolation and characterization of full length rat glucocorticoid receptor cDNA clones has made it possible to begin an analysis of wild-type and mutant receptors expressed in stably and transiently transfected cells to determine the structure-function relationships dictating enhancement activity. One approach has been to use in vitro mutagenesis to construct various internal and terminal receptor deletions, to identify the minimal region required for GRE enhancement in transiently transfected COS and CVI cells. Specific carboxy-terminal deletions result in the synthesis of receptors that no longer require hormone binding for enhancer activation, thereby functioning as constitutive activators. Stably transfected cell lines expressing some of these same amino terminal and internal deletions have also been constructed, allowing us to estimate the specific activities of receptor mutants with regard to enhancement. By measuring the level of induced mRNA/mole receptor protein, we find that the residual enhancement activity (20-60%) is sufficient for 5-10 fold induction of several different endogenous genes. By these methods we have identified a minimum overlap of 85 amino acids (aas) which delineates the enhancement activity. Moreover, a severely deleted receptor protein of only 150aas (including this overlap segment) is capable of GRE enhancement in CVI cells. Since the DNA binding domain has been functionally mapped to this same 85aa region (Rusconi and Yamamoto, submitted), we have initiated a series of gene-fusion experiments to determine the connection between DNA binding and enhancer activation. As an alternative approach to understanding receptor-dependent GRE enhancement, we have analyzed genetically selected receptor mutants isolated from a murine lymphoma cell line (increased nuclear transfer, nt^1, mutants) that seem to bind steroid normally, but display altered DNA binding and enhancement activities. The nucleotide sequence and functional expression of nt^1 cDNA clones that we recently isolated from two independent mutant cell lines, will likely be informative in elucidating the requirements for enhancement and DNA binding.

B 005 STRUCTURE OF THE PROGESTERONE GENE. B.W. O'Malley, O. Conneely, M.A. Carson, A. Dobson, & W.T. Schrader. Baylor Col. Med., Houston, TX 77030.

The chicken progesterone receptor is encoded by a single gene. Northern analysis demonstrates the existence of at least three mRNA species and asmany as five. The mRNA's are tissue specific for oviduct and inducible by estrogens. PR contains a cysteine-rich region (C1) which is conserved among the steroid receptors and v-erb A. The C1 region encodes two putative "fingers" which may be involved in metal binding; these two structures are coded for by separate exons. A second region of significant homology (C2) is conserved among the steroid receptors but not v-erb A. Sequence analysis suggests it may play some role in steroid binding and/or steroid hormone activation. Two variants, one in the C1 region and the other in the C2 region were obtained from cDNA libraries. The C1 variant is derived from an alternate polyadenylation site. The C2 variant is generated by a point mutation at the C2 region which creates a BamH1 site - it may be an allele of the gene. The human progesterone receptor protein sequence is highly conserved relative to the avian molecule as determined by sequence analysis of the cloned cDNAs. Mutational analysis of the receptor protein will be carried out using transfection protocols and presented. The vitamin D receptor also has been cloned and sequence comparisons will be made.

Steroids in Non-Mammalian Species

B 006 ECDYSONE-RESPONSIVE GENES, P. Cherbas, L. Cherbas, A. Bieber, J. Rebers, K.S. Lee, R.A. Schulz, A. Andres, L. Li, R. Moss, Department of Biology, Indiana University, Bloomington, IN 47405 and Department of Cellular & Developmental Biology, Harvard University, Cambridge, Ma 02138.

We have been studying early responses to ecdysone in the Drosophila Kc cell line. Kc cells, although of undefined tissue origin, are useful because of their dramatic and robust morphological, proliferative, and biochemical responses to the steroid. Detectable early changes in protein synthesis are confined to a small set of ecdysone-inducible polypeptides of 28, 29, and 40 kilodaltons. These are the products of two genes, the Eip28/29 gene (cytogenetic location 71CD) and the Eip40 gene (55E). The Eip28/29 and Eip40 transcripts are induced in parallel, their levels being detectably above basal within 20 min and maximal (5-15 x basal) by the end of 3 hrs. The results of pulse-labeling experiments are compatable with the idea that induction is transcriptional. Ecdysone does not alter the position of the cap site, nor the structure or stability of the mature transcripts. Thus the Eip28/29 and Eip40 genes appear to be bona fide analogs in Kc cells of the early puff sites known from studies of salivary gland chromosomes.

Transcripts of both genes are present basally in all Drosophila cell lines we have tested and there is a strong correlation between the magnitude of inductin (and the final, induced titer) and the strength of the morphological and proliferative responses. Likewise these transcripts are developmentally regulated in flies, though the precise structures of the transcripts and the details of their regulation are as yet unknown. It is remarkable that both genes reside at sites known to puff early in the salivary gland response to ecdysone.

Although the functions of the gene products are unknown, the Eip40 product is strongly homologous (ca. 40% identical) to the products of the metB and metC genes of Escherichia coli.

By transforming Drosophila cell lines with altered Eip gene constructs, we have begun to identify sequences required for hormonal regulation.

B 007 EVIDENCE FOR STEROID HORMONE RECEPTORS IN FUNGI

David Feldman, Stanford University School of Medicine, Stanford, California 94305.

We have demonstrated the presence of steroid binding proteins in simple eukaryotic organisms such as yeast. Candida albicans, a pathogenic yeast, possesses a corticosteroid binding protein (CPB) which exhibits high affinity (5 nM) for corticosterone and progesterone. CBP sediments at 4 S on sucrose gradients and has an apparent molecular weight (M_r) ~ 43,000 daltons by gel chromatography. Saccharomyces cerevisiae, simple baker's yeast, possesses an estrogen binding protein (EBP) with high affinity for estradiol (1 nM) but a low affinity for diethylstilbestrol. Saccharomyces EBP sediments at 5 S on sucrose gradients and has an M_r ~65,000. Paracoccidioides brasilienses, the pathogenic fungus causing paracoccidioidomycosis (South American blastomycosis) also possesses an EBP. This EBP exhibits a high affinity for estradiol (15 nM), sediments at 4.4 S on sucrose gradients and has an M_r ~60,000. Since paracoccidioidomycosis is predominately a disease of males (80:1) and since exposure rates of males and females is equivalent as measured by skin testing, we hypothesized that the high estrogen milieu in potential female hosts somehow inhibited the infectivity of the fungus. We could not detect any effect of estradiol on growth of the organism. However, this dimorphic fungus is inhaled as a mycelial-form organism and converts to a yeast-form organism (M → Y conversion) in the lungs in order to become invasive. In vitro assay of M → Y conversion demonstrated dramatic inhibition of yeast-form production by estradiol. The effect was dose-responsive with substantial inhibition by as little as 1 nM estradiol. Steroids that failed to bind to the EBP did not inhibit M → Y conversion. In summary, several yeasts and fungi possess binding proteins which exhibit high affinity for mammalian steroid hormones. We postulate that these binding proteins represent hormone receptors in these simple organisms which have analogy to steroid receptors in higher organisms. In the case of P. brasiliensis, estradiol additionally inhibits the M → Y conversion in the fungus. We believe this action is mediated by the EBP and hypothesize that the high estrogen milieu of the female hosts inhibits this critical step in fungal pathogenesis. This hypothesis provides a molecular mechanism for the epidemiologic findings of very low incidence of disease in women and high incidence in men.

Steroid Receptor Structure

B 008 RECEPTOR MEDIATED ACTIONS OF THE VITAMIN D HORMONE, Mark R. Haussler, D.J. Mangelsdorf, B.S. Komm, E.A. Allegretto, K. Yamaoka, J.W. Pike, D. McDonnell and B.W. O'Malley, University of Arizona, Tucson, AZ 85724 and Baylor College of Medicine, Houston, TX 77030

The action of the hormonal metabolite of vitamin D, namely $1,25(OH)_2D_3$, to induce calcium binding protein (CaBP) in chick intestine as well as to promote cell differentiation of leukemia (HL-60) cells to macrophages, is mediated by a receptor protein that concentrates the hormone in the nucleus. We have further characterized the receptor via proteolytic mapping, immunochemical analysis and in vitro translation of its mRNA. Mammalian $1,25(OH)_2D_3$ receptors have molecular weights ranging from 52,000 daltons (human) to 54,000 daltons (mouse, pig) and are both qualitatively modified by phosphorylation at serine residues and upregulated when cultured cells are treated with the $1,25(OH)_2D_3$ hormone. The avian $1,25(OH)_2D_3$ receptor monomer is a 60,000 dalton protein (with a minor species of 58,000 daltons) that binds $1,25(OH)_2D_3$ near the C-terminus and possesses a zinc stabilized, DNA binding domain near the N-terminus. Combined with previous evidence (Pike, J.W., Biochem. Biophys. Res. Commun. 100:1713,1981) for sulfhydryl groups essential to DNA binding, these data suggest that the $1,25(OH)_2D_3$ receptor contains a similar motif to other steroid receptors, comprised of cysteines configured into a DNA-binding structure - perhaps via tetrahedrally coordinated zinc. This conclusion is supported by our recent isolation of cDNA clones of the avian receptor by monoclonal antibody screening of an expression vector library. $1,25(OH)_2D_3$ receptor cDNAs verified by hybrid selection possess the erb-A-like consensus sequence highlighted by nine of nine conserved cysteines and an overall homology in this region between the receptor and v-erb-A of 49%. Receptor cDNAs are currently being used both to select for larger cDNAs containing the hormone binding region and to probe receptor mRNA via Northern analysis. Additional screening of a chicken intestinal cDNA library with polyclonal antibody to CaBP generated several cDNA clones. Northern blot analysis disclosed that one class of clones hybridized to vitamin D-dependent transcripts of 2000, 2900 and 3300 bp. Sequence analysis indicates that the cDNA (760 bp) to these vitamin D-induced mRNAs codes for CaBP, starting at amino acid #27. Thus, we have cloned cDNAs to the avian $1,25(OH)_2D_3$ receptor and obtained a cDNA in the coding region of the major $1,25(OH)_2D_3$ induced protein--CaBP. These cDNAs should be valuable in elucidating the molecular action of vitamin D, especially if genomic clones of these avian proteins can be isolated. It is also tempting to speculate that the $1,25(OH)_2D_3$ receptor complex may function in the differentiation of tumorigenic cells like HL-60 by competitively inhibiting the antimaturation effect of putative v-erb-A coded proteins at the level of DNA transcription.

B 009 RABBIT PROGESTERONE RECEPTOR : STRUCTURE AND MOLECULAR CLONING. H. Loosfelt, M. Atger, M. Misrahi, A. Guiochon-Mantel, F. Logeat, A. Bailly, M. Applanat and E. Milgrom. Unité de Recherches Hormones et Reproduction (INSERM U. 135), Faculté de Médecine Paris-Sud, 94275 Le Kremlin-Bicêtre Cedex, France

The rabbit progesterone receptor cDNA was cloned from λgt11 and λgt10 libraries. Sequence analysis allowed to deduce the primary structure of the protein which consists of 930 amino acids and contains, between residues 568 and 645, a cysteine-rich basic region highly homologous to its counterpart in glucocorticoid and estrogen receptors and v-erbA. Homology with the two other steroid hormone receptors, specially the glucocorticoid receptor is also encountered in the C-terminal region while the N-terminal region is totally different in size and sequence and contains a very high proportion of proline residues (1).

Post-translational modifications of the receptor include two different phosphorylation reactions (2): one basal and one hormone-dependent. The latter provokes a characteristic "upshift" in the electrophoretic mobility of the receptor. Both reactions involve serine residues.

The receptor binds with high affinity to specific regions of the uteroglobin gene. The purified receptor binds to the same sites, giving identical footprints whether in the presence or absence of hormone. Such binding is dependent on the presence of hormone in intact cells or crude cellular extracts. It is thus probable that in the nucleus the hormone-freereceptor is stabilized in a non-activated (non-DNA binding) form by a non-identified factor which is lost during purification (3).

REFERENCES:

1. Loosfelt H., Atger M., Misrahi M., Guiochon-Mantel A., Mériel C., Logeat F., Benarous R. and Milgrom E. DNA. Proc. Natl. Acad. Sci. USA, 1986, in press.
2. Logeat F., Le Cunff M., Pamphile R. and Milgrom E. Biochem. Biophys. Res. Commun., 1985, 131, 421-427.
3. Bailly A., Le Page C., Rauch M. and Milgrom E. EMBO J., 1986, in press.

B010 TRANSFORMATION OF GLUCOCORTICOID RECEPTORS TO THE DNA BINDING STATE, William B. Pratt, Deptartment of Pharmacology, The University of Michigan Medical School, Ann Arbor, MI 48109.

Incubation of L cell cytosol containing untransformed, non-DNA binding glucocorticoid receptors with monoclonal antibodies that react only with the 100-kDa glucocorticoid binding phosphoprotein causes the immuno-specific adsorption to protein-A-Sepharose of both the 100-kDa glucocorticoid receptor and a 90-kDa phosphoprotein that does not bind steroid. The 90-kDa receptor-associated protein has been identified as the 90-kDa murine heat shock protein (hsp90). Incubation of cytosol containing steroid-bound receptors at 25°C causes transformation of the steroid-receptor complex to the DNA-binding state. This transformation is accompanied by conversion of the receptor from a 9S to a 4S form and by dissociation of the anionic hsp90 from the 100-kDa steroid binding protein. Temperature-mediated 9S to 4S conversion, dissociation of hsp90, and acquisition of DNA binding activity are all steroid-dependent events. In contrast to temperature-mediated transformation, transformation by a salt, such as ammonium sulfate, does not require the presence of steroid. These observations are consistent with the proposal that dissociation of the anionic hsp90 from the receptor occurs during transformation of the receptor to the more cationic DNA-binding form. The observation that dissociation of the complex by salt in the absence of ligand yields a form of the unoccupied receptor that binds with high affinity to DNA-cellulose (demonstrated by Western blotting) suggests that the only role performed by the steroid may be to permit temperature-dependent dissociation of the heteromeric receptor-hsp90 complex. Thiol groups on the receptor are absolutely required for the untransformed receptor to bind steroid and for the transformed receptor to bind to DNA. Thiol groups are also required for transformation. If cytosol containing untransformed, steroid-bound receptor is treated with hydrogen peroxide and then incubated 25°C, conversion of the receptor from a 9S to 4S form and dissociation of hsp90 are prevented. If DTT is added to reverse the peroxide effect and cytosol is incubated a second time at 25°C, hsp90 dissociates and the receptor is converted to the 4S, DNA-binding form. The transformation that occurs during the second incubation step is both temperature-dependent and inhibited by molybdate. Thus, critical sulfur moieties in the receptor must be reduced in order for the temperature-mediated dissociation component of receptor transformation to occur.Both cytosolic and nuclear receptor from ^{32}P-labeled L cells have been resolved by immunoadsorption and SDS-PAGE, followed by immunoblotting and direct autoradiography of the immunoblots. No difference in the extent of ^{32}P-labeling of the receptor can be detected with temperature-mediated transformation and nuclear binding in intact cells or with temperature transformation and binding to DNA-cellulose in cytosol preparations.

B011 STRUCTURAL ANALYSIS OF THE AVIAN PROGESTERONE RECEPTOR USING ANTIBODY PROBES, David O. Toft, William P. Sullivan, David F. Smith, Christopher J. Krco and Daniel J. McCormick, Departments of Biochemistry and Molecular Biology, and Immunology, Mayo Medical School, Rochester, MN 55905.

Studies on the composition of purified avian progesterone receptor indicate three major protein components; the A and B receptor forms which bind hormone and a 90 kDa protein that has been identified as a major heat shock protein, hsp 90. Sedimentation and immune precipitation studies with monoclonal antibodies against hsp 90 show an association of this protein with A and B receptors, individually, when they are in the nontransformed 8S state. This complex is disrupted during in vitro transformation of receptor. Three antibodies against hsp 90 react with the antigen only when disrupted from receptor and apparently recognize epitopes that are masked by receptor association. One of these antibodies recognizes a highly conserved epitope that exists in hsp 90 from several mammalian species as well as some lower eukaryotes. Five monoclonal antibodies were prepared against progesterone receptor; four react with both A and B receptors and one reacts only with the B form. This latter antibody also reacts with several mammalian progesterone receptors. All five antibodies recognize epitopes in the amino terminal half of the progesterone receptor indicating that the A and B receptors are structurally similar in this region. The five antibodies all react with nontransformed and transformed receptor and do not interfere with receptor binding to DNA or progesterone. In addition, rabbit antiserum was prepared against a synthetic peptide corresponding to part of the highly conserved receptor region with homology to the erb A protein. This antiserum recognizes the purified A and B receptor forms using Western blotting procedures indicating that both receptor forms contain this conserved region. However, the antiserum does not react with native progesterone receptor in either the transformed or nontransformed state indicating that the epitopes are masked or in a conformation that is not recognized by the antiserum.

Regulation of Cell Function and Differentiation

B 012 STEROID REGULATION OF PROTEIN MATURATION AND COMPARTMENTALIZATION, Gary L. Firestone, Department of Physiology-Anatomy & Cancer Research Laboratory, University of California at Berkeley, Berkeley, CA 94720. The biological control of post-translational reactions that operate upon proteins during transport to their final cellular destinations is crucial for regulation of cellular function. Using the expression of mouse mammary tumor virus (MMTV) glycoproteins and phosphoproteins as sensitive molecular probes in the viral infected rat hepatoma cell line M1.54, we have discovered and documented two novel posttranslational regulatory circuits under control of dexamethasone, a synthetic glucocorticoid. One steroid-regulated pathway controls the posttranslational sorting of three cell surface-associated MMTV glycoproteins and one extracellular species that are derived from a common glycosylated precursor while a second distinct pathway regulates the proteolytic maturation of a viral phosphorylated precursor polyprotein. The regulated post-translational reactions require de novo RNA and protein synthesis, receptor function, do not require a critical concentration of MMTV polyprotein substrates and can be genetically uncoupled from each other as well as the glucocorticoid stimulation in MMTV transcription. Immunoprecipitation of cell surface-specific and intracellular MMTV glycoproteins revealed that the MMTV glycoproteins expressed at the cell surface in the presence of dexamethasone are located in an intracellular fraction in uninduced cells. Analysis of oligosaccharide processing demonstrated that the glucocorticoid-regulated branchpoint in glycoprotein sorting occurs in the medial Golgi after the acquisition of endo H resistant side chains but before the attachment of galactosyl residues to the carbohydrate moiety and palmitic acid to the polypeptide backbone. Mutant derivatives of M1.54 which fail to express cell surface MMTV glycoproteins were selected by complement mediated cytolysis. One such clonal isolate, CR4, displayed normal rates of proliferation, viral transcript synthesis and expression of the precursor polyproteins, a constitutive glycosylated maturation product and the regulated phosphosproteins. However, the glucocorticoid regulated sorting pathway responsible for the production of galactosylated and palmitylated cell surface MMTV glycoproteins was selectively defective in this variant. Intraspecific heterokaryons were formed by fusion of CR4 and uninfected HTC cells and the expression of cell surface viral glycoproteins monitored by indirect immunofluorenscence. Uninfected HTC cells complemented the defect in CR4 only after exposure to dexamethasone demonstrating that cellular-encoded glucocorticoid-regulated gene products can control the trafficking of a specific subset of cell surface-associated glycoproteins. We are currently attempting to identify and isolate the glucocorticoid regulated components that mediate this posttranslational regulatory circuit. This work was supported by the National Cancer Institute (CA 35547) and a National Science Foundation PYI Award (DCB 8351884).

B 013 HORMONAL CONTROL OF ADIPOCYTE DIFFERENTIATION, Gordon M. Ringold, Alger B. Chapman, David M. Knight, Marc Navre and Frank M. Torti, Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.
The environmental cues that control developmental decisions often take the form of hormonal signals. We have been characterizing the roles that glucocorticoid hormones and certain monokines and growth factors play in controlling the decision of fibroblast-like preadipocytes to differentiate into mature adipocytes. In this regard we have found that glucocorticoids stimulate the precocious differentiation of the TA1 cell line and that tumor necrosis factor (TNF) will block and reverse their differentiation. We have recently isolated a glucocorticoid inducible gene (clone 5) from TA1 cells that is implicated in the triggering of adipocyte (and perhaps myocyte) differentiation. Most impressively, the clone 5 gene can be rapidly shut-off by treatment of cells with fibroblast growth factor (FGF) a condition that prevents TA1 cell differentiation. This and other observations have led us to the hypothesis that a critical level of clone 5 gene product may be required to trigger differentiation; those hormones that induce it (e.g., glucocorticoids) stimulate differentiation whereas hormones or environmental conditions that repress it (e.g., FGF or low cell density) inhibit differentiation. Additional studies detailing various aspects of the hormonal control of clone 5 gene expression will be presented.

Regulation of Gene Expression - I

B 014 DIFFERENTIAL REGULATION OF GENE EXPRESSION BY GLUCOCORTICOIDS AND PROGESTINS, Jutta Arnemann, Georgos Chalepakis, Emily Slater, and Miguel Beato, Institut für Molekularbiologie und Tumorforschung, Philipps-Universität, D-3550 Marburg, F.R.G.

The hormone regulatory element (HRE) of mouse mammary tumour virus (MMTV) can mediate transcriptional induction of an adjacent promoter by glucocorticoids and progestins (1). The receptors for both hormones interact with the same region of the MMTV long terminal repeat (LTR), between -200 and -54 (2), although there are differences in the relative affinity of each receptor for the individual binding sites within the HRE. Differential binding of the glucocorticoid and progesterone receptors has also been observed within the regulatory region of the chicken lysozyme gene (3). Using a combination of deletion mutants, linker scanning mutants, point mutations and synthetic oligonucleotides we show that different sequence motives of the HRE contribute very differently to gene regulation by glucocorticoids and progesterone. In addition, the topological state of the transfected DNA, influences differentially the hormonal inducibility of a heterologous promoter. From the results of competition studies and transfection experiments with cloned receptor cDNAs we begin to understand the interplay between different hormone receptors on their DNA regulatory elements.

REFERENCES
1. Cato, A.C.B., Miksicek, R., Schütz, G., Arnemann, J. and Beato, M. (1986) EMBO J. 5, in press
2. Ahe, D. von der, Janich, S., Scheidereit, C., Renkawitz, R., Schütz, G. and Beato, M. (1986) Nature 313, 706-709
3. Ahe, D. von der, Renoir, J.M., Buchou, T., Baulieu, E.-E. and Beato, M. (1986) Proc. Natl. Acad. Sci. USA 83, 2817-2821

B 015 REGULATION OF TRANSCRIPTION BY STEROID HORMONE RECEPTORS, TISSUE SPECIFIC TRANS-ACTING FACTORS AND PHORBOL ESTERS. Michael Karin, Masayoshi Imagawa, Robert Chiu, Mordechai Bodner, Sharon Dana, Christophe Lefevre and Richard Imbra, Division of Pharmacology M-036 University of California, San Diego, La Jolla, CA 92093.

The glucocorticoid hormone receptor was shown to bind at specific sites (GRE's) on responsive genes, such as human metallothionein II_A ($hMT\text{-}II_A$) and growth hormone (hGH) and stimulate their transcription. While $hMT\text{-}II_A$ responds to glucocorticoids in most cell types that contain a reasonable number of receptors, genes like hGH respond to hormones only in cell types permissive for their expression. Thus, the presence of glucocorticoid receptors and their binding sites are necessary but not sufficient for hormonal induction of tissue specific genes. To investigate the role of other factors in expression and regulation of the hGH gene family we analyzed in detail the hGH-N gene. We found that this gene contains a tissue specific promoter element active only in pituitary tumor cells. This promoter is recognized by at least three different trans-acting factors, only one of which is specific to GH expressing cells. This factor, GHF-1, recognizes an upstream promoter element, required for expression in-vivo and in-vitro. In addition, one or two other factors bind to an enhancer element found further upstream. These factors, present in all cell types, could conceivably interact with a glucocorticoid receptor bound to a nearby GRE. However, the enhancer, like the GRE, is incapable of stimulating transcription of the hGH-N promoter unless the tissue specific factor GHF-1 is present. These experiments suggest that the first and most important step in activation of the hGH-N promoter is binding of the tissue specific factor, GHF-1.

In addition to regulation of gene expression by steroid hormones, we are studying the control of transcription by phorbol ester tumor promoters. We will present data demonstrating that, unlike steroid hormones, which act via specific trans-acting factors (i.e. the receptors), involved only in hormonal induction, phorbol esters regulate gene expression via the same factors which are responsible for maintaining a basal level of transcription. Increased phosphorylation of these factors by protein kinase C is being considered as a likely mechanism for phorbol ester stimulated transcription.

B 016 ANALYSIS OF REGULATORY ELEMENTS IN ANDROGEN-RESPONSIVE GENES, Malcolm Parker, Paul Webb, Tim Thompson, Roger White, John Mills and Maurice Needham, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

We have studied the role played by steroid hormones in regulating promoter activity in mouse mammary tumour virus (MMTV) and several androgen-responsive prostate genes. The MMTV promoter is stimulated by androgens, glucocorticoids and progestins in the human mammary tumour cell line ZR-75 but by progestin alone in the T47D #11 cell line. There is no obvious correlation between androgen receptor content and fold stimulation, since T47D #11 cells have relatively high androgen receptor concentrations (220 fmoles per mg protein) that are known to be functional by other criteria. The androgen response element has been mapped to overlap with the glucocorticoid and progesterone response elements within 200 nucleotides of the start of transcription. Although this hormone response element acts on heterologous promoters we have mapped downstream viral sequences which are required for normal viral expression.

Similarly, DNA sequences from prostate genes, which have been shown to bind androgen receptors selectively, were analysed for their affect on both the homologous and heterologous promoters in prostate tumour cell lines. Although the androgen response element within MMTV was shown to be functional, none of the prostate DNA sequences acted as an androgen response element in such homologous cells. The only condition we have found for maintaining specific prostate gene expression is to reimplant embryonic or neonatal prostate cells as ectopic subcapsular renal grafts in normal syngeneic adult hosts. Cells from 5 or 10 day-old rats produced near normal (85%) concentration of mRNA for prostatic steroid binding protein, but thereafter there was age-dependent decline in expression so that prostate from 37 day-old rats produced less than 1% normal levels after reimplantation. These results suggest two programmes are involved in controlling transcription, one to produce normal ontogenesis and another to maintain specific gene expression.

B 017 GLUCOCORTICOID REGULATION OF EXPRESSION OF THE TYROSINEAMINOTRANSFERASE GENE. Günther Schütz, Hans-Michael Jantzen, Uwe Strähle, Gerd Klock, Bernd Gloss, Francis Stewart, Peter Becker. Institute of Cell and Tumor Biology, German Cancer Research Center, Im Neuenheimer Feld 280, D-6900 Heidelberg, Federal Republic of Germany.

Transcription of the tyrosineaminotransferase (TAT) gene in liver parenchymal cells is controlled by glucocorticoids. In gene transfer experiments two glucocorticoid response elements (GRE) located 2500 bp upstream of the transcription initiation site were identified. These elements are binding sites of the glucocorticoid receptor as evidenced from in vitro and in vivo footprinting experiments. The two GREs induce expression of the TAT gene in a cooperative manner, the proximal element which in itself has no inducing capacity will strongly enhance glucocorticoid induction in the presence of the other. Cooperativity of these two elements is maintained when located upstream of a heterologous promoter which is not regulated by glucocorticoids. An oligonucleotide of 15 bp representing one of the footprint regions is sufficient to confer glucocorticoid inducibility to the thymidine kinase promoter. Interestingly the same oligonucleotide mediates induction by progesterone in progesterone receptor containing cells. Local alterations in the structure of chromatin at the GREs take place as evidenced from DNase I hypersensitive site mapping experiments. Using the genomic footprinting technique it is shown that changes in in vitro protein/DNA interactions at the GREs can only be detected after hormone treatment in hepatoma cells, but not in fibroblast cells, in which the TAT gene is not expressed. These data support the concept that glucocorticoids increase the affinity of the receptor to its target sequences. An assay to map cleavages produced by topoisomerase I with camptothecin, an inhibitor of this enzyme, has been developed. The cleavages map predominantly in the transcribed region of the TAT gene. Cleavage frequency increases with induction of the gene by glucocorticoids or cAMP. Induction of TAT mRNA synthesis is prevented if the topoisomerase I inhibitor is administered with the inducer. The cleavage sites are characterized by association of a protein to the 3' end of the fragments as expected for topoisomerase I. These observations imply an intimate relationship between topoisomerase I activity and TAT gene transcription.

B 018 REGULATION OF GENE EXPRESSION BY DIOXIN, James P. Whitlock, Jr., Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305.
2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD, dioxin) is a widespread, potent environmental contaminant that produces a diverse set of biological responses, which, in some cases, reflect the altered expression of specific genes. An intracellular receptor protein, which binds TCDD saturably and with high affinity, mediates several of TCDD's biological effects. In mouse hepatoma cells, TCDD induces aryl hydrocarbon hydroxylase activity by activating the transcription of a specific cytochrome P-450 gene. Studies of receptor-defective variant cells indicate that the activation of cytochrome P-450 gene transcription requires functional TCDD receptors. Analysis of the DNA that flanks the 5'-end of the cytochrome P450 gene reveals at least three control regions: a promoter, an inhibitory element, and a dioxin-responsive element (DRE). Therefore, expression of the cytochrome P-450 gene represents a balance between negative and positive control. Analysis of the DRE reveals that it confers responsiveness to TCDD upon a heterologous promoter and gene. The function of the DRE is relatively independent of both its location and orientation with respect to the promoter. The DRE requires TCDD receptors for its function. Together, the DRE and TCDD receptors constitute a dioxin-responsive enhancer system. The DRE contains two discrete, non-overlapping DNA domains that respond to TCDD. Each TCDD-responsive domain acts independently of the other, each requires TCDD receptors for function, and each has the properties of a transcriptional enhancer. The region of chromatin that contains the DRE is hypersensitive to nucleases. TCDD does not alter the pattern of DNase hypersensitivity in the 5'-flanking region of the cytochrome P-450 gene. Exposure of cells to TCDD results in the protection of a specific DNA domain from exonuclease digestion. This protection requires TCDD receptors. The boundaries of the protected domain coincide with those of the DRE.

Regulation of Gene Expression - II

B 019 THE PRO-OPIOMELANOCORTIN GENE: A MODEL FOR NEGATIVE REGULATION OF TRANSCRIPTION BY GLUCOCORTICOIDS, Jacques Drouin, Jean Charron, Lucie Jeannotte, Mona Nemer, Richard K. Plante and Orjan Wrange*, Institut de recherches cliniques de Montréal, Montréal, Canada, H2W 1R7 and *Karolinska Institute, Stockholm, Sweden.
The gene encoding pro-opiomelanocortin (POMC) offers an interesting model system to study negative control of transcription in eucaryotes. Indeed, glucocorticoid hormones specifically inhibit transcription of the POMC gene in the anterior pituitary. Expression and regulation of the POMC gene is highly tissue-specific. For example, while the POMC gene is expressed in both the anterior and intermediate lobes of the pituitary gland, only anterior pituitary POMC transcription is inhibited by glucocorticoids and stimulated by corticotropin releasing hormone (CRH).

In order to identify DNA sequence elements responsible for tissue-specific transcription and inhibition by glucocorticoids, chimaeric genes constituted of POMC promoter fragments fused to bacterial sequences encoding neomycin resistance (neo) were tested by electroporation into cell lines of pituitary and fibroblastic origin. The POMCneo hybrid gene is transcribed efficiently from the correct start site in the POMC-expressing pituitary cells, AtT-20. In contrast, the POMC promoter is inactive in L cells where only random initiation of transcription is observed. Deletion analysis indicates that sequences up to position -478 bp are required for tissue-specific transcription. rPOMC upstream sequences (-706 to -135 bp) behave as an enhancer element since they increase transcription when inserted upstream in either orientations or downstream of an heterologous promoter (HSV thymidine kinase); however, this enhancer activity does not appear to be tissue-specific.

In order to precisely localize DNA segments involved in DEX inhibition of transcription, we tested directly for binding of purified rat glucocorticoid receptor (GR) to rat POMC promoter sequences. Three GR binding sites are present in the POMC promoter fragments used in electroporation experiments. Glucocorticoid (DEX) treatment of pools of AtT-20 cells electroporated with pPOMCneo plasmids decreases transcription of the electroporated POMCneo gene to the same extent as the cellular POMC gene. DEX similarly inhibits transcription from a POMC fusion gene present on an episomal BPV vector. Deletion analysis indicates that a GR binding site which overlaps a putative "CCAAT" box sequence is essential for DEX inhibition of POMC transcription. Glucocorticoid inhibition of POMC transcription may, therefore, result from receptor binding in the "CCAAT' box region and impaired "CCAAT" box function.
(Supported by MRC and NCI of Canada)

B 020 SPECIFIC BINDING OF TWO ESTRADIOL DEPENDENT TRANS-ACTING FACTORS TO AVIAN VITELLOGENIN GENE AND THE ESTRADIOL DEPENDENT DEMETHYLATION OF THESE BINDING SITES.Jean-Pierre Jost and Hanspeter Saluz, Friedrich Miescher Institut P.O. box 2543 CH-4002 Basel,Switzerland.
The in vitro secondary stimulation of vitellogenin gene in isolated nuclei was used as an assay for putative regulation factors. The system shows that the reactivation of the silent gene needs polyamines and protein factors. Only protein factors isolated from the liver of estradiol treated animals reactivate the gene. The specific DNA-protein interaction of these factors is described. Preferential binding of one of them to vitellogenin gene was tested in vitro by DNA competition and gel retention and by exonuclease III protection experiments. The factor binds with a Kd of 3.5×10^{-10}M to a dyad symmetry structure 5' GTCTTGTTCCAAAC 3' of the third intron of the gene. The binding of the factor to DNA is sequence specific on single and double stranded DNA.The second factor: estradiol-receptor complex (ER) binding site was studied by DNA-cellulose binding/competition assay and by DNAase I footprinting. The binding site of ER overlaps a glucocorticoid binding site and contains also a dyad symmetry structure 5'GGTCAGCGTGACC 3'.genomic sequencing was used to study the estradiol dependent demethylation of four CpGs. Three of these sites, two of which lie within the ER binding site and one on a short stretch of alternating purine/pyrimidine were initially fully methylated. Analysis of liver DNA revealed that estradiol treatment to immature roosters resulted in a demethylation of these sites which occured initially only on one DNA strand. This demethylation correlated well with the induction of vitellogenin mRNA synthesis.The demethylation of the second strand lagged 24 hours behind. The 4th CpG located within the overlapping glucocorticoid receptor binding site was already hemimethylated at the onset of the experiment. The demethylation of this site also occured with kinetics similar to the rate of vitellogenin mRNA synthesis. All 4 CpGs remained demethylated even after cessation of gene transcription.Comparison of the methylation state of these CpGs indicate a clear dependence of the demethylation on estradiol.

B 021 THE ESTROGEN RESPONSIVE DNA ELEMENT: STRUCTURE AND INTERACTION WITH THE ESTROGEN RECEPTOR, Gerhart U. Ryffel, Ludger Klein-Hitpaß, Petra Drüge and Andrew C.B.Cato, Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, Postfach 3640, D-7500 Karlsruhe 1, FRG.
In the liver of Xenopus the vitellogenin genes are under strict estrogen control (1). A stable transfected A2 vitellogenin gene of Xenopus is estrogen regulated in the human breast cancer cell line MCF-7. Using vitellogenin CAT fusion genes we have mapped an estrogen responsive element (ERE) in the 5' flanking region. This ERE is able to confer estrogen inducibility to the HSV thymidine kinase promoter (2). The fact that the ERE acts in both orientation and over a distance of at least 2500 nucleotides classifies this element as an estrogen dependent enhancer. The 13 bp palindrome 5'-GGTCACAGTGACC-3' is sufficient for estrogen control and a single point mutation destroys hormonal regulation. In such a mutant estrogen control can be reestablished by linking a functional glucocorticoid responsive element, suggesting that various steroid hormone receptors may cooperate. We could show that the ERE preferentially binds estrogen receptor using DNA-cellulose competition assays. For this binding the 13 bp palindrome is sufficient and a point mutation within the palindrome abolishes preferential binding of the receptor. Estrogen responsiveness was introduced as a new characteristic into various cell lines by transfection of HEO, an expression vector coding for the human estrogen receptor (in collaboration with S.Green, G.Stack and P.Chambon). Estrogen responsiveness was proven by analyzing the estrogen inducibility of a cotransfected vitellogenin-CAT gene containing the ERE (Drüge et al. submitted). In conclusion all our data establish that the ERE, a 13 bp palindrome, is recognized as the cis-element by the trans-acting estrogen receptor.

1. Wahli and Ryffel (1985) Oxford Surveys on Eukaryotic Genes 2, 96.
2. Klein-Hitpaß et al. (1986) Cell 46, 1053.

Steroids and Regulation of Cancer - I (joint session)

B 022 pS2, OESTROGEN AND PROGESTIN RECEPTOR GENE EXPRESSION IN BREAST CANCERS.
P. Chambon[1], J.P. Bellocq[2], M. Berry[1], J.P. Briand[3], B. Gairard[4], A. Krust[1], S. Jakowlew[1], A.M. Nunez[1], R. Renaud[4], M.C. Rio[1], M. Roberts[1] and J. Wallace[1]. [1]LGME/CNRS and U.184/INSERM, Faculté de Médecine, 67085 Strasbourg; [2]Dept. Anatomie Pathologique, CHU, Strasbourg; [3]IBMC/CNRS, Strasbourg; [4]Dépt. Gynécologie I, CHU, Strasbourg - France.
We have previously reported the cloning and sequencing of the cDNA of the human pS2 gene (1,2) whose transcription is controlled by oestrogens in the human breast cancer cells MCF-7 (3,4,5). Using an antibody against a synthetic peptide whose sequence was deduced from that of the cDNA, we have now found that the pS2 protein is synthesized by MCF-7 cells grown in the presence of oestradiol (but not in the presence of tamoxifen) and secreted in the medium, as expected from the existence of a putative signal sequence in the cDNA-deduced protein sequence. The existence of an oestrogen-responsive element (ERE) in the pS2 gene promoter has been demonstrated by cotransfection in HeLa and MCF-7 cells of a vector expressing the human oestrogen receptor (hER) and recombinants containing the reporter E.coli CAT gene and various segments of the pS2 gene promoter region. Northern blot analyses of human normal and tumoral cells and biopsies have revealed that pS2 mRNA is present exclusively in a subset of breast cancers. These results were confirmed by the selective presence of the pS2 protein in these cancers, as assayed by immunohistochemistry. A large number (> 200) of human breast cancer biopsies have been analyzed by Northern blotting for the presence of pS2, hER and progestin receptor (hPR) mRNAs, as well as by immunohistochemistry for the presence of pS2 and hER proteins. ER and PR levels were also determined in these biopsies by using conventional binding of labelled hormones. It appears from these studies that the pS2 gene is specifically expressed in a subset of the human breast cancers which express the hER gene. Interestingly, the pS2 gene was not expressed in all of the breast cancers which express simultaneously the hER and hPR genes. The possible value of pS2 gene expression as an additional marker for further defining oestrogen-dependent breast cancers will be discussed.

1) P. Masiakowski et al., NAR (1982) 10, 7895; 2) S.B. Jakowlev et al., Nucl. Acids Res. (1984) 12, 2861; 3) A.M.C. Brown et al. (1984) PNAS 81, 6344; 4) B. Westley et al., JBC (1984) 259, 10030; 5) P. Chambon et al. in "Recent Progress in Hormone Research" (1984) vol. 40, 1.

B 023 ROLES OF PDGF/SIS-LIKE PROTEINS IN THE AUTOCRINE GROWTH REGULATION OF SSV-TRANSFORMED FIBROBLASTS, T. F. Deuel[1/2], G. F. Pierce[1/3], H.-J. Yeh[1], L. K. Shawver[1], P. G. Milner[1], Departments of Medicine[1], Biological Chemistry[2], Pathology[3], Jewish Hospital at Washington University Medical Center, St. Louis, MO 63110
The platelet-derived growth factor (PDGF) is highly homologous to the transforming protein of the Simian Sarcoma Virus (SSV), $p28^{v\text{-}sis}$. PDGF-like molecules are expressed in SSV-transformed cells and a PDGF-like growth factor is secreted into conditioned media from some but not all SSV-transformed cells. The secreted growth promoting activity interacts with PDGF cell surface receptors, activates the PDGF receptor tyrosine kinase, and appears capable of stimulating the autocrine growth of SSV-transformed cells. The secreted protein thus resembles $p28^{v\text{-}sis}$ but has not been established as the product of the v-sis gene. Immunoelectron microscopy has been used to localize PDGF/sis-like antigens in endoplasmic reticulum/Golgi, suggesting alternate potential sites for the interaction of $p28^{v\text{-}sis}$ with its cell surface receptor as each is processed. PDGF-like antigens of 66, 65, and 44 kDa also have been identified and partially purified from the cell nucleus, suggesting these proteins may represent a family of PDGF/sis-like molecules potentially important in different roles of growth regulation in SSV-transformed cells.

GROWTH REGULATION OF HUMAN BREAST CANCER BY SECRETED GROWTH FACTORS.

B 024 Marc E. Lippman, Robert B. Dickson, Edward P. Gelmann, Cornelius Knabbe, Neal Rosen, Eva Valverius, Diane Bronzert, Susan Bates, Sandra Swain. Medical Breast Cancer Section, Medicine Branch, National Cancer Institute, NIH, Bethesda, MD.
Our laboratory has been investigating the significance of growth factor production by human breast cancers. We have demonstrated that human breast cancer cells produce insulin-like growth factor 1 (IGF-1); transforming growth factor alpha-like activity (TGF alpha); platelet derived growth factor (PDGF); transforming growth factor beta (TGF beta); and an un-named high molecular weight growth factor activity. IGF-1 has the following properties: breast cancer cells produce radio-immunoassayable IGF-I. The IGF-I material co-purifies with authentic human IGF-I. Breast cancer cells contain mRNA which hybridizes to an authentic human cDNA probe for IGF-I. Breast cancer cells express IGF-I cell surface receptors and are growth stimulated by the addition of exogenous IGF-I in vitro. Estrogens stimulate and antiestrogens inhibit IGF-I secretion. Preliminary studies with antisense IGF-I genes suggest an autocrine role for IGF-I. Transforming growth factor alpha-like activity has the following properties. The major secreted species is a 30 KDa peptide. A cDNA for human TGF alpha recognizes a 4.8 Kb and a 1.6 Kb mRNA species. Radioreceptor activity, radioimmunoassay activity, transforming activity and mRNA are all coordinately induced by estrogens. Breast cancer cells express TGF alpha/EGF cell surface receptors; many lines are growth stimulated by TGF alpha/EGF. EGF infusion into the nude mouse stimulates transient tumor development of coimplanted MCF-7 cells in the absence of estrogen. Preliminary experiments with anti EGF receptor and anti TGF antibodies suggest an autocrine role for TGF alpha. PDGF has the following properties: human breast cancer cells produce fibroblast "competency" activity. Immunoprecipitation with PDGF specific antibodies identifies peptides of expected size (28 KDa unreduced, 15 and 16 KDa reduced). Studies with cDNA directed against PDGF A and B chain mRNA reveal that breast cancer cells express a variable pattern of the expected A chain (2.5, 2.9Kb) and/or B chain (4.1Kb) RNA species. PDGF production is estrogen regulated in an estrogen dependent cell line. Since breast cancer cells are not known to have PDGF receptors, PDGF may act as a paracrine growth factor in breast cancer. Infusion of growth factor concentrates into nude mice bearing estrogen dependent tumors is capable of partially replacing estrogen as a proximate stimulator of breast cancer growth. In addition, conversion to the estrogen independent phenotype is associated with increased growth factor production. Taken together, these experiments strongly suggest a critical role of secreted growth factor activities in the regulation of human breast cancer growth and progression.

B 025 AUTOCRINE AND PARACRINE ACTIVITIES OF AN ESTROGEN-INDUCED PROTEASE SECRETED BY BREAST CANCER CELLS, Henri Rochefort , Françoise Capony, Marcel Garcia, Muriel Morisset, Gilles Freiss and Françoise Vignon, Unité d'Endocrinologie Cellulaire et Moléculaire, INSERM U 148, and University of Montpellier, 60 Rue de Navacelles, 34100 Montpellier France.

We have studied estrogen-regulated proteins in an attempt to understand the mechanism by which estrogens stimulate cell proliferation and mammary carcinogenesis. In estrogen receptor positive human breast cancer cell lines (MCF7, ZR75-1) estrogens specifically increase the production into the culture medium of a 52,000 daltons (52K) glycoprotein. Several high affinity monoclonal antibodies to the partially purified secretory 52K protein have allowed to purify to homogeneity this protein and its cellular processed products. The 52K protein has been identified as the secreted precursor of a cathepsin-D like protease bearing mannose-6-phosphate signals. The protein is processed intracellularly into a 48 and 34K forms which accumulate in the lysosomes. The precursor 52K displays an in vitro autocrine mitogenic activity on estrogen deprived MCF7 cells, can be taken up by these cells via mannose-6-phosphate receptors and is able to degrade basement membrane and proteoglycans following its activation. The cellular related proteins, as detected by immunohistochemistry and immunoassay are more concentrated in proliferative mammary ducts than in resting ducts and their concentration in breast cancer cytosol appears to be more correlated with lymph nodes invasion than to estrogen or progesterone receptors level. The protein is also produced constitutively by ER-negative cell lines, while in some antiestrogen resistant variants, it becomes inducible by tamoxifen, contrary to the wild type MCF7 cells. The structure of the protein from MCF7 cells has been compared to that of cathepsin-D(s) prepared from normal tissues. This estrogen-induced protease, in addition to other estrogen regulated growth factors, may have important autocrine and/or paracrine functions in stimulating the growth and invasion of hormone-dependent and independent breast cancer.

Regulation of Transcription (joint session)

B 026 CHROMATIN STRUCTURE AND GLOBIN GENE EXPRESSION, G. Felsenfeld, B. Emerson, D. Jackson, T. Kimura, B. Kemper, M. Lieber, J. Hesse and J. Nickol, Lab. of Molecular Biology, NIDDK, NIH, Bethesda MD 20892.
Chromatin structure near transcriptionally active genes is often marked by domains that are hypersensitive to nucleases. We have examined a number of such domains in the 3' and 5' flanking regions of the globin gene family in chicken erythrocytes. The hypersensitive domain in the 5' flanking region of the β^A-globin gene is lacking a normal nucleosome, but within the nucleosome-free domain (about 200 base pairs long) at least three specific factors are observed to bind both in vitro and in vivo. Some of these binding sites play a role in regulation of the expression of the gene. In the 3' flanking region of the gene we have identified a sequence with the properties of an enhancer. We have shown that the enhancer is specific both with respect to cell type and developmental stage. It is located within a hypersensitive domain, and we have identified factors that bind to the domain; some of the factors may themselves be developmentally regulated. In other experiments, we have examined the properties of hypersensitive domains in an embryonic β gene and an adult α gene. These domains have an architecture similar to those described above; some of the factors binding to the domains are novel, but it appears that some regulatory elements may be shared.

B 027 CONTROL OF TRANSCRIPTION IN PROKARYOTES AND EUKARYOTES--A COMMON MECHANISM, Mark Ptashne, Department of Biochemistry and Molecular Biology, Harvard University, 7 Divinity Avenue, Cambridge MA 02138.
Various experiments bearing on the questions of how regulatory proteins recognize specific sequences in DNA and turn genes on and off in prokaryotes and eukaryotes will be described.

1. Hochschild, A. and Ptashne, M. 1986. Cooperative binding of λ repressors to sites separated by integral turns of the DNA helix. Cell 44, 681-687.

2. Griffith, J., Hochschild, A., and Ptashne, M. 1986. DNA loops induced by cooperative binding of λ repressor. Nature 322, 750-752.

3. Ptashne, M. 1986. Gene regulation by proteins acting nearby and at a distance. Nature 322, 697-701.

4. Hochschild, A., Douhan III, J., and Ptashne, M. 1986. How λ repressor and λ cro distinguish between O_R1 and O_R3. Cell 47 (in press, December 5).

Steroids and Regulation of Cancer - II (joint session)

B 028 GLUCOCORTICOOID-INDUCED RESPONSES IN LYMPHOMA AND FIBROSARCOMA CELL LINES, Suzanne Bourgeois, Michel Crepin and Douglas C. Dean, The Salk Institute, Regulatory Biology Laboratory, San Diego, California 92138.

Glucorticoids induce lysis of T-lymphomas and the synthesis of fibronectin in the human fibrosarcoma cell line HT1080.

The mechanism of hormone-induced cytolysis of T-lymphomas is still obscure. We have demonstrated earlier the existence of a "lysis" function required for glucocorticoid-induced T-cell killing (1). The "lysis" gene is not expressed in the glucocorticoid-resistant cell line SAK8, but its expression (in the presence of dexamethasone) is activated by DNA demethylation in those cells (2). Following up on several recent reports that glucocorticoids induce DNA fragmentation in sensitive T-cells, we have examined the possibility that lymphocytolysis might result from glucocorticoid-induced nuclease activity(ies). We have demonstrated that several calcium-dependent nuclease activities begin to appear after 1 h of dexamethasone treatment in a glucocorticoid sensitive cell line, P3-95, derived from SAK8 cells by treatment with the DNA demethylating agent 5-azacytidine, but are not detected in SAK8 cells. We conclude that this induction of nuclease activities precedes the cytolysis process and is directly dependent on the glucocorticoid-sensitive phenotype of the T-cell. Therefore, the gene(s) which encodes them is a possible candidate for the "lysis" gene.

Fibronectin (FN) is a large glycoprotein which serves to anchor cells to a substratum. Synthesis of FN is strongly inhibited upon neoplastic transformation which may account, at least partially, for alterations in morphology and adhesion which are observed in transformed cells. In addition, the synthesis of FN is stimulated by glucocorticoid hormones (3). As a first step in the study of how expression of the FN gene is controlled, we have isolated genomic clones containing the 5'-end and flanking sequences of the human fibronectin gene. The 5'-end of the FN gene is found on a 3.7 kb EcoRI fragment which contains about 2.7 kb of flanking sequence. The first exon is 414 bp long and contains a 5'-untranslated region of 267 bp. Based on the position of the translational initiation codon, FN is synthesized with a 31 amino acid extension on its amino terminus which seems to consist of both a signal peptide and a propeptide. The sequence ATATAA is found at $^{-}$25 and the sequence CAAT is present at $^{-}$150. Sequences exhibiting homology to the binding sites for the transcription factor SP1 are present.

1. Gasson, J.C. and Bourgeois, S., J. Cell Biol. 96, 1983, 409-415.
2. Gasson, J.C., Ryden, T. and Bourgeois, S., Nature 302, 1983, 621-623.
3. Oliver, N., Newby, R.F., Furcht, L.T. and Bourgeois, S., Cell 33, 1983, 287-296.

B 029 STEROID EFFECTS ON CULTURED MAMMARY TUMOUR CELL LINES, Roger J.B. King, James F. Glover, Pirkko Harkonen and Philippa D. Darbre, Imperial Cancer Research Fund, P.O. Box 123, Lincoln's Inn Fields, London WC2A 3PX, U.K.

The biology of steroid action on breast cancer cells indicates that multiple transducing mechanisms must be involved.

Proliferation From studies on the regulation of S115 mouse- and ZR-75 human mammary tumour cell lines by androgen and oestrogen respectively, we formulated the idea that steroids changed the cell phenotype from normal (-hormone) to transformed state (+hormone). With both cell lines, multiple effects of steroid were noted, some of which could be independently regulated. The relevant steroid (testosterone/S115 cells, oestradiol/ZR-75 cells) stimulated proliferation during log-phase growth and increased saturation density in monolayer culture and markedly increased growth as spheroids in suspension culture (anchorage independence). With both cell lines, glucocorticoids (dexamethasone) inhibited log-phase proliferation in monolayer. In S115 cells, but not ZR-75 cells, glucocorticoids also increased saturation density and anchorage independence. Thus by judicious choice of steroid and cell line, one can divorce the different types of response. What is clear is that no simple model of modulation of one oncogene/growth factor can explain these data.

In the mouse system, steroid effects on density regulation and suspension growth could be mediated by a protein from the 3' long terminal repeat of MMTV; both androgens and glucocorticoids induce a 16S mRNA from this region of the virus. On the other hand, the opposite effect of these two classes of steroid on log phase/monolayer growth would tend to rule out an MMTV contribution to this facet of growth regulation.

Transfection experiments have confirmed that androgen, as well as glucocorticoids, can act directly on the long terminal repeat (LTR) of MMTV in S115 cells. The possibility that this important regulatory element of DNA can be under multiple steroid regulation may be relevant in defining biological action.

Transition from hormone responsive to unresponsive state S115 cells lose their androgen sensitivity when cultured in the absence of androgen. Loss of sensitivity is not accompanied by loss of androgen receptor as evidenced by androgen sensitivity of transfected chaemeric genes coupled to MMTV LTR. The loss of sensitivity is a multistage process, the later stages of which involves hypermethylation of at least the MMTV LTR.

B 030 MOLECULAR AND GENETIC ANALYSIS OF GLUCOCORTICOID ACTIONS IN HUMAN LEUKEMIC CELLS, E. Brad Thompson[a], R. Evans[b], M. G. Rosenfeld[c], Y-S. Yuh[a], B. Gametchu[a], J. Ashraf[a], and J. M. Harmon[d]. [a]Univ. of Tex. Med. Branch, Galveston, TX. 77550, [b]The Salk Insti., LaJolla, CA., [c]Univ. Calif. at San Diego School of Med., and [d]U.S. Univ. of the Health Sciences, Bethesda, MD.

Glucocorticoids inhibit the growth of and even kill many types of human leukemic cells. In CEM cells, grown from childhood acute lymphoblastic leukemia, occupancy of receptors by glucocorticoids causes cell lysis. Unlike results from mouse model systems, selection for resistant CEM cells yields two broad classes of mutant phenotypes: without prior mutagenesis one with "activation-labile" receptors; with prior mutagenic treatment, one with a paucity of receptors (r^-). A third, spontaneously resistant phenotype lacks the lysis function (ly^-) but has receptors (r^+). Somatic cell hybridization between the receptor mutants shows no evidence for trans-active resistance factors and no complementation. However there was complementation between the r^- cells and the r^+ly^- cells, so that complete sensitivity to glucocorticoids was restored in the hybrids. Monoclonal antibodies to glucocorticoid receptors were prepared and used to analyze the normal and resistant CEM cells. Both activation-labile and r^- cells contain high quantities of normal, 95 KDa, immunoreactive receptor. Northern blot analysis of the mRNA from the receptor mutants with several probes derived from the original alpha-human glucocorticoid receptor cDNA showed the same ∿7 and 4.5 kb forms of receptor mRNA as did the wild-type cells. Sensitivity of oncogene transcripts to glucocorticoids in hormone-sensitive and -resistant cells is being investigated.

Steroids in Physiology

B 031 STUDIES OF STEROID 21-HYDROXYLASE DEFICIENCY, Maria I. New, Susan Drucker, Perrin C. White and Phyllis W. Speiser, Division of Pediatric Endocrinology, The New York Hospital-Cornell Medical Center, New York NY 10021.

Defective steroid 21-hydroxylation is the most common of the biochemical defects causing hyperplasia of the adrenal cortex. Steroid 21-hydroxylase deficiency is a monogenic autosomal recessive disorder with three clinical forms: the simple virilizing and salt-losing classical variants, and the more recently recognized nonclassical (attenuated) form, which shows a wide clinical range of effects and whose characterization emerged from coordinated hormonal testing and family studies.

Test programs have shown the feasibility of neonatal screening for 21-hydroxylase deficiency by blood-spot hormonal assay for elevated 17-hydroxyprogesterone. Prenatal detection of disease currently depends on HLA serotyping of cultured amniocytes jointly with measurement of amniotic 17-hydroxyprogesterone (at 13-18 wk gestation).

Molecular genetic studies have started to identify specific mutations altering 21-hydroxylase activity. The gene for the enzyme steroid 21-hydroxylase, a cytochrome P-450, is situated within the major histocompatibility complex (HLA) on the short arm of human chromosome 6, between the B and DR antigen loci. The 21-hydroxylase enzyme is encoded for by one of two highly homologous gene sequences, the other of which has recently proven to be a pseudogene. Linkage disequilibria between certain B and DR alleles and classical 21-hydroxylase deficiency have permitted the use of HLA genotyping in conjunction with hormonal evaluation for diagnosis of this disorder and for identification of carrier haplotypes in population studies. The most significant associations for the classical disorder are Bw47(;DR7) for both forms, B60(40) for salt-losing, and B51(5) for simple virilizing (all positive); and B8(;DR3) (negative).

Certain mild 21-hydroxylase deficiency allotypes involved in the nonclassical disorder are also genetically linked with HLA, exhibiting the distinct positive antigen associations B14(;DR1). It is now also known that distinct nonclassical disease phenotypes result genotypically from the conjunction of two mild 21-hydroxylase defects or from a mild defect occurring with a severe (classical) defect.

The classical defect (severe) associated with the the HLA-Bw47;DR7 haplotype is the result of a deletion of the active 21-hydroxylase gene, while the nonclassical (mild) defect is associated with a gene duplication of the pseudogene when the haplotype contains HLA-B14;DR1.

B 032

REGULATION OF STEROID HYDROXYLASE GENE EXPRESSION, Michael R. Waterman and Evan R. Simpson, Departments of Biochemistry and OB/Gyn and Cecil H. and Ida Green Center for Reproductive Biology Sciences, University of Texas Health Science Center, Dallas, TX 75235.

Steroid hydroxylases, located in steroidogenic tissues including the adrenal cortex, testis and ovary, are necessary for the biosynthesis of steroid hormones including sex hormones, glucocorticoids and mineralocorticoids. We have found that steroid hydroxylase gene expression is regulated by three distinct mechanisms, presumably involving three different sets of regulatory proteins. The best understood of these mechanisms is the cAMP-dependent regulation which is mediated by peptide hormones derived from the anterior pituitary; ACTH, LH, FSH. These peptide hormones bind to specific cell-surface receptors (i.e. ACTH in the adrenal cortex) activating adenylate cyclase activity leading to elevated levels of intracellular cAMP. This leads to synthesis of labile steroid hydroxylase inducing proteins (SHIP) which enhance the expression of steroid hydroxylase genes at the transcriptional level. This action of cAMP is essential for maintenance of optimal steroidogenic activity throughout life. A second mechanism by which steroid hydroxylase gene expression is regulated can be defined as tissue-specific. There appear to be two subclasses within this category. One instance involves all-or-none expression of these genes such that 11β-hydroxylase (P-45011β) and 21-hydroxylase (P-450C21) genes are expressed only in the adrenal cortex while cholesterol side chain cleavage cytochrome P-450 (P-450scc) and 17α-hydroxylase (P-45017α) are expressed in a variety of steroidogenic tissues including adrenal cortex, testis and ovary. A second type of tissue specific expression is observed in the ovary where under certain physiological conditions both P-450scc and P-45017α are expressed (pre-ovulatory follicle) and under other conditions only P-450scc is expressed (corpus luteum). A third type of mechanism which regulates expression of steroid hydroxylase genes is that which leads to fetal imprinting and is cAMP-independent. We have found in both anencephalic human fetal adrenals and mid-gestational (85 day) sheep fetal adrenals, conditions during which ACTH is undetectable in fetal blood, the steroid hydroxylase mRNA levels are as high as those in normal human fetal adrenals or late-gestational (145 day) sheep fetal adrenals, respectively. During these latter conditions ACTH is readily detectable in fetal blood. It is our hypothesis that expression of steroid hydroxylase genes by this cAMP-independent mechanism precedes the development of the cAMP-dependent mechanism. The long range goal of this laboratory is to elucidate the details of these three mechanisms of regulation of steroid hydroxylase gene expression, including the nature of the trans-acting proteins involved.

Steroid Receptor Structure

B 100 IDENTIFICATION OF AN ENDOGENOUS RNA ASSOCIATED WITH THE TRANSFORMED GLUCOCORTICOID RECEPTOR, Masarrat Ali and Wayne V. Vedeckis, Dept. Biochem. & Mol. Biol., L.S.U. Medical Center, New Orleans, LA.

The glucocorticoid receptor (GR) from mouse AtT-20 pituitary tumor cell, when transformed using a variety of in vitro protocol, yields a DNA-binding, RNA containing 6S form. In order to better understand the physiological role of RNA interaction with the transformed GR we have isolated and purified the putative RNA from AtT-20 cells. The GR associated RNA (GR-A-RNA) was purified by a combination of DEAE-cellulose, hydroxylapatite and oligodT-adsorption and preparative sucrose gradient procedures. The GR-A-RNA was homogenous on formaldehyde-agarose gel. The GR-A-RNA showed following characteristics: i) it can reconstitute the 6S native GR when added to the 4S form ii) it can be covalently cross-linked to the 4S GR using formaldehyde and the resulting GR-RNA complex sediments at the density of a ribonucleoprotein iii) it has a sedimentation coefficient similar to tRNA (4S) iv) accepts amino acid to form aminoacyl-tRNA (GR-A-RNA) complex which is also active in binding to the 4S form to generate the 6S GR v) the analysis of GR-A-RNA on high resolution acrylamide-urea sequencing gel revealed that it contained a major tRNA of 76 nucleotide no minor tRNA species of 74 and 78 nucleotides. On the basis of amino acid acceptor activity, GR-A-RNA mainly contained $tRNA_{arg}$ (52%), $tRNA_{lys}$ (17%), and $tRNA_{his}$ (9%). These results conclusively demonstrate that the transformed GR binds to the endogenous tRNAs as identified above. The GR-tRNA interaction may be important in the regulation of genes controlled by glucocorticoids. It is proposed that tRNA bidning to the 4S GR may be involved in the degradation of the 4S form via ubiquitin mediated pathway.

B 101 CLONING OF AN Ah RECEPTOR REGULATORY GENE F.F.Chu, F.Sander and O.Hankinson; Lab of Biomedical and Environmental Sciences, UCLA, Los Angeles, CA 90024

The Ah receptor regulates the induction of several drug metabolizing enzymes. The best studied enzyme in mouse hepatoma cells is the cytochrome P_1-450. A potent ligand for this receptor is 2,3,7,8-tetrachlorodibenzo-p-dioxin abbreviated as TCDD. Similar to the receptor for glucocorticoid hormone, the Ah receptor upon binding to ligand will translocate into the nucleus and then activate the genes. We intend to clone the gene regulates the nuclear translocation of the Ah receptor by using the C mutant of Hepa-1 cell, this C mutant has normal level of cytosolic Ah receptor but no nuclear translocation.

DNA obtained from HepG2 (a human hepatoma cell) is used to transfect the C mutant. Selection for the receptor translocation gene or the C gene is based on the expression of the P_1-450 activity. Four secondary transfectants have been obtained from two primary transfectants, they share similar although not identical human sequences. We have cloned fragments of the human DNA into lambda EMBL3 and GT10; one is 20 kb the other is 3 kb. The two clones do not overlap. We intend to use these clones to screen cDNA libraries to obtain a cDNA clone of the gene responsible for the translocation of the Ah receptor.

B 102 Characterization of Chicken Progesterone Receptor Complimentary DNA's. Orla M. Conneely, Alan D.W. Dobson, Clark Huckaby, Mary Anne Carson, Tanya Zarucki-Schulz, Geoffrey L. Greene, David O. Toft, Ming-Jer Tsai, William T. Schrader and Bert W. O'Malley. Baylor College of Medicine, Houston, Texas 77030.

cDNA clones encoding the A & B forms of the chicken progesterone receptor (CPR) have been isolated from chicken oviduct cDNA libraries. Analysis of the complete amino acid sequence of PR deduced from the cDNA clones revealed two areas of striking homology between the progesterone, estrogen and glucocorticoid receptors which correspond to the DNA and hormone binding domains of the steroid receptors. The chick progesterone receptor is encoded by at least three mRNA species. Variant cDNAs encoding at least two forms of polypeptide have been identified. The origin of the variant cDNAs, and of the A and B forms of the receptor will be indicated. In addition, functional analysis of the structural domains of the various receptor forms will be presented.

B 103 MUTATIONAL ANALYSIS OF THE GLUCOCORTICOID RECEPTOR, Mark Danielsen, Jefferey Northrop, Gordon M. Ringold, Stanford Medical Centre, Stanford, CA 94305

We have cloned a full length cDNA for the mouse glucocorticoid receptor (GR). Expression of this cloned cDNA in receptor-deficient Cos 7 cells yields a fully functional GR protein. In addition, both the DNA binding and hormone binding mutant receptors from the S49.22R (nt-) cell line have been cloned. The DNA binding deficient receptor has an $arg_{484} \rightarrow his$ change which maps within the cys, lys, arg rich domain of the protein. The hormone binding deficient receptor has a $glu_{546} \rightarrow gly$ change. These single amino acid changes indicate that the cys, lys, arg rich region is indeed involved in DNA binding and that the c-terminal 25kd is involved in hormone binding.

In order to provide additional information on the domain structure of the GR we have created altered forms of the GR using in vitro mutagenesis. These results indicate that: a) the N-terminal half of the receptor is not required for gene activation, b) the hormone binding domain is contained within the c-terminal 25kd c) the cys, lys, arg rich DNA binding domain acts as a constituitive activator when the hormone binding domain has been removed.

B 104 RECEPTOR INTERCONVERSION AS A MODEL OF ESTROGEN ACTION. Nooshine Dayani, Reid McNaught and Roy G. Smith, Baylor College of Medicine, Houston TX 77030

A model is presented in which the receptor can be interconverted between three states (R_x, R_y, R_{nb}). The interconversion is monitored by Scatchard analysis, sucrose density gradient analysis, and affinity labeling using [^{3}H]tamoxifen aziridine followed by receptor purification with estrogen receptor monoclonal antibody affinity chromatography and SDS gel electrophoresis. The results are consistent with each state existing in different conformations having a common molecular weight of approximately 66,000. The R_x conformation is stabilized by antiestrogens. The conversion to the steroid binding form is induced by ATP, ADP and GTP. Cyclic nucleotides are ineffective. There is a specific requirement for Mg^{2+}; neither Ca^{2+} nor Mn^{2+} will substitute. Nonhydrolyzable nucleotide analogues were tested for their relative efficiency to convert R_{nb} to R_y. Conversion occurred with AMP-CPP, but AMP-PCP and AMP-CP were inert. Thus, activation of R_{nb} to form R_y appears to be catalyzed by an event requiring the loss of the terminal phosphoryl moiety from either ATP or ADP. The kinase is present in chick and hen oviduct cytosol and has been substantially purified. Using the purified kinase, and ^{32}P γ ATP, under conditions in which R_{nb} is converted to R_y, phosphorylation of the immunoprecipitated receptor can be demonstrated. Since R_y can be recycled from R_{nb}, an estrogen mediated increase in estrogen receptor concentrations, which does not occur with antiestrogens, can be explained without involving an increase in receptor synthesis rates. This receptor interconversion model may have general application to hormone action, and may explain agonist/antagonist activity. (Supported by NIH Grant #HD17727).

B 105 HEPATITIS B VIRUS DNA INTEGRATION IN A GENE HOMOLOGOUS TO v-erb-A AND STEROID RECEPTOR GENES IN A HEPATOCELLULAR CARCINOMA. Hugues de Thé, Agnès Marchio, Pierre Tiollais and Anne Dejean. [1]Unité de Recombinaison et Expression Génétique, (INSERM U163, CNRS UA271), Institut Pasteur, Paris, France.

Hepatitis B virus (HBV) is clearly involved in the aetiology of human hepatocellular carcinoma (HCC). The finding of HBV DNA integration into human liver DNA in almost all HCCs studied suggested a possible role of these integrated viral sequences in liver oncogenesis. We have cloned and sequenced a single HBV integrated sequence present in a HCC sample and the corresponding unoccupied allele from the non-tumorous part of the same liver. HBV integrated in a cellular DNA open reading frame (ORF) assigned to chromosome 3, with a consequent microdeletion of genomic DNA. We identified within the deduced translation product of the ORF a region of striking homology with both the v-erb-A oncogene protein and the supposed DNA-binding domain of the human glucocorticoid receptor (hGR), the human oestrogen receptor (hER) and the chicken progesterone receptor (cPR). Remarquably the viral genome integrated a few nucleotides upstream from this v-erb-A, hGR, hER and cPR-homologous region so that a readthrough transcription could occur from a viral promoter into a cellular gene. We suggest that this gene, became inappropriately expressed as a consequence of HBV integration thus contributing to the cell transformation. The characterization of this new v-erb-A related gene that we referred to as hep, is in progress.

B 106 IMMUNOCHEMICAL COMPARISON OF A GLUCOCORTICOID RECEPTOR FRAGMENT PRODUCED BY NEUTROPHIL ELASTASE WITH A NUCLEAR TRANSFER INCREASED MUTANT RECEPTOR. Clark W. Distelhorst, Case Western Reserve University, Cleveland, OH 44106

We recently found that neutrophil elastase (NE) digests glucocorticoid receptors (GR) in the cytosol of human leukemia cells to produce fragments that are similar in size to the nuclear transfer increased mutant (nt^i) GR in S49 mouse lymphoma cells. The present study is an immunochemical comparison of these receptor fragments and the nt^i GR. GR in wild type and nt^i S49 cells were affinity labeled with ^{3}H-dexamethasone mesylate (DM) and analyzed by SDS-PAGE. The M_r of wild type and nt^i GR were 97 kDa and 48 kDa respectively. Limited digestion of wild type GR with NE and chymotrypsin produced 50 kDa and 40 kDa DM-labeled fragments respectively. Chymotrypsin converted the 50 kDa fragment produced by NE to a 40 kDa fragment but did not digest the nt^i GR. Both the 50 kDa fragment produced by NE and the nt^i GR bound to DNA-cellulose and eluted from DNA-cellulose at the same salt concentration. The 50 kDa fragment produced by NE reacted with monoclonal anti-receptor antibodies (BUGR2) in an immunoadsorption assay using protein A-sepharose but the nt^i GR did not.

We conclude that: (a) NE digests the GR at a different site than chymotrypsin to produce a 50 kDa fragment that contains both the steroid-binding and the DNA-binding domain. (b) The 50 kDa fragment produced by NE and the nt^i GR are derived from the same region of the GR but the nt^i GR appears to be missing a BUGR2 reactive site and a chymotrypsin sensitive site.

B 107 ACTIVATION LABILE GLUCOCORTICOID RECEPTOR MUTANTS, Laura P. Eisen, Marian S. Elsasser and Jeffrey M. Harmon, Uniformed Services University, Bethesda, MD 20814. Spontaneously arising glucocorticoid receptor mutants isolated from the human leukemic cell line CEM-C7 have been shown to contain significant amounts of glucocorticoid receptor which have a decreased ability to translocate the nucleus after incubation with dexamethasone. Previous studies have demonstrated that the receptors from these clones are unable to form and/or maintain a stable activated form of the steroid-receptor complex. They have therefore been defined as activation labile (act^l). To more clearly define the nature of the receptor defect in these clones we have examined their glucocorticoid receptors by DNA binding, *in vivo* and *in vitro* affinity labeling, and immunoblotting with anti-human glucocorticoid receptor antibodies. Affinity labeling in both intact and broken cell preparations demonstrated that the steroid-binding protein of the act^l receptors was 92 kDa both before and after activation, indicating that the instability of the act^l receptors is not the result of proteolysis. These results were confirmed by immunoblotting of both covalently labeled and non-covalently labeled receptors. In addition, DNA-cellulose chromatography of affinity labeled act^l receptors after attempted activation revealed that covalently labeled complexes were capable of DNA binding. The affinity of this binding appeared normal. These results suggest that unlike mouse nt$^-$ mutants which have altered affinity for DNA, act^l mutants appear to result from the inability of the receptors to retain ligand when activated. Thus, it appears that retention of ligand after activation is necessary for normal receptor function. This work was supported by PHS grant CA32226.

B 108 A NOVEL EFFECT OF MOLYBDATE ON PARTIALLY PURIFIED BRAIN TYPE I RECEPTORS FOR [^{3}H]ALDOSTERONE. S.M. Emadian and W.G. Luttge, Dept. of Neurosci. & Center for Neurobiological Sciences, University of Florida, Coll. of Med., Gainesville, FL 32610.

We have recently reported that although molybdate reduces temperature-induced losses in the binding capacity of unoccupied Type I receptors in crude brain cytosol preparations, this stabilization is accompanied by a dose-dependent loss of binding capacity at 0 and 22 C [Emadian et al., J. Steroid Biochem. 24(5):953, 1986]. Here we report that the addition of 2 mM molybdate during, or subsequent to, gel filteration of Type I receptors, results in a significant increase in the binding capacity of these receptors over and above that measured in crude cytosol preparations.

Brain cytosol from Adrex-Ovex CD-1 female mice was prepared in 20 mM HEPES buffer (pH 7.60). Partially purified unoccupied Type I receptors were obtained by filtering crude cytosol through Sephadex G-25 fine columns at 4-5 C. Cytosol was then incubated with 10 nM [^{3}H]aldosterone and 5 uM RU 26988 (to block Type II receptors). In comparison to results with crude cytosol preparations, partial purification led to a significant loss in Type I receptor binding capacity. However, in the presence of 2 mM molybdate, partial purification increases the specific binding of Type I receptors to levels markedly higher than those seen in crude preparations. Furthermore, although the addition of 2 mM molybdate protects [^{3}H]aldosterone binding capacity in crude cytosol aged at 22 C, this reagent fails to prevent heat-induced binding losses in partially purified preparations.

The effects of molybdate on partially purified receptors clearly differs from those seen in crude cytosol preparations, as well as those reported for other steroid receptors.

B 109 CHARACTERISATION OF A 29K DALTON OESTROGEN RECEPTOR-RELATED PROTEIN (p29), Joanne R. Finley, Arnold I. Coffer and Roger J.B. King, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX, U.K.

D5, a monoclonal antibody raised against a partially purified fragment of the human oestrogen receptor (ER) recognises a 29K dalton non-steroid binding protein. p29 will complex with activated (salt, temperature, phosphatase-treated) ER. Activation is inhibited by molybdate or GTP. p29 is a cytoplasmic, phosphoprotein which contains a GTP binding site. In normal tissues it is qualitatively and quantitatively related to ER content. It is absent from ER negative breast tumours; most positive tumours (67%) contain p29 (immunoassay and histochemical assay). The presence or absence of p29 is a good indicator of response to hormonal therapy in advanced breast cancer. Tumours which are ER+ p29- (24% of total) have a low response rate (12%). In endometrium, p29 levels are increased fivefold by oestrogen and decreased by progestin. Hormone regulation of p29 is currently under investigation in a ZR-75 mammary carcinoma cell culture system.

p29 has been purified to homogeneity from ZR-75 cells by immunoaffinity purification and the amino acid sequence is being determined. Sequence data will confirm the identity of cDNA clones from a ZR-75 λgt11 expression library screened with D5 antibody. Whether p29 represents a previously unknown part of the ER machinery or is a product of it, remains to be established. Whichever model is correct, the ability of p29 to bind GTP suggests that it may play an important role in cell function.

B 110 HIGH LEVEL EXPRESSION OF A GLUCOCORTICOID RECEPTOR FRAGMENT IN E. COLI AND ITS USE IN THE GENERATION OF NOVEL MONOCLONAL ANTIBODIES, Leonard P. Freedman and Keith R. Yamamoto, University of California, San Francisco CA 94143.

A primary objective of this laboratory is to understand the mechanism of action of the glucocorticoid receptor protein (GR). We know that after binding hormone, GR is activated to a state in which it can bind to specific DNA sequences with high affinity and in some unknown way enhance the transcription of genes adjacent to these sequences. By cloning the cDNA for GR and expressing full length (795 amino acids) and deletion derivatives, our laboratory has accumulated considerable information regarding the functional domains of the protein. In order to examine the structure and function of the intact GR, we have begun to raise monoclonal antibodies (Mab) against the protein in mice. To date, all but one Mab available from other sources appear to recognize determinants on the N-terminal half of GR, the so-called "C-domain", far from the DNA and steroid binding domains. This strongly suggests that the C-domain is immunodominant. To circumvent this problem, we cloned a GR fragment containing only the DNA and steroid binding domains (from amino acid 440 to 795) into a T7 expression vector (provided by F. W. Studier, Brookhaven National Laboratory) and obtained high levels of this protein, called M27, in *E. coli*. M27 was purified from gels and used to immunize Balb/c mice. Having generated a very strong immune response from one mouse, spleen and myeloma Sp2/0 cells were fused and the supernatants from resulting hybridomas screened by ELISA using purified rat liver GR as antigen. Several positive clones were identified; they have been subcloned and their classes determined. One antibody appears to recognize a determinant in the steroid binding domain of GR, and should be useful, for example, in the study of ligand-mediated activation of this protein.

B 111 IDENTIFICATION OF LYMPHOMA CELL MEMBRANE PROTEIN MONOCLONAL ANTIBODY TO THE THE GLUCOCORTICOID RECEPTOR: CORRELATION TO GLUCOCORTICOID INDUCED CELL LYSIS, B. Gametchu, University of Texas Medical Branch, Galveston, TX 77550.

Plasma membrane receptor protein from S-49 mouse lymphoma cells was detected using direct immunofluorescent labeling with monoclonal antibody to glucocorticoid receptor(s) (GR). Cellular heterogeneity in GR content was evident. Using immunoadsorption to antibody-coated tissue culture plates, the cells were separated into membrane antigen-positive (100%) and membrane antigen-deficient (38%) populations. Gel electrophoresis and specific immunoblot analysis of the membrane proteins from the membrane antigen-positive group revealed multiple protein bands ranging in size from 85 to 145 kDa. Furthermore, comparison of the glucocorticoid sensitivity between these groups of cells showed complete lysis in the membrane antigen-positive cells and only partial lysis in the antigen-deficient group, suggesting that the lysis response of cells of glucocorticoids is mediated by a plasma membrane GR.

B 112 IMMUNOLOGIC ANALYSIS OF HUMAN PROGESTERONE RECEPTORS: STRUCTURE, PHOSPHORYLATION, AND NUCLEAR PROCESSING. KB Horwitz, DP Edwards, DO Toft, PL Sheridan, University of Colorado Health Sciences Center, Denver, CO 80262.

We have purified progesterone receptor (PR) B-proteins from T47D human breast cancer cells using an immunoaffinity column prepared with an anti-chick PR antibody (PR-6 of D. Toft). Four monoclonal antibodies directed against human PR have been generated, one of which (AB-52) cross-reacts with two progestin binding proteins of Mr 120,000 (B-protein) and 94,000 (A-protein). The other three are B-specific. Using the antibodies, and in situ photoaffinity labeling, we have shown that both A and B are intracellular proteins, and that they form separate 8S holoreceptor complexes and do not associate as dimers. We have also shown that B-receptors are good DNA-binding proteins. To study PR phosphorylation, T47D cells were incubated with [^{32}P]orthophosphate under two conditions -- without hormone treatment to study untransformed receptors, and after hormone treatment to study chromatin-bound receptors. In T47D, PR are substrates for two phosphorylation reactions: once while they are untransformed, when protein B but not protein A is phosphorylated; then again in nuclei when both B and A are phosphorylated. These data suggest that phosphorylation of untransformed B-receptors is confined to the NH_2-terminal end of the molecule and does not confer hormone binding capacity, since A-proteins can bind hormone. Nuclear polyphosphorylation occurs at a site common to A and B. It may precipitate processing since immunoblot analyses show that following hormone treatment and polyphosphorylation PR undergo this down-regulation step leading to a true loss of receptor protein. Restoration of receptors appears to involve protein resynthesis and is chronically blocked by synthetic progestins.

B 113 CHARACTERIZATION OF THE CHROMOSOMAL CHICKEN PROGESTERONE RECEPTOR GENE. Clark S. Huckaby, Wanda G. Beattie, Alan D.W. Dobson, Orla M. Conneely, Ming-Jer Tsai, and Bert W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston Texas 77030.

Chicken progesterone receptor (PR) cDNA's recently cloned in this laboratory (Conneely et al., 1986, Science 233: 767-770) hybridize to a single chick gene in high-stringency genomic Southern blots. At this stringency, cDNA probes selected four over-lapping cosmid clones from a chick oviduct genomic library; the clones represent over 80 kb of chromosomal DNA. Restriction mapping and Southern analyses suggest that the entire PR gene (~45 kb) is represented. Sequence comparison between cDNA and genomic clones is in progress and has revealed that the cDNA region encoding the conserved putative DNA-binding domain of the PR is interrupted by a 13-kb intron. A 150-bp exon encodes the first of two putative DNA-binding "fingers" in the complete receptor. The sequence ACTAAA resides about 150-nt into the ajoining 13-kb intron and is apparently sometimes recognized as an alternative polyadenylation signal. Hence, cDNA's have been found which contain 23 nt plus an in-frame stop codon that match the sequence of the 5' end of the 13-kb intron. The biological role of the variant gene product, which contains the first part of the DNA-binding consensus but none of the steroid binding region, is under investigation. The overall structure of the PR gene will be presented.

B 114 PROTEINS ASSOCIATED WITH UNTRANSFORMED ESTROGEN RECEPTOR: INFLUENCE OF HYDROPHOBIC INTERACTIONS UPON TRANSFORMATION, T. William Hutchens, Chee Ming Li and Paige K. Besch, Reproductive Research Lab, St. Luke's Episcopal Hospital and Department of Obstetrics and Gynecology, Baylor College of Medicine, Houston, TX 77005.

Estrogen receptors from calf uteri have been analyzed by high-performance size-exclusion chromatography using conditions designed to evaluate hydrophobic interactions between the steroid binding subunit and other receptor-associated proteins. The single, large (untransformed) species of soluble estrogen-receptor complex consistently (n = 9) found in calf uteri displayed a change in Stokes radius from 8.0 nm to 3.5 nm rapidly upon exposure to elevated ionic strengths (0.4 M KCl). In contrast, exposure to 6 M urea resulted in conversion of the untransformed receptor (8.0 nm) to a 6.0-6.5 nm receptor form not previously observed in either hypotonic or hypertonic buffers. In the presence of both 6 M urea and 0.4 M KCl, the untransformed estrogen-receptor complex was converted to a smaller receptor form intermediate in size (4.5-5.0 nm) to that observed in 6 M urea or 0.4 M KCl alone. The formation of this 4.5-5.0 nm receptor form was estrogen-dependent as determined by parallel analyses of unliganded receptor in urea/KCl buffer. The normally high affinity of receptor for estradiol (Kd = 0.4 nM) was relatively preserved in the presence of 6 M urea (Kd = 3.5 nM). Furthermore, the urea-induced change in apparent size (8 nm to 6.0-6.5 nm) at low ionic strength was accompanied by a complete exposure of the DNA-binding site as evidenced by nearly quantitative (>95%) interaction with DNA-agarose. The urea-induced interaction between receptor and DNA was of high affinity (Kd = 0.2 nM) and was found to be salt-resistant. Thus, hydrophobic interactions within or between the steroid binding subunit and other receptor-associated proteins are significant in vitro, and may reflect the mechanism and potential for as yet undisclosed biospecific associations in vivo.

B 115 DETECTION OF PROTEIN KINASE ACTIVITY ASSOCIATED WITH IMMUNOPURIFIED ESTROGEN RECEPTOR ISOFORMS, S.M. Hyder, N. Sato and J.L. Wittliff, Hormone Receptor Laboratory, J. Graham Brown Cancer Center, University of Louisville, Louisville, KY 40292
Recently, we have shown that high-performance hydrophobic interaction chromatography (HPHIC) may be used for rapid separation of steroid receptors which retain their biological activity (Hyder et al. J. Chromatogr. 327: 237, 1985; ibid 1986, in press). Another report published from our laboratory demonstrated that estrogen receptor from human breast cancer cells (MCF-7) exhibited protein kinase activity (Baldi et al. BBRC 135: 597, 1986). Thus the estrogen receptor appears to be a far more complicated regulatory molecule than previously envisioned. Here we report the separation and characterization of estrogen receptors from individual human breast tumors and rat uteri using a Beckman CAA-HIC, a non-ionic polyether bonded hydrophobic column. This separation mode resolved receptor proteins into two isoforms (R_t = 22 min and 27 min) without the need for organic solvent and with near 100% recoveries. Maximum resolution was obtained when a descending salt gradient of ammonium sulphate (2-0 M) in phosphate buffer, pH 7.4 was used. Purification of estrogen receptor of ~ 5-20 fold was obtained with a single pass. Receptor purified by HPHIC retained ligand binding capacity and exhibited protein kinase activity which was dominant in the R_t = 22 min ER isoform when immunoprecipitated to essentially homogeneity with monoclonal antibody D547. This rapid method of purifying estrogen receptors with retention of steroid binding and protein kinase activities, is helping to elucidate the complicated nature of this regulatory molecule. Supported in part by USPHS Grant CA-42154 from the NCI and the Phi Beta Psi Sorority. S.M.H. is a Research Fellow of the Graduate School and N.S. is a Visiting Investigator from Iwate Medical University in Japan.

B 116 IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CHICK PROGESTERONE RECEPTOR
J.Isola, T.Ylikomi, P.Tuohimaa University of Tampere,BOX 607,33101 Tampere FINLAND
Progesterone receptor (PR) of the chick oviduct and ovary were localized immunoelectron microscopically by using a highly specific polyclonal anti-PR antibody, IgG-RB (1). Light microscopy of immunostained paraffin and frozen sections favored nuclear localization of PR (2). Electron microscopically PR was localized by using a pre-embedding technique and peroxidase-anti-peroxidase (PAP) or immunogold methods for detection (2,3). By PAP-technique nuclear localization of PR was found. Only weak cytoplasmic immunostaining was observed in the endoplasmic reticulum. By using an immunogold method the localization of PR was further characterized. Inside the nucleus PR was widely dispersed. The strongest labelling for unoccupied PR was in the dense chromatin. After progesterone treatment in vivo more labelling was observed on the dispersed chromatin and especially on the border zone of dense and dispersed chromatin, which is known to be the transcriptionally most active site in the nucleus.. The change was statistically significant (4). In conclusion, we suggest that chick PR is a nuclear protein, which is localized in the cytoplasm (endoplasmic reticulum) probably due to recent synthesis. Our results suggest conformational changes in the structure of PR-containing chromatin after receptor occupation. REFERENCES: 1.Tuohimaa P. et al. BBRC 119:433,1984. 2. Isola J. et al. Histochemistry 1986 (in press). 3. Isola J. et al. J. Steroid Biochem. 1987 (in press). 4. Isola J. (submitted).

B 117 COVALENT LABELING AGENTS FOR THE ESTROGEN RECEPTOR, John A. Katzenellenbogen, Kathyrn E. Carlson, Jonathan F. Elliston, Francesco G. Salituro, David M. Simpson, and Jeffrey A. Zablocki, Departments of Chemistry and Physiology and Biophysics, University of Illinois, Urbana, IL 61801.
The estrogen receptor can be labeled covalently by aziridine derivatives of the anti-estrogens tamoxifen (TAZ), desmethylnafoxidine (NAZ), and iododesethyltamoxifen (I-TAZ), and the non-steroidal estrogen hexestrol. The covalent labeling properties of TAZ have been described previously (J. Biol. Chem. 258, 3487 [1983]): it labels covalently a 65 kDa-protein from estrogen target tissues efficiently and selectively, and it can be used both in cell-free preparations, whole cells, and tissues. Compared to TAZ, NAZ, which embodies a phenolic hydroxyl group, affords somewhat higher apparent binding affinity and provides unambiguous stereochemistry during synthesis because of its cyclic "B-ring". I-TAZ reacts with ER with a selectivity somewhat less than that of TAZ, but affords the advantage of iodine labeling. Several hexestrol derivatives were prepared in which the aziridine function was linked to the hexestrol side chain through ketone, ester, or thioether functionality. The rate and efficiency of the irreversible binding of these derivatives to the estrogen receptor depends in detail upon the structure of this linkage: several of these analogs react rapidly and efficiently, and one of them is an agonist. These agents will be useful in characterizing the properties and structure of the estrogen receptor and in probing for receptor-gene interaction sites.

B 118 PURIFICATION OF A KINASE INVOLVED IN RECEPTOR CONVERSION FROM A NON-STEROID BINDING TO THE LOWER AFFINITY ESTROGEN BINDING FORM. R.W. McNaught, N. Dayani and Roy G. Smith, Baylor College of Medicine, Houston, TX 77030.

Two high affinity estrogen receptors, R_x and R_y, can be identified in the estrogen stimulated chick oviduct on the basis of their kinetic properties (R_x: Kd 0.1 nM; R_y 1 nM) and sedimentation coefficients (R_x: 4.2S; R_y: 3.5S). Additionally we have demonstrated in oviduct cytosol prepared following short term withdrawal from DES stimulation (2-3d) that R_y but not R_x exists in a non-steroid binding form (R_{nb}). R_{nb} can be converted to R_y during assay under exchange conditions (30C/3h) in the presence of 1-5 mM ATP or ADP and Mg^{2+}. This augmentation of binding sites has been shown by Scatchard assay, sucrose density gradient, competition studies and affinity labeling/PAGE analysis to be authentic lower affinity estrogen receptor R_y. Ammonium sulphate fractionation (30%) of soluble oviduct preparations allows the separation of the receptor (R_y/R_{nb}) entities from the kinase activity that catalyzes the R_{nb} to R_y conversion. The activity is stable to freeze/thaw but is destroyed by exposure to heating at 100C. It has also been identified in chick kidney and adult hen oviduct. Isolation of the kinase, by ion exchange and HPLC chromatography results in a 3,000 fold level of purification. This is the first report describing purification of a specific enzyme involved in the preferential recycling of the lower affinity receptor type (R_y) between a non-binding and steroid binding form. (Supported by NIH Grant #HD17727).

B 119 ANALYSIS OF GLUCOCORTICOID RECEPTOR GENE EXPRESSION IN THE HORMONE RESPONSIVE DDT_1 MF-2 CELL LINE: *Comparison to the DDT_1 GR1 glucocorticoid resistant variant.* Norris JS[1,2], MacLeod SL[1] Cornett LE[2] Smith RG[3] Department of Medicine[1], and Physiology[2] University of Arkansas Medical School, Little Rock 72205 and Departments of Urology and Cell Biology[3] Baylor College of Medicine, Houston, Texas 77030.

The DDT_1 MF-2 (*wild type*) cell line has previously been shown to contain androgen and glucocorticoid receptors. The mitogenic action of androgens on *wild type* cells is completely blocked by glucocorticoids. Conversely the DDT_1 GR1 (*variant*) derived from *wild type* cells is slightly responsive to the mitogenic action of androgens but is not growth inhibited by glucocorticoids. Furthermore, a marker protein, p29 is glucocorticoid inducible in *wild type* but not in *variant* cells. These data correlate with a >80% reduction in glucocorticoid receptor protein in the *variant*. Analysis of glucocorticoid receptor poly A^+ RNA isolated from the *wild type* reveals 5.7 Kb transcripts that are inducible with cycloheximide and reduced by glucocorticoid treatment. In the *variant*, poly A^+ RNA levels are greatly reduced and are not glucocorticoid regulated. Restriction mapping of *variant* genomic DNA reveals a rearrangement or deletion in the 3' noncoding trailer region of the glucocorticoid receptor which is not observed in the *wild type*. Therefore, comparison of the 3' noncoding trailer region of *wild type* verses *variant* glucocorticoid receptor cDNA may suggest how glucocorticoid receptor poly A^+ RNA is stabilized in *wild type* cells. Supported by GM 30669.

B 120 THE *Ah* RECEPTOR: A CLOSE RELATIVE OF STEROID RECEPTORS WHICH BINDS TOXIC FOREIGN CHEMICALS. Allan B. Okey*, Lynn M. Vella* & Michael S. Denison#. *Division of Clinical Pharmacology, Hospital for Sick Children, Toronto, Ontario CANADA; #Department of Pharmacology, Stanford University School of Medicine, Stanford, California 94305.

The *Ah* (aromatic hydrocarbon) receptor binds toxic halogenated compounds such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and certain carcinogenic polycyclic aromatic hydrocarbons such as 3-methylcholanthrene (MC). No endogenous ("physiologic") ligand for the *Ah* receptor has yet been demonstrated. The physicochemical properties of the *Ah* receptor and the general mechanism by which the receptor functions in gene regulation are very similar to the structure and mode of action of receptors for steroid hormones. However, the *Ah* receptor is not identical to any of the previously characterized steroid receptors. No known steroid competes with [^{3}H]TCDD or [^{3}H]MC for high-affinity binding sites on the *Ah* receptor. The *Ah* receptors from mouse or rat liver cytosol bind [^{3}H]TCDD with a K_d ≈ 1 nM and a B_{max} of 50-200 fmol/mg cytosol protein. These receptors sediment ≈ 9*S* in conditions of low ionic strength and have Stokes radii of ≈ 7 nm corresponding to an M_r of 250,000-280,000. In conditions of high ionic strength the rat *Ah* receptor readily dissociates to a 5.6*S*/5.2nm ligand-binding subunit with an M_r ≈ 121,000. Mouse *Ah* receptor is resistant to salt-induced dissociation into subunits, but with prolonged exposure to 0.4 M KCl it can be partially converted into a 4.9*S*/5.2nm subunit with an M_r ≈ 105,000. After binding ligand the *Ah* receptor:ligand complex is transformed to a state which binds with high affinity to DNA. It is not yet clear whether the form of *Ah* receptor which binds to DNA corresponds to the subunits produced by exposure to high salt. Molybdate partially stabilizes both mouse and rat cytosolic *Ah* receptors in the 9*S* form in the presence of 0.4 M KCl; molybdate also slows the rate of thermal inactivation of receptor. However, molybdate is less effective at stabilizing *Ah* receptor in rodent liver than it is at stabilizing glucocorticoid receptor in the same tissues. The *Ah* receptor now has been detected and characterized in several tissues and cell lines of rodent and human origin. The structural properties of the *Ah* receptor and its general mode of action suggest that the *Ah* receptor is a member of the steroid receptor family but that the *Ah* receptor is a separate and distinct member of this family.

B 121 SULFHYDRYL GROUPS CONTENT OF CHICKEN PROGESTERONE RECEPTOR: EFFECT OF OXIDATION ON DNA BINDING ACTIVITY. S. Peleg, W.T. Schrader and B.W. O'Malley, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Sulfhydryl groups are required for steroid receptor-DNA interaction. Receptor amino acid sequences share a concensus cysteine-rich region which is thought to be part of the DNA binding domain. Based on this information we examined the effect of reducing agents on receptor DNA-binding activity and quantified SH groups by labeling with [^{3}H]N-ethyl maleimide. Receptor proteins A (M_r = 79K) and B (M_r = 108K) are present in equal amounts, but over 70% of A molecules bind DNA whereas only 10 to 15% of B molecules have this activity. We found different sensitivities of the DNA sites to reducing agents; subunit A is stabilized preferentially compared to B. Dithiothreitol was the best stabilizer; β-mercaptoethanol and 1-thioglycerol were less effective. We speculated that subunit B is oxidized more easily, and thus quantified sulfhydryl groups on both DNA$^+$ and DNA$^-$ receptor forms after immunopurification and resolution on SDS gels. Labeling of DNA$^+$ receptors with [^{3}H]NEM showed 21-23 SH groups on either A or B when the proteins were reduced and denatured. The same number was seen without reduction if DNA$^+$ receptors were tested. In contrast, the DNA$^-$ receptor B had only 10-12 SH groups detectable without reduction. We conclude that progesterone receptors unable to bind DNA contain 10-12 oxidized cysteine residues, likely present as disulfide bonds. These cysteines are reduced in the DNA$^+$ state. We could not reduce the oxidized disulfides when the native DNA$^-$ proteins were treated with DTT. Thus we speculate that irreversible loss of DNA binding activity in vitro is due to oxidation of cysteines which are inaccessible to DTT in the native state.

B 122 INVOLVEMENT OF MICROTUBULE ELEMENTS IN THE NUCLEAR TRANSLOCATION OF ESTROGEN RECEPTORS: K.G.RAJENDRAN, Tulio LOPEZ* and Indu PARIKH*. Dept. Molecular Biology, Wellcome Research Laboratories and *Dept. Biochemistry, Glaxo Inc., Research Triangle Park, North Carolina, 27709.

The role of microtubule elements in the transmission of molecular information from the cytoplasm to the nucleus of estrogen target cells was investigated. Serially subcultured human breast cancer cells (MCF-7) and uteri from immature Sprague Dawley rats were used. Cytosol was prepared from cell or tissue homegenate either at high speed (105000xg for 1 h) or at low speed (9000xg for 15 min) in PBS containing 1 mM PMSF and 1 mM DTT. Progesterone receptor (PR) synthesis in MCF-7 cells is modulated by estradiol, effecting an increase of over 500% within 48 h of estradiol (1 nM) treatment. PR synthesis was inhibited by colchicine in a dose-dependent manner, achieving complete inhibition at 1 μM, but not by lumicolchicine. Other agents which prevent tubulin polymerization such as podophyllotoxin, vinblastine and vincristine also inhibited PR synthesis. Moreover, nuclear translocation of estradiol was inhibited 60% after 16 h exposure to colchicine. When antitubulin antibodies were added to low-speed cytosol from rat uterine tissue and immunoprecipitated with Protein A, ER level in the supernatant decreased by 80%. This effect could be neutralized with exogenous tubulin. Copolymerization of tubulin from cytosol also reduced ER levels while appropriate controls showed that this is not due to spontaneous aggregation of ER. Taken together, these data implicate microtubule elements in the mechanism of estrogen action and in the subcellular distribution of ER.

B 123 ATP INDUCED ACTIVATION OF PURIFIED RAT HEPATIC GLUCOCORTICOID RECEPTORS, Thomas J. Schmidt and Edward E. Diehl, Department of Physiology and Biophysics, The University of Iowa, Iowa City, IA 52242.

Although numerous reports have indicated that ATP stimulates the activation/ transformation of crude rat hepatic glucocorticoid receptors, the question of whether this effect involves a cytoplasmic mediator(s) or represents a direct interaction with the receptor protein itself has remained unanswered. Utilizing unactivated rat hepatic glucocorticoid receptor complexes purified by a three-step scheme which includes affinity chromatography, we have shown for the first time that ATP interacts directly with the receptor protein in stimulating activation as reflected by an increase in DNA-cellulose binding as well as by a shift in the elution profile of the receptor complexes from DEAE-cellulose. ATP stimulates activation in a dose-dependent manner (maximally at 10mM) and there appears to be no nucleoside specificity since GTP, CTP and UTP as well as ADP and GDP also stimulate activation significantly. All of these observations closely parallel data obtained from activation experiments with crude hepatic receptors. ATP does not appear to stimulate activation of receptors (crude or purified) by initiating a phosphorylation reaction since hydrolysis-resistant analogues of ATP and ADP are also effective. Although pyrophosphate (PPi) can mimic the effects of ATP, neither of these "activating" effects can be explained in terms of increased ionic strength. Although the precise mechanism(s) underlying this in vitro effect of PPi on the purified receptors remains unclear, this result does not rule out the possibility that ATP may directly influence in vivo activation. (Supported in part by NIH AM 34490 and ACS grant BC 464).

B 124 **NON-STEROID BINDING FORMS OF GLUCOCORTICOID RECEPTOR IN DEXAMETHASONE TREATED HELA S_3 CELLS** C.M. Silva, C.M. Jewell and J.A.Cidlowski, University of North Carolina, Chapel Hill, NC 27514

Chronic exposure of HeLa S_3 cells to dexamethasone causes a pronounced down regulation of steroid binding sites and a decrease in responsiveness to hormone as seen by the inability to induce alkaline phosphatase. In order to better understand this adaptation of cells to chronic steroid exposure, we have studied receptors from cells which have been under chronic dexamethasone ($1x10^{-6}$ M dex) exposure for up to 2 years. As detected by whole cell saturation binding assays, these cells contain only 0-20% of the receptor content of untreated cells. Furthermore, analysis of [^{3}H]dexamethasone mesylate labeled extracts on one- and two- dimensional gels reveals detectable receptor only in control, non-treated cells. In contrast, analysis of similar extracts by the recently developed Southwestern blot procedure (Silva et al, PNAS in press) which involves renaturation of proteins after electrophoresis and probing with ^{32}P-labeled MMTV DNA, results in detection of receptor in both non-treated and down-regulated cells. Northern blot analysis of poly $(A)^+$ mRNA from non-treated and down-regulated cells reveals a 7.5 kb glucocorticoid receptor messenger RNA in both groups of cells. These results provide evidence of a non-steroid binding population of receptors present in down-regulated cells which is able to bind selectively to DNA. The function of these non-steroid binding forms of receptor is currently under investigation.

Supported by AM 32988, AM 33016, AM 33060.

B 125 AFFINITY LABELING OF CYS-656 IN RAT GLUCOCORTICOID RECEPTOR, S. Stoney Simons, Jr. Janet G. Pumphrey, Stuart Rudikoff, and Howard J. Eisen, NIH, Bethesda, MD 20892. The identity of the amino acid(s) of rat HTC cell glucocorticoid receptor that are affinity labeled by dexamethasone 21-mesylate (Dex-Mes) was investigated. The specificity of Dex-Mes labeling of proteins was determined using bovine serum albumin (BSA) as a model. About 90% of Dex-Mes reaction occurred at the one non-oxidized cysteine of BSA. This reaction was specifically blocked by nearly stoichiometric amounts of methyl methanethiolsulphonate (MMTS). Thus both Dex-Mes and MMTS react very selectively with thiols in protein. MMTS was equally efficient in preventing both [^{3}H]dexamethasone binding to HTC cell glucocorticoid receptors and [^{3}H]Dex-Mes labeling of the 98K receptor protein. These results indicate that Dex-Mes labeling of the receptor involves covalent reaction with at least one cysteine in the steroid binding site. In order to determine which of the 20 cysteines of the glucocorticoid receptor are labeled by Dex-Mes, small (~1600 daltons) radiochemically pure fragments of the [^{3}H]Dex-Mes labeled 98K receptor were generated by proteolysis with trypsin, chymotrypsin, and S. aureus V8 protease. Analysis of these [^{3}H]Dex-Mes labeled fragments on SDS-polyacrylamide gels revealed that all of the covalent [^{3}H]Dex-Mes was located on one or a few cysteines in an approximately 18 to 26 amino acid long region of the receptor. Sequential Edman degradation of each fragment gave directly the number of amino acid residues between the NH_2-terminus proximal cleavage site and a single [^{3}H]Dex-Mes labeled cysteine. A comparison of these amino acid spacings with the amino acid sequence of the HTC cell glucocorticoid receptor (Miesfeld et al., Cell, 389 [1986]) uniquely identified Cys-656 as the one cysteine labeled by [^{3}H]Dex-Mes.

B 126 THE ESTROGEN RECEPTOR DIMER IS RESPONSIBLE FOR HIGH AFFINITY BINDING TO TOTAL AND SPECIFIC DNA, D.F. Skafar, R.A. Maurer* and A.C. Notides, Univ. of Rochester, Rochester, NY 14642 and *Univ. of Iowa, Iowa City, IA 52242.

The 5S estrogen receptor dimer is required for positively cooperative binding of [^{3}H]estradiol. Current results suggest that the dimer is also required for high affinity binding of the calf uterine estrogen receptor to total and specific DNA. The dependence on receptor concentration of the affinity of the [^{3}H]estradiol-bound receptor for DNA was measured using DNA-Sepharose chromatography in Tris buffer, pH 7.4, containing 0.2 M KCl. The association constant for binding both crude and 1000-fold purified estrogen receptor to DNA is 1.8×10^5 M^{-1} DNA bp at low receptor concentrations and increases to 5.8×10^5 M^{-1} bp at high concentrations. The number of salt bridges between the [^{3}H]estradiol-bound receptor and DNA (m') was measured at low and high receptor concentration as an indicator of the receptor's conformation. At concentrations $\geq$ 6 nM, m' = 12-14; at concentrations $<$ 2.4 nM, m' $\cong$ 2. If the conformation of the monomer remained the same after dissociation at low receptor concentrations, 6-7 salt bridges would have been expected. The 2 salt bridges observed indicates the free monomer and the monomers within the dimer have different conformations. Binding of the highly purified estrogen receptor to a specific DNA sequence 1 kb upstream of the prolactin gene also depended on receptor concentration. As measured by a nitrocellulose filter assay, very little binding to DNA occurred at receptor concentrations $<$ 1.3 nM. Binding increased sharply at receptor concentrations $>$ 1.3 nM. These data are consistent with the concept that the estrogen receptor dimer is required for high-affinity binding to DNA.

CHARACTERIZATION OF ESTROGEN RECEPTORS IN MOUSE MAMMARY TUMORS

B 127 Mels Sluyser, Bruno Moncharmont and Stefan Zotter, Division of Tumor Biology, The Netherlands Cancer Institute, Amsterdam, The Netherlands

Studies using anti-estrogen receptor (anti-ER) monoclonal antibody JS34/32 (ab) show that in GR mouse mammary tumors the nuclear binding of ER is linear with the radiolabeled E2 binding. The soluble fraction (cytosol) of GR tumors apparently contains two types of ER, one of which binds both E2 and ab, whereas the other binds E2 but not ab. These ERs have been characterized by sucrose gradients, ion exchange chromatography and electrophoresis. Hormone independent GR mammary tumors contain low but significant levels of ER as shown by ab and radiolabeled E2 binding. This ER also has been characterized. Differential immunoperoxidase staining of human and mouse mammary tumor cells in tissue sections using JS34/32 will be demonstrated.

IMMUNOLOGICAL ANALYSIS OF STRUCTURE AND LOCALIZATION OF AVIAN PROGESTERONE RECEPTOR

B 128 **P. Tuohimaa, J. Isola, T. Ylikomi, Univ. Tampere Box 607, 33101 Tampere FINLAND**

A rabbit antiserum against the highly purified B-subunit of chick progesterone receptor, (PR) IgG-RB (Tuohimaa et al., BBRC 119:433, 1984) and monoclonal antibody BF4 (Radanyi et al., PNAS 80:2854, 1983) were used to characterize and localize different forms of PR in the oviduct. Nuclear, cytosolic and microsomal PR were produced by fractional ultracentrifugation. 8S,5S,4S and mero forms of PR were made as described by Tuohimaa et al. (J. Steroid Biochem. 20:429, 1984). Antibody-PR complexes were analyzed by using protein A, sucrose gradient ultracentrifugation HPLC and Western blotting Immunohistochemistry and -electron microscopy were performed as described by Isola et al. (Histochemistry 1986, in press).

8S-form of PR appears to contain either B- or A-subunit redognized by IgG-RB and 90K Ca-dependent kinase recognized by BF4. 4S form contains either A- or B-subunit only. Mero-receptor is proteolytic fragment of A- and B-subunits. Practically all the binding forms of PR are intranuclear, only during the receptor synthesis significant amounts of the receptor are seen in the cytoplasm. Microsomal PR is immunologically similar with nuclear PR, it may represent partially the newly synthetized PR and partially the receptor escaped from the nucleus during the homogenization. 8S and cytosol PR appear to be artefactual receptor forms produced by the homogenization and redistribution of the soluble nuclear proteins.

CHARACTERIZATION OF STEROID HORMONE RECEPTOR POLYMORPHISM.

B 129 J. L. Wittliff, S. M. Hyder, N. A. Shahabi, L. Myatt, A. van der Walt and W. B. Mujaji, Hormone Receptor Laboratory, University of Louisville, Louisville, KY 40292

A major focus of our research is to elucidate the mechanism by which structurally relatively simple molecules, steroid hormones, initiate a wide variety of cellular events via their receptors. Polymorphism of estrogen (ER) and progestin (PR) receptors was detected employing HPLC in size exclusion (HPSEC), chromatofocusing (HPCF), ion-exchange (HPIEC) and hydrophobic interaction (HPHIC) modes using normal and neoplastic breast and uterus. [^{3}H]R5020 and [^{3}H]ORG-2058 were used to determine PR while [^{125}I]iodoestradiol-17β and [^{3}H]tamoxifen were used for ER. Excess unlabeled steroid was employed to assess non-specific association. Tris-HCl and phosphate buffers with and without 10 mM sodium molybdate were used. HPLC was performed in a multi-dimensional manner employing flow-through apparatus for measuring conductivity, pH, UV absorption and [^{125}I]radioactivity. Using TSK-3000 SW and TSK-4000 SW columns for HPSEC, ER exhibited 2-3 isoforms while PR from human tissues separated into 2-5 isoforms. In contrast to size distinguished isoforms, both HPIEC and HPCF distinguished 2-4 isoforms of ER and only 1-2 isoforms of PR. HPIEC detected 2 isoforms of ER in most tissues regardless of the source. HPHIC consistently separated ER into 2 isoforms based upon hydrophobicity. Unexpectedly, immunopurified ER isoforms exhibited protein kinase activity suggesting another factor contributes to receptor polymorphism. Properties of these isoforms suggests an interrelationship of physiological importance such as precursor-product. Supported in part by the Marie Overbey Memorial Grant from the ACS, the Phi Beta Psi Sorority and USPHS Grant 42154 from the NCI.

B 130 A 56K PROTEIN IS RELATED TO THE HUMAN ANDROGEN RECEPTOR. Klaus Wrogemann, Eduardo Rosenmann, Fred Pereira, Kimberly Duerksen, Barbara Nickel, Anne Schwartz, Morris Kaufman and Leonard Pinsky. Departments of Biochemistry and Human Genetics, University of Manitoba, Winnipeg, Manitoba, and Lady Davis Institute, Jewish General Hospital, Montreal, Quebec, Canada.

We have discovered by two-dimensional gel electrophoresis a 56K protein in genital skin fibroblasts (Nature 298, 563 (1982); 304, 740 (1983). The protein is water soluble and appears as doublet spots with pI's of 6.7 and 6.5, which are electrophoretic variants of the same protein. The presence of the protein in general correlates with androgen receptor activity: it is present in male and female genital skin, but not detected in non-genital skin fibroblasts. It is also present in prostatic cancer. It is absent in genital skin cells from 12 of 14 patients with androgen resistance (AR) syndrome due to negligible androgen receptor binding activity. The presence of the protein in the two other AR cell strains virtually rules out that it is androgen induced. Photolytic labeling of intact cells with ^{3}H-methyltrienolone followed by 2-D electrophoresis marks exclusively the two 56K protein spots and is suppressed by excess cold MT but not by triamcinolone acetonide. Nonspecific labeling of other proteins is only detected after 5-10 fold longer exposure times. The data indicate that the protein molecules in the 56K spots cannot represent only the high affinity low capacity receptor. Are we possibly dealing with precursors of the androgen receptor or with "post receptor" molecules waiting to become recycled or degraded? (Supported by the MDAC, MRC, MHRC and NSERC).

B 131 **PROGESTERONE RECEPTOR EXPRESSION IN THE CHICKEN BURSA OF FABRICIUS.** **T.Ylikomi,J.Isola,P.Tuohimaa University of Tampere Box 607, 33101 Tampere FINLAND**

Bursa of Fabricius (BF) is the site of for B lymphocyte maturation in birds. Progesterone receptor (PR) in chick BF was characterized and quatified by steroid binding studies, HPLC, sucrose gradient analysis and immunoblotting. Its cellular localization was studied by immunohistochemistry and combined technique of autoradiography and immunohistochemistry. The PR containing cells were characterized by immunoelectron microscopy and by different histochemical techiques. The antibody used in the study was polyclonal and directed to the chicken oviduct PR. The estrogen sensitivity of the organ and PR expression was studied from early embryonic stages until the involution of the BF. Two estogen sensitive, PR expressing cell types were detected: subepithelial-interfollicular stromal cells and muscle cells linig the BF. PR was not detected inside the lymphoid follicles or in the epithelial cells. The PR was inducible by estrogen administration in these cells from the early stages of development in both sexes and was spontaneously expressed at the age of 10-15 weeks posthatching only in the females.

It is concluded that the bursa of Fabricius is a sex steroid sensitive organ very early during the development. Endogenous estrogens, however, are not able to express PR until after the onset of sexual maturation. This implies that estrogen and progesterone might affect the structural organization through the stromal cells and the function through the muscle cells, but probably not before the onset of puberty.

B 132 MOLECULAR FORMS OF THE ANDROGEN RECEPTOR:CHARACTERIZATION WITH A POLYCLONAL ANTISERUM, Charles Y-F. Young, Michael P. Johnson, David R. Rowley and Donald J. Tindall, Baylor College of Medicine, Houston, Texas 77030

Antibodies to steroid receptor proteins have proven to be powerful probes for elucidating molecular structure. Recently, we discovered a human serum which contained polyclonal auto-antibodies that recognized the androgen receptor, but not other steroid receptors or steroid binding proteins in body fluids. These antibodies were exclusively IgG class and kappa light chain specific. The antiserum interacted with the 4.8S, 54Å monomeric form of the receptor in the presence of high salt (0.4M KCl), sedimenting at 16.2S on a 10-50% sucrose density gradient and eluting in the void volume of an agarose A1.5m gel filtration column. It also reacted with the 9S oligomeric form of the receptor under low salt conditions to form heavier immune complexes. In addition to the 4.8S, 54Å intact form of the receptor, fragments of 3.0S, 20Å, and 3.9S, 35Å could be generated reproducibly from rat ventral prostate or Dunning R3327 H tumor. The generation of these fragments was due to the actions of endogenous proteases. The antiserum reacted with both the 20Å and the 35Å fragments to form intermediate-sized 7-12S complexes in 10-50% sucrose density gradients and complexes greater than 60Å in A1.5m agarose gel filtration columns. Taken together, these data suggest that the androgen receptor provides more than one epitope for antibody interaction, and that the 20Å meroreceptor contains at least one antigenic determinant. Further analysis of the structure and function of the androgen receptor using these antibodies is in progress. (Supported by grants CA32387 and DK37688 from NIH and DCB8416979 from NSF)

Regulation of Gene Expression

B 133 A DNA-BINDING PROTEIN THAT RECOGNIZES THE REGULATORY SEQUENCES OF A RAT α_{2u} GLOBULIN PROMOTER, William Addison[1] and David Kurtz[2], Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Transcription of rat α_{2u}-globulin genes can be induced by glucocorticoids. This induction is a secondary response to the hormone ie. ongoing protein synthesis is required for the hormone to exert its effect. We have recently identified a sequence between -115 and -160 of a cloned α_{2u} gene that is required for this regulation (Mol. Cell. Biol. 6, 2334-2346 (1986)). The sequence's most striking feature is the presence of dyad symmetry; a six bp sequence GAACCG is separated by 22 bp from its inverted complement. We have detected a protein in crude extracts of rat liver, rat kidney, and cultured mouse L-cell nuclei that binds to the elements of dyad symmetry in the α_{2u} regulatory region. Mutant promoters which cannot bind the protein at one or the other site in the regulatory region show impaired induction of α_{2u} in response to dexamethasone. While the protein seems to be required for the induction, it itself does not appear to be regulated by glucocorticoids.

[1] present address: Dept. of Genetics, University of Alberta, Edmonton, Alta., Canada.

[2] present address: Dept. of Pharmacology, Medical University of South Carolina, Charleston, South Carolina.

B 134 COMPARATIVE BINDING OF THE GLUCOCORTICOID- AND ANTIGLUCOCORTICOID-RECEPTOR COMPLEXES TO A REGION UPSTREAM FROM THE RAT TYROSINE AMINOTRANSFERASE GENE.

Gisèle Beck, Sylvette Chasserot and Geneviève Genot. IBMC-CNRS, Strasbourg F67084.

Glucocorticoid (dexamethasone) and antiglucocorticoid (RU38486) bind to the same receptors but from competition experiments it appears that the resulting complexes behave differently for the binding to acceptor sites in the nucleus. The difference in the nuclear binding between the agonist- and the antagonist-receptor complexes may trigger the machinery of antagonistic action.

To gain further information, the interactions of dexamethasone- or RU38486-receptor complexes with cloned fragments from the 5'-flanking region of the rat tyrosine-aminotransferase gene (TAT gene) were investigated. The receptor-DNA binding assays were run by competition of unlabeled DNA fragments with DNA-cellulose for binding labeled complexes in cytosol. Two fragments were tested, fragment A, located between 0 and 1.3 Kb and fragment B, located between 1.3 and 3 Kb upstream of the transcription initiation site. Only the fragment B was able to bind the agonist-receptor complexes. This observation is in agreement with the characterisation of glucocorticoid control elements located 2500 bp upstream from the transcription initiation site of the TAT gene (Schutz et coll. 1986, Biol. Chem. Hoppe Seylers n°367 Suppl. p 84). On the other hand, the antagonist-receptor complexes bind indifferently to the fragments A and B. These results favour the hypothesis that the antiglucocorticoid-receptor complexes recognize two different nuclear acceptor sites simultaneously or sequentially.

B 135 GLUCOCORTICOID REGULATION OF PLASMINOGEN ACTIVATORS (PAs) IN TWO MAMMARY CELL LINES, *N.Busso,**D.Belin, C.Failly-Crépin and *J.D.Vassalli,
Institut de Recherches Scientifiques sur le Cancer. Villejuif, France. *Institut d'Histologie et d'Embryologie and **Département de Pathologie. Centre Médical Universitaire, Geneva, Switzerland.

We have studied the plasminogen-dependent proteolytic activity of two human mammary cell lines, MDA-MB-231 and HBL-100. The two lines differed both in their constitutive pattern of PA production, and in their response to the synthetic glucocorticoid dexamethasone. A zymographic analysis of the PAs produced showed the presence of u-PA in cell extracts and culture media, whereas t-PA was detected only in the culture media of both cell lines. Additional bands of activity (Mw >100 Kd) were detected in the culture media. These were produced by complexes between PAs and PA inhibitors. Treatment with dexamethasone (10^{-7} M) decreased u-PA activity in both cell lines, and increased t-PA activity only in HBL-100 cells. The inhibition of u-PA and the stimulation of tPA activities were accounted for by changed patterns of enzymes synthesis, as indicated by immunoprecipitation of (^{35}S) cysteine-labeled proteins with specific antibodies. Hormonal modulation was associated with changes in the steady-state levels of PA mRNAs as quantified using u-PA and t-PA ^{32}P-labeled c-RNA probes . After a 4h incubation in presence of dexamethasone , the steady-state level of u-PA mRNA was decreased 3 fold in MDA-MB-231 and 2 fold in HBL-100 cells; t-PA mRNA level was increased 2 fold in HBL-100 cells only . Cycloheximide did not prevent these effects, suggesting a direct action on the transcription of PA genes. In addition to PAs, the two cell lines also secreted a PA inhibitor immunologically related to that produced by the endothelial cells. This inhibitor was increased by dexamethasone in MDA-MB231 cells exclusively. The effect of the synthetic antiglucocorticoid RU 38486 has been assessed in this system by nuclear run-on transcription: the action of dexamethasone on u-PA and tPA gene transcription was reversed by the antiglucocorticoid.

B 136 CELL-SPECIFIC AND HORMONAL REGULATION OF HUMAN GROWTH HORMONE AND CHORIONIC SOMATOMAMMOTROPIN GENES, Peter A. Cattini, John D. Baxter and Norman L. Eberhardt, University of California, San Francisco, CA 94143.

Human growth hormone (hGH) and chorionic somatomammotropin (hCS) genes belong to a family of related genes. Despite extensive sequence homology (>90%), the hGH and hCS genes are differentially expressed in the pituitary and placenta, respectively. Whereas rat (r) GH gene expression is known to be regulated by triiodothyronine (T_3), such regulation of the hGH and hCS genes is unknown. We have used stable and transient gene transfer techniques to study hGH-1 and hCS-1 gene expression and regulation by T_3. Both hGH-1 and hCS-1 genes were expressed at comparable levels in stably trasfected rat pituitary (GC) cells and the transcripts were of the expected size (~ 1.1 kb). Whereas hCS and endogenous rGH mRNA levels were increased by T_3 treatment, hGH mRNA levels were decreased. Hybrid genes containing hGH-1 and hCS-1 gene 5'-flanking sequences, including the promotor, fused upstream of the chloramphenicol acetyl transferase (CAT) gene, were used to transiently transfect GC cells and non-pituitary cell lines. The hGH-1 and hCS-1 gene hybrids were preferentially expressed in GC cells. Expression of the hCS-1 gene or gene hybrid in GC cells may be related to the high sequence homology it shares with the hGH-1 gene, homology not shared between the rGH and rCS genes. Accordingly, tissue-specific elements in the hGH-1 and hCS-1 5'-flanking DNA might be related to those present in the rGH gene which account for pituitary-specific expression. T_3 treatment resulted in a 0.7-fold decrease in CAT activity with the hGH-1 gene hybrid but a 4.0-fold increase with the hCS-1 gene hybrid, indicating that T_3 responsive elements are contained in their 5'-flanking DNA.

B 137 IDENTIFICATION OF A NEW GLUCOCORTICOID-INDUCED LYSIS GENE PRODUCT, Mark M.Compton and John A. Cidlowski, University of North Carolina, Chapel Hill NC 27514.

Studies in our laboratory and others indicate that glucocorticoid-mediated internucleosomal DNA degradation plays a central role in steroid-mediated lymphocyte cell death. To further elucidate the mechanism of this nucleolytic process we have sought to identify the gene product(s) involved. Adrenalectomized rats were treatec *in vivo* with dexamethasone, sacrificed 5 hours later and nuclear thymocyte proteins extracted with 0.6 M NaCl. Nuclease activity of specific nuclear proteins was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis in gels containing DNA. DNase activity was measured by degradation of DNA in the gel matrix and visualized by ethidium bromide staining. Glucocorticoid treatment resulted in the induction of two major protein families, a 30-32kD doublet and a series of 3-4 proteins of 12-19kD, both of which expressed prominen DNase activity. Quantitation of this protein induction revealed an increase of 137±6% in the high molecular weight family and a more dramatic 342±24% increase in the lower molecular weight family. Induction of this nuclease was demonstrated by *in vivo* labelling with [^{35}S]methionine. This response was both time and steroid concentration dependent and specific for the glucocorticoid class of steroid hormones. Furthermore, nuclease induction could be blocked by the glucocorticoid antagonist RU 486, indicating a receptor mediated process. Incubation of nuclear extracts from dexamethasone treated rats with isolated nuclei from glucocorticoid resistant cells resulted in internucleosomal DNA degradation, whereas extracts from control animals were virtually inactive. Moreover, the kinetics of nuclease induction parallel those for glucocorticoid-mediated DNA degradation. These findings are consistent with the concept that glucocorticoids mediate a nucleolytic lysis gene product responsible for lymphocytolysis. Supported by NIH AM 32078.

B 138 A PRIMARY ECDYSTERONE-CONTROLLED GENE LOCUS IN CHIRONOMUS TENTANS CODES FOR MULTIPLE TRANSCRIPTS. Karoline Dorsch-Häsler, Kurt Amrein, Beat Lutz, Alicja Stasiak, and Markus Lezzi, Federal Institute of Technology, 8093 Zürich, Switzerland.

The primary ecdysterone-activated gene I-18C of Chironomus tentans has been cloned and characterized. Three different transcripts (4.6. kb, 4.55 kb and 1.8 kb), produced by differential splicing, were shown to have a common transcription initiation site. The three transcripts differ in their metabolism and in their tissue distribution. While transcription of all three transcripts could be stimulated by the steroid moulting hormone ecdysterone in an epithelial cell line, only the 4.6 kb and 4.55 kb transcripts were detected in RNA isolated from salivary glands of prepupae. Expression of the 1.8 kb transcript in the epithelial cell line could also be stimulated by heat shock. Accordingly, steroid receptor-binding sequences and heat shock regulatory elements are located in the 5' upstream region of the gene. The 1.8 kb transcript was found to be polysome-associated and it was shown to code for a 16.6 kD protein. In contrast, the 4.6 kb and 4.55 kb RNA species appear in the non-polysomal fraction as ribonucleoprotein particles.

B 139 **DIFFERENTIAL REGULATION OF TWO TCDD-INDUCIBLE GENES** Tracy J. Dunn and Henry C. Pitot, McArdle Laboratory for Cancer Research, Univ. of Wisconsin, Madison, WI

TCDD and certain other aromatic hydrocarbons are believed to regulate a specific subset of genes by acting through the Ah receptor using a mechanism analogous to that proposed for steroid hormone action. We have used cDNA clones which identify the mRNAs of two TCDD-inducible xenobiotic metabolizing enzymes in the rat, cytochrome P-450c and TCDD-aldehyde dehydrogenase, to show that the regulation of these two genes by TCDD differs greatly in their dose-response relationships, induction kinetics, and tissue distribution. The TCDD-aldehyde dehydrogenase requires at least a 20-fold higher dose of TCDD to reach maximal induction in the liver than does cytochrome P-450c and the transcriptional reponse of TCDD-aldehyde dehydrogenase does not plateau until between 4 and 8 days after intraperitoneal administration of TCDD, although the transcriptional response of the cytochrome P-450c gene reaches its maximum level as early as 12 to 24 hours after dosing. The induced transcriptional responses of both genes then remain at the plateau level until at least 22 days after administration of TCDD. The tissue distribution in both TCDD-induced and uninduced rats for both cytochrome P-450c and TCDD-aldehyde dehydrogenase was investigated. Significant differences in the regulation of the two genes in various tissues suggest non-coordinate regulation of these two genes. The expression of either of these two genes can only be demonstrated in tissues possessing the Ah receptor, but cannot be correlated with the level of receptor present. We present data which suggests that TCDD can regulate the transcription of specific genes in different ways and that complex regulatory mechanisms are likely involved in mediating this differential gene regulation at the level of transcription.

B 140 IN SITU DETECTION OF TCDD RECEPTOR BINDING TO A REGULATORY REGION OF THE CYTOCHROME P_1-450 GENE. Linda K. Durrin and James P. Whitlock, Jr., Dept. of Pharmacology, Stanford University School of Medicine, Palo Alto, CA 94305.

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces transcription of the cytochrome P_1-450 gene in mouse hepatoma cells through binding and activation of an intracellular receptor protein. We have utilized the in situ exonuclease III protection method of Wu (Nature 309, 229, 1984) to probe for proteins bound to a constitutive nuclease hypersensitive region in chromatin flanking the cytochrome P_1-450 gene. Restriction enzyme cutting at a unique site within the hypersensitive region followed by exonuclease III digestion detects the presence of a bound protein -1650 bp upstream from the transcription start site. This region has been previously demonstrated to behave as a TCDD-inducible enhancer in gene-transfer experiments. Bound protein is found in nuclei isolated from TCDD treated cells but not control cells, and is a primary response to the inducer as indicated by the rapidity with which it appears (within 1 h after TCDD treatment) and its insensitivity toward cycloheximide inhibition of protein synthesis. Furthermore, a variant cell line defective in the nuclear uptake of TCDD receptors fails to accumulate the bound protein in response to TCDD although it maintains the nuclease hypersensitive region. These results are consistent with a TCDD-dependent interaction of the TCDD receptor with a regulatory sequence flanking the cytochrome P_1-450 gene. We postulate that preformed chromatin conformations such as nuclease hypersensitive regions may be a prerequisite for accessing regulatory sequences to trans-acting factors involved in transcriptonal activation.

B 141 HORMONAL REGULATION OF THE CHICKEN OVALBUMIN GENE IN TRANSGENIC MICE. Alex Elbrecht, Francesco J. DeMayo, Ming-Jer Tsai and Bert W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.

Six independent strains of transgenic mice carrying the natural chicken ovalbumin gene were generated by microinjection of single cell embryos with linearized DNA fragments containing the entire ovalbumin coding region, with 4 Kb of 5'-flanking DNA and 500 bp of 3'-flanking sequence. To date we have examined two of the strains (OV20 and OV33) for expression. Since the ovalbumin gene is induced by steroid hormones, initial screening with Northern blots was done after treatment with estradiol-17β. Strain OV20 did not express the ovalbumin gene. Strain OV33 contained 2 independent integrations of approximately 2 and 8 copies of the gene. In mice containing only the 2 copy integration the ovalbumin gene is expressed and the RNA is correctly processed. The highest levels are in the testes and are at least partially androgen dependent. Thus, DNA elements involved in transcription and hormone regulation of the chicken ovalbumin gene are encoded in the injected DNA fragment and are recognized by homologous factors in the mouse.

B 142 DIFFERENTIAL EXPRESSION OF HUMAN KERATIN GENES IN EPIDERMIS. E. Fuchs, G. Giudice and A. Tyner, The University of Chicago, Chicago, IL 60637. The keratins (40-70kd) are the major structural proteins of the epidermis. There are two distinct types (I and II) of keratins based on sequence and hybridization studies. Both types of keratins seem to be important for filament assembly, and all epidermal cells express at least one member of each type. In the basal layer, a single type I (K14) and a single type II (K5) keratin are expressed. During the course of terminal differentiation, new mRNAs encoding keratins K10 and K11 (type I), and K1 and K2 (type II) are synthesized. This differentiative process can be induced in tissue culture by reducing the level of vitamin A in the medium. This induction may be relevant in vivo, where terminally differentiating cells are continuously moving away from the supply of vitamin A in the blood vessels of the dermis. When skin is wounded, a set of keratins (K6, K16 and K17) is transiently expressed. These keratins can be induced in vitro when skin is placed in culture medium. Recently, we have shown that the induction of these hyperproliferation-associated keratins may at least initially be at the posttranscriptional level. We have now isolated and sequenced a number of the human epidermal keratin genes. Using gene transfection studies, we have begun to examine the molecular mechanisms underlying the regulation of different sets of epidermal genes. In addition, we have examined a) the role of both types of keratins in filament assembly, and b) the functional significance of the differential expression of different sets of keratins.

B 143 DNA METHYLATION DEPRESSES RAT GROWTH HORMONE GENE EXPRESSION, Marcia Gaido and Jeannine Strobl, West Virginia University, Morgantown WV 26506. There is a tissue specific pattern of methylation at a CGCG sequence 144 basepairs upstream of the rat growth hormone (rGH) transcription initiation site. We have now directly tested the effect of site specific methylation at this site on rGH promoter activity. 1.5 kilobasepairs of rGH promoter sequences were inserted in front of two bacterial indicator genes, Neo and CAT. A CGCG specific methylase (M-BsuE) was isolated from B.subtilis ISE15 and used to methylate rGH-CAT and rGH-Neo. Methylated and unmethylated fusion genes were transferred into GH_3 rat pituitary tissue culture cells by calcium-phosphate coprecipitation or electroporation. Cells transfected with rGH-Neo were harvested after 2 days, replated at 5 x 10^5 cells/10cm^2 dish in selective media (400 µg/ml G418), and counted after 2 weeks. Cells were harvested 24 hours after transfection with rGH-CAT and acetylation of ^{14}C-chloramphenicol measured in whole cell extracts. Methylation of rGH-Neo resulted in a 60-80% decrease in rGH promoter activity. Methylated rGH-CAT exhibited a 68% decrease in promoter activity. In contrast, methylation of RSV-Neo and RSV-CAT fusion genes resulted in a 37% decrease and 50-70% increase, respectively, in promoter activity. We conclude that methylation of fusion genes does not result in a general depression of promoter activity and demonstrate for the first time a direct relationship between site specific methylation of the rGH promoter and a decrease in rGH promoter activity.

B 144 **NUCLEAR FACTOR(S) RECOGNIZE THE HCMV 1EI ENHANCER, Peter Ghazal, Henryk Lubon & Lothar Henninghausen. NIH, NIDDK, Bethesda, MD 20892**

The major immediate-early gene I (1EI) of the human cytomegalovirus (HCMV) contains one of the strongest known enhancers upstream of its promoter. We report here results from in vitro transcription and competition experiments (in crude Hela cell nuclear extracts) which indicate that trans-acting factor(s) are responsible for the CMV enhancer activity in vitro. The crude nuclear extract was fractionated over successive columns of heparin agarose, DEAE Sepharose and phophocellulose. Each column was stepped washed with increasing concentrations of salt. In vitro transcription reconstitution experiments were performed from the various chromatographic fractions. These data demonstrate that in addition to the PolII fraction at least two other fractions (the heparin agarose flow through (HAO.1) and the phosphocellulose 1M KCl stepwash (P11-1) are also necessary for efficient in vitro transcription of the HCMV 1EI gene. On the basis of two criteria, mobility shift assays and DNAseI protection assays, three sites of specific protein - DNA sequence interactions have been determined for the HAO.1 and P11-1 fractions. These are located for the HAO.1 at nucleotide positions -440 to -420 and for the P11-1 at -473 to -443 and -335 to -315 within the HCMV enhancer region.

B 145 STUDIES ON TWO CHOLESTEROL REGULATED PROMOTERS IN ANIMAL CELLS, Gregorio Gil, Timothy Osborn, Joseph L. Goldstein and Michael S. Brown, UTHSCD, Dallas, Texas 75235.

We are interested in the role of negative feedback regulation in the maintenance of cholesterol homeostasis. To this end we are studying the process whereby excess intracellular cholesterol inhibits transcription of the genes encoding 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase and HMG CoA synthase, the enzymes that catalyze the rate limiting steps in the biosynthesis of cholesterol. We have already demonstrated that transcriptional inhibition in vivo is a property of the 5' flanking region of HMG CoA reductase gene. Using in vitro transcription and foot printing assays, we have now defined specific and unique DNA-protein interactions in the 5' flanking-promoter region that are essential to basal promoter activity. The relationships between the different DNA-protein interactions and the mechanism of cholesterol mediated suppression of transcription will be discussed.

B 146 STEROID-DEPENDENT BINDING OF TRANSCRIPTION FACTORS TO MMTV CHROMATIN in vivo. Gordon L. Hager, Anna Tate Riegel and Michael G. Cordingley, National Cancer Institute, Bethesda, MD 20892 USA.

We have employed an exonuclease III-resistance assay to detect the interaction of factors with the glucocorticoid-inducible promoter of MMTV in vivo. The promoter was amplified on bovine papilloma virus minichromosomes to approximately 200 copies per cell, permitting high-resolution analysis of ExoIII-resistant boundaries on MMTV sequences. Two separate factors are shown to bind to the promoter in response to induction. Access of ExoIII is unaffected by hormone treatment, indicating that the hormone-dependent boundaries result from actual binding of the proteins, rather than from altered enzyme access. Receptor binding at the hormone response element therefore induces the binding of at least two putative transcription factors to the MMTV promoter. High-affinity binding to DNA can be demonstrated for both factors in nuclear extracts, and their apparent concentration is unaffected by hormone treatment of cells prior to extract preparation. We also find that nucleosomes are positioned over specific sequences in the MMTV LTR. The second member of this phased array appears to be selectively lost during transcriptional activation. We discuss the ramifications of these observations for the mechanism of steroid hormone action.

B 147 IDENTIFICATION OF AN ANDROGEN RESPONSE ELEMENT IN THE 5'-FLANKING REGION OF TWO RAT SEMINAL VESICLE GENES WHOSE EXPRESSION IS DEPENDENT UPON TESTOSTERONE, Stephen E. Harris, Wallace L. McKeehan and Marie A. Harris, W. Alton Jones Cell Science Center, Inc., Lake Placid NY 12946.

The 5'-flanking regions of the two androgen responsive rat seminal vesicle genes, SVS IV (-523 to -10) and SVS VI (-300 to +216) were cloned into the Hind III site of pSV0-CAT. These 5'-SVS-CAT fusion constructions (CAT = chloramphenicol acetyltransferase) were then used in transient gene transfer CAT expression assays using the androgen responsive hamster tumor cell line, DDT1, which has high levels of androgen receptor. In the SVS IV-CAT and SVS VI-CAT fusion genes, testosterone (T) (10^{-8} M) added to a serum-free medium for 24 hr resulted in 13-fold and 4-fold induction in CAT activity, respectively. pSV0-CAT and pSV2-CAT served as negative and positive controls. With the SVS IV-CAT fusion gene, using BAL 31 nuclease deletions, the androgen response element(s) has been mapped to the -350 to -90 region using the DDT1 tumor cells. Further mapping is underway. We are also using primary rat seminal vesicle and prostate epithelial cell cultures for transfection and CAT assays using the SVS-CAT constructions. The primary cells are grown in a serum-free WAJ404 media, with various growth factors ± T. With the SVS IV-CAT fusion gene transfected into primary seminal vesicle cells we have observed a 3-4-fold increase in CAT activity with T. A BAL 31 deletion (Δ -60 to -380) of the SVS IV-CAT fusion gene, gives no increase in CAT activity with T. Rat seminal vesicle cells grown on extracellular matrix (ECM) where the seminal vesicle epithelial cells seem to "differentiate" are now being tested in transient gene transfer experiments with the SVS-CAT fusion genes and deletions.

B 148 GLUCOCORTICOID REGULATION OF GENE EXPRESSION IN HUMAN PITUITARY ADENOMAS, Randi E. Isaacs, Paul R. Findell, Charles B. Wilson, Pamela Mellon and John D. Baxter, University of California, San Francisco, CA 94143.

We have made use of human pituitary adenomas as a novel in vitro system to study multihormonal regulation of the mRNAs encoding the anterior pituitary hormones. Study of the genes for human growth hormone (hGH), prolactin (Prl), adrenocorticotropin (ACTH) and α-and β-subunits of thyroid stimulating hormone (TSH), luteinizing hormone (LH) and follicle stimulating hormone (FSH), has been limited by the lack of human cell lines expressing the endogenous genes. Messenger RNA levels and GH secretion from primary cell cultures of four hGH-secreting pituitary adenomas were stimulated 2- to 4-fold (P<.05) by the glucocorticoid dexamethasone (Dex, 10μM). Insulin (5nM) had no significant effect on either mRNA or protein, but when added concurrently with Dex it significantly inhibited the Dex induction of both, suggesting that the pathways for insulin and Dex regulation of hGH gene expression may be linked. Dex caused a 2-fold stimulation of Prl mRNA from one lactotropic adenoma. ACTH secretion from a corticotropic adenoma demonstrated a 66% inhibition of protein secretion by Dex (P<.05) and a small stimulation (25%) with insulin treatment. Dex plus insulin was not significantly different from Dex alone. These findings were also reflected in the mRNA levels, demonstrating that adenomatous corticotropes retain glucocorticoid sensitivity despite their tumor tissue origin. Dex significantly inhibited α-subunit mRNA from a TSH-secreting adenoma, while it stimulated α-subunit mRNA from the one non-secreting adenoma expressing the gene. These are the first demonstrations of hormone regulation of anterior pituitary peptide gene expression in an endogenous system.

B 149 MOLECULAR CLONING OF cDNA AND GENE FOR THE CHICKEN AVIDIN, M.S. Kulomaa, R.A. Keinänen, P.A. Kristo[1], M.L. Gope[2], T. Zarucki-Schulz[2] and B.W. O'Malley[2], Univ. Tampere, Tampere, Finland; [1]Res. Lab. Alko Ltd., Helsinki, Finland; [2]Baylor College of Medicine, Houston, Texas, USA.

Three cDNA clones for the chicken avidin were isolated from the oviduct cDNA library by screening with antibodies and synthetic oligodeoxynucleotides. Amino acid sequence derived from the cDNAs was identical to the known sequence of avidin. A 597 bp clone, λAV4, containing the entire coding region was isolated using a cDNA as a probe. A signal peptide of 24 amino acids with typical structural determinants for cleavage was found to precede the first amino acid residue of avidin. The insert contained a noncoding region of 43 and 84 bp at the 5' and 3'-end respectively. A predicted polyadenylation site was located 20 bp prior to the poly(A)-tail. The size of ∿700 nucleotides was detected for the avidin mRNA by RNA hybridization (Northern) analysis. A specific induction of the avidin mRNA by progesterone was observed in the oviduct, but not in the liver or kidney. More precise studies on hormonal regulation of induction have now been initiated. Only a single fragment was detected when genomic DNA from the spleen was digested with BamHI and examined by DNA hybridization (Southern) analysis suggesting a single copy gene for avidin. A clone, λAVg12201, with an insert of ∿10-12 kb was detected when a genomic library was screened by a cDNA. The clone was digested with EcoRI and subcloned into a plasmid vector. One of the subclones, pAVg1.8, was found to contain the 3'-end of the avidin gene within the insert of 1.8 kb. The detection of a subclone containing the 5'-end and sequencing analysis of the avidin gene are now in progress.

B 150 VARIANT CELL LINES WITH ALTERED GLUCOCORTICOID RESPONSIVENESS SELECTED BY WAY OF AN MMTV PROMOTER FUSION WITH A GENE ENCODING A SELECTABLE MARKER, Wen-Liang Kuo and David O. Peterson, Department of Biochemistry and Biopysics, Texas A&M University, College Station, Texas 77843.

A genetic approach was designed to select variant cell lines with altered responsiveness to glucocorticoid hormones. The *Escherichia coli* xanthine-guanine phosphoribosyl transferase gene (*Ecogpt*) was used as the selectable marker and was linked to the glucocorticoid-responsive promoter of mouse mammary tumor virus (MMTV) to form plasmid pMTVgpt. This plasmid and a purified thymidine kinase gene from herpes simplex virus were cotransfected into mouse Ltk$^-$ cells. Stable tk$^+$ transformants were selected, and independent clones were analyzed with regard to sequence organization of pMTVgpt DNA and expression of *Ecogpt*. Several transformants in which *Ecogpt* expression was induced by glucocorticoids were identified and served as parental cell lines for selection of variants. Mycophenolic acid was used to select variant cells which could constitutively express *Ecogpt* in the absence of glucocorticoids, and 6-thioxanthine was used to select against expression of *Ecogpt*. No obvious changes in pMTVgpt sequence organization were observed in the variant cells. A simple *cis/trans* test was performed using a transient expression assay in which pLC1, a plasmid containing the MMTV promoter fused to the coding sequences for bacterial chloramphenicol acetyltransferase (CAT), was introduced into each variant cell line. Transiently expressed CAT activity in some variant cell lines was altered relative to parental cells, suggesting that *trans*-acting factor(s) might be involved in altering glucocorticoid responsiveness in the variants. The glucocorticoid receptor protein is one such *trans*-acting factor, and several biochemical assays of receptor activity have been performed with the variant cells.

B 151 ANDROGEN-RESPONSIVE SEQUENCES OF THE MOUSE SEX-LIMITED PROTEIN GENE. Fabrizio Loreni, Martha Kalff and Diane M. Robins, Columbia University, N.Y., NY 10027. To study DNA sequences involved in hormonal regulation, we have compared murine C4 and Slp genes as they show extensive homology but are differentially expressed. C4 is the fourth component of complement; Slp, or sex-limited protein, lacks complement activity and is regulated by androgen, unlike C4. The cloned full-length Slp gene shows testosterone-enhanced expression following transfection into a mammary carcinoma cell line, while C4 does not. Gene fusion constructs were made that link upstream and promoter regions of Slp or C4 to the bacterial chloramphenicol acetyltransferase (CAT) gene. A sequence within 3 kb upstream of the Slp gene confers androgen-inducible expression on the CAT gene in transient transfection assays. In particular, this element serves as an inducible promoter when placed in an inverted orientation directly in front of the CAT gene. CAT fusion mRNA transcription starts at multiple sites within this Slp-derived DNA. Therefore, this element seems to mediate androgen-responsiveness independently of position or orientation. While both C4 and Slp promoter fragments drive CAT expression efficiently in fibroblasts, neither does so in the hormone responsive cell line. These and other results suggest that a negative element blocks regulation by the hormone-responsive sequences in non-homolgous cells. Presumably in vivo the negative effect is overcome in expressing tissues (liver). Thus at least two distinguishable DNA regions are involved in hormonal and tissue-specific regulation of the Slp gene.

B 152 Structure and Expression of a Gene which is Androgen- and Zinc-Regulated in Rat Prostate. R.J. Matusik, P. McNicol, and A.M. Spence. Dept. of Physiology, U. of Manitoba, Winnipeg, Manitoba, R3E 0W3. We previously reported the cloning of a cDNA (pM-40) to an androgen-regulated, zinc-inducible mRNA which is expressed at high levels in the dorsolateral prostate of the rat. The sequence of pM-40 encodes a secreted protein of Mr 20.5 Kd or a non-secreted protein of 19 Kd. Two proteins, of Mr 19 Kd and 20.5 Kd are produced when synthetic transcripts of M-40, produced from a linked bacteriophage promoter, are translated in a rabbit reticulocyte lysate. These results suggest that the M-40 protein is synthesized in secreted and intracellular forms in vivo. A cloned fragment of rat genomic DNA reveals that the gene is comprised of seven exons spanning approximately 17 kbp. The exons and their flanking regions have been sequenced. Canonical CCAAT and TATAAA boxes are present 48 and 27 bp, respectively, upstream of the 5′ end of the mRNA, which was located by S1 mapping. Two elements in the 5′ flanking region bear striking homology to the metal regulatory elements which precede metallothionein genes. Nuclear runoff transcription experiments demonstrate that zinc induces M-40 expression at the level of transcription. Androgens, in contrast, exert primarily a post-transcriptional effect on M-40 expression. (Supported by the Medical Research Council of Canada.)

B 153 **REGULATION OF SURFACTANT APOPROTEIN GENE EXPRESSION IN FETAL LUNG,** Carole R. Mendelson and Vijay Boggaram, Univ Tex Hlth Sci Ctr, Dallas TX 75235. **Pulmonary surfactant, a developmentally- and hormonally-regulated lipoprotein, reduces surface tension at the alveolar-air interface. The major apoprotein of surfactant, a sialoglycoprotein, $M_r \simeq 35{,}000$, is believed to serve an important role in surfactant function. Previously, we found that surfactant apoprotein gene expression is initiated in fetal rabbit lung tissue after day 26 of gestation. Cortisol and cyclic AMP analogues increase the levels of surfactant apoprotein and its mRNA in lung explants from 21-day fetal rabbits. To investigate further the regulation of surfactant apoprotein gene expression in fetal lung tissue, we have isolated a cloned cDNA specific for the major rabbit surfactant apoprotein. This cDNA hybridizes to two major species of mRNA (2.0 and 3.0 kb in length) that are coordinately induced in rabbit lung tissue during development and with hormonal treatment. The two mRNA species appear to be encoded by a single gene. Cortisol treatment of lung explants from 21-day fetal rabbits caused an induction of surfactant apoprotein mRNA levels that was first observed after 24-48 h of incubation. Bt_2cAMP caused a marked induction of surfactant apoprotein mRNA levels by 4-6 h of incubation; a stimulatory effect of cyclic AMP was often detectable as early as 2 h after its addition to the culture medium. Cycloheximide (CHX, 2μg/ml) markedly reduced the levels of surfactant apoprotein mRNA in both control and Bt_2cAMP-treated explants after 4 h of incubation. This inhibitory effect of CHX was reversed within 6 h of its removal from the medium. These findings are suggestive that a protein with a relatively short half-life mediates the expression of the surfactant apoprotein gene and its induction by cyclic AMP.**

B 154 A GLUCOCORTICOID RECEPTOR BINDING SITE IS NOT SUFFICIENT TO CONFER HORMONAL RESPONSE TO THE HERPES SIMPLEX THYMIDINE KINASE PROMOTER.
David D. Moore and Mark A. Malia, Dept. Molecular Biology, Mass. General Hospital, Boston, MA 02114.

We have inserted a 24 base pair oligonucleotide containing a glucocorticoid receptor binding site into a number of positions near heterologous promoters. In all cases the cloned site retains full capacity to bind receptor in vitro. Inserting a single binding site upstream of truncated versions of the human metallothionein IIA or mouse metallothionein I promoters confers hormonal response equal to or greater than that resulting from the endogenous binding site in the intact human metallothionein IIA promoter. However, in contrast to results of others using MMTV restriction fragments, insertion of the site upstream of the herpes thymidine kinase promoter at -109 or -197 does not confer any glucocorticoid responsiveness. Insertion of the murine sarcoma virus enhancer at either position greatly increases expression and confers glucocorticoid responsiveness. A simple interpretation of these results is that the mammalian promoters and viral LTRs bind factors which are required in addition to receptor for response to glucocorticoids. Preliminary results support the resultant prediction that addition of binding sites for such factors should make the oligo/TK constructs hormonally responsive.

B 155 GLUCOCORTICOID REGULATION OF PRONATRIODILATIN GENE EXPRESSION,
Mona Nemer, Stefania Argentin, Jean-Pierre Lavigne, Michel Chamberland and Jacques Drouin, Institut de recherches cliniques de Montréal, Montréal, Canada H2W 1R7.
Pronatriodilatin (PND) is the protein precursor to atrial natriuretic factor (ANF), a 28 AA peptide hormone with potent natriuretic, diuretic and vasorelaxant activities. Northern blot analysis using a rat PND cDNA clone indicates that PND mRNAs of similar size are found in heart atria and ventricles. However, PND mRNA concentration is 150 fold lower in ventricles. In previous studies, we established that changes in salt intake and extra cellular fluid volume alter PND mRNA levels. We have now undertaken to investigate the hormonal control of atrial and ventricular PND gene expression. Since ANF inhibits adrenal steroidogenesis, we tested the putative feedback action of adrenal steroids on PND gene expression. Administration of dexamethasone (DEX) to adrenalectomized rats increases atrial PND mRNA levels 5 fold; ventricular PND mRNA levels are similarly stimulated. Glucocorticoid stimulation of cardiac PND mRNA concentration also occurs in primary cultures of atrial and ventricular cardiocytes, indicating that the DEX effect is at the heart level. Thus glucocorticoid stimulation of PND gene expression may account for many cardiovascular effects of glucocorticoids. In order to investigate the mechanism of this glucocorticoid stimulation, we have obtained stable transfectants containing the entire rat PND gene or a hybrid gene constituted of the rat PND promoter fused to coding sequences for bacterial neomycin resistance (neo). RNase mapping analysis indicates correct initiation of transcription from both constructs. These transfectants are used to identify glucocorticoid responsive sequences in the PND gene. (Supported by MRC, QHF and FCAR, Canada)

B 156 GENETIC STUDIES ON REGULATION OF GENE EXPRESSION BY GLUCOCORTICOID HORMONES, Mukund Nori and Michael R. Stallcup*, Biol. Dept., Univ. S. Carolina, Columbia, S.C. 29208 & *Dept. of Pathology, USC Med. School, Los Angeles, CA 90033.
A murine T lymphosarcoma cell line infected with mouse mammary tumor virus (MMTV) has been used for studies on regulation of gene expression by glucocorticoid hormones. These cells, upon exposure to dexamethasone (dex), exhibit increased levels of MMTV RNA and MMTV encoded proteins on the plasma membrane and, eventually, cell death. Variants that do not express viral proteins on the cell surface have been isolated using complement killing with anti-MMTV antiserum or with a monoclonal anti-gp52 antibody. These variants have functional receptors, since they have retained their cytolytic response to glucocorticoids. Thus, the failure to induce MMTV proteins implies a lesion other than in the receptor. In cytodot assays, most clones exhibit the same hormone-induced increase in MMTV RNA levels as the parent line, indicating post-transcriptional lesions in the hormone response. In addition, there appear to be a few clones which show no induction of MMTV RNA, indicating a possible lesion in the transcriptional step of the inductive process. Initial studies on the characterization of the variants suggests abnormal membrane turnover rate and/or nonretention of the major viral envelope glucoprotein on the cell surface of these cells.

B 157 1,25-DIHYDROXYVITAMIN D_3 AND CALBINDIN-D_{28K}: STUDIES ON THE MOLECULAR ARCHITECTURE OF ITS GENE AND REGULATION OF ITS EXPRESSION AT THE TRANSCRIPTIONAL LEVEL. A. W. Norman, G. Theofan, P. P. Minghetti, Y. Fujisawa and L. Cancela, Div. Biomed. Scs. and Dept. Biochem., Univ. Calif., Riverside, CA 92521.

The hormonally active form of vitamin D_3 [1,25(OH)$_2$D$_3$] has been shown to modulate the transcription of several genes; calbindin, a vitamin D-induced 28K calcium-binding protein is an example of such a gene. We have carried out in vitro nuclear transcription (run-off) assays to determine whether the induction of calbindin-D_{28K} in the chick intestine by 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$] is occurring at the genomic level. Rachitic chicks were dosed with 6.5 nmoles of 1,25(OH)$_2$D$_3$ and calbindin-D_{28K} gene transcription, 1,25(OH)$_2$D$_3$ nuclear uptake, and 1,25(OH)$_2$D$_3$ receptor occupancy levels were measured at various times. Calbindin-D_{28K} gene transcription was significantly induced by 15-30 min following hormone administration, reached a peak by 2 hr, and started to decline by 4 hr. This time course was closely paralleled by the rate of uptake of 1,25(OH)$_2$D$_3$ into nuclei and by 1,25(OH)$_2$D$_3$ receptor occupancy levels. These results indicate that 1,25(OH)$_2$D$_3$ acts like other steroid hormones to turn on transcription of specific genes via a receptor mediated mechanism. In order to establish the molecular structure and to define the vitamin D-dependent regulatory elements of this gene, we have screened a chicken genomic library to ascertain the DNA in question. Radioactive hybridization probes were prepared from cDNAs which spanned the 3' untranslated region of the calbindin mRNA. Two lambda clones were found which contained 23-24 Kb of chromosomal DNA. This insert DNA has subsequently been subcloned, mapped and is currently being sequenced in our laboratory. [Supported in part by USPHS grant AM-09012-022]

B 158 MECHANISM OF GLUCOCORTICOID RECEPTOR mRNA REGULATION. Sam Okret, Lorenz Poellinger, Yu Dong and Jan-Åke Gustafsson. Dept of Medical Nutrition, Karolinska Institute, Huddinge Hospital F69, S-141 86 Huddinge, Sweden

A cDNA clone for the rat glucocorticoid receptor (GR) was used to study mechanisms of GR mRNA regulation. The regulation seems to be complex since a continious treatment of rat hepatoma cells with dexamethasone caused a cyclic variation in GR mRNA levels. After 6hr of treatment a 1.5-2-fold increase in GR mRNA was seen followed by a 50-80% reduction after 24-48hr compared to the initial level. The initial level of GR mRNA was restored after 72hr. The down regulation after 24hr seemed to be a primary response since it also occured in the presence of the protein synthesis inhibitor, cyclohexamide. However, a 4-fold increase in GR mRNA levels was seen both in the presence and absence of dex, suggesting a GR-gene suppressor activity and/or stabilization of GR mRNA. The above result might indicate that the GR protein in itself is involved in regulation of GR mRNA levels by i.e. binding to its own gene. In line with the latter we demonstrate by an immunoprecipitation assay, DNase I and methylation protection experiments that the GR interacts within a region of the cDNA that represents a fragment of the 3'nontranslated region of the GR mRNA, which exists in the GR gene as a single exon. The GR binding region contains concencus octanucleotides derived from the mouse mammary tumor virus and shown to be important for GR binding. The presence of GR binding sites in the 3'nontranslated region might be of importance since this region in addition to the coding sequence is evolutionary highly conserved. Transfection experiments and transcriptional assays will provide further insight.

B 159 ANALYSIS OF TRANSCRIPTION REGULATORY ELEMENTS UPSTREAM OF THE HUMAN GROWTH HORMONE GENE. L.N. Peritz, P.A. Cattini, J.D. Baxter, N.L. Eberhardt. Metabolic Research Unit, University of California, San Francisco, CA 94143.

We have defined several elements that regulate expression of the human growth hormone (hGH) gene. A series of 5'-flanking deletions was generated with endpoints within 450 bp upstream of the start of transcription of the hGH gene and was fused to the bacterial chloramphenicol acetyltransferase (CAT) gene. These constructions were transiently introduced into either rat pituitary tumor (GC) or Hela cells. In GC cells, CAT activity was high when expressed from constructions with 213 bp or more of 5'-flanking DNA, but it decreased when only 196 bp were present. A different expression pattern emerged when the same plasmids were introduced into HeLa cells. CAT activity was poorly expressed when the gene was downstream from 450 bp of hGH 5'-flanking DNA; the activity increased when the upstream DNA was deleted to nucleotide (nt) -291, but fell again upon deletion to nt -260 and remained low with further deletion. These results suggest the presence of a negatively acting element between nts -450/-291 and a positively acting element between nts -291/-260. DNase I footprinting experiments indicate the presence of factor(s) that bind to the region between nts -300/-250. In summary, 3 transcription elements have been identified upstream of the hGH gene. One, located between nts -213/-196 upstream of the start of transcription, acts specifically in GC (pituitary) cells. Two others, one negative (nts -450/-291) and one positive (nts -291/-260), regulate expression in HeLa cells. These two elements may not be functional in GC cells due to the dominance of the downstream cell-specific element (nts -213/-196).

B 160 DIFFERENTIAL ACTIVITY OF A HORMONE-RESPONSIVE ENHANCER IN VARIOUS CELL LINES, Magnus Pfahl and Kun C. Wu, La Jolla Cancer Research Foundation, La Jolla CA 92037.

We have investigated the hormone responsiveness of the mouse mammary tumor virus (MMTV) hormone-responsive enhancer (HRE) in three different cell lines. Constructs containing various portions of the MMTV long terminal repeat (LTR) ligated to the Herpes simplex virus thymidine kinase (tk) gene were transfected into Rat-2 cells (tk^- rat embryo fibroblasts), CHO tk^- cells, and F9 tk^- (embryonal carcinoma) cells. Tk^+ clones were selected in HAT medium in the presence of 10^{-6} M dexamethasone (dex). All Rat-2 clones selected under these conditions were dependent on the presence of the hormone for their tk^+ phenotype. A minimum of 2.5×10^{-9} dex was necessary to allow growth of the clones in HAT medium. Dot blot analysis of tk mRNA of these clones showed that the tk gene was 20- to 50-fold inducible by dex. Other steroid hormones such as progesterone, estrogen, testosterone, aldosterone, and vitamin D_3 were unable to induce the tk gene. From CHO cells, we only obtained clones which produced the tk enzyme at a constitutive level and grew equally well in HAT medium with and without dex. Dot blot analysis of these clones also did not reveal any hormonal induction of tk mRNA. From F9 tk^- cells, we were unable to obtain any tk^+ clones, although we were able to obtain clones which carried stably integrated copies of our MMTV-tk constructs by co-transfection with the neo gene. It therefore appears that the MMTV-HRE is silent in F9 cells, constitutive in CHO cells, and glucocorticoid-responsive in Rat-2 cells. Our *in vitro* measurements showed that all three cell lines contained approximately 7×10^4 glucocorticoid receptors per cell.

B 161 DNA SEQUENCES RESPONSIBLE FOR GLUCOCORTICOID INHIBITION OF PRO-OPIOMELANOCORTIN GENE EXPRESSION, Richard K. Plante, Lucie Jeannotte, Jacques Drouin and Orjan Wrange*, Institut de recherches cliniques de Montréal, Montréal, Canada H2W 1R7 and *Karolinska Institute, Stockholm, Sweden.

Glucocorticoids specifically inhibit the transcription rate of the pro-opiomelanocortin gene in the anterior pituitary. We have studied this negative regulation using gene transfer, as well as *in vitro* binding of the purified glucocorticoid receptor (GR) to the rat POMC gene. Six GR binding sites were characterized by DNAaseI, exonucleaseIII and DMS footprinting. DNA sequences responsible for POMC transcription inhibition were identified by electroporation of AtT-20 cells (mouse pituitary tumor cells expressing POMC) with plasmid constructs containing POMC promoter fragments (spanning -706 to +63) fused to the bacterial gene coding for neomycin resistance (neo). As shown by RNase mapping analysis, the POMCneo transcripts present in electroporated cells are initiated at the same site as in the pituitary and their level is inhibited by glucocorticoids (DEX) to the same extent as endogenous POMC mRNA. Deletion analysis of the 5'-flanking region of the POMC promoter indicates that no more than two glucocorticoid receptor (GR) binding sites are required for inhibition of POMC transcription. A GR binding site which overlaps the "CCAAT" box sequence is essential for DEX inhibition of transcription. In order to elucidate the mechanism by which GR binding results in inhibition of transcription, the interaction between this site and GR is analyzed by site-directed mutagenesis for comparison with GR binding sites in up-regulated genes. The importance of the position of POMC GR binding sites relative to other promoter elements are also currently under investigation. (Supported by MRC and NCI of Canada)

B 162 ANDROGEN REGULATES C-MYC EXPRESSION IN RAT VENTRAL PROSTATE V. E. Quarmby, E. M. Wilson, and F. S. French, Laboratories for Reproductive Biology, University of North Carolina, N.C. 27514

The c-myc protein is thought to play a key role in cell growth control. Androgens stimulate the growth of the rat prostate gland. We have investigated the role of c-myc expression during postnatal growth, castration-induced involution and androgen-stimulated regeneration of the rat ventral prostate. Adult male rats were castrated and maintained for periods up to 7 days with or without androgen treatment. Total RNA from ventral prostates was analyzed by northern blot hybridization using ^{32}P-labeled c-myc DNA and cDNAs for prostatein and actin. In the intact rat, steady state levels of c-myc mRNA (2.3kb) were low, whereas strong positive signals were obtained for prostatein and actin. Within one day post-castration, c-myc mRNA increased 5 fold and remained elevated for 7 days. In contrast, prostatein mRNA levels descreased dramatically upon castration, while actin mRNA levels changed only slightly. Testosterone administration to rats 4 days after castration decreased c-myc mRNA levels within 20-30 hr, while it increased prostatein mRNA within 4 hr. Cycloheximide, given ip 2 hr before sacrifice to prevent mRNA degradation, increased c-myc mRNA 7 fold, but had little effect on the levels of prostatein or actin mRNA, indicating that these mRNAs turnover more slowly than c-myc. With cycloheximide treatment, the level of c-myc mRNA was less after testosterone restimulation than in the untreated castrate, suggesting that androgen decreased c-myc mRNA by inhibiting its transcription. Supported by research grants HD04466 and CA32458.

B 163 NUCLEAR FACTORS BINDING TO 5'-FLANKING SEQUENCES OF THE RABBIT UTEROGLOBIN GENE, V.C. Rider and D.W. Bullock, Baylor College of Medicine, Houston TX 77030. Progesterone stimulates the transcription of the uteroglobin gene in rabbit uterus. We have used nondenaturing electrophoresis to identify specific binding of proteins, extracted with 0.4 M KCl from endometrial nuclei of progesterone-treated rabbits, to the contiguous 5'-flanking fragment (-395 to 9) of the uteroglobin gene (UG400), excised by digestion with Bam HI. Binding reactions were carried out for 30 min at 22°C, using 0.6 ng end-labelled UG400 and 10 µg nuclear extract, adjusted to 0.1 M KCl, in the presence of 12 µg poly dI-dC to reduce nonspecific binding. Protein binding was detected by retarded migration of the labelled DNA on electrophoresis in 45 mM Tris-borate, 1 mM EDTA, pH 7.8. Six distinct bands (NP1-NP6) were seen, whose electrophoretic shifts were abolished by proteinase K (50µg/ml) but not by RNase A (50 µg/ml). Similar shifts were seen using the end-labelled Eco RI-Bam HI fragment (375 bp) of pBR 322, but this fragment did not compete for binding to UG400. Binding to UG400 was also resistant to competition by the 527-bp Hpa II fragment of pBR 322 or the 148-bp Ava I subfragment (-395 to -248) of UG400, at up to 100-fold molar excess. Incubation with the 203-bp Ava I subfragment (-194 to 9) of UG400, however, specifically inhibited the binding of NP3, NP5 and NP6 to labelled UG400. The results indicate that extracts from nuclei actively transcribing the uteroglobin gene contain proteins that bind specifically to sequences in putative control regions within 194 bp upstream of the transcription start site. (Supported by funds from NIH Grant HD09378)

B 164 CHROMATIN CONFIGURATIONS ASSOCIATED WITH ANDROGEN REGULATION OF MOUSE SEX-LIMITED PROTEIN, Diane M. Robins and Cynthia Hemenway, Columbia University, N.Y., NY 10027. We have investigated the chromatin structure of two closely related genes located in the mouse MHC in order to correlate specific DNA sequences and chromatin structures with developmental, hormonal and tissue-specific gene expression. The C4 gene encodes the fourth component of complement, while the product of its neighboring homologous gene Slp, or sex-limited protein, lacks complement activity and is regulated by androgen. The major site of synthesis for both proteins is the liver. DNaseI digestion of liver chromatin reveals four major sites of hypersensitivity in the 5' regions of the C4 and Slp genes. Two of these sites, located 2.0 and 2.3 kb upstream of the start-site of transcription, map specifically to the Slp gene and are associated with developmental and hormonal regulation. They appear prior to Slp expression in the male, reflecting an early gene committment event, and can be induced in the female by androgen administration. The stability of these sites upon testosterone withdrawal is markedly different in males and females which may indicate differences in the establishment and/or maintenance of hypersensitivity. Chromatin patterns from mice carrying different regulatory alleles of Slp and C4 correlate with the mode of gene regulation rather than the absolute levels of transcription. DNA sequences that correlate with far upstream Slp hypersensitive sites have been studied by transfection assays, in which they have been shown to confer testosterone-responsiveness on the bacterial chloramphenicol acetyltransferase gene.

B 165 REGULATION OF MILK PROTEIN GENE EXPRESSION. J.M. Rosen, C.A. Bisbee, K.F. Lee, & L. Yu-Lee, Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030 Milk protein gene expression in mammary epithelial cells is regulated by the complex interplay of several peptide and steroid hormones at both the transcriptional and post-transcriptional levels. Recent sequence analysis of a number of milk protein genes has resulted in the identification of a putative 30 bp mammary consensus sequence usually located at position -110 to -140 relative to the CAP site. We have attempted to define by DNA-mediated gene transfer cis-acting regulatory sequences required for the control of milk protein gene expression. Two general strategies have been employed: First, the flanking regions of the casein genes have been linked to a readily assayable reporter gene. Second, the entire milk protein gene or a functional minigene has been introduced into stable transfectants using a BPV-derived eukaryotic shuttle vector. Several conclusions can be drawn from these experiments: 1. The 5' flanking regions of the ß- and gamma-casein genes(including at least 2.3 kb of flanking DNA and in some experiments the first exon and a portion of the first intron)are relatively weak promoters and not sufficient to elicit a hormonal response under conditions where the endogenous genes are markedly induced. Constructions containing exon I show a prefential increase in expression in mammary epithelial cells as compared to fibroblasts 2. Amplified, unrearranged copies of the ß- and α-casein genes and minigenes, respectively, have been identified in transfected cell lines, and in both cases strand-specific gene expression has been observed. However, no hormonal regulation has been observed in these transfectants and very little of the correctly sized mature mRNA is detected. To complement these approaches we have also generated several transgenic animals containing the entire ß-casein gene.(Supported by grant NIH-CA16303).

B 166 THE GLUCOCORTICOID RECEPTOR MEDIATES REPRESSION OF TRANSCRIPTIONAL ENHANCERS FROM THE BOVINE PROLACTIN GENE. Dennis D. Sakai[1], Sherron Helms[2], Keith R. Yamamoto[1], and Fritz M. Rottman[2], [1]Dept. of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, CA 94143; [2]Dept. of Molecular Biology and Microbiology, Case Western Reserve University, Cleveland, OH 44106.

Transcription from the bovine prolactin (BPRL) promoter is repressed by glucocorticoid hormone. We have analyzed the DNA sequences required for repression by transient transfection experiments and by in vitro footprinting. Like genes which are positively regulated by glucocorticoids, the 5'-flanking region of the BPRL gene contains a number of specific binding sites for purified glucocorticoid receptor protein. Deletion analysis indicates the three most promoter-proximal binding sites are most important for both constitutive promoter activity and glucocorticoid regulation. Deletion of any one of these three sites reduces correctly initiated transcription substantially while eliminating the hormone response. Fusion of individual GR binding sites to heterologous promoters increases basal expression from the promoters and confers hormone repressibility. Glucocorticoid regulatory elements (GREs) from hormone-inducible genes such as mammary tumor virus are enhancers whose function is strictly dependent upon the presence of hormone and receptor. In contrast, the BPRL receptor binding sites appear to act as enhancers in the absence of glucocorticoids or receptor. Glucocorticoid treatment suppresses this enhancement. We designate these BPRL sequences repressible (-)GREs to distinguish their properties from inducible (+)GREs.

B 167 GLUCOCORTICOID MEDIATED MODULATION OF PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR GENE EXPRESSION IN THE HUMAN FIBROSARCOMA LINE HT-1080. Wolf-Dieter Schleuning, Robert L. Medcalf and Eva van den Berg*. Central Hematology Laboratory, University Hospital Center, CHUV, 1011 Lausanne, Switzerland and *Gaubius Institut TNO, 2313 AD Leiden, The Netherlands.

Plasminogen activator (PA) biosynthesis is characteristically associated with biological processes such as cell migration, tissue remodeling or malignant growth (Danø et al. (1985) Adv. Cancer Res. 44,139-266). Two functionally similar but genetically distinct PAs are known: tissue-type PA (t-PA) and urokinase-type PA (u-PA). Recently two likewise genetically distinct but functionally similar specific protein inhibitors of PA have been isolated and characterized: plasminogen activator inhibitor (PAI) 1 and plasminogen activator inhibitor (PAI) 2. We have obtained cloned probes for all four proteins and studied the regulation of their biosynthesis on the transcriptional level using Northern blot hybridization and nuclear "run on" transcription assays. u-PA is constitutively expressed in the HT-1080 cell line at high levels. Dexamethasone almost completely suppresses u-PA transcription whereas PAI-1 transcription is strongly induced. Dexamethasone also leads to a slight increase of t-PA mRNA, whereas no effect on PAI-2 mRNA is observed. These results indicate that u-PA and PAI-1 are oppositely regulated, glucocorticoid dependent transcription units and lend weight to the hypothesis that proposes a role of u-PA in extracellular matrix turn over during inflammatory disease and malignant growth.

B 168 FUNCTIONAL IDENTIFICATION OF DNA SEQUENCES ESSENTIAL FOR ESTROGEN INDUCTION OF VITELLOGENIN GENE EXPRESSION

Anne Seiler-Tuyns, Ernest Martinez, Philippe Walker, Anne-Marie Mérillat, Stephen Green*, Pierre Chambon* and Walter Wahli. Institut de Biologie animale, Université de Lausanne, CH-1015 Lausanne, Switzerland. *Institut de Chimie biologique, Faculté de Médecine, F-67085 Strasbourg Cedex, France.

In Xenopus laevis, vitellogenin gene expression in the liver is strictly controlled by estrogen. Therefore these genes should be useful to identify estrogen-responsive elements present in the DNA sequence. The expression of a chimeric gene, formed by the fusion of the vitellogenin gene B1 upstream region and the CAT gene, is regulated by estrogen when this gene is transfected into the hormone-responsive human cell-line MCF-7. Deletion mapping has shown that a 13 bp palindromic element, present at position -334 is essential for estrogen inducibility in these cells. This element was previously detected by sequence comparison of several liver inducible genes.
We have also cotransfected a human estrogen receptor cDNA present on an expression vector and the chimeric vitellogenin-CAT genes into the Xenopus kidney cell-line B-32. The results are essentially identical, which may indicate that a common mechanism of regulation could exist for Xenopus and Man.

B 169 FEASIBILITY OF USING BOVINE PAPILLOMAVIRUS VECTORS TO STUDY THE ROLE OF CHROMATIN STRUCTURE IN ESTROGEN REGULATED PROLACTIN GENE EXPRESSION.
Mark Seyfred and Jack Gorski, University of Wisconsin, Madison WI. 53706

We have constructed vectors containing bovine papillomavirus (BPV) DNA and the 5' upstream region of the prolactin gene. These vectors were transfected into estrogen responsive GH_3 and MCF-7 cells as well as estrogen non-responsive HeLa cells. The BPV-Prl constructs exist as stable episomes at a level of 5-10 copies per cell and appear largely unrearranged. The minichromosomes have been extracted away from the bulk genomic DNA by using low ionic strength buffers. Studies are currently underway to examine the chromatin structure of the minichromosomes as well as the interaction of estrogen receptors with the minichromosomes.

B 170 RETINOID REGULATION OF TRANSGLUTAMINASE GENE EXPRESSION IN MOUSE PERITONEAL MACROPHAGES, Joseph P. Stein, Ennio A. Chiocca and Peter J.A. Davies, University of Texas Medical School, Houston, Texas 77225.
Retinoids are a class of monocyclic isoprenoid molecules, structurally related to vitamin A, that have dramatic effects on the growth and differentiation of both normal and neoplastic cells. These properties form the rationale for the current testing of synthetic retinoids as chemopreventative and chemotherapeutic agents. Although retinoids are presumed to exert their regulatory effects on cellular growth and differentiation through specific cytoplasmic binding proteins, experimental evidence in support of this model is lacking. Indeed, the molecular events by which retinoids alter cellular functions are quite poorly understood. We have recently demonstrated that retinoic acid (RA) acts as a direct and acute regulator of the expression of a specific enzyme, tissue transglutaminase (TGase), in mouse and human myeloid cells. Exposure of resident mouse peritoneal macrophages to RA causes a significant increase in the amount of translatable Tgase mRNA within 30 minutes. This activation is completely blocked by actinomycin D, but not affected by cycloheximide. In order to investigate the molecular mechanism of Tgase induction a lambda gt11 library was prepared from RA stimulated macrophages. Six cDNA clones were isolated by screening with an infinity purified Tgase antibody. A 750 bp cDNA insert from one phage clone was subcloned into the bacterial plasmid pBR328. DNA sequencing of this clone has yielded a partial amino acid sequence of mouse tissue transglutaminase. The cDNA (pmTG700) hybridizes to a mouse mRNA species of 4 Kb, whose levels increase 50-fold within six hours after RA stimulation. This cDNA probe will be used to assess the affect of retinoic acid on the transcriptional rate of the mouse macrophage Tgase gene, as a first step toward understanding the molecular basis of retinoid action in myeloid cells.

B 171 TRANSCRIPTION FACTOR BINDS SPECIFICALLY TO THE GTCAAAGG (COUP) SEQUENCE OF THE OVALBUMIN GENE. Ming-Jer Tsai, Sophia Y. Tsai, Heng Wang, Lee-Ho Wang, Milan Bagchi, and Bert W. O'Malley. Department of Cell Biology, Baylor College of Medicine, Houston, Texas 77030 USA.

COUP box transcription factors has been isolated and partially purified from HeLa cell and oviduct nuclear extracts. This factor is required for the efficient transcription of the ovalbumin gene but not the SV40 early gene. COUP transcription factor binds to the Chicken Ovalbumin Upstream Promoter sequence (COUP) spanning between -70 to -90. Binding competition experiments were carried out to determine the relative affinity of COUP factor to various promoters. We found that promoter DNA fragment, isolated from the ovalbumin gene, competes better than those isolatd from the ovomucoid, Y and β-actin genes. In contrast, the β-globin gene, SV40 early gene and adenosine deaminase gene promoters do not compete. Thus COUP factor is different from the CAAT box transcription factor which binds very well to the CCAAT box consensus of the β-globin gene. Detailed information on the interaction of COUP transcription factor to its binding sequence was obtained by DNaseI footprinting, gel retardation and methylation and ethylation interference studies. Another factor, S300-II, has also been isolated which has no apparent DNA-binding specificity to the COUP region of the ovalbumin gene. However, a partially purified fraction can prolong the half-life of the COUP box transcription factor-DNA complex. This result is consistent with the notion that S300-II may stabilize the COUP factor-DNA complex and thus enhances the transcription of the ovalbumin gene.

B 172 CORTISOL INDUCTION OF GLUTAMINE SYNTHETASE IN EMBRYONIC CHICKEN RETINA, Lily Vardimon, Lyle E. Fox and A.A. Moscona, The University of Chicago, Chicago, IL. 60637

The enzyme glumatine synthetase (GS) is a differentiation marker in embryonic retina. The chicken GS gene has been cloned and used to investigate regulation of GS expression during retina development. Accumulation of GS mRNA in the retina tissue is low at early embryonic stages. The level of the major GS transcript (3Kb) begins to rise sharply on day 15 of development, subsequent to systemic elevation in adrenal corticosteroid hormones. The increase in GS mRNA accumulation correlates with an increase in the amount of the GS enzyme. When cortisol is prematurely added to organ-cultures of retinas from embryos of different ages it induces precocious increases in the levels of GS mRNA and of GS enzyme. However, the increases are significantly greater at later than at earlier embryonic ages, suggesting that competence to transcribe or stabilize GS mRNA in response to stimulation with cortisol increases with development. Addition of cortisol to retina tissue on day 13 of development induces an increase in GS mRNA accumulation which plateaus after four hours. By separating the two cell types of the retina, neurons and glia, we have demonstrated that accumulation of GS mRNA and precocious induction of GS mRNA by cortisol are restricted to glia cells. When retina cells are plated in culture dishes as a monolayer under conditions that favor the cells remaining monodispersed, induction by cortisol of GS mRNA accumulation is greatly reduced. We are using an in vitro nuclear transcription assay to investigate whether cortisol induces GS mRNA accumulation in retina cells by affecting the rate of GS mRNA transcription or stabilization.

Biology and Physiology

B 173 CHARACTERIZATION AND ESTROGEN REGULATION OF AN ACID EXTRACTED GROWTH FACTOR FROM UTERUS. Candace Beck and Charles W. Garner, Dept. of Biochemistry, Texas Tech Univ. Health Sciences Center, Lubbock, TX 79430.

An acid stable uterine-derived growth factor (UGF) extracted from uteri of several species (rat, bovine and rabbit) stimulates DNA synthesis as measured by ^{3}H-thymidine incorporation into uterine carcinosarcoma (UCS) cells. These extracts also stimulate DNA synthesis in cultures of NMuMG (mouse mammary gland) and H4-II-E-C (rat hepatoma) cells. 3T3 Swiss fibroblasts and USC cells respond at lower concentrations of extract. GH3 cells are unresponsive to stimulation by acid extracts. This suggests that UGF can stimulate growth in some but not all E_2-target tissues as well as non-target tissues. Uterine extracts prepared from E_2 treated ovariectomized rats show an increase in growth factor activity within 24 hours after injection. The activity remains elevated with subsequent injections. UGF-like activity is also found in liver, kidney, spleen, skeletal muscle, and heart. E_2 treatment of ovariectomized rats stimulates UGF activity in the uterus (250% of control) and liver and kidney (150% of control each). E_2 did not stimulate UGF activity in heart, spleen and skeletal muscle. The increased stimulation seen in E_2 responsive tissues suggests regulation of UGF by steroids. Uterine extracts are also capable of stimulating glucose transport and glucose-6-phosphate dehydrogenase activity in UCS cells. UGF stimulates growth of UCS cells in the absence of serum and steroid hormones. Since E_2 is known to stimulate DNA synthesis, glucose transport, and G-6-PDH in vivo, it is possible that UGF is the actual mediator of these E_2 actions. Gel filtration shows molecular weight heterogenity with a major peak eluting at M.W. 12kD. UGF binds to heparin-agarose in the absence of salt and elutes with 0.5M NaCl.

B 174 MODULATION OF ANDROGEN RECEPTOR BINDING TO NUCLEAR ACCEPTOR SITES, James H. Bryan and Edward J. Keenan, The Oregon Health Sciences University, Portland, OR 97201.
Androgens produce both hyperplasia and hypertrophy in the prostate (an androgen-dependent tissue) while in the kidney (an androgen-sensitive tissue) androgens produce only hypertrophy. The molecular basis by which these distinct steroid actions are produced is unknown. While cytosolic androgen receptors (AR) obtained from both tissues are similar, the nature of their interaction with the nucleus is poorly understood. Since the differential actions of steroids are most likely modulated at the nuclear level, this study examined the ability of AR from prostate (P) and kidney (K) to interact with prostatic and renal nuclear acceptor sites. P and K were obtained from adult rats 24h post-castration. Nuclei were purified and nuclear matrices (NM) were prepared by standard procedures. Cytosolic AR were equilibrated (16h, 0°C) with ^{3}H-R1881 (5nM) ± unlabeled R1881 (500nM) then incubated with NM or nuclei from P or K for 1.5h. Both P and K-NM bound 80-100% of the total prostatic AR added. In contrast, less than 20% of the added renal AR bound to either NM. Studies conducted using whole nuclei also revealed significant binding of prostatic but not renal AR. When a 1:1 mixture of P and K cytosols was labeled with ^{3}H-R1881 and equilibrated with nuclei, the binding of the prostatic AR was reduced by 60-70%. Incubation of nuclei with unlabeled K cytosol prior to addition of labeled P-AR failed to alter the interaction of the P-AR with the nuclei. These studies indicate that prostatic AR readily associate with nuclear acceptor sites in both prostate and kidney. In contrast, AR from kidney associate poorly with both types of nuclei. It appears that cellular factors in the kidney may act directly on the AR to modulate binding to nuclear acceptor sites.

B 175 ANDROGEN RECEPTORS IN GOLDFISH BRAIN: CHARACTERISTICS AND SEASONAL CHANGES. G.V. Callard and M. Pasmanik, Boston University, Boston, MA 02215.
Circulating testosterone (T) exerts its actions on the brain and pituitary via androgen receptors (AR) or, following aromatization to estradiol, via estrogen receptors. In teleost fish aromatase levels in brain are 1000 times higher than in mammals, a rate expected to limit T actions via AR. Experiments were carried out to determine whether goldfish have AR and, if present, whether they have unusual binding properties. Preparation of subfractions and measurement of binding have been described (Callard and Mak, PNAS 82:1336, 1985). Both cytosol and nuclear extracts bound [^{3}H]T with high affinity (Kd = 1.6 nM), but the number of binding sites was unusually high for a receptor (Bmax = 1.4 nM). DHT was an effective competitor but estradiol, progesterone and 11-keto T, a principal plasma androgen in teleosts, were not. This binding component could be distinguished from the TeBG of goldfish blood by its dissociation half-time (6 h, 4 C), adherence to DNA-cellulose (elution maximum ≅ 0.1-0.2 M NaCl), and sedimentation profiles (9-10S in low vs 5S in high salt). [^{3}H]T binding was tissue-specific: pituitary = forebrain >> mid/hindbrain > testis; spleen, gill, muscle = 0. In nuclear extracts activity was seasonal with a peak at spawning (April) and a nadir during reproductive inactivity in July (8.0 vs 0.5 pmol/g), corresponding exactly to seasonal changes in brain aromatase. We conclude this protein is an AR, not heretofore identified at this phylogenetic level. It differs from mammalian AR only in its relative abundance (1000-fold greater), a feature which may somehow be related to high levels of aromatase. This is the first report to show a correlation between brain AR and reproductive cyclicity in any species (NSF DCB85-19737).

B 176 A PUTATIVE ESTROGEN RECEPTOR FROM SNAKE LIVER WITH UNUSUAL PROPERTIES? Ian P. Callard and Deborah Riley, Boston University, Boston, MA 02215.
Understanding of steroid receptors is derived largely from the mammalian uterus and avian oviduct. This laboratory has sought to characterize subavian steroid receptors in relationship to natural cycles. Here we report a putative hepatic estrogen receptor associated with the vitellogenic cycle in the snake, Nerodia. Although extracts have high affinity (K_d 10^{-9}-10^{-10}), specificity for estradiol, association time ~ 45 mins (20°C), and nuclear binding increases after estradiol, unlike classical receptors, the Nerodia receptor does not bind to DNA-cellulose or DEAE Sepharose, nor does it bind the synthetic estrogen, DES. In addition, sedimentation rate is unaffected by KCl concentration.
Nuclear location of the receptor is indicated by extraction of increasing amounts of receptor by KCl concentrations up to 0.5 M, 50% of binding being extracted by 0.16 M KCl. Both cytosolic and nuclear estrogen binding was target organ specific, binding being low to non-detectable in lung, skeletal muscle and intestine, present in liver, oviduct and kidney. Significant changes in total hepatic and nuclear receptor correlate with vitellogenic stage, and similar trends were seen in cytosol. Thus, nuclear receptor (in fmol/g tissue) varies as follows: early vitellogenic:1933 ± 234;mid-vitellogenic:2155 ± 412;late vitellogenic: 1034 ± 325;pregnant:313 ± 165. In summary a novel hepatic estrogen receptor which is probably associated with vitellogenesis is present in the snake, Nerodia. It is likely that receptor moieties which do not conform to the classical mammalian pattern are physiologically important in a variety of non-mammalian species. NSF PCM 81-04144 and DCB 85-18296 (IPC).

B 177 THE BETA THALASSEMIC MOUSE: GENETIC DEFECT AND COMPENSATION, M. Joan Curcio, Daniel Kuebbing, W. French Anderson and Brian Safer, Laboratory of Molecular Hematology, NHLBI, NIH, Bethesda, MD 20892. A mouse model of the inherited hemoglobin disorder, beta-thalassemia, results from a deletion including the entire beta major gene. The breakpoints of this deletion have been isolated. A 3709 (+/-2) bp region, including the entire beta major globin gene and 2 kb of 5' flanking region, is deleted. A novel 66 (+/-2) bp sequence, ending in a stretch of 25 dA:dT base pairs, was found to bridge the deletion. In the thalassemic mouse, there is a compensatory increase in synthesis of beta minor globin, resulting in a beta minor:alpha globin ratio of 0.7 in the homozygous thalassemic mouse, as compared to 0.2 in the normal homozygous diffuse mouse. The increase in beta minor globin synthesis occurs at translation rather than at transcription. RNase T1 analysis of reticulocyte mRNA reveals that the beta:alpha ratio of globin mRNA is 0.3, significantly lower than the globin synthetic ratio of 0.7. However, the beta:alpha ratio of mRNA on polysomes is higher than unassociated mRNA, demonstrating that beta minor mRNA is preferentially translated. Elevated synthesis of beta minor globin is maintained during in vitro translation in thalassemic reticulocyte lysate. In this system, partial inhibition of translational elongation by cycloheximide decreases the beta minor:alpha globin synthetic ratio, while partial inhibition of initiation by hemin deficiency increases the beta minor:alpha synthetic ratio. This suggests that beta minor mRNA competes with alpha mRNA at initiation of translation for a limiting mRNA binding factor. Addition of a partially purified translation factor tentatively identified as eIF-4A to beta thalassemic mouse reticulocyte lysate lowers the ratio of beta minor to alpha globin synthesis by stimulating alpha globin synthesis.

B 178 Prognostic Relevance of Receptor Status and Proliferative Activity in Node Negative Breast Cancer Patients. G. DiFronzo, D. Coradini, V. Cappelletti, G.F. Scavone, M.G. Daidone, R. Silvestrini. Istituto Nazionale Tumori, Milano, Italy.

Many prognostic factors have been used for breast cancer: number of histologically involved axillary lymphnodes, tumor diameter, estrogen(ER) and progesterone receptor (PgR) status and tumor growth kinetic variables. Each factor has been assessed independently with respect to its prognostic relevance. We have already reported that at 3 years of follow-up, ER + and node-negative women have a significantly lower recurrence rate than ER- ones. Among ER+ patients, subsets of women who are PgR- have a poor prognosis. Similarly in a previous work (R.Silvestrini *et.al.* 8th Annual San Antonio Breast Cancer Symposium, Nov. 1985) we have investigated the prognostic relevance of proliferative activity, expressed by in vitro labeling index (LI), in association with ER status. Proliferative activity is significantly correlated with the disease free survival (DFS) in the general population. We found that this was also true among women with ER+ tumors. Starting from these results, we studied a new series of 179 patients with the aim of investigating the predictive relevance on the 3 years-DFS based on the combination of all the three prognostic variables LI, ER, PgR. Both ER and PgR status of the tumors allowed us to discriminate patients relapsing at 3 years, whereas no difference in DFS was found between slow and fast proliferating tumors. Moreover, in the subset of fast proliferating tumors the knowledge of ER and PgR status was no more able to predict the prognosis at 3 years of follow-up. However, ER and PgR, either alone or in combination predicted the DFS in patients with low proliferating tumors. Clinical implications of the present results respect to the planning of adjuvant therapies will be discussed.

B 179 GLUCOCORTICOID EFFECTS ON CARBOHYDRATE, PROTEIN AND ARACHIDONIC ACID METABOLISM IN MICROVASCULAR ENDOTHELIAL CELLS, Mary E. Gerritsen and Catherine A. Partridge, New York Medical College, Valhalla, NY 10595.

The direct actions of glucocorticoids on vascular endothelial cells are poorly understood, although the potent inhibitory actions of steroids on the inflammatory response in the microvasculature has been recognized for several decades. Using isolated and cultivated rabbit coronary microvessel endothelial (RCME) cells, the actions of these steroids on endothelial metabolism were determined. Binding studies using [^{3}H] dexamethasone (DEX) demonstrated high affinity, glucocorticoid-specific binding sites in RCME cells. DEX pretreatment inhibited basal, A23187, thrombin, and ATP stimulated PGI_2 and PGE_2 synthesis, and inhibited A23187-stimulated arachidonic acid (AA) release but did not inhibit the metabolism of exogenous AA. These inhibitory effects were prevented by pretreatment with cylcoheximide or actinomycin D. Related glucocorticoids (triamcinolone, cortisol) exhibited effects similar to DEX, while other steroids (progesterone, aldosterone, 5-dihydrocortisol, 5-dihydrotestosterone) had little or no inhibitory activity. DEX pretreatment had no significant effect on [^{3}H] leucine or [^{3}H] methionine incorporation into protein, nor was there any dramatic alteration in the distribution of [^{3}H] methionine-labeled proteins as examined by two dimensional SDS-PAGE. These studies indicate that although glucocorticoids exert potent inhibitory effects on the release of inflammatory mediators (prostaglandins) from RCME cells, they have relatively minor effects on carbohydrate and protein metabolism. Supported by the American Heart Association and NIH HL 35739.

B 180 HUMAN GENE FOR HEAT SHOCK PROTEIN 108, Denis R. Headon and Ronan Power, Department of Biochemistry, University College, Galway, Ireland.

Eucaryotes and procaryotes respond to environmental stress by the production of a defined set of proteins known as heat shock or stress proteins. These have been extensively studied in Drosophila melanogaster where the largest of 8 major heat shock proteins has a molecular weight of 83,000. In Hela cells,proteins having molecular weights of 85-90,000, 100,000 and 110,000 have been induced by heat shock. The gene for a protein of molecular weight 108,000 which copurifies with the progesterone receptor B-subunit of chick oviduct has been cloned from mRNA preparations. This cDNA has been used to screen a human genomic library in the charon 4A vector. The average insert size in this library is 15 kb. In excess of 400,000 plaques were screened and 36 positive clones were identified on primary screening. The five strongest positives from tertiary screening were used in subsequent analysis. A clone of 16.5 kb prepared by Eco.R1 excision from the vector was analysed by Southern blotting at highest stringency using 1.9 kb fragment corresponding to the 3' end of the cDNA. This 16.5 kb fragment has been cloned into Eco.R1 site of pBR 322.

B 181 CHARACTERIZATION OF THE EFFECT OF ALDOSTERONE INFUSION ON ADRENAL COMPENSATORY HYPERTROPHY (ACH) IN THE HAMSTER, James W. Ingram and William E. Grizzle, Depts. of Pathology and Surgery, University of Alabama at Birmingham & Veterans Adm. Medical Center, Birmingham, AL 35294.

We have reported that exogenous infusion of aldosterone at 80 ng/hr (Alzet pumps implanted in the peritoneal cavity at initial surgery) in the Syrian hamster blocks the compensatory growth of the remaining adrenal gland that follows left unilateral adrenalectomy. We have investigated the effect on ACH of varying the infused dose of aldosterone from 0 ng/hr to 1000 ng/hr. ACH was calculated by comparing the average right adrenal weight of unilateral left adrenalectomized (ULA) animals with that of sham (S) animals 3 days post-operatively. In each experiment ACH in ULA and S animals receiving only vehicle was compared with ACH in ULA or S animals receiving a known dose of aldosterone. Infusion of vehicle (13.5 to 26.6% growth) or of aldosterone at 1 ng/hr (11.5% growth) does not block ACH. Infusion of aldosterone at doses between 36 ng/hr to 1000 ng/hr effectively blocked ACH (5.3% to 0% growth). Dosages of 100 ng/hr of aldosterone did not elevate serum levels of aldosterone; however, 400 ng/hr did elevate serum aldosterone. We conclude aldosterone effectively blocks ACH in the hamster over a wide range of infusion rates.

Supported by Veterans Administration Medical Research funds and by UAB Department of Pathology.

B 182 COMPLEMENTATION OF VARIANT GLUCOCORTICOID-REGULATED GLYCOPROTEIN TRAFFICKING IN TRANSIENT HETEROKARYONS, Nancy J. John, Omar K. Haffar and Gary L. Firestone, Department of Physiology-Anatomy & Cancer Research Laboratory, University of California at Berkeley, Berkeley, CA 94720. We have previously shown glucocorticoid hormones to regulate the posttranslational maturation of mouse mammary tumor virus (MMTV) precursor polyproteins in M1.54, a stably infected rat hepatoma (HTC) cell line. Utilizing complement mediated cytolysis, we have recovered mutant derivatives of M1.54 which fail to express hormone inducible cell surface viral glycoproteins. One such clonal isolate, CR4, is similar to wild-type with respect to synthesis of MMTV RNA's, production of the MMTV glycoprotein precursor ($gPr74^{env}$) and a glycosylated maturation product (gp51), but fails to express three viral cell surface glycoproteins (gp78, gp70, and gp32) and one extracellular species (gp70s) which derive from $gPr74^{env}$ in glucocorticoid-treated wild type cells. To begin to define the genetic lesion responsible for the highly selective deficit in the hormone-regulated glycoprotein maturation pathway in CR4, heterokaryons were formed by fusing CR4 cells and B2 cells (the uninfected parental HTC) with each other. Indirect immunofluorescence analysis performed 40 hrs after fusion on dexamethasone induced cells revealed the complementation of cell surface glycoprotein transport in these "transient" heterokaryons, in which the nuclei do not fuse. We conclude that uninfected HTC cells contain normal trans-acting regulatory factors that control the steroid regulated trafficking of cell surface glycoproteins. (Supported by ACS PF-2892, NIH NRS training grant CA09041, and NCI CA35547.)

B 183
Abstract Withdrawn

B 184 PROLIFERATION OF ESTROGEN-RESPONSIVE MCF-7 HUMAN BREAST CANCER CELLS GROWN IN THE SHORT-TERM AND LONG-TERM ABSENCE OF ESTROGENS, Benita S. Katzenellenbogen, Kari L. Kendra, and Yolande Berthois, Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.

We have shown previously that phenol red, the pH indicator in tissue culture media, is a weak estrogen (Proc. Natl. Acad. Sci. USA 83, 2496 [1986]). MCF-7 cells grown in phenol red-free medium with charcoal dextran-treated serum for periods up to 1 month show a reduced rate of cell proliferation. Estradiol then stimulates proliferation markedly and reproducibly and antiestrogen does not suppress control cell growth although it inhibits estrogen-stimulated proliferation. In these short-term estrogen withdrawn cells, which show a slow rate of cell proliferation, antiestrogens appear as partial agonists/antagonists. Antiestrogens at low concentrations stimulate proliferation but show no stimulation at high concentrations where they fully inhibit estrogen-stimulated proliferation. Cells maintained for several months in the apparently complete absence of estrogens (no phenol red and with charcoal dextran-treated calf serum) increase their rate of cell proliferation and estrogen then is unable to further increase this maximal rate of proliferation, but antiestrogen is again able to decrease proliferation. This growth "adaptation" is not due to changes in cellular estrogen receptor levels but may reflect modifications in which the cells become supersensitive to possible low levels of estrogens, or more likely, that in the absence of estrogen and antiestrogen, there is altered regulation of growth factor production. A model will be presented to explain this reversible adaptation process. (Supported by NIH grant CA18119).

B 185 ATTACHMENT AND PROLIFERATION OF PRIMARY CULTURES OF NORMAL RAT PROSTATIC EPITHELIAL CELLS ON COLLAGEN OR PLASTIC SURFACES AND THE ROLE OF STEROID HORMONES, [1]R.J. Keating, [2]R.K. Tcholakian, [1]A.C. von Eschenbach, [1]Dept. of Urology, M.D. Anderson Hosp. & Tumor Inst., [2]Dept. of OB-Gyn & Reprod. Sci., U.T. Med. Schl., Houston, TX 77030.

Primary cultures of prostatic epithelial cells are a means of studying their biological properties, but are difficult to grow routinely under in vitro conditions. In this study we determined the effect of a biological substrate, rat tail collagen (RTC), on attachment, growth, dihydrotestosterone (DHT) content and acid phosphatase (AcP) production in primary cultures of prostatic epithelium. Rat ventral prostates were collagenase digested, sieve filtered, Percoll separated, and epithelial cultures initiated in supplemented Hams F-12 with regular sera (RS), stripped sera (SS) with and without testosterone (T) or estradiol (E_2). Cells were harvested and counted on 3, 5, 7 & 10 d. DHT was measured by RIA and AcP by staining techniques. A cell attachment index (AI; attached/total) showed addition of RTC significantly improved cell anchorage ($P > 0.05\%$) compared to plastic by 7d in culture. RTC often significantly ($P > 0.05\%$) enhanced culture growth. There was no improvement in cell proliferation with T while DHT, an indicator of 5α reductase activity (5α RA), was detected only in cultures incubated in the presence of T regardless of culture conditions. AcP was present in cultures grown on both surfaces and in all culture media. The data presents evidence that 5α RA is dependent upon T and that AcP, as well as cell growth, do not require androgens. The enhanced cell anchorage and proliferation with RTC represents an improved culture model for investigating regulatory mechanisms of normal prostate epithelium at the cellular level.

B 186 DEVELOPMENT AND HORMONE REGULATION OF ANDROGEN RECEPTOR LEVELS IN THE SEXUALLY DIMORPHIC LARYNX OF XENOPUS LAEVIS, Darcy Kelley, David Sassoon and Neil Segil, Columbia University, New York, New York, 10027.

The larynx of adult South African clawed frogs, Xenopus laevis, has high levels of androgen receptor (Segil et al., 1983); receptor content in males is three times that of females. To determine when sex differences in receptor level arise, we assayed [^{3}H]-dihydrotestosterone (DHT) binding activity in cytosolic preparations of larynges from metamorphic and postmetamorphic male and female frogs. Scatchard analyses and competition studies of binding activity indicate that DHT is binding to a saturable component (androgen receptor) of the cytosol with high affinity and specificity. At metamorphosis, male and female juveniles have receptor levels of 262 and 269 fm/mg cytosolic protein respectively, approximately 7 to 20 times adult values. At 3 months post-metamorphosis (PM), sexually dimorphic levels are observed. Receptor levels decrease gradually in females from metamorphosis to 10 months. In males, levels remain high through 6 months, and then decrease by 10 months. Administration of exogenous DHT to 3 months PM juveniles downregulates receptor levels from 180 (male) or 74 fm/mg (female) to 33.5 fm/mg (both sexes). Testosterone has a less pronounced effect on receptor levels in males and is ineffective in females. Thus we conclude that sexually dimorphic adult levels of receptor in larynx arise by decrease from initially high, sexually monomorphic levels and that high levels of circulating androgens normally present at 6 months PM are responsible for the marked decrease in receptor levels observed during laryngeal development.

B 187 GLUCOCORTICOID EFFECT ON CASEIN ACCUMULATION IN MAMMARY EPITHELIAL CELLS IS PARTIALLY MEDIATED BY STROMAL ELEMENTS IN COMBINED CELL/EXPLANT CULTURE, Brett K. Levay-Young and S. Nandi, Department of Zoology and Cancer Research Lab, University of California, Berkeley CA 94720.

Previous work has indicated that glucocorticoid is required for synthesis of casein by mammary epithelial cells in serum free explant culture. Results from cell culture on this point have been equivocal. To address this we have cultured mammary epithelial cells from adrenalectomized/ovariectomized virgin mice in collagen gel with a serum free medium; casein accumulation was assayed by ELISA and protein blotting. The cells responded to insulin (I) and prolactin (PRL) with or without added cortisol (F); addition of linoleic acid to I + PRL with or without F greatly enhanced casein accumulation. In neither case was there a reproducible F effect, and F was not required for casein accumulation under these conditions. The casein accumulation was dose responsive to and reasonably specific for linoleic acid. Seeking to confirm this effect in fragment organ culture, we found that explants from midpregnant mouse mammary glands showed no response to I, PRL and linoleic acid, although the explants responded normally to I, PRL and F. Attempting to reconcile these results, we cocultured cells in collagen gel as above with explants of mammary fat pads cleared of epithelium (i.e. with only stroma). The epithelial cells in the collagen gel showed no enhanced response over control to I and PRL in the presence of the explants, but addition of F caused a 2 fold increase in casein accumulation in the epithelial cells. This demonstrated that the glucocorticoid responsiveness for casein accumulation had been restored by some factor(s) released by the stromal elements of the explants. This investigation was supported by Public Health Service grant numbers CA09041 and CA05388 awarded by the National Cancer Institute, DHHS.

B 188 GLUCOCORTICOID SENSITIVITY OF OVCA 433 HUMAN OVARIAN CARCINOMA CELLS: INHIBITION OF PLASMINOGEN ACTIVATORS, CELL GROWTH AND MORPHOLOGICAL ALTERATIONS, Bruce A. Littlefield, Waheeda Amin and Beth Y. Karlan, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT 06510

OVCA 433 human ovarian carcinoma cells (R.C. Bast, Jr. et al, J. Clin. Invest. 68,1331-1337, 1981) secrete large amounts of both urokinase (UK) and tissue-type plasminogen activator (tPA). Extracellular UK and tPA activities are 95% inhibited by treatment of cells with 100 nM dexamethasone (Dex) for 3 d. Dex inhibition of both PA types is time and concentration dependent, with half-maximal inhibition occurring with 1 nM Dex after 3 d, or with 100 nM Dex after 1.5 d. Dex treatment leads to a 30% inhibition of cell growth and a pronounced flattening and enlargement of the cells. Other classes of steroid hormones - estrogens, progestins, androgens and mineralcorticoids - are without effect on UK and tPA activities, cell growth, or morphology. OVCA 433 cells contain about 14,000 nuclear glucocorticoid receptors (GR) per cell (measured at 37°C), with an average affinity (Kd) for [^{3}H]Dex of 6.6 nM. Only active glucocorticoids compete with [^{3}H]Dex for nuclear GR binding sites. Our results demonstrate for the first time glucocorticoid sensitivity of ovarian carcinoma cells, a tumor type not usually considered hormonally responsive. Since almost 90% of ovarian carcinoma tumor samples contain GR (M.C. Galli et al, Cancer 47,1297-1302, 1981), it is possible that glucocorticoid sensitivity could be exploited and/or augmented by appropriate combination chemotherapy, particularly following the development of resistance to the more conventional chemotherapeutic drugs.

B 189 RAPID CELLULAR RESPONSES INDUCED BY ESTRADIOL AND EGF IN HUMAN MAMMARY TUMOR CELLS: S6-PHOSPHORYLATION, PROTEIN SYNTHESIS AND c-myc EXPRESSION.

I.Novak-Hofer,W.Küng, D.Fabbro, I.Lützelschwab and U.Eppenberger
Depart. of Research, Laboratory of Biochemistry-Endokrinology, University Clinic Medical School CH-4031 Basel, Switzerland

The proliferation of the human mammary tumor cell line ZR-75-1 is stimulated by estradiol (E_2) and Epidermal Growth Factor (EGF) or α-Transforming Growth Factor (α-TGF) to a similar extent and in an additive manner. Whereas EGF and α- TGF led to rapid activation of 40S ribosomal protein S6 kinase and S6 phosphorylation, E_2 did not induce these responses. The rates of onset of overall protein synthesis during the first 4 h of stimulation were distinctly slower with E_2 than with EGF. Northern analysis of RNA from EGF (α-TGF) and E_2-treated ZR-75 cells using a human c-myc probe revealed distinctly slower induction of c-myc expression by E_2 compared with EGF (α-TGF).

B 190 PROTO-ONCOGENE EXPRESSION DURING ESTROGEN STIMULATION OF CELL PROLIFERATION IN CHICK OVIDUCT. S.A. Rempel, and R.N. Johnston, University of Calgary, Alberta, Canada.

The expression of certain proto-oncogenes (eg *c-myc*) is enhanced in cells stimulated to divide by such extracellular proteins as platelet-derived growth factor and epidermal growth factor. However, it is not known whether cell proliferation induced by steroid hormones is also associated with regulation of expression of identified proto-oncogenes. We have examined this potential mode of regulation in the chick oviduct which begins intense cell proliferation within 24 hours of estrogen administration *in vivo*. Continued steroid treatment results in the differentiation of epithelial cells, and in the secretion of specific proteins such as ovalbumin. Therefore, estrogen treated chick oviduct provides an *in vivo* system in which to study steroid hormone regulation of proto-oncogene expression during normal cell proliferation and differentiation.

Five day old chicks were injected daily with estrogen, and sacrificed on days 0, 1, 7 and 14 of treatment. Wet weight measurements verified the onset of tissue-specific, hormone-induced growth of the oviduct tissue, but not in control liver and heart tissue. RNA samples from these tissues were used in Northern blot hybridizations with radiolabelled control and proto-oncogene probes. Abundant ovalbumin transcripts were detected in day 7 and 14 oviduct, indicating normal oviductal differentiation. Actin transcripts were detected in all tissues, and increased in abundance during oviduct maturation with the appearance of smooth muscle cells. Glyceraldehyde 3-phosphate dehydrogenase transcripts did not vary in abundance, as expected with a constitutively expressed gene.

In contrast, c-*myc* transcript abundance was greatly increased in day 1 oviduct tissue, and transcripts were present in elevated levels on days 1, 7 and 14. Nuclear run-off measurements of c-myc transcription rates indictate a 10-fold increase by day 1, with a return to baseline by day 14. Hybridizations with c-*fos* probe also indicated increased transcripts on days 1, 7 and day 14 in oviduct tissue. Neither of these transcripts was comparably increased in control tissues.

These results are consistent with the activation of proto-oncogene expression during the normal proliferation of cells. Furthermore, these results provide new evidence that steroid hormones may directly or indirectly regulate the expression of proto-oncogenes *in vivo* during this cellular process.

B 191 DIFFERENTIAL REGULATION OF GLUCOCORTICOID RECEPTORS IN THE LIVER AND SKELETAL MUSCLE OF IMMATURE AND MATURE MALE RATS, R. Sharma and P.S. Timiras, University of California, Berkeley, CA 94720.

Glucocorticoids stimulate enzymes and increase protein and glycogen synthesis in liver whereas they decrease synthesis and increase degradation of protein and RNA in skeletal muscle. These responses are controlled partly by binding the hormone to specific intracellular receptors followed by translocation of hormone-receptor complexes to nuclear acceptor sites. Adaptive alterations in responsiveness to hormonal and other biochemical stimuli are age-related phenomena. Specific binding of [^{3}H] dexamethasone to cytosol and translocation of bound receptor complexes to purified nuclei were studied in the liver and skeletal muscle of immature (3-week) and mature (26-week) Long-Evans male rats. A marked decrease in the specific binding sites with no apparent change in dissociation constant (Kd) was observed in both the tissues of mature rats compared to immature. This decrease was more pronounced in the skeletal muscle than liver. Heat activation (25°C for 45 min) significantly enhanced nuclear binding of steroid-receptor complexes in the skeletal muscle to the same extent at both ages. In the liver, enhancement was significantly higher in the mature rats than immature. Cross-mixing experiments (i.e., binding of activated cytosol from mature rats to nuclei of immature and vice-versa) show an age-related receptor specificity. Ca^{2+} activated (0°C for 45 min with 20mM Ca^{2+}) nuclear translocation was significantly higher in both tissues of immature rats compared to mature. Thus, the level of glucocorticoid receptor and some of its physiochemical properties change differentially with age in the tissues studied.

B 192 **EFFECT OF INTERLEUKIN-1 ON GLUCOCORTICOID ACTION IN REUBER H35 HEPATOMA CELLS.** **R.D.Stith, M.R. Hill and R.E. McCallum, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190.**

Interleukin-1 (IL-1) appears to be an important monokine released during acute infection and inflammation. Previous work in this laboratory has shown that IL-1 interferes with glucocorticoid-regulated liver metabolism by decreasing hepatic cytosolic glucocorticoid binding, inhibiting the induction of phosphoenolpyruvate carboxykinase (PEPCK), the rate-limiting enzyme in gluconeogenesis, and decreasing plasma glucose levels in C3H/HeJ mice. The purpose of this study was to examine the effect of recombinant IL-1(human, β) on hepatic glucocorticoid function in Reuber H35 hepatoma cells (RHC). RHC were incubated with various concentrations of IL-1 ranging from 10^{-9} M to 10^{-12} M. The smallest concentration lowered specific ^{3}H-dexamethasone binding in the cytosol to 72.3±3.0 % of control. Scatchard analysis of the binding data revealed a significant reduction in Bmax from 201.9 fmol/mg protein in control incubations to 144.9 fmol/mg protein in IL-1 treated cells, with no change in values of Kd (control, 1.0 nM; IL-1 treated, 1.14 nM). The perturbation seen in glucocorticoid binding due to IL-1 was reflected in reduced activity of PEPCK. PEPCK induction by dexamethasone (2 μM) and cAMP(0.5 mM) was inhibited by IL-1 to 86% of control. These data suggest IL-1 perturbs glucocorticoid action and alters gene expression in hepatoma cells.

Supported by NIH grant #AI20322 from the National Institute of Allergy and Infections Diseases.

B 193 DECREASED GLUCOCORTICOID RECEPTOR BINDING SITES BY 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN (TCDD) IN RAT LIVER CYTOSOL, Geoffrey I. Sunahara, George W. Lucier and Karen G. Nelson, The National Institute of Environmental Health Sciences, Research Triangle Park, NC, 27709.

Human exposure to halogenated aromatic hydrocarbons such as TCDD (an environmental contaminant) has led to clinical reports describing chloroacne and hepatic dysfunction. TCDD is also a potent teratogen and liver tumor promoter in certain animal models. Studies have indicated that adrenalectomized (ADX) rats are more sensitive to TCDD exposure than sham-operated controls. The exact mechanism remains unclear. To determine whether alterations in circulating glucocorticoids and/or its receptor (GRc) pathway are involved in some of the TCDD effects, we performed time course and dose-response studies using sham-operated and 10-13 days post-ADX adult female Sprague-Dawley rats treated with TCDD or the corn oil vehicle. TCDD exposure (≥ 10 µg/kg) maximally reduced hepatic cytosolic [^{3}H]-dexamethasone binding levels in sham-operated, Kd= 0.52 ± 0.05 (SEM) nM; Bmax= 137 ± 5 fmol/mg protein and ADX animals (Kd= 0.68 ± 0.04 nM; Bmax= 495 ± 23 fmol/mg) compared to corn oil treated sham-operated (Kd= 0.67 ± 0.04 nM; Bmax= 307 ± 19 fmol/mg) and ADX controls (Kd= 0.78 ± 0.06 nM; Bmax= 682 ± 80 fmol/mg), as early as 6 hrs following a single administration of the dioxin by gavage. This effect was dose-dependent in intact animals (ED50= 3-5 µg/kg); moreover, ADX rats showed maximal reduction in GRc levels at TCDD doses as low as 1 ng/kg. These results suggest that circulating glucocorticoids may be important in modifying the animal's response to TCDD. Futhermore, modification of the GRc pathway may have a role in some of the actions of TCDD in the liver.

B 194 GLUCOCORTICOID REGULATION OF PHOSPHOPROTEIN MATURATION IN RAT HEPATOMA CELLS, Anne K. Vallerga and Gary L. Firestone, Department of Physiology-Anatomy and Cancer Research Laboratory, U.C. Berkeley, Berkeley, Ca., 94720. Mouse mammary tumor virus (MMTV) infected rat hepatoma cells were utilized to study glucocorticoid regulated maturation of the MMTV phosphoproteins and to identify posttranslational regulatory mechanisms under steroid control. Previous results demonstrated that the glucocorticoid regulated maturation of the MMTV phosphorylated polyprotein, Pr74, into several protein products results from an independent hormone response that requires receptor function and de novo RNA and protein synthesis. Comparison of MMTV phosphoproteins expressed in parallel [^{35}S]methionine and [^{32}P]phosphate pulse-chase radiolabeling experiments revealed that the phosphorylation of Pr74 is stabilized in the presence of dexamethasone and rapidly turned over with a half-life of 1.5 hrs in uninduced cells. Exposure to actinomycin D, prior to dexamethasone did not alter the phosphorylation state of Pr74 but prevented protein processing of this precursor. Initial cell fractionation studies have shown that Pr74 is associated with the intracellular membrane fraction and can be salt extracted suggesting that maturation of the viral phosphoproteins occurs on the cytoplasmic face of the membrane. Using in vitro translated polyproteins as substrates we have shown that cellular components in extracts from dexamethasone treated cells can mature the phosphoproteins in vitro. We are using this cell-free maturation assay to identify and isolate hormone-induced componants that mediate the glucocorticoid-regulated phosphoprotein maturation. (Supported by NIH NRS training grant CA09041, and NCI CA35547).

B 195 BIOLOGICAL ACTIVITY OF NOVEL PURE ANTIOESTROGENS, Alan E. Wakeling, Imperial Chemical Industries, PLC, Pharmaceuticals Division, Macclesfield, Cheshire. England.

A complete understanding of the role of oestradiol in control of the growth of hormone-responsive tissues, including breast tumours, has proved elusive. Currently available antagonists, for example tamoxifen, are of limited use in this respect because they are all partial agonists and their physiological effects are diverse. The partial-agonist antioestrogens stimulate uterine growth in rats and mice and are correspondingly unable to block completely the uterotrophic action of oestrogens. The novel 7α-substituted analogues of oestradiol described here are devoid of uterotrophic activity in both rats and mice and will eliminate uterine growth responses to both endogenous and exogenous oestradiol. These compounds also block the uterotrophic response to partial-agonist antioestrogens. They competitively inhibit receptor binding of oestradiol and are potent, reversible, inhibitors of breast tumour cell growth in vitro. These compounds thus appear to satisfy the pharmacological criteria of pure antioestrogens and offer, for the first time, the opportunity to study in an unequivocal manner the physiological and biochemical consequences of the complete blockade of oestrogen action.

B 196 ESTRADIOL INDUCES ACTIVATION OF fos PROTO-ONCOGENE TRANSCRIPTION IN RAT UTERUS IN VIVO. Alessandro Weisz and Francesco Bresciani, Istituto di Patologia generale ed Oncologia, Ist Medical School, University of Naples, 80138 Naples, Italy.

We tested wether a correlation exists between estrogen induction of uterine cells proliferation and proto-oncogene expression. Groups of castrated adult female rats were injected both with estradiol-valerate in sesame oil (100 μg/Kg; I.M.) and 17β-estradiol in 10% ethanol (15 μg/Kg; I.P.). The relative transcription rate of 20 proto-oncogenes (abl, bas, erb A, erb B, ets, fms, fos, fps/fes, mos, myb, myc, raf, Ha-ras, Ki-ras, rel, sis, src, N ras, N myc, B lym) was then measured, at different time intervals from the hormone administration, in uterine and liver nuclei with a nuclear run-on assay. Transcription of fos proto-oncogene begins to increase steadily in the uterus 9-12 hrs after estrogen treatment, reaches a maximum (4 to 6 fold increase) at 23-33 hrs and then decline to reach at 54 hrs the same value measurable at 9 hrs. Transcriptional activation of fos proto-oncogene does not occurs in the liver of the same animals, and in the uterus of rats injected with the vehicles alone. Increased levels of fos mRNA were also found in the uterus of estrogen treated rats, as detected by Northern blot analysis. Maximum DNA synthesis, measured by ^{3}H thymidine incorporation and H4 histone genes transcriptional activation, followed by 33 to 45 hrs the estrogen administration only in the uterus but not in the liver. Supported by a grant of the Italian National Research Council, Special Project "Oncology", Contract no. 85.02053.44.

B 197 SPECIFIC FACTORS REGULATING RAT GROWTH HORMONE GENE EXPRESSION, Brian L. West, Daniel F. Catanzaro and Tim L. Reudelhuber, Univ.of Calif.,San Francisco, CA 94143

Rat growth hormone (rGH) gene expression is pituitary specific and is regulated by both glucocorticoid and thyroid hormones. When the rGH 5'-flanking region is linked to the chloramphenicol acetyltransferase(CAT) gene, it functions in a tissue-specific, hormone-inducible fashion. We have focused on this region of the rGH gene to identify DNA elements and nuclear factors which are important for its expression. By monitoring the DNAse1 footprinting of GC pituitary cell extracts onto the rGH flanking DNA, two separate binding regions were identified. These are located between bases -62 and -94 and between bases -105 and -140, with respect to the RNA start site. The factors which bind at these positions, which we refer to as GC1 and GC2, are tissue specific, since they are undetectable in the non-pituitary cells tested. A series of 5' deletion mutations were made, and the two binding regions were also altered by site-directed mutagenesis. These mutant 5'-flanking sequences were placed in front of the CAT gene, to determine which positions were required for directing the transient expression of this enzyme in GC cells. Mutating or deleting both the GC1 and GC2 binding regions caused significant decreases in the CAT expression. DNA elements further upstream of -140 were also shown to be important by the deletion analysis. Therefore, the GC1 and GC2 binding activities appear to represent factors which are important for the tissue-specific expression of the rGH gene. Possible interactions of these factors with the thyroid hormone and glucocorticoid hormone receptors are now being investigated.

B 198 MULTISTEROID REGULATION OF PROTEIN MATURATION IN VIRAL INFECTED RAT HEPATOMA CELLS, Sandra D. Winguth and Gary L. Firestone, Department of Physiology-Anatomy & Cancer Research Laboratory, University of California, Berkeley, CA 94720. Dexamethasone, a synthetic glucocorticoid,is required for full posttranslational maturation of mouse mammary tumor virus(MMTV) glycoproteins and phosphoproteins in M1.54 cells, a viral infected rat hepatoma cell line. Pulse-chase radiolabeling with [^{35}S]methionine revealed that glucocorticoids (dexamethasone and hydrocortisone) and mineralcorticoids with known glucocorticoid activity (11-dexoycorticosteron and aldosterone) regulated the maturation of MMTV glycoproteins and phosphoproteins in a manner that correlates with their occupancy and affinity for M1.54 cell glucocorticoid receptors and their induction of cellular encoded alpha$_1$-acid glycoprotein, tyrosine aminotransferase and glutamine synthestase. Exposure to RU38486, a synthetic glucocorticoid antagonist, prevented induction of the steroid-regulated posttranslational response as well as the other tested glucocorticoid responsive genes. Significantly, progesterone induced the posttranslational maturation of MMTV phosphoproteins but antagonized the glucocorticoid-regulated MMTV glycoprotein maturation and all other tested M1.54 cell glucocorticoid responses. Exposure to suboptimal concentrations of progesterone and dexamethasone synergistically regulated phosphoprotein maturation while treatment with actinomycin D, an inhibitor of de novo RNA synthesis, prevented both the progesterone and glucocorticoid mediated postranslational responses. Taken together, our results suggest that two distinct classes of steroid hormones can alter the posttranslational maturation of a specific subset of protein substrates by inducing the synthesis of cellular components in a receptor dependent process.(Supported by NCI CA 35547 and NIH NRS training grant CA 09041).

B 199 THE RETINAL-SPECIFIC GLUCOCORTICOID HORMONE INDUCTION OF GLUTAMINE SYNTHETASE mRNA IN THE CHICK IS A PRIMARY RESPONSE, Anthony P. Young and Gerald Patejunas; University of Illinois at Chicago; Chicago, IL 60680. During terminal differentiation of the chick, glutamine synthetase(GS) enzyme activity rises dramatically in retina and in brain, but is expressed constitutively in liver. We have isolated cDNA clones that encode chick retinal GS. The clones detect a 3.2 kb RNA that is expressed in retina, brain, and liver. Expression of this RNA in the three tissues parallels the developmental profiles of GS enzyme activity. Injection of hydrocortisone 21-phosphate into the yolk sac of chicks at embryonic day 10 results in a retinal-specific induction of GS mRNA, assayed 4 days after injection. Southern blotting data demonstrate that the RNA detected in the three tissues is the product of one gene, indicating that tissue-specific regulatory mechanisms must modulate expression of this complex gene. A readily measurable increase in GS mRNA is observed within 2 hr of incubation of retinal organ cultures with hydrocortisone. Incubation of these cultures with 2 µg/ml cycloheximide, a concentration that inhibits protein synthesis by 93%, affects neither the basal level nor the hydrocortisone-mediated induction of GS mRNA. These data raise the possibility of a synergistic mechanism, involving tissue-specific regulatory molecules in addition to the glucocorticoid hormone receptor, to explain the retinal-specific steroid induction of GS mRNA.

B 200 THE EFFECT OF ESTROGENS ON HUMAN T CELLS FROM POSTMENOPAUSAL WOMEN WITH AND WITHOUT ENDOMETRIAL CARCINOMA. I. Yron[1], I. Lidor[2], Y. Pardo[2], J. Ovadia[2], I.P. Witz[1], and B. Sredni[3]. [1]Tel Aviv University, Tel Aviv; [2]Beilinson Medical Center, Petah Tikva; [3]Bar Ilan University, Ramat Gan, Israel.

Endometrial carcinoma (EC) is known to be associated with long term exposure to estrogens unopposed by progestogens. We studied some characteristics of peripheral blood T cells from Stage I EC patients and from women with various degrees of hyperplasia of the endometrium, being the preneoplastic stages of the disease, and the in-vitro and the in vivo effect of estrogens on T cell responses. We have previously shown an altered T cell response in EC patients. We present here data suggesting that suppessor T cells respond to estrogens and may be one of the mechanisms responsible for the deficiency reported above. A small proportion of Con-A activated PBL form rosettes with autologous and allogeneic erythrocytes (designated autorosette forming cells - ARFC). These cells are known to suppress various T cell functions probably by producing suppressor factors. Our data show that the frequency of ARFC in patients with proliferative endometrium and with EC was 2-5 fold higher than that of healthy controls or of patients with stage I of non-hormone dependent cancer. The suppressor factor produced by patients' ARFC was more active as well. Futhermore, the in vitro addition of estradiol to Con-A stimulated PBL from healthy donors increased the frequency of ARFC to levels found in PBL from EC patients. Tamoxifen (an anti estrogen drug), reduced the frequency of the estrogen-stimualted ARFC to the original low level. These results thus indicate that estrogen may play an in vivo inhibitory role on certain T cell functions.

B 201 DEXAMETHASONE INHIBITS COLLAGEN SYNTHESIS BY A DIRECT EFFECT ON THE COLLAGEN GENE PROMOTER. M.A. Zern, M.J. Czaja, R. Tur-Kaspa and F.R. Weiner, Albert Einstein College of Medicine, Bronx NY 10461.

Glucocorticoids have been shown to be useful in the treatment of certain types of chronic liver disease, and we have recently shown that detoxified alcoholics with high endogenous cortisols have a trend towards better liver function. We previously demonstrated that dexamethasone (DEX) inhibits collagen synthesis by decreasing the transcription of the types I and IV collagen genes in in vitro and in vivo systems. To delineate more precisely the mechanism whereby steroids affect collagen gene transcription, we evaluated the effects of DEX on the mouse $\alpha 2(I)$ collagen gene promoter in a transient expression vector system. The mouse $\alpha 2(I)$ collagen gene promoter was linked to the structural gene for chloramphenicol acetyl transferase (CAT) and the SV40 enhancer in the recombinant plasmid pAZ1009 (kindly provided by Dr. B. de Crombrugghe NIH). Collagen gene promoter activity was then determined by transfecting this plasmid into mouse fibroblast cell lines and measuring the resulting CAT expression of the cells grown in the presence or absence of 10^{-6}M DEX. In cells transfected with the construct containing the collagen promoter pAZ1009 and grown in DEX, CAT activity was decreased by 62% ($p < .05$) compared to cells transfected with the same plasmid and grown in the absence of DEX. No DEX effect was noted with either the positive, pSV_2CAT, or the negative control, $pA_{10}CAT$, both of which lack the collagen gene promoter. Review of the nucleotide sequence from the promoter region of the mouse $\alpha 2(I)$ collagen gene revealed a sequence at map position -323 to -328 which is similar to the glucocorticoid consensus sequence. In conclusion these data suggest that DEX exerts one of its inhibitory effects on collagen synthesis through a direct effect on the collagen gene promoter possibly through a glucocorticoid responsive-like element. Studies of deletion mutants of this collagen gene promoter are presently underway to better define the basis for this inhibition.

B 202 CLONING AND CHARACTERIZATION OF THE 1,25 DIHYDROXY VITAMIN D_3 RECEPTOR. McDonnell, D.P., Manglesdorf, D.J. Pike, J.W. Haussler, M.R. and O'Malley, B.W. Baylor College of Medicine Houston, TX 77030 and University of Arizona Health Science Center. Tuscon, AZ 85724.

We have cloned the receptor for 1,25 Dihydroxy vitamin D_3 from a chicken intestinal λgt11 expression library. The initial clone was obtained using a rat monoclonal antibody to the receptor to screen $3X10^6$ recombinants in a randomly primed library. In this screen a single immunopositive clone was isolated. We authenticated this clone by showing that it crossreacted with a second independent monoclonal antibody to the receptor. The clone contained a small insert of 310bp. This insert was subcloned and was used to hybrid select a specific message from intestinal poly A^+ mRNA which when translated gave a 60kd protein which co migrated with the receptor and was also immunoprecipitable. Northern analysis also gave the predicted results. The clone hybridized to a message of 3.5kb that was present in the intestine and kidney (the main target tissues) but did not hybridize to messages in the liver, a tissue in which there is no detectable receptor. The size of the message also correlated with that predicted from in vitro translation and immunoprecipitation of receptor mRNA sized across a sucrose gradient. We sequenced a series of clones obtained by hybridization and deduced the amino acid sequence of the receptor. When this was compared to the amino acid sequence of the chicken progesterone and estrogen receptors it was shown to be extremely homologous in the putitive DNA binding regions of these receptors. It was found also to be the most homologous of all the receptors to v-erbA.