METAL ION/MOLECULAR BIOLOGY INTERFACE

Organizers: Vincent Pecoraro, Steve Lippard and Michael Karin

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Poster Sessions

Molecular Biology Tutorial

CD 001 METHODS FOR DETERMINING STRUCTURAL DETAILS OF PROTEIN-DNA COMPLEXES IN SOLUTION, Thomas D. Tullius, Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

Several structural motifs that proteins use for binding to DNA have now been identified, mainly through X-ray crystal structures of protein-DNA complexes. Among these are the helix-turn-helix, zinc finger, and b-ZIP (leucine zipper) motifs. Each interacts with DNA in a different way, and forms a complex of distinct architecture. For example, proteins containing the helix-turn-helix motif tend to bind to one face of the DNA helix, while the X-ray structure of a protein with three zinc fingers reveals that the fingers wrap around DNA in the major groove. Despite the differences in architecture of these DNA-protein complexes, conventional footprinting methods would be hard-pressed to distinguish them. By way of illustration, the DNase I footprint of either a zinc finger- or a helix-turn-helix-DNA complex is a continuous blank on the gel, even though the two structures are quite different. In this tutorial I will describe work in my laboratory that is aimed at providing new experimental approaches to determining structural details of protein-DNA complexes in solution. We make use of the basic methodology of footprinting, which has several advantages over conventional spectroscopic or crystallographic methods. These advantages include high sensitivity (femtomole amounts of protein-DNA complex are feasible), and the ability to study complicated protein-DNA complexes consisting of hundreds of base pairs of DNA bound by several proteins. Our goal is to make the highest-resolution footprints possible of a DNA-protein complex, so that different structural types can be distinguished. To this end we use the smallest available chemical probe of DNA, the hydroxyl radical (•OH). This radical has the desirable property of cleaving every nucleotide of a DNA molecule at nearly the same rate. Nucleotides that are covered by protein are protected from attack by the radical. The footprint reveals, at single-nucleotide resolution, where the diminutive •OH can contact DNA. The hydroxyl radical footprint thus represents a high-resolution *image* of the protein bound to the DNA. An alternative experimental strategy gives information on the energetically-important *contacts* that are made by a protein with the nucleosides of DNA. In this experiment the DNA is first treated with the hydroxyl radical, which serves to prepare a randomly-gapped collection of DNA molecules. Protein is then added to the gapped DNA, and a mobility-shift gel is used to separate protein-bound from free DNA. The single-nucleoside gaps that interfered with protein binding (and thus the nucleosides that make energetically-significant contacts) are revealed by electrophoresis of the extracted bands on a DNA sequencing gel. I will describe the application of these methods to a variety of DNA-protein complexes, to illustrate the different structural phenotypes that are revealed by such experiments.

Biophysical Methods Tutorial

CD 002 ELECTRON PARAMAGNETIC RESONANCE TECHNIQUES IN PHOTOSYNTHESIS RESEARCH, John H. Golbeck, Department of Biochemistry, University of Nebraska, Lincoln, NE 68593-0718.

