Su-Pos466

A HIERARCHY OF RELAXATION PHENOMENA OBSERVED IN HEMOGLOBIN EMBEDDED IN A ROOM TEMPERATURE TREHALOSE GLASS. ((M. Yang, J. Wang, A. Sheikh, D.S Gottfried, E.S Peterson and J.M. Friedman)) Department of Physiology and Biophysics, Albert Einstein College of Medicine, Bronx, NY 10461

Upon photodissociation COHb exhibits a wide range of nonequilibrium relaxation phenomena starting within some fraction of a picosecond and extending out to tens of microseconds. In addition equilibrium fluctuations result in conformational averaging. All of these dynamics can have an impact on ligand rebinding. In an effort to better understand the relationship between conformational dynamics and ligand binding reactivity, we have embedded COHbA in a room temperature trehalose sugar glass [Hagen et al., *Science* **269**,959 (1995)] in order to decouple solvent dependent and solvent independent dynamics as well as reducing the amplitude for large scale conformational fluctuations. Ligand rebinding kinetics are followed as a function of temperature (60-300K) and compared to those observed in water-glycerol solvents (50-95% glycerol:water). Time resolved resonance Raman is used to determine the effect of the trehalose glass on the fast relaxation of the heme iron-histidine unit. Time correlated single photon counting fluorescence lifetime measurements are used to probe the overall dynamics of the globin as reflected in the distribution of lifetimes for the tryptophan residues in HbA. The results show that the trehalose glass does not impede the initial fast relaxation of the iron-histidine unit but does dramatically impede conformational averaging and completely eliminates ligand escape at all temperatures examined. The fluorescence studies show that when COHbA is embedded in the room temperature trehalose glass, the picosecond Trp lifetimes are nearly unchanged, but there is a complete absence of the nanosecond fluorescence decay (observed in aqueous solutions), which is replaced by a decay of 600-800 ps and an amplitude of approximately 1%. We ascribe this change in the fluorescence decay to a significant decrease in the coupled structural dynamics of the A (Trp's α14,β15) and E helices that normally allow transient opening of the distal heme pocket.

Su-Pos467

CRYSTAL STRUCTURE OF THE C-TERMINAL DOMAIN OF GELATINASE A. ((A. M. Libson[1], A. G. Gittis[1], I. E. Collier[2], B. L. Marmer[2], G. I. Goldberg[2], E. E. Lattman[1])) [1]Johns Hopkins School of Medicine, Baltimore MD 21205. [2]Washington University School of Medicine, St. Louis MO 63110.

Gelatinase A is one of a family of extracellular matrix metalloproteases (MMP) which play a role in normal physiological processes such as cell morphogenesis and tissue repair. Overexpression of gelatinase A has been shown to promote tumor invasion and metastasis in certain malignant cells. Understanding the mechanism of spatial and temporal regulation of gelatinase A is critical in describing and controlling these processes. Regulation of gelatinase A has been shown to be mediated by its hemopexin-like C-terminal domain. Here, we report the crystallization and completed X-ray crystal structure of the hemopexin-like C-terminal of gelatinase A (gA-Ctd) determined at 2.15 Å. The gA-Ctd structure is one of the first reported examples of a four-bladed β-propeller protein comprised of four homologous, anti-parallel β-sheet domains which are symmetrically arrayed about a central pseudo four-fold axis. The four-fold axis forms a large channel-like opening in gA-Ctd in which a metal ion, Ca^{2+}, and a Na^+ and Cl^- ion pair are bound. The Ca^{2+} ion is coordinated exclusively by main chain carbonyl oxygens and water molecules representing a novel metal binding motif in proteins. Also, a Zn^{2+} metal ion is found coordinated by two symmetry related gA-Ctd molecules. With this structure, we have modeled a complete active gelatinase molecule which includes the catalytic and the fibronectin-like domains. Given the homology among MMPs, the overall fold of other MMP hemopexin domains is expected to be similar to that of gA-Ctd. The high degree of internal symmetry found in gA-Ctd suggests that it may have evolved from a gene duplication event.

Su-Pos468

SPATIAL DYNAMICS AND PATTERN FORMATION DURING CLOT GROWTH *IN VITRO*. ((F.I.Ataullakhanov, G.T.Guria, V.I.Sarbash, R.I.Volkova and V.Zarnitcina)) National Research Center for Hematology, Moscow, Russia, 125167.

We studied the spatial and temporal characteristics of a growing clot *in vitro* by simultaneous monitoring of the production of thrombin and polymerized fibrin. Following contact with an activating surface thrombin concentration near the surface grows exponentially and propagates from the activating surface in a form of a thrombin wave. The amplitude and speed of the thrombin wave remain constant up until the wave stops abruptly at some distance from the activating point. Fibrin polymerization mimics the thrombin propagation after a small delay. The developed clot becomes surrounded by an "inactivation zone", in which coagulation is strongly suppressed. When coagulation threshold is decreased, the "stratified structures" can be formed, in which solid layers alternate with liquid blood.

We interpret the observed regimes of clot growth in terms of the hypothesis that blood is a new type of active medium. When coagulation is initiated two active waves propagate from a point of activation. Interaction of these autowaves determines the dynamics and size of a clot.

We constructed and analyzed the phenomenological and kinetic models of clot formation and thrombin production according to the reactions of the intrinsic coagulation pathway. The models agree well with the experimental data.

CHEMOSENSORY TRANSDUCTION AND CHEMOTAXIS

Su-Pos469

AN APPROACH TO A SPATIAL DISTRIBUTION OF OLFACTORY RECEPTOR NEURONS WITH IDENTIFIED TUNING SPECIFICITIES. ((J. Hirono, T. Sato and M. Takebayashi)) Life Electronics Research Center, Electrotechnical Lab., Amagasaki, 661 Japan.

In odor responses to n-aliphatic odorants with a straight chain of 3-9 carbons, the relationship between spatial distances and odorant tuning specificities were examined in mouse olfactory receptor neurons (ORNs). The ORNs were isolated on a Cell-Tak coated cover glass by the tissue-printing method which was expected to reserve their relative spatial distance in intact tissue within about 200 µm. Odor responses were recorded optically by measuring intracellular calcium increases with fura-2. Tuning specificities were dependent on both the carbon chain length and the functional group (carboxyl and hydroxyl). In most ORNs, the sensitivity was maximal at a single carbon chain length, therefore, the value of similarity for their tuning specificities could be evaluated from the difference between the lengths. We found that ORNs responsive to n-aliphatic odorants tended to be distributed within close proximity to each other according to the increase in the similarity of the tuning specificities and there was a correlation between the spatial distance and the value of similarity . On the other hand, many ORNs did not respond to n-aliphatic odorants in the neighborhood of ORNs sensitive to subsets of the odorants. Our results also indicated that ORNs with quite different tuning specificities were distributed in the neighborhood.

Su-Pos470

SEPARATION OF WHITE BLOOD CELLS FROM RED BLOOD CELLS IN A MICROFABRICATED LATTICE ((R.H. Austin and Shirley S. Chan)), Dept. of Physics, Princeton University, Princeton, NJ 08544

Leukocytes (WBCs) are relatively rare in a healthy person: approximately 1 out of every thousand cells in the blood are WBCs, the rest are predominantly the un-nucleated erythrocytes (RBCs). Chemotactic control of WBC invasion of small openings (one micron or less) is a critical element in understanding, and perhaps someday controlling, the invasive and lethal transformation of WBCs in leukemia. Quantitative study and understanding of the physical phenomena involved in chemotaxis requires the ability to both capture from blood the relatively rare WBCs and then to challenge them with a precise array of variable sized openings in the presence of a known chemical gradient. We have recently been able to show that a suitably chosen opening in microlithographically constructed array structures can with 100% efficiency sort rare white blood cells from red blood cells and hold them for optical observation under a transparent glass lid. We have developed sensitive fluorescence and dark-field techniques to observe the signal transduction and motile response to both hydrodynamic shear fields and chemotactic patterns. Videos of this work will be shown.

Su-Pos471

RAPID ALTERNATE MEASUREMENT OF MEMBRANE POTENTIAL AND INTRACELLULAR CALCIUM IN CELL ENSEMBLES. M.M. Zviman, Y. Hayashi, J.G. Brand, J.H. Teeter and D. Restrepo. [1]Monell Chemical Senses Center; [2]Department of Physiology, University of Pennsylvania; and [3]Veterans Affairs Medical Center, Philadelphia, PA 19104.

Multiparamter optical measurements have played a key role in understanding the behavior of multicellular systems. We have studied the spectral properties of the voltage-sensitive dye di-8-ANEPPS and the Ca^{2+}-sensitive dye fura-2 in azolectin liposomes and in isolated taste buds from mouse. We find that the fluorescence excitation spectrum of di-8-ANEPPS and fura-2 are largely non-overlapping allowing alternate ratio measurements of membrane potential and $[Ca^{2+}]_i$ in the same preparation using excitation wavelengths of 340 and 360 nm for fura-2, and 440 and 500 nm for di-8-ANEPPS. There is a small spillover of di-8-ANEPPS fluorescence into the excitation ranges used for fura-2 (340 and 360 nm, 12.5 nm bandwidth). However, voltage-induced changes in fluorescence of di-8-ANEPPS excited at the fura-2 wavelengths are small. In addition, di-8-ANEPPS fluorescence is localized to the membrane while fura-2 fluorescence is localized to the cytoplasm Because of this, under the appropriate conditions the effect of spillover of di-8-ANEPPS fluorescence in the $[Ca^{2+}]_i$ estimate is <1 %. Moreover, we find that in double-labeled cells the ratio of fluorescence emitted when excited at 360 nm divided by fluorescence emitted when excited at 440 nm (F_{360}/F_{440}) provides an empirical parameter to quantify the extent of the error due to di-8-ANEPPS spillover. We have applied this method to study the response of taste cells in multiple cells within isolated taste buds. We show that membrane potential and $[Ca^{2+}]_i$ can be measured alternately in isolated taste buds from mouse. Stimulation with glutamate and glutamate analogues indicates that taste cells express both metabotropic and ionotropic receptors. Alternate optical measurement of membrane potential and $[Ca^{2+}]_i$ is a technique that should be applicable to a variety of multicellular preparations.

Work supported by NIH grants DC01838 and DC00566.

Su-Pos472

PROTEOLYSIS STUDIES OF DYNAMIC STRUCTURE IN BACTERIAL CHEMOTAXIS RECEPTORS ((S.K. Seeley, O.J. Murphy III, K.L. Ouellette, R.M. Weis, and L.K. Thompson)) Department of Chemistry, University of Massachusetts, Amherst, MA 01003.

The transmembrane receptors of bacterial chemotaxis consist of a ligand-binding periplasmic domain, two membrane-spanning segments, and a cytoplasmic domain which interacts with cytoplasmic proteins to control swimming behavior. Although the cytoplasmic domain plays key roles in both excitation and adaptation, it is the least structurally understood region of the receptor. We have recently demonstrated global mobility in a 31 kDa cytoplasmic fragment (c-fragment) of the aspartate receptor which retains partial function. These findings have led us to question whether the unusual flexibility observed in the c-fragment is also a property of the native functioning receptor, and whether changes in flexibility play a role in signal transduction. Proteolysis experiments were used to compare the accessibility of the c-fragment *vs.* the cytoplasmic domain of the intact receptor. Results suggest that the intact receptor possesses flexibility similar to that of the c-fragment. Experiments which probe how proteolysis of the receptor changes upon binding of ligand or binding of the cytoplasmic proteins are in progress to probe the role of changes in flexibility in the mechanism of transmembrane signaling.

Su-Pos473

PROBING THE LIGAND-BINDING SITE OF THE TRANSMEMBRANE SERINE RECEPTOR USING SOLID-STATE NMR. ((Y.S. Balazs, E. Del Federico, J. Wang, and L.K. Thompson)) Department of Chemistry, University of Massachusetts at Amherst, Amherst, MA 01003.

The mechanism by which the membrane-bound serine receptor of bacterial chemotaxis relays environmental information across the cell's inner membrane to the cytoplasmic region, triggering a cascade of protein responses which control the swimming behavior of the cell, is unknown. The serine receptor is a 60kDa protein consisting of three general domains: a periplasmic ligand-binding domain, two membrane-spanning regions, and a cytoplasmic domain. Solid-state NMR can be used to directly probe the ligand binding site of the intact, membrane-bound serine receptor to identify any deviations from a computer generated model (1) based on the crystal structure of the homologous aspartate receptor soluble ligand-binding domain fragment (2). The 13C-NMR chemical shift anisotropy of the serine ligand carboxyl indicates that it is bound in a deprotonated state with hydrogen bonding consistent with a carboxylate to a guanidino group. In addition, ligand binding perturbs the arginine side chain nitrogen resonance in a uniformly 15N-labeled receptor, compatible with modeled interactions between bound ligand and the arginine side chains of the ligand-binding site. REDOR experiments are in progress to measure ligand-receptor distances and further test the structural model.

1. Jeffrey, C., and D. E. Koshland Jr. 1993. *Prot.Sci.* 2:559-566.
2. Milburn, M.V. *et al.* 1991. *Science.* 254:1342-1347.

GENE REGULATION AND TRANSCRIPTION

Su-Pos474

FLUORESCENCE SPECTROSCOPIC STUDY OF TATA BINDING PROTEIN INTERACTING WITH ITS ASSOCIATED TAFS (yTAF130 AND BRF1) USING INTRINSIC TRYPTOPHAN AND 5-HYDROXY TRYPTOPHAN. ((G. M. Perez-Howard, Y. Bai, K. E. Gerrish, P. A. Weil, J. M. Beechem)) Vanderbilt University, Nashville, TN 37232.

Many molecular biological studies have shown that protein-protein interactions between the TATA binding protein (TBP) and its TATA associated factors (TAF). 5-hydroxytryptophan (5HW) has been placed into TBP and has been successfully expressed and purified. Similar techniques are being applied to the yTAF's. A His-6 tag was placed in the carboxyl end of the protein in the purification procedure and results in the ability to obtain highly purified protein containing 5HW. 5HW has additional absorption bands to the red of tryptophan, providing the ability of monitoring the binding of TBP to its associated TAFs which is free of the interference due to the intrinsic tryptophans which may be present in any of the other associated proteins. Presently, the direct TBP binding of both the BRF1 factor in the pol III class and the yTAF 130 of the pol II transcriptionary machinery are being investigated. The 5HW has been inserted into a number of TBP protein regions, and also into many of the TAFs. Time-resolved anisotropy experiments reveal that there is indeed direct binding of TBP and TAF130 and other more complex effects on TBP conformation are also evident. The kinetics of the TBP/TAF interactions and the effect of prebound TAF on DNA binding rates will also be examined.

Su-Pos475

lac REPRESSOR: CORRELATION OF OVER 4000 SINGLE SITE MUTATIONS WITH THE 3-DIMENSIONAL STRUCTURE.((H.C. Pace, M. A. Kercher, M. Lewis and P. Lu)) Department of Biochemistry & Biophysics and Department of Chemistry, University of Pennsylvania, Philadelphia, PA 19104 ((J. H. Miller)) Department of Microbiology & Molecular Genetics, University of California, Los Angeles, CA 90024

The 3-dimensional structures of (1) the intact *lac* repressor, (2) the *lac* repressor bound to the gratuitous inducer 1-isopropyl-beta-D-thiogalactoside (IPTG), and (3) the *lac* repressor complexed with a 21 base-pair symmetric operator DNA provide the framework for understanding a wealth of biochemical and genetic information. A comprehensive site specific substitution analysis on the *lac* repressor protein has resulted in over four thousand single amino acid replacements of known phenotype between codons 2 through 329 of the *lacI* gene†. We have made an initial correlation of the site-specific mutational analysis with the 3-dimensional structures. There is an unexpectedly large number of proline and glycine tolerant sites, especially in the middle of alpha-helices and beta-sheets, which have no effect on repression, except in the DNA-binding domain itself, and in the most buried regions of the inducer binding domain. The most acceptable amino acid, however, is alanine which can be inserted at 90% of the positions with no effect on phenotype. Locations where these residues are not tolerated suggest either functional or folding roles. An overview of the effects caused by changes in residue surface area and hydophobicity will be presented. (Supported by the USARO)

†P. Markeiwicz, L. Kleina, C. Cruz, S. Ehret, J. H. Miller, *J. Mol. Biol.* **240**, 421-433 (1994).

Su-Pos476

ROLE OF AROMATIC STACKING INTERACTION IN HELIX-5 ON OPERATOR SITE DISCRIMINATION BY PHAGE λ *c*I REPRESSOR.
((Y.T. Huang, E. Russinova, J.B.A. Ross & D.F. Senear)) Dept. of Molecular Biology & Biochemistry, University of California, Irvine 92717 and Dept. of Biochemistry, Mount Sinai School of Medicine, NY, 10093.

Sequence specific DNA binding by regulatory proteins provides the basis for transcriptional regulation. Two levels of specificity are critical to regulation of the bacteriophage λ lysogenic to lytic switch. First, like all transcriptional regulators, *c*I repressor dimers distinguishes operator from non-specific DNA. Specific H-bonding constituting direct readout of the DNA sequence is considered the well understood mechanism for this. However, differential affinity for O_R1, O_R2 and O_R3 provides opposing regulation of divergent promoters that is equally critical to the switch. Site specificity at this level is poorly understood. Conformational adaptation by both the repressor and the different operators appears to be involved. To evaluate whether subunit:subunit interactions mediate the repressor conformational adaptations, we investigated the effects on both dimer stability and operator binding of amino acid substitutions at the contacts between symmetrically related helices-5 in the dimer interface. Substitutions for Y88 (A, C, F, S, & W) greatly perturb differential operator affinity without affecting operator versus non-operator specificity. The pattern of effects suggests that the geometry of the face-to-face aromatic stacking interaction between symmetrically related Y88's, a group in the dimer interface but far removed from the DNA binding interface, plays a critical role in operator discrimination. By contrast, the significant effect of I84S substitution is to greatly decrease affinity for all three operators. Presumably, the altered packing of the dimer interface moves the two helix-turn-helix motifs out of register with successive DNA major grooves. Supported by NIH GM41465.

Su-Pos477

BINDING OF *E. COLI* LEXA REPRESSOR TO THE *RECA* OPERATOR SEQUENCE
((Elisabeth S. Jenuwine, Nand K. Relan and Sandra L. Shaner)) Department of Chemistry, Wayne State University, Detroit, MI 48202

The *E. coli* LexA repressor coordinately controls about 20 genes comprising the bacterial SOS response, an inducible DNA repair system. The position of the operators relative to the promoters is highly variable among the SOS operons. While most possess only one operator, several have two operator sites with variable spacing between their dyadic centers, and one gene has been shown to have three operator sites. This suggests a highly intricate regulatory scheme to optimize the cellular response to DNA damage by adjusting the balance of the SOS proteins induced. We are attempting to understand this by studying the equilibrium binding of *E. coli* LexA repressor to some of its operators by the polyacrylamide gel mobility shift assay as a function of solution conditions. In the presence of NaCl at 22°C, there is a significant salt dependence in the binding to the *recA* operator, typical for protein-nucleic acid interactions with some electrostatic contributions to the binding free energy. In preliminary experiments in which we vary the anion of the Na^+ salt from chloride to fluoride, we see little change with anion identity. This indicates that the salt effect on the binding interaction arises solely from the polyelectrolyte effect, not from anion binding or release by the protein upon complex formation. Increasing the temperature to 37°C changes the binding affinity for complex formation at any given salt concentration and results in a change in the sensitivity of complex formation to NaCl concentration.

Su-Pos478

ION EXCHANGE IN THE BINDING OF AN HIV-1 TAT PEPTIDE TO TAR RNA ((M.G. Fried and D.F. Stickle)) Department of Biochemistry and Molecular Biology, Pennsylvania State University College of Medicine, Hershey, PA 17033

The equilibrium association constant for the binding of HIV-1 Tat-derived Tfr38 peptide to ΔTAR RNA (K_{obs}) depends strongly on salt concentration. Negative values of ∂logKobs/∂log[NaCl] are found at high salt concentrations, consistent with models that predict the displacement of cations from the environment near the RNA as the peptide binds. Dramatic increases in ∂logKobs/∂log[NaCl] at low salt concentrations suggest the involvement of additional ion exchange processes. The binding data of Weeks and Crothers ((1992) Biochemistry 31, 10281-10287) were analyzed according to models in which ion binding and release are consequences of the transfer of peptide from bulk solution to the higher cation concentrations and lower anion concentrations that exist in the vicinity of the RNA. Non-cooperative models for ion binding and release cannot account for the dependence of K_{obs} on [NaCl], but good correspondence is obtained when these reactions are modeled as cooperative processes. The results suggest that for complex formation at low [NaCl], the release of cations from ΔTAR RNA can be offset by the cooperative uptake of cations by Tat peptide. Supported by NSF grant DMB-91-96154.

Su-Pos479

DETERMINING EQUILIBRIUM CONSTANTS FOR THE BINDING OF NUCLEOTIDES TO Eσ^{70} RNAP-PROMOTER OPEN COMPLEXES FROM NUCLEOTIDE STABILIZATION EXPERIMENTS. ((K.L. McQuade‡, P.E. Schlax, Jr.† and M.T. Record, Jr.‡*†)) Departments of Biochemistry‡ and Chemistry* and Biophysics Program†, University of Wisconsin, Madison, WI 53706. (Spon. by John L. Markley)

At 10°C, Eσ^{70} RNA polymerase-λP_R promoter complexes cannot be completely driven to the open form, even in a vast excess of holoenzyme. Adding the initiating dinucleotide CpA (complementary to positions -1 and +1 of the transcribed strand) increases the equilibrium fractional occupancy $\theta^{\circ}_{RP_o}$ and decreases the first order dissociation rate constant k_d in a concentration-dependent manner. At a fixed concentration of CpA, adding UTP (+2,+4) has a similar effect on both parameters. Likewise, adding both UTP and GTP(+3) enhances this effect. We propose that the nucleotide-dependent increases in equilibrium occupancy and stability at λP_R result from abortive cycling by open complexes, which shifts the distribution of complexes toward the open form. We also present quantitative analyses of the dependences of $\theta^{\circ}_{RP_o}$ and k_d on the concentrations of initiating nucleotides that can be interpreted in terms of equilibrium constants for the binding of these nucleotides to open complexes and rate constants for phosphate bond formation and abortive transcript release.

Su-Pos480

EFFECT OF α-THIO-ATP ON THE STEADY-STATE KINETICS OF NUCLEOTIDE INCORPORATION CATALYZED BY THE WILD-TYPE AND ACTIVE-SITE MUTANT T7 RNA POLYMERASES.
((P. A. Osumi-Davis and A-Y. M. Woody)) Department of Biochemistry and Molecular Biology, Colorado State Univ., Fort Collins, CO 80523.

S_p-ATPαS is a substrate for T7 RNA polymerase and the reaction proceeds with inversion of configuration at the α-phosphorous (Griffiths et al., Nucl. Acid Res. 15, 4145, 1987). We have obtained steady-state kinetic parameters for ATP and S_p-ATPαS incorporation into a trimer (GGA) and a pentamer (GGACU) in the presence Mg^{2+} on a 22 bp template by the wild-type enzyme and the mutants, D537E, K631R, Y639F, H811Q and D812E. For the wild-type enzyme, no significant change in the $(k_{cat})_{app}$ (S_p-ATPαS) was observed compared to $(k_{cat})_{app}$ (ATP) implying that the rate limiting step is other than the chemical step. Interestingly all the mutants studied exhibited thio-effect of ~ 10-fold decrease in $(k_{cat})_{app}$ (S_p-ATPαS) suggesting that phosphodiester bond formation might be a rate-determining step. The results of these studies as well as the results of studies in progress using Mn^{2+} as a transcription activator will be discussed. A decrease in K_m(S_p-ATPαS) values was observed relative to K_m(ATP) for the wild-type and all the mutants. The implication of these results that the interaction between enzyme and nucleotide appears enhanced when sulfur is substituted for oxygen on the α-phosphate group in S_p-ATPαS will be discussed. Supported by NIH Grant GM23697 (A-YMW).

Su-Pos481

DNA BINDING DETERMINANTS OF THE α-SUBUNIT OF RNA POLYMERASE: A NOVEL DNA BINDING DOMAIN ARCHITECTURE.
((Jia[1], X., Krishnan[1], V.V., Gaal[2], T., Ross[2], W., Blatter[3], E.E., Tang[3], H., Ebright[3], R. H., Gourse[2], R.L., and Assa-Munt[1], N.)) [1]La Jolla Cancer Research Foundation, 10901 N. Torrey Pines, Rd. La Jolla, CA 92037. [2]Department of Bacteriology, University. of Wisconsin. Madison, WI 53706. [3]Department of Chemistry, Rutgers University. New Brunswick, NJ 08855. (spon by W. Chazin)

The *E. coli* RNA polymerase α-subunit binds via its C-terminal domain (αCTD) to a recognition element (the UP element) in certain promoters. We used biophysical, genetic and biochemical techniques to identify the residues in αCTD important for UP element-dependent transcription and DNA-binding. These residues occur in two regions of αCTD, close to, but distinct from, residues important for interactions with certain transcription activators. We used multidimensional NMR spectroscopy to determine the solution structure of αCTD. αCTD contains a non-standard helix followed by four α-helices. The two regions of αCTD important for DNA binding correspond to the first α-helix and the loop between the third and the fourth α-helices. The αCTD DNA-binding domain architecture is unlike any DNA-binding architecture identified to date. We propose that αCTD has a novel mode of interaction with DNA. Our results suggest models for αCTD-DNA and αCTD-DNA-activator interactions during the initiation of transcription. (The biophysical work was supported by grant MCB-9506933 to N.A.M. and the Lucille P. Markey Charitable Fund to LJCRF)

Su-Pos482

INTERACTIONS OF THE VITAMIN D RECEPTOR WITH THE RETINOID X RECEPTOR AND A VITAMIN D RESPONSE ELEMENT MONITORED BY FLUORESCENCE ANISOTROPY

((John J. Hill, Steve A. Strugnell*, Chris Perman, Bridgette Wiefling*, Hector F. DeLuca* and Catherine A. Royer)) School of Pharmacy and *Department of Biochemistry, University of Wisconsin, Madison, WI 53706

The vitamin D receptor (VDR) and retinoid X receptor (RXR) are members of the steroid hormone receptor superfamily. Previous studies that employed these receptors in extracts from a baculovirus expression system illustrated that VDR and RXR, at low salt, can form high affinity, cooperative homo and heterocomplexes at a DRE. In this study, interactions of purified, full length RXR and VDR with the rat osteocalcin DRE were monitored by the anisotropy of emission of a fluorescence probe covalently bound to the 5'-end of the DNA oligo. Cooperative, high affinity binding (midpoint of the binding profile ≈ 20 nM) of RXR to DRE was observed at salt concentrations between 10mM and 100mM with the stoichiometry of the complex decreasing with increasing salt. VDR, however, illustrated weaker binding to the DRE than RXR at the higher salt concentrations. The binding of the VDR receptor as a heterocomplex with RXR increases the affinity of VDR for the DRE. The response of the receptors binding to DNA in the presence of ligands, such as, 1,25-dihydroxyvitamin D_3 and 9-*cis*-retinoic acid will be presented.

Su-Pos483

FUNCTIONAL COMPARISON OF RETINOIC ACID RECEPTORS WITH HETEROLOGOUS LIGAND BINDING DOMAINS

((S.M. Pemrick, P. Abarzua, C. Kratzeisen, L. J. Sturzenbecker, A. A. Levin, W. Hunziker, M.S. Marks, J.A. Medin, K. Ozato, J. F. Grippo)) Depts. Metabolic Diseases and Oncology, Hoffmann-La Roche Inc., Nutley, NJ and Laboratory of Cell Biol. NCI, NIH, Bethesda, MD.

Chimeric receptors were constructed which contain the DNA-binding domain (DBD) of the retinoic acid receptor (RARα) and the ligand-binding domain (LBD) of the vitamin D receptor (VDR) or the estrogen receptor (ER). These chimeras exhibit high affinity binding to the hormone predicted from their LBD. Fold induction of ligand-mediated transcriptional activity of RARα/VDR is similar to RARα from thymidine kinase-luciferase reporter genes containing the following hormone response elements: β(RARE)3; RXREs (Apo A1 and CRBP II); TREpal; (TRE3)3. RARα/ER and ER/RARα/ER fail to mediate β-estradiol-induced transcriptional activity from the RARE or the RXREs. ER/RARα/ER is transcriptionally active from the (TRE3)3 response element; whereas, RARα/ER was only marginally active from either TREpal or (TRE3)3 All receptors except ER/RARα/ER bind to the RARE, and form heterodimers with RXR. Heterodimerization with RXR, and appropriate interactions between the LBD and DBD within and between each receptor member of the heterodimer determine the functional properties of these chimeric receptors.

Su-Pos484

TRANSCRIPTIONALLY ACTIVE REOVIRUS CORE PARTICLES VISUALIZED BY ELECTRON CRYO-MICROSCOPY AND IMAGE RECONSTRUCTION

((Mark Yeager[c], Scott G. Weiner[c], and Kevin M. Coombs[d])) [c]The Scripps Research Institute, Dept. of Cell Biology, La Jolla, CA 92037 USA. [d]University of Manitoba, Dept. of Medical Microbiology, Winnipeg, Manitoba, Canada. (Spon. by J. A. Tainer)

Reovirus is a non-enveloped, double-capsid icosahedral virus (diameter ~850Å) with a segmented dsRNA genome. Early steps in reovirus infection are characterized by stages of controlled disassembly. Within the gut lumen of the host, outer capsid proteins of the virus are removed by proteolysis to generate infectious subvirion particles (ISVPs) which enter cells. Within the cytoplasm, ISVPs are further uncoated to generate core particles that manifest RNA-dependent RNA polymerase (transcriptase) activity. Gradient-purified core particles also manifest transcriptase activity in a buffer containing Mg^{++}, ATP and nucleotide substrates. The (-) strand of the parental dsRNA serves as template for the synthesis of the capped (+) strand RNA. Proteins involved in this process include λ3, which is the putative catalytic subunit of the transcriptase, and λ2, which projects as pentameric "turrets" centered at each vertex and which manifests guanylyltransferase activity. Electron cryo-microscopy (ECM) and icosahedral image processing were previously used to determine the low resolution 3-D structures of virions, ISVPs and inactive core particles [Dryden *et al., J. Cell Biol.* 122: 1023-1041 (1993)]. ECM is a powerful method for examining dynamic processes such as *in vitro* transcription. Core particles were incubated in transcriptase buffer at 37°C for ~10 min. Aliquots were placed on holey carbon grids, blotted, and then immediately plunged into an ethane slush. Grids were maintained at -178°C, and images were recorded using minimal dose conditions. A 3-D map at 32Å resolution was computed by icosahedral image processing methods. The density maps of the inactive and active core particles display no significant changes in either the nodular surface or the external appearance of the λ2 "turrets." However, active cores did show additional density within the central channel of the λ2 "turrets," which presumably represents exiting nascent RNA. In addition, shifts in density at radii near the protein/RNA interface (230-250Å) may represent conformational changes associated with transcription.

Su-Pos485

DEVELOPMENT OF A NOVEL ADENOVIRUS COMPONENT SYSTEM THAT TRANSFECTS CULTURED CARDIAC CELLS WITH HIGH EFFICIENCY

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Although it is clear that gene transfection is a potentially valuable approach to study cardiac cell function and differentiation, classical transfection methods are limited by their poor efficiencies in cardiac cells. Recently studies show that recombinant replication-defective human adenovirus constructs can transfect primary cardiac cultures with near 100% efficiency. Since such recombinants are time consuming to prepare, the goal of this study was to develop a plasmid/viral transfection system that would capitalize on the advantages of the adenovirus. We have found that a "component system" formed by preincubation of dl312 adenovirus, poly-L-lysine, and an expression plasmid (*lacZ* reporter gene under control of the HCMV promoter), can transfect cultured cardiac cells. Optimal conditions were determined by quantitating *lacZ* production. Histochemical analysis of cultures revealed that the component system transfected 80% of the cells under these conditions. *LacZ*-positive myocytes could be identified in intact myocytes with the fluorescent substrate C_{12}-fluorescein di-β-galactopyranoside. Functional studies with such cells indicated that contractile behavior was maintained in transfected cardiocytes. In summary, this powerful and simple approach can mediate the transfer of a variety of genes that can be studied at the biochemical and cellular level in cardiac cells.

DNA REPLICATION

Su-Pos486

VACCINIA TOPOISOMERASE I (TOPO I): DNA CLEAVAGE AND SUPERCOIL RELEASE ARE NOT TIGHTLY COUPLED

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Topo I catalyzes site-specific DNA strand cleavage and religation by forming a phosphotyrosyl linkage between the DNA and Tyr-274, resulting in the release of DNA supercoils. We have shown that linear DNA substrates are cleaved (k_{cl} = 0.06 ± 0.02 s^{-1}) and religated (k_r = 0.66 ± 0.2 s^{-1}) such that the cleavage equilibrium (k_{cl} /k_r = 0.1) favors uncleaved E•DNA (Biochem. **33**, 327). For type I topos, two mechanisms have been proposed for supercoil release, *strand passage* where a single supercoil is removed per cleavage/ligation event with multiple intermediates and late product formation, or *free swiveling* with few intermediates and early product formation. To determine the mechanism, single-turnover experiments were done with supercoiled pUC19 DNA under conditions where topo I cleaves predominanly at a single site. The concentrations of substrate, intermediate topoisomers, and relaxed products *versus* time showed few intermediates and early product formation, consistent with a *swivel* mechanism. From these data, the rate constants for cleavage (0.15 s^{-1}), religation (2 s^{-1}), and the cleavage equilibrium (0.075), agree with those obtained with linear substrates, while supercoil release is fast (~70 supercoils/s). Thus, the number of supercoils removed for each cleavage event depends on kinetic competition between religation and fast supercoil release.

Su-Pos487

EXPLORING THE DYNAMICS AND REACTION MECHANISM OF THE BACTERIOPHAGE T4 DNA POLYMERASE: DUPLEX DNA COMPLEX: A Time Resolved and Stopped Flow Fluorescence Quenching Study.

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Fluorescence quenching studies with the nucleotide 2-aminopurine (AP) on the primer terminus of duplex DNA have been performed to investigate the local environment of DNA within the exonuclease active site in the Bacteriophage T4 DNA polymerase:DNA (E:DNA) complex. AP is extremely fluorescent as a free nucleotide but this fluorescence intensity is quenched dramatically (≈ 25-125 fold) upon incorporation into DNA. However, when DNA with AP in the primer terminal position is bound by T4 pol, its fluorescence is enhanced to nearly that of the free nucleotide, allowing direct observation of intermediate E:DNA complexes during the rapid excision reaction. Quenching studies with acrylamide have shown that AP on the primer terminus within the E:DNA complex is as protected from solvent as are the interior tryptophans within many proteins(Ksv=1.8 M^{-1}). However, time resolved studies showed that while the quenching of free AP (Ksv=34.8 M^{-1}) is entirely dynamic, that of AP within the E:DNA complex is >85% static. Real-time exonuclease reactions monitored by AP release (total-intensity and anisotropy) reveal a complex multi-phasic excision reaction. We are going to exploit the differential quenchability of free AP and E:DNA complexes in order to directly isolate those transitions associated with the bound E:DNA complex.

Su-Pos488

CD OF THE Y34F MUTANT fd GENE 5 PROTEIN IS UNCHANGED ON BINDING POLY[rA]. ((B. L. MARK and D. M. GRAY)) Program in Molecular & Cell Biology, University of Texas at Dallas, Richardson, TX 75083-0688.

The mutant Y34F gene 5 protein does not exhibit the canonical decrease in magnitude of the tyrosine L_a band at 229 nm that is observed for the wild type protein upon binding to ssDNA or poly[dA] (B. L. Mark *et al.* (1995) *Biochemistry 34*, 12854). A decrease in the 229 nm band of the wild type protein is also observed on binding to poly[rA] (B.-C. Sang & D. M. Gray (1989) *Biochemistry 28*, 9502). When complexed with either the wild type or Y34F gene 5 proteins, poly[rA] has a CD spectrum in the 250-300 nm region that is essentially like that of free poly[rA] at a high temperature. Since the CD spectrum of a Y34F•poly[rA] complex is dominated by the nucleic acid in the 250-300 nm region, we were able to fit this region of the spectrum with basis vectors derived from a singular value decomposition of spectra of free poly[rA] as a function of temperature. Using coefficients derived from this fit, the contribution of poly[rA] to the CD spectrum of the Y34F•poly[rA] complex could be calculated and subtracted in the 180-250 nm region. Our results indicate that there is no detectable change in the 180-250 nm region of the CD spectrum of the Y34F protein, or in its secondary structure, upon binding poly[rA]. This raises the possibility that the Y34F protein will be useful in deciphering the short-wavelength CD changes that occur for other nucleic acid sequences when complexed with this ssDNA binding protein.

Supported by NSF Grant MCB-9405683 and Robert A. Welch Grant AT-503.

Su-Pos489

EFFECT OF DNA STRUCTURE ON POLYMERIZATION CATALYZED BY HIV-1 REVERSE TRANSCRIPTASE: MECHANISM OF FRAMESHIFT MUTAGENESIS. ((Guixian Jin and Kenneth A. Johnson*)), Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

Frameshifts may be generated via a premutational intermediate involving slippage of either the template or the primer strand to produce misalignment (T. A. Kunkel 1990. *Biochem.istry* 29: 8003-8011). In order to provide direct mechanistic evidence for slippage-mediated frameshift error pathways occuring during catalysis by HIV-1 RT, we have examined the kinetics of extension over synthetic premutational intermediates having an unpaired extrahelical base (A) in the template at the 3rd(23/33(3A)-mer), 4th(23/33(4A)-mer), 5th(23/33(5A)-mer) and 6th(23/33(6A)-mer) positions from the 3'-primer terminus. Equilibrium dissociation constants for DNAs with or without embedded extrahelical A are similar, all on the order of 10^{-8} M. However, the dissociation constants ($K_{d,dTTP}$) for dTTP binding to RT•23/33(3A)-mer, •23/33(4A)-mer and •23/33(5A)-mers are increased by factors of 57, 23 and 11, respectively. The maximum polymerization rates k_p only decrease by factors of 5, 1.7, 2.8. $K_{d,dTTP}$ and k_p for dTTP incorporation into 23/33(6A)-mer are equal to the values for normal duplex DNA. Correct nucleotide binding and incorporation even during slippage might be a reflection of the frameshift error frequency.

Su-Pos490

IS THE DNA POLYMERASE AN EFFECTOR OF DNA HELICASE CATALYZED DNA WINDING? ((Kevin J. Hacker and Kenneth A. Johnson)) Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802

While the role of the DNA helicase in driving replication fork movement has been well studied, the role of the DNA polymerase in aiding the action of the DNA helicase has not. Using the T7 DNA replication system, we have developed an assay were the T7 DNA polymerase - helicase - DNA replication fork (three annealed oligonucleotides) ternary complex is formed in the presence of dTMP-PNP (a nonhydrolyzable helicase substrate). DNA synthesis and helicase movement are intiated by the addition of excess dNTP. Polymerase and helicase movement is then detected by following primer elongation, since the helicase is required for rapid DNA synthesis. Helicase movement is also independently monitored by following the displacement of one of the oligonucleotides. The rate of unwinding of the 25 base-pair DNA duplex region of the fork in the absence of the polymerase and the rate for elongation of the primer by 25 bases were the same (0.1 sec^{-1}). This rate is more than 100-fold slower than expected. Since no partially elongated primers were detected in our reactions, we believe that initation of DNA unwinding is the rate limiting step. Currently, we are determining the effect of the DNA polymerase on the number of nucleotides hydrolyzed by the helicase per base-pair unwound.

Su-Pos491

E. COLI PRIMARY REPLICATIVE HELICASE DnaB PROTEIN. CONFORMATIONAL TRANSITIONS AND INTERACTIONS WITH DNA. ((M. J. Jezewska and W. Bujalowski)) Department of Human Biological Chemistry and Genetics, The University of Texas Medical Branch at Galveston, Galveston, TX 77555-1053

The interactions of the *E. coli* primary replicative helicase DnaB protein with ssDNA and the structure of the complexes have been studied using the fluorescence spectroscopy and analytical centrifugation technique. We show that, in the presence of the ATP nonhydrolyzable analog AMP-PNP, the DnaB helicase binds polymer ssDNA with the site-size of 20 ± 3 nucleotides per protein hexamer. Thermodynamic studies show that the hexamer has only a single, strong binding site for ssDNA. Moreover, photo-cross-linking experiments indicate that only a single subunit is primarily in contact with ssDNA. These results preclude the possibility of extensive wrapping of the ssDNA around the hexamer and formation of the complex in which all six protomers are simultaneously bound to ss nucleic acid. These results indicate that long-range allosteric interactions occur on the level of the quaternary structure of the hexameric enzyme, leading to the selection of a limited set of subunits as a binding site for ssDNA. We provide the first direct evidence of dramatic global conformational changes of the DnaB hexamer, induced by nucleotide cofactors and ssDNA binding, and the presence of multiple conformational states of the enzyme.

Su-Pos492

THE EFFECT OF RNA SECONDARY STRUCTURE ON THE KINETICS OF POLYMERIZATION CATALYZED BY HIV-1 REVERSE TRANSCRIPTASE ((Zucai Suo and Kenneth A. Johnson*)), Department of Biochemistry and Molecular Biology, The Pennsylvania State University, University Park, PA 16802

The effect of template secondary structure on the kinetics of polymerization catalyzed by HIV RT is not clear. In this study, we designed and synthesized an RNA template with a stable hairpin structure from the HIV genome. The heteroduplexes of this template with two DNA primers are substrates for HIV-1 RT. The burst rates for incorporation of dNTPs, catalyzed by RT, decreased even though the affinity of one heteroduplex (20/66-mer) for RT was not affected by the hairpin structure. The hairpin structure was unwound and read through by HIV-1 RT slowly. RNase H activity was not important for RT to read through the secondary structure of template since wild type and RNase H-deficient RT had similar activities in the time span from 6 ms to 15 min. The accumulated intermediate products suggest that RT pauses during elongation of the primers. The pausing sites were dependent on template sequence, not on the primer positioning. The assumption that HIV nucleocapsid has helicase-like unwinding activity in HIV reverse transcription was not true since it inhibit full-length product synthesis slightly. AZT resistant mutant and wild type RT gave rise to the similar product distribution patterns in processive polymerization including AZTTP. This means the RNA secondary structure has no effects on the AZT inhibition *in vitro*. The mismatches of AZTTP•G (template) and AZTTP•C (template) were detected on the sequencing gels.

Su-Pos493

INTERACTIONS AMONG ELEMENTS OF THE T4 DNA REPLICATION COMPLEX VISUALIZED BY CRYOELECTRON MICROSCOPY. ((Mingwu Wang[1], Feng Dong[2], Peter von Hippel[2] and Edward Gogol[1])) [1]School of Biological Sciences, University of Missouri-Kansas City, Kansas City, MO and [2]Institute of Molecular Biology, University of Oregon, Eugene, OR

The bacteriophage T4 DNA polymerase holoenzyme consists of a core polymerase (gp43) and two accessory protein complexes (gp45 and gp44/62) that respectively form and catalyze the assembly of a "sliding clamp" that endows the polymerase with its required processivity. Replication of double-stranded templates also requires a helicase (gp41) and primase (gp61) encoded by T4. Our previous cryoelectron microscopy work has identified the complex formed by gp45 and DNA cofactors as a recognizable structure, and has documented the nucleotide-dependent assembly of gp41 into hexamers that presumably reflect the active state of the helicase. We have extended these studies to visualize the core polymerase in association with DNA, and to examine the interactions of the elements of the replication machinery with each other in functionally relevant complexes.

Supported by NSF grant MCB-9496306 (to EG) and NIH grants GM-15792 and GM-29158 (to PH).

Su-Pos494

KINETIC MECHANISM OF DNA BINDING AND DNA-INDUCED DIMERIZATION OF THE *E.COLI* REP HELICASE ((Keith P. Bjornson, Keith J. M. Moore, and Timothy M. Lohman)) Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, St. Louis MO 63110

The monomeric *E. coli* Rep protein undergoes a DNA-induced dimerization upon binding either single stranded (ss) or duplex DNA and the dimer is the active form of the Rep helicase. Using stopped-flow fluorescence, we have determined a minimal kinetic mechanism in which Rep monomer (P) binds to ss oligodeoxynucleotides ($dN(pN)_{15}$) (S) by a two-step mechanism to form PS*, which can then dimerize with P to form P_2S. This mechanism is supported by four independent studies in which the kinetics were monitored by changes in fluorescence intensity of three different probes: Rep Trp fluorescence, the fluorescence of $d(T_5(2\text{-AP})T_4(2\text{-AP})T_5)$, containing the fluorescent base, 2-aminopurine (2-AP), and $dT(pT)_{15}$ labeled at its 3' end with fluorescein ($3'\text{-F-}dT(pT)_{15}$). Simultaneous (global) analysis of the time courses of $d(T_5(2\text{-AP})T_4(2\text{-AP})T_5)$ (100 nM) binding to a range of Rep monomer concentrations (25 nM - 400 nM) yields the following rate constants: $k_1 = 3.3\pm0.5 \times 10^7\ M^{-1}\ s^{-1}$; $k_{-1} = 1.4\pm0.4\ s^{-1}$; $k_2 = 2.7\pm0.9\ s^{-1}$; $k_{-2} = 0.21\pm0.06\ s^{-1}$; $k_3 = 4.5\pm0.3 \times 10^5\ M^{-1}\ s^{-1}$; $k_{-3} = 0.0027\pm0.0008\ s^{-1}$. This mechanism provides direct evidence that Rep monomers can bind ss DNA and that ss DNA binding induces a conformational change in the Rep monomer that is likely required for Rep dimerization. The implications of these results for the mechanism of Rep-catalyzed DNA unwinding will be discussed.

Su-Pos495

DIPOLE BOUND, NUCLEIC ACID BASE ANIONS STUDIED VIA NEGATIVE ION PHOTOELECTRON SPECTROSCOPY

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The anions of the nucleic acid bases, thymine and uracil, were generated in the gas phase using a supersonic expansion nozzle ion source and studied by means of negative ion photoelectron spectroscopy. Both anions exhibit spectroscopic signatures indicative of the excess electron being bound to the dipolar field of the molecule. The adiabatic electron affinities of uracil and thymine were determined to be 93 ± 7 meV and 69 ± 7 meV, respectively. No spectroscopic evidence for conventional (valence) anions of uracil or thymine was observed.

Su-Pos496

The Formation and Methylation of the "Slippage Structures" *in vitro*: the Molecular Basis of the Fragile-X Syndrome. *by Xian Chen[1,2], S.V.S. Mariappan[1], R. Ratliff[3], R. K. Moyzis[3], S.S. Smith[4], E.M. Bradbury[2], and Goutam Gupta[1]*

Theoretical Biology and Biophysics[1], Life Sciences Division [2], and Center for Human Genome Studies[3] of Los Alamos National Lab, NM 87545; Dept. of Cell and Tumor Biology of City of Hope National Medical Center[4], CA 91010.

DNA triplets, (GCC)n(GGC)n, are located on the 5' control region of the FMR-1 gene. Expansion and hypermethylation of these triplets cause gene inactivation and onset of the fragile X syndrome. Previously, we have shown that the individual strands of the fragile X repeat form hairpin structures under physiological conditions. By using biophysical and molecular biological approaches, We now show that the preference of hairpin formation by the GCC-strands leads to hairpin-based slippage structures (essentially three-way junctions) that may act as structural intermediates during replication and account for repeat expansion. The enzymatic studies indicate that the presence and the associated mobility of the hairpins in the slippage structures make them excellent substrates for methylation by the human methyltransferase. We propose that the formation of the slippage structures causes the massive expansion of the repeat and the hypermethylation of the CpG sites inside the repeat, furthermore, upon methylation this region may interact with the methyl-CpG binding proteins and inactivate the gene.

TRANSLATION/TRANSFORMATION/PROLIFERATION

Su-Pos497

FLUORESCENCE STUDY ON THE INTERACTION OF THE WHEAT GERM PROTEIN SYNTHESIS INITIATION FACTOR eIF-2 WITH 3-o-(ANTHRANIOYL)-GTP and 3-o-(ANTHRANIOYL)-GDP ((J. Ren, C-C. Wei, and D. J. Goss)) Department of Chemistry, Hunter College, CUNY, New York, NY 10021

The first step in eukaryotic protein synthesis is the binding of Met-tRNA to initiation factor eIF-2 and successive transfer to the ribosome 40S subunit. This process is GTP/GDP dependent. Two fluorescent modified guanosine nucleotides Ant-GTP and Ant-GDP, were synthesized. The binding reaction of eIF-2 to GDP and GTP was studied by direct fluorescence intensity and anisotropy. It was found that the fluorescent guanosine nucleotides bind to eIF-2 at the same binding site as unmodified guanosine nucleotides. The binding constant of eIF-2 with GTP/GDP was measured and it was found that eIF-2:GDP K_a was 900 nM and eIF-2:GTP K_a was 150 nM. An eIF-2B like protein has not been found in the wheat germ system. eIF-2 reactivation may bypass eIF-2B by controlling the concentration ratio of GTP/GDP. Quenching experiments indicated that GTP/GDP bound to eIF-2 was surface exposed making it accessible for nucleotide exchange.

Su-Pos498

THE EFFECT OF THE 3'-UNTRANSLATED REGION ON THE TRANSLATIONAL EFFICIENCY AND STABILITY OF THE mRNA IN A CELL-FREE SYSTEM FROM WHEAT GERM. ((E.A. Matveeva,* Yu.B. Beresin, V.S. Skosyrev, Yu.B. Alakhov and S.V. Matveev)) *Institute of Cell Biophysics, Branch of Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, Pushchino, Moscow Region, 142292, Russia. (Spon. by G. Filatov)

Five constructs were prepared for studying the effect of the poly(A) tail and the tRNA-like tail on the translation efficiency of the obelin template.Additionally, the stability of the mRNAs was measured throughout the translation process.The poly(A) region represents the 7335-7491 domain of the 3' poliovirus Sabin1 tail.The tRNA-like region represents a cysteinyl tRNA from yeast.The constructs differed by the presence or absence of one of these regulators and their alignment in relation to the 3' mRNA tail. Our results showed that the mRNA stability increases along the constructs set: mRNA*tRNA-mRNA-mRNA*poly(A). The mRNA stability was measured by determining the native in the protein synthesis template which remains intact after the desired time of the translation process.We showed that the mRNA stability does not depend on the mRNA concentration. On the other hand, the activity of the mRNA in protein synthesis depends on its concentration strongly.The quality of synthesized obelin per unit of added mRNA (translation efficiency for short) using mRNA*tRNA is six times more than the translation efficiency of mRNA or mRNA*poly(A) for the template concentrations of 10 nM or lower.The translation efficiency decreases with an increase in the template concentration in all cases.The translation efficiency becomes biggest for mRNA*poly(A) concentrations of 100 nM or more. We showed that the distinction in translation efficiency is not connected with initiation ability of these constructs. Therefore, we have concluded that the different mechanisms of reinitiation occurred according to the regulator present in the construct.

Su-Pos499

SEQUENCE-STRUCTURE THREADING OF TYROSYL- AND TRYPTOPHANYL-TRNA SYNTHETASE SEQUENCES: THE EVOLUTION OF SEVERAL SIMILAR ENZYMES. ((C.W.V. Hogue, and S.H. Bryant))
National Center for Biotechnology Information, NIH, Bethesda, MD 20894.

The two enzymes from *B. stearothermophilus*, tyrosyl- and tryptophanyl-tRNA synthetases (TyrRS and TrpRS) share isomorphous structures (Doublié, Bricogne, Gilmore and Carter Jr., 1995 Structure 3:17), despite an insignificant level of sequence identity (less than 15%). Eucaryotic cytoplasmic TrpRS and TyrRS (ecTrpRS and ecTyrRS) are distinctly different enzymes from their bacterial counterparts, despite sharing the common motifs, HIGH and KMSKS found in their ATP-binding sites. The ATP binding sites of ecTrpRS and ecTyrRS share significant sequence similarity, and can be considered to have diverged from a common ancestor, separate from the bacterial enzymes. In addition there are two distinct sequence forms of bacterial TyrRS. The implication of these sequence differences are that TrpRS or TyrRS activites originated very late in the emergence of protein synthesis, with TrpRS activity emerging at least twice, and TyrRS activity emerging at least three times in evolution. Sequence-structure threading was performed using the crystal structures of *B. stearothermophilus* TrpRS and TyrRS, and all the available TrpRS and TyrRS sequences. Identical structural core definitions were chosen for the two enzymes, and the threading was computed with a Gibbs-sampling algorithm lacking gap-penalties. The probability (p) of computed threads being a chance event was computed by comparison with control threading experiments using randomly shuffled versions of the same sequences. With the exception of yeast ecTyrRS, all the TrpRS and TyrRS sequence-structure threads had significant p-values, indicating they all share a common structure. This has allowed the creation of a master alignment of TrpRS and TyrRS enzymes and the subsequent comparison of these enzymes from the perspective of structural evolution. Yeast cytoplasmic TyrRS likely has a unique c-terminal domain. Both convergent and divergent evolution is observed in this family of enzymes.

Su-Pos500

A MODEL FOR THE AMBIVALENT ACTION OF 5-HYDROXYTRYPTAMINE ON MITOGENIC SIGNAL TRANSMISSION IN CULTURED FIBRABLASTS
((Mary N. Stamatiadou)) Institute of Biology, NCRNS Research Center "Demokritos", Aghia Paraskevi, Athens, Greece.

Lateral controlling filters in mitogenic signal transduction in cultured fibroblast cells may cause the cells to be locked in the prereplicative phase in which they remain quiescent but fully competent to resume progression to mitosis following stimulation by growth factors. Regulation of intacellular glutamine synthesis by various exogenous agents, including 5-hydroxytryptamine (5-HT), may constitute such a lateral controlling filter in mitogenic signal transmission. Excess glutamine causes a decrease of the response to growth factors, while glutamine deficiency limits cell growth. 5-HT inhibits the endogenous process of glutamine synthesis through action on the *de novo* synthesis of the enzyme glutamine synthetase (EC 6.3.1.2). This inhibitory effect exhbits a threshold dosage (1 mM), above which the inhibiory effect on endogenous glutamine synthesis is coupled to an inhibitory effect on cell proliferation which is totally eliminated in the presence of 3 mM 5-HT. However, at lower concentrations (0.1-0.5 mM), below the above threshold dose, 5-HT stimulates cell proliferation while it has no effect on intracellular glutamine synthesis. A model is presented in which the ambivalent action of 5-HT on cultured fibroblast cell proliferation may arise from effects on primary signal transmission versus effects on lateral controlling filters involving the network of nutrient requirements of the cell, including the system of endogenous glutamine synthesis.

Su-Pos501

MECHANISM OF MEMBRANE DEPOLARIZATION IN PROLIFERATING VASCULAR SMOOTH MUSCLE CELLS. ((L.J. Hymel, V. Ruiz-Velasco, G.G. Schofield, and E. Songu-Mize)) Tulane University School of Medicine and Louisiana State University Medical Center, New Orleans, LA 70112.

Previously we have observed that rapidly proliferating A7r5 cells appear to be chronically depolarized based on the binding affinity of dihydropyridines. We have applied two independent techniques, whole-cell current-clamp and electrophoretic equilibration of [^{3}H]tetraphenylphosphonium (TPP$^+$), to quantify resting membrane potential (E_m) as a function of the growth stage of A7r5 cells. Our current clamp experiments confirm a gradual hyperpolarization from -38.9 $\pm$ 1.1 mV at 3 days (proliferative stage) to -42.7 $\pm$ 1.0 mV at 5 days (confluent) and -50.3 $\pm$ 1.0 mV at 10 days (postconfluent). We employed the TPP$^+$ equilibration technique to define the dependence of E_m on $[K^+]_o$, and found significantly greater deviation from E_K at lower $[K^+]_o$ in proliferating cells. The results revealed a dramatic elevation of P_{Na}/P_K in proliferating cells (0.39) compared to confluent (0.11) and postconfluent cells (0.06). Replacement of extracellular Na^+ with NMG^+ or blockade of Ca^{2+} channels with 10 μM Cd^{2+} or 100 μM verapamil did not significantly alter E_m at any growth stage; thus, increased Na^+ or Ca^{2+} influx cannot explain the depolarization of proliferative cells. However, both 2 mM ouabain- and 10 mM TEA^+-sensitive $^{86}Rb^+$ uptake were each reduced by about 4-fold in proliferative cells. Therefore, we believe that both steady-state Na^+,K^+-ATPase and K^+ channel activities are reduced in proliferative VSMC, leading to chronic membrane depolarization. Since this depolarization is in the range above the threshold for activation of voltage-gated Ca^{2+} channels, the activity of which is required to maintain VSMC proliferation, regulation of E_m via Na^+,K^+-ATPase and K^+ channel activities may be an important determinant of VSMC proliferation.

Su-Pos502

HYDROLYSABLE HYDROPHOBIC TAXOL DERIVATIVES. ((Shaukat Ali, Eric Mayhew, Andrew Peters, Sharma Minchey, Andrew Janoff)) The Liposome Company, Inc., Princeton, NJ 08540.

A series of taxol derivatives with α-bromoacyl chains attached at the 2'-position of taxol, varying from six to sixteen carbons in length, were synthesized and characterized. In vitro studies showed that acyl chain length was inversely related to the growth inhibitory effects of the derivatives. Taxol derivatives with six, eight and twelve carbon lengths had potencies very similar to taxol. GI_{50} [concentration necessary to inhibit cell growth 50%] was 4 nM for derivatives *vs.* 2 nM for taxol on MCF7 [human breast cancer] cell line). Increasing the acyl chain length to 14 or 16 carbons resulted in a significant increase in GI_{50} to 70 or 200 nM (or decrease in potency), respectively. Acyl derivatives lacking the α-bromine were relatively less active, suggesting that the α-bromine atom facilitated the hydrolysis of acyl chains to yield free taxol, although the rate of hydrolysis was controlled by the acyl chain length. These differences in rate of hydrolysis and growth inhibitory activities may indirectly reflect differences in the susceptibility of the α-bromine to hydrolysis after association of the derivative with cell membranes.

Su-Pos503

PROXIMITY OF CD4 AND TcR/CD3 ON ACTIVATED AND RESTING T4 CELLS. ((P.S. Pine, J.L. Weaver and A. Aszalos)) Division of Research and Testing, CDER, FDA, Laurel, MD .

Several anti-HIV drugs block the binding of the coat protein gp120 to the CD4 receptor on T4 cells. Those drugs which act upon the CD4 might also hinder the reported activation induced association of TcR and CD4 molecules on the cell surface (Julius et al.,Immunol. Today,14:177,1993). To address this question, we measured distances between the CD4 and the TcR molecules on human peripheral blood lymphocytes with or without activation and with or without cross-linking of CD4. Using the photobleaching fluorescence resonance energy transfer method (Szabo, et al.,Biophys J.,61:661,1992), we have found that the distance between OKT3-FITC and anti-Leu3a-biotin-streptavidin-PE labeled mAb bound to TcR and CD4, respectively, does not change significantly during activation with OKT3 and with cross-linking of CD4 molecules. The distance remains between 134 and 144Å. Similar results were obtained when we measured distance between anti-Leu4-FITC and anti-Leu3a-biotin-streptavidin-PE. Reversing the donor (FITC) and acceptor (PE) positions yielded the same results. However, distance measurements between OKT4E-FITC and anti-Leu4-biotin-streptavidin-PE indicated closer proximity with activation and with or without cross-linking.

Su-Pos504

THERMODYNAMICS OF PHOSPHOTYROSINE PEPTIDE LIGAND BINDING TO GRB2-SH2 DETERMINED BY ISOTHERMAL TITRATION CALORIMETRY. ((C. McNemar, A. Prongay, R. Zhang, J. Durkin, M. Snow, P. Mui, S. Black, O. Wilson, H. Le, P.C. Weber and W.T. Windsor)). Schering-Plough Research Institute, 2015 Galloping Hill Rd., Kenilworth, N.J. 07033

Binding between the SH2 domain of Grb2 and the phosphotyrosine sequence on SHC is one of the critical pathways for stimulating Ras-dependent cell proliferation and differentiation. We have studied the molecular interactions between the SHC phosphotyrosine (Y317) peptide Ac-S_{-1}pY$V_{+1}N_{+2}V_{+3}Q_{+4}$-NH_2 (SHC peptide) and the SH2 domain of Grb2 by performing isothermal titration calorimetry measurements to determine the thermodynamics of binding. The dissociation constant (Kd) for the SHC peptide is ~ 0.2 μM (ΔG ~ -9 kcal/mol) and the interaction is driven thermodynamically by the change in enthalpy of binding (ΔH ~ -7.5 kcal/mol) at 20°C. Binding studies were also performed with modified SHC peptides that replaced each of the six native residues with an alanine residue. While these studies confirmed that pY and N_{+2} are critical for binding, substitutions at the other sites produced only a 2-3 fold decrease in affinity. These thermodynamic results and those from a temperature dependent study were compared to computational values derived from a three-dimensional homology model of the Grb2-SH2 / SHC peptide structure.

Su-Pos505

THERMODYNAMIC STUDIES OF THE BINDING OF ODORANTS TO BOVINE OBP. (G.Bains and L. M. Amzel) Department of Biophysics and Biophysical Chemistry, Johns Hopkins University School of Medicine, Baltimore, MD 21205. (Sponsored by G.Bains)

The binding of several odorants to the bovine odorant binding protein (OBP) has been studied by isothermal titration calorimetry. Recombinant OBP has been purified in a fusion protein construct with the maltose binding protein where it retains all of its binding activity. The energetics of binding to the fusion protein have been measured for the odorants 1-isobutyl-2-methoxy pyrazine, l-carvone, menthone, jasmone, and cinnemaldehyde. The odorants IBMP, cinnemaldehyde, and l-carvone bind with a stoichometry of 1 odorant per OBP dimer and affinities of 9.5E4, 3.4E5 and 7.5E5 M^{-1} respectively. Measured enthalpies of binding for these compounds are 2.7, 0.72 and -6.7 kcal/mol respectively. In the cases of menthone and jasmone, the binding isotherms are clearly biphasic suggestive of 2 very different sites for binding. Titrations with menthone, which possesses similar functional groups to carvone however located in different positions on a cyclohexane ring, reveal a reaction heat which is initially endothermic and eventually becomes exothermic as a second site saturates (k1=2.3E5 M^{-1}, k2=2.9E4 M^{-1}, Δh1=2.6kcal/mol, Δh2=-3.6kcal/mol). These results present a challenge for the development of models which can explain the diversity in the reaction energetics in the binding of small organic molecules to proteins.

Su-Pos506

AN IMPROVED METHOD FOR ANALYSIS OF INTERACTIONS OF TIGHT-BINDING LIGANDS ON TARGET PROTEIN MOLECULES. ((A. Takai)) Dept of Physiol., Sch of Med., Nagoya Univ., 466 Nagoya, Japan

It has been reported that okadaic acid (OA) and other tight-binding protein phosphatase inhibitors including microcystin-LR, calyculin-A and tautomycin prevent each other from binding to protein phosphatase-2A (PP2A) [see e.g., McKintosh *et al.* (1990). *FEBS Lett.* **264**, 187-192]. Recently we have introduced an improved procedure for evaluating the extent to which the affinity of an enzyme to a labeled tight-binding ligand is reduced by binding of a non-labeled tight-binding ligand to the enzyme [Takai *et al.* (1995) *Biochem. J.* 306, 657-665]. By applying the procedure we have analyzed the dose-dependent reduction of the PP2A binding of [24-^{3}H]OA by addition of non-labeled tight-binding inhibitors. The results obtained indicate that the binding of the non-labeled inhibitors to the PP2A molecule causes a drastic (10^6 to 10^8-fold) increase in the dissociation constant associated with the interaction of [24-^{3}H]OA and PP2A. This means that the absolute values of the standard free-energy ($\delta\Delta G^{0'}$) for the binding of PP2A with [24-^{3}H]OA decreases by 34-48 kJ·mol^{-1} when the enzyme is bound to one of the non-labeled ligands. These estimated $\delta\Delta G^{0'}$ values amount to 60 - 80 % of the absolute value of the standard free-energy change of the PP2A binding of OA [60 kJ·mol^{-1}; Sasaki *et al.* (1994) *Biochem. J.* 298, 259-262]. The protein phosphatase inhibitors may share the same binding site on the PP2A molecule.

Su-Pos507

DETERMINATION OF MEMBRANE PARTITION COEFFICIENTS USING A POTENTIOMETRIC METHOD

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The membrane partition coefficient of a drug can be determined by potentiometry in multilamellar vesicles (MLV) using the PCA101 Pka Log P Analyzer designed and manufactured by Sirius Analytical Ltd. (Forest Row, East Sussex, U.K.). This non-radiolabel method is a derivation of the logP function in octanol which is a specialized application of pK_a determination using this apparatus. The PCA101 uses volumetric analyses where precisely known volumes of standardized strong acid (0.25 M HCl) or base (0.25 M KOH) are added to a solution of protogenic substance, during which the pH is continuously measured with a combination glass electrode within the interval of pH 2-11 at a constant temperature of 25°C. Drugs that are weak acids or bases partition into a model membrane (cholesterol: Egg Phosphatidylcholine at different molar ratios) and the titration curve shifts resulting in a $p_{[MLV]}K_a$. The larger the difference between the pK_a and the $p_{[MLV]}K_a$ the larger the $logP_{[MLV]}$. This method has reliably determined $logP_{[MLV]}$ for drugs ranging from 1-6. A significant correlation has been shown between the $LogP_{[MLV]}$ determined by a radiotracer method and the potentiometric method under a variety of cholesterol:Egg PC ratios. For the first time, drug-membrane design QSARs can be easily obtained and several examples will be provided.

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NEW FACILITIES FOR MACROMOLECULAR STRUCTURE ANALYSIS

Su-Pos508

BIOCAT - A NEW FACILITY FOR BIOLOGICAL X-RAY DIFFRACTION AND SPECTROSCOPY AT THE ADVANCED PHOTON SOURCE. **((G. B. Bunker & T. C. Irving)) Dept. Biological, Chemical and Physical Sciences, Illinois Institute of Technology, Chicago IL 60616.**

The Biophysics Collaborative Access Team, BioCAT, is an NIH funded ($8.6 M, first 5 years) initiative to develop and operate a national user facility at the Advanced Photon Source to study the structure and dynamics of biological systems at the molecular level, with a focus on partially ordered samples such as membranes, fibers, and solutions. The primary research techniques supported by the proposed facilities will be resonant (anomalous) and non-resonant X-ray diffraction (XRD), and X-ray absorption fine structure (EXAFS) spectroscopy, with emphasis on time-resolved studies, polarized XAFS, hybrid diffraction/spectroscopic techniques, and development of novel techniques that exploit the unique properties of the APS. BioCAT equipment, software, and laboratories will be made available to a large research community comprising a wide variety of subspecialities and having diverse interests in structural and molecular biology. The BioCAT beam line will be the brightest facility dedicated for this purpose in the world capable of delivering ~10^{14} photon/s into a minimum focal spot of order 40 μm x 250 μm. Horizontal and vertical focussing will be independent, allowing custom tailoring of beam size and divergence to the sample being studied. The first experiments will occur in fall of 1997.

Su-Pos509

DEVELOPMENT OF A CRYO SCANNING TRANSMISSION X-RAY MICROSCOPE AT THE NSLS. ((J. Maser, C. Jacobsen, A. Kalinovsky, J. Kirz, A. Osanna and S. Wang) State University of NewYork at Stony Brook, Stony Brook, NY 11794-3800)

A scanning transmission x-ray microscope (STXM) has been in operation at the National Synchrotron Light Source at Brookhaven National Laboratory for some time. The current system is capable of imaging hydrated objects of several micrometer thickness including whole cells at a spatial resolution of 50nm. The distribution of different major chemical constituents in the object can be mapped by combining multiple images taken at different wavelengths. Radiation damage has been recognized as a limiting factor to obtaining higher resolution images or to taking multiple images from radiation sensitive objects. Cryo electron microscopy has shown improved structural stability of cryo-fixated samples. To take advantage of this, we are currently developing a cryo-STXM which is designed to image frozen hydrated objects at a temperature of below -160°C. This should allow us to improve the resolution in a single image of a radiation sensitive sample. It should furthermore allow us to take several high-resolution images of the same object area to obtain chemical contrast, or to allow spectral analysis of small sample areas. Finally, we want to take large data series required to reconstruct a 3D image of an object. Therefore, cryo-STXM is designed to allow tilting of the sample in excess of +/-60°. Efforts towards first use of the cryo-STXM will be presented.