Among the various spectroscopic techniques used to study the electron donor and acceptor molecules involved in photosynthesis, one method has particular relevance: electron paramagnetic resonance spectroscopy. The reason for this is twofold: 1) photochemistry is inherently a one-electron event in which organic molecules can exist in a cationic, anionic or semiquinone form, and hence exist as a paramagnetic species, and 2) photosynthetic complexes contain a number of transition metals which inherently contain unpaired electrons. In photosynthesis, the unpaired electron is usually generated by chemical oxidation, a brief flash of saturating light, or continuous illumination to bring about photochemical turnover, and on the detection of the unpaired electron as it shuttles between the organic and metal-containing cofactors in the reaction centers. The electron paramagnetic resonance spectrometer consists of an electromagnet and a radio-frequency generator that produce fields that converge on a sample tube, a diode detector to convert the paramagnetic spins to an electric current, a phase-sensitive amplifier, and a digitizer. The sample is illuminated directly in the microwave cavity and the paramagnetism is detected at discrete combinations of electric and magnetic fields. The main advantage of ESR spectroscopy is that since only paramagnetic species are detected, photochemical events can be easily seen in samples containing many proteins, cofactors and chromophores. In other words, the presence of a high concentration of diamagnetic species is largely irrelevant, and a low-concentration paramagnetic species can be detected among the other-wise interfereing molecules. Moreover, since metalloproteins, such as iron-sulfur centers, show a unique pattern of resonances in EPR spectroscopy, multiple species can be easily distinguished. The drawback is that that the spin relaxation properties of metals such as iron make it necessary to cool the sample to temperature approaching liquid helium in order to observe the spectrum. In other metals, such as nickel, copper, and cobalt, have less pronounced spin relaxation properties and can be observed at 77 K and above. Metals such as manganese and most organic radicals can be observed at room temperature. Electron paramagnetic spectroscopy, in combination with other methods, including circular dichroism, optical rotary dispersion, Mössbauer, extended X-ray atomic fine structure, absorption spectroscopy and fluorescence spectroscopy, have led to a rather consistent model of electron transfer in photosynthesis. For example, as the electron moves among the various acceptors of Photosystem I, the backreaction kinetics between $P700^+$ and the acceptor radical is related to the identity of the electron acceptor and the spectrum during continuous illumination provides information concerning the nature of the electron donor or acceptor molecules. In this picture, the primary donor and acceptor chlorophyll molecules P700 and A_0 undergo charge separation upon absorption of light, producing a cation ($P700^+$) and an anion (A_0^-) radical pair. These can be seen optically by transient absorbance changes in the visible and IR portions of the spectrum, and by ESR spectroscopy at $g \sim 2.002$. Following charge separation, the electron is stabilized by transfer to the intermediate electron acceptor A_1 (a phylloquinone), and then to the first iron-sulfur center F_X (a [4Fe-4S] cluster). The electron is then stabilized by transfer to the terminal iron-sulfur centers, F_A/F_B (two [4Fe-4S] clusters). These centers can be determined directly by monitoring the *g*-values of 2.05, 1.94 and 1.86 for F_A and 2.07, 1.92 and 1.89 for F_B.

CD 003 ELECTROSTATIC CONTROL OF REDOX POTENTIALS AND pK'S: APPLICATION TO PHOTOSYNTHETIC REACTION CENTERS, M.R. Gunner and Barry Honig, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168 St., New York, NY, 10032.

The factors that determine redox potentials in proteins are discussed. The factors include the direct effects of liganding groups, the electrical potential produced by charged amino acids, the potential produced by polar amino acids, and the reaction field of the surrounding solvent. Each of these contributions can be calculated by solving the Poisson-Boltzmann equation for the protein in question. This is accomplished using the DelPhi program.

Our first application has been to the four hemes of bound cytochrome of the *R. viridis* reaction center. All four hemes have redox potentials that are significantly shifted from that of a model heme in solution, but among themselves they also span a range of about 500 mV. We have used DelPhi to account for this enormous variation which must be due to specific interactions between the hemes and the surrounding protein. The calculations produced excellent agreement with experimental data accounting for both the shifts with respect to the model heme in solution but also for the redox potentials of the individual hemes. Electrostatic interactions with charged groups provided the largest contribution to the observed redox potentials although the bound ligands also had important effects. Interactions with the solvent as well as the potential produced by polar groups in the protein were found to be of lesser importance.

An important component of the calculations is the correct assignment of the charge state of ionizable amino acids. However, the ability to calculate pK's is of far more general importance since electron transfer is frequently coupled to proton uptake and release. Some general rules concerning the magnitude of the contributions expected from different interactions will also be discussed. Finally, the contribution of ionizable amino acids to proteins stability will be briefly considered.

De Novo Design of Metalloproteins and Metal Assemblies

CD 004 DESIGN AND DEVELOPMENT OF METAL SWITCHES TO CONTROL ENZYME ACTIVITY, Charles Craik, Robert Fletterick, Barry Haymore*, Jeffrey Higaki, Mary McGrath and Scott Willett, Departments of Pharmaceutical Chemistry and Biochem/Biophys, Univ. of CA at San Francisco, San Francisco, California 94143-0446, *Central Research Laboratory, Monsanto Company, St. Louis, MO 63137.

A rigorous test of one's understanding of structure-activity relationships in proteins is the use of those relationships in altering the function of a protein in a prescribed fashion. In recent years, the field of protein engineering has progressed to the point where the rational design of proteins is now providing an avenue for the development of proteins as reagents with a practical use. This is especially true of proteins whose activities and/or structures are dependent upon coordinating metal ions. The database of information on metalloproteins is developed to the extent that basic rules governing protein/metal interactions can be formulated and applied in engineering metalloproteins for specific purposes. An essential requirement for any system involving a proteolytic enzyme is the regulation of hydrolytic activity. A novel approach to regulating the activity of a protease in a metal-dependent fashion is by introducing a neighboring amino acid residue that assists in reversibly repositioning an essential active-site amino acid residue. We have computer-modelled and engineered several such sites into trypsin. Recombinant trypsins were designed whose catalytic activity can be regulated by varying the concentration of Cu^{+2} in solution. A bidentate site was formed by substitution of Arg96 with a His in rat trypsin (trypsinR96H) which places a new imidazole group on the surface of the enzyme near the essential active site His57. The unique spatial orientation of these His side chains results in the formation of a stable, metal-binding site that chelates divalent first row transition metal ions. Modelling studies predicted that occupancy of this site by a metal ion prevents the imidazole group of His57 from participating as a general base in catalysis. As a consequence, the primary effect of the transition metal ion is to inhibit the esterase and amidase activities of trypsin R96H in a velocity modulated fashion. The apparent K_i for this inhibition is in the micromolar range for copper, nickel and zinc, the tightest binding being to Cu^{+2} at 21μM. The apparent K_i for Ni^{+2} is 49μM and for Zn^{+2} is 128μM. This is the same order predicted based on experimentally determined association constants for metal binding to imidazole. Trypsin R96H activity can be fully restored by removing the bound Cu^{+2} ion with EDTA. Multiple cycles of inhibition by Cu^{+2} ions and reactivation by EDTA demonstrate that reversible regulatory control has been introduced into the enzyme. Twelve X-ray data sets were collected and analyzed for trypsin R96H crystals which had been treated with various concentrations of $CuCl_2$ and under different conditions. The 2.3Å crystal structure shows that His57 is displaced from its normal position to form a coordination site with His96 and that Tris is also a copper ligand. This structure provides evidence for the inhibition of the enzyme by Cu and verifies the design principles. Two other bidentate sites with less affinity and two tridentate binding sites with greater affinity for the transition metals have also been created and will be discussed. The introduction of a coordination complex into trypsin involving the active-site His 57 illustrates how a metal switch can be engineered into a protease for the purpose of regulating its function. This is a useful step in protein design which may not be limited to proteases since histidine is one of the most common amino acids at the active site of a protein.

CD 005 MODEL BUILDING OF PROTEIN CONFORMATIONS, Ken Smith, Anthony Nicholls, and Barry Honig, Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168 St., New York, NY 10032

Attempts to "engineer" metal binding sites into proteins, to design proteins *de novo*, and to predict protein conformations by homology would be greatly facilitated by the existence of a rapid and accurate means of evaluating conformational free energies. Most current attempts to construct models of small sections of proteins are based on intuitive model building rather than on objective criteria as to what comprises an acceptable structure. This talk will summarize recent progress in deriving a computer algorithm that evaluates conformational free energies based on a rapid but reliable treatment of all the factors that determine protein conformation

The first step in any "objective" design procedure is the generation of a large number of possible conformations. A number of algorithms designed to accomplish this task have been reported in the literature and will be discussed briefly. The focus of the talk will be the second stage of the process; choosing among a large number of trial conformations that have been generated but which clearly are not all equally stable. Molecular dynamics simulations offer one possibility but given the important role of solvation effects in determining conformation, it would be necessary to include a large number of water molecules and ions in the simulations before their predictions could be viewed as reliable. Given the vast computational demands involved in such calculations, they do not at present appear to offer a viable option as a tool in protein design.

Our own approach is based on a description of the system involving an atomic level treatment of the protein and a continuum treatment of the solvent and ionic strength. For the purposes of obtaining conformational energies, a standard vacuum force field is used to calculate all the bonding and van der Waals interactions in the protein. A term which scales as surface area is introduced to account for the effects of van der Waals interactions between protein and solvent. Electrostatic interactions, including hydrogen bonding, are treated with DelPhi, a program which solves the Poisson-Boltzmann equation numerically. Hydrophobic interactions are accounted for using an algorithm which calculates hyrophobic surface tension including the effects of local curvature . The hydrophobic strength that is used is approximately 50 cal/mole/$Å^2$, i.e. about twice as large as the traditional value. The increase above the traditional value results from a new method to extract free energies of transfer from experimental partition coefficients. Free energies currently in the literature are "incorrect" in that they contain a contribution that should not be used in evaluating conformational or binding energies.

Our new algorithm has been tested by calculating the energies of loops on proteins whose conformations are known. In each case the crystal structure emerges as the structure of lowest free energy. In contrast, more standard potential functions do not succeed in identifying the crystal structure using energetic criteria. A complete evaluation of the free energy of each conformation takes about 2 minutes on a Convex C2 computer.

CD 006 DESIGN AND SYNTHESIS OF FOUR-HELIX BUNDLE CHANNEL PROTEINS, Anne Grove [1], John M. Tomich[2], Takeo Iwamoto[2], G.L. Reddy[1], Stephan Marrer[1], Myrta S. Montal[1], and Mauricio Montal[1], [1]Departments of Biology & Physics, University of California San Diego, La Jolla, CA 92093- 0319, [2]University of Southern California, Childrens' Hospital, Los Angeles, CA 90054-0700.

A central goal in membrane protein science is to understand how channel proteins work in terms of the underlying protein structures. Channel proteins are transmembrane oligomers organized around a central aqueous pore. Among the best characterized are the voltage-gated channels and the ligand-gated channels, responsible for two fundamental properties of the brain namely, electrical excitability and synaptic transmission. Knowledge of the physiology of the molecule and its primary structure is leading us to design proteins that by imitating the natural sequence tend to fold predictably into stable structures and reproduce the targeted biological activity. The success in this endeavor is beginning to establish the occurrence of a unifying structural-motif that accounts for the pore function of this class of proteins (1). The strategy involves molecular dynamics calculations and molecular graphics, solid-phase protein synthesis, recombinant DNA technology and single-channel recordings from lipid bilayers. Proteins which emulate the pore properties of authentic voltage-gated and ligand-gated channels were designed and synthesized. These encompass two voltage-gated channels, the sodium channel and the dihydropyridine-sensitive calcium channel, and two ligand-gated channels, the nicotinic acetylcholine receptor – a cation-selective channel and the glycine receptor – an anion-selective channel. The designed proteins consist of bundles of four amphipathic α-helices with sequences corresponding to specific segments of the authentic proteins, arranged such that charged or polar residues line an aqueous pore. Molecular models suggest that such structures satisfy geometric and functional requirements to constitute the pore-forming element of channel proteins. The synthetic proteins mimic the ionic conductance, selectivity and pharmacological properties of the authentic channel proteins. Synthetic proteins representing segments of the authentic proteins not predicted to line an aqueous pore do not form channels (2-4). The availability of the structure models and of designed pore proteins should prove helpful in understanding the mode of action of drugs that act by blocking channels and in the design of new blockers that might be of therapeutic value.

([1]*FASEB J.* 4:2623-2635; [2]*Proc.Natl.Acad.Sci.USA* 87:6929-6933; [3]*Proc.Natl.Acad.Sci.USA* 88:6418-6422; [4]*Proteins* 8:226-236). Supported by NIH (NIMH, ADAMHA, NIGMS), ONR, DAMR.

Metal Regulation of Gene Expression (Transition Metals)

CD 007 STRUCTURE OF mRNA ENCODING A METALLOPROTEIN: THE FERRITIN IRE (IRON REGULATORY ELEMENT), Elizabeth C. Theil, Lin Peng-Nain, David J. Dix and R. Ann McKenzie, Department of Biochemistry, North Carolina State University, Raleigh, NC 27695-7622.

Ferritin mRNA function is regulated by the concentration of intracellular iron. At low iron concentrations, initiation of ferritin mRNA is low and translation to ferritin synthesis occurs at low rates. Iron releases the block to inhibition and ferritin is translated at high rates. In animals, a ferritin mRNA specific binding protein has been identified and characterized called IREBP/IRP or P-90; plant extracts appear to be deficient in P-90.

A sequence of 28 nucleotides (IRE-iron regulatory element) is conserved in the noncoding regions of both ferritin and transferrin receptor mRNAs which allows coordinate regulation of the synthesis of the two proteins by iron. Both the position of the IRE and the surrounding sequences differ in the two mRNA allowing two different mechanisms of regulation (translation vs degradation). Sequences which base-pair (FL) flank the IRE in ferritin mRNA causing the entire structure (IRE+FL) to be close to the 5'methylated cap crucial to the initiation of translation for all eukaryotic mRNAs. The IRE is required for negative control of ferritin mRNA (repression by P-90/IREBP/FRP) and positive control (high rate of translation in the absence of the regulatory protein).

Metal coordination complexes and enzymes have been used as nuclease probes of IRE+FL structure of the red cell ferritin H chain mRNA (bullfrog) model. Secondary structure analysis predicts a hairpin loop (IRE) connected by a bulge to the base-paired FL. 1,10-Phenanthroline (Cu), which cleaves at single-stranded open regions of RNA, shows magnesium-dependent (0-5 mM) conformational transitions in the loop and stem regions of the IRE and the FL, suggesting magnesium-dependent transitions between conformers. Fe-EDTA, which reflects solvent accessibility, showed that the IRE has regularly spaced hypersensitive regions along both the IRE and FL, suggesting tertiary folds. The Fe-EDTA "footprint" of P-90/IRE-BP was confined to the IRE (Harrell et al. Proc Natl Acad Sci.USA 88:4166, 1991). Protein nucleases specific for single or double-stranded (stacked) sequences cleaved very few sites in the IRE and FL; the importance of long-range interactions was indicated by the large number of nuclease sites in the FL when a synthetic 55-mer corresponding to the IRE+FL was examined. A conformational change in the FL, when P-90/IREBP binds to the IRE, indicates the active role of mRNA and the IRE+FL structure in regulation. (NIH-DK20251).

CD 008 **TRANSCRIPTIONAL REGULATION OF METAL HOMEOSTASIS GENES IN YEAST,**

Dennis J. Thiele, Pengbo Zhou, Mark S. Szczypka, Tomasz Sosinowski and Katherine Tamai, Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, Michigan 48109-0606.

Copper homeostasis in eukaryotic cells is a dynamic process which must balance the essential requirement for this metal with its known potent cytotoxicity. Our laboratory has been investigating the molecular mechanisms by which two eukaryotic microorganisms, the baker's yeast *Saccharomyces cerevisiae* and the opportunistic pathogenic yeast *Candida glabrata*, regulate the expression of genes which play important roles in copper homeostasis. In *S. cerevisiae*, the single metallothionein (MT) gene, denoted *CUP1*, encodes a small cysteine-rich protein which efficiently and tenaciously binds copper to protect cells from copper poisoning. *CUP1* gene transcription is induced by high levels of environmental copper through the action of a copper-activated sequence-specific DNA binding protein, ACE1, which binds to multiple sites within the CUP1 promoter. ACE1 also activates the expression of other *S. cerevisiae* genes, such as copper, zinc superoxide dismutase (*SOD1*), which provides another line of defense against copper toxicity. Furthermore, we have demonstrated that a number of other physiological signals activate transcription of *CUP1*, suggesting that these conditions alter the intracellular levels of bioavailable copper and other metals.

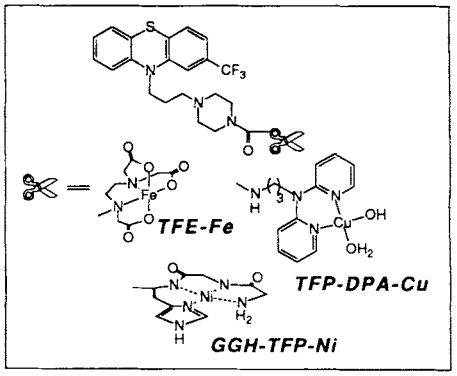
C. glabrata also provides an interesting system for investigations of metal homeostasis in eukaryotic cells since, like higher organisms, *C. glabrata* contains an MT gene family. Using surrogate genetics, we have cloned the *C. glabrata* gene encoding a copper-activated DNA binding MT *trans*-activator denoted *AMT1*. We report on the mechanisms by which AMT1 senses high environmental copper levels and transmits this signal to activate transcription of the *C. glabrata* MT gene family. Studies with these two eukaryotic microorganisms suggest that yeast provide experimentally tractable and informative model systems with which to dissect the molecular mechanisms underlying metal homeostasis.

DNA Cleavage Chemistry

CD 009 **METAL ION COMPLEXES AS PROBES OF PROTEIN STRUCTURE, FUNCTION AND DYNAMICS,**

Alanna Schepartz, Department of Chemistry, Yale University, 225 Prospect Street, New Haven, CT 06511.

In contrast to the rapid development of molecules capable of nucleic acid cleavage, synthetic reagents for the localized cleavage of proteins are virtually unknown. Protein cleavage reagents have utility in the study of protein-protein interactions, protein conformational changes, and for mapping drug binding sites on protein surfaces. We have identified three metal ion complexes capable of cleaving proteins under physiological conditions. Initially, we described TFE-Fe, which consists of the protein cleavage reagent EDTA-Fe linked to the calmodulin inhibitor trifluoperazine (TFP). By using a known calmodulin inhibitor to direct redox active iron to the protein's active site, TFE-Fe cleaves calmodulin in the TFP binding pocket to generate several discrete cleavage fragments. Two other protein cleavage reagents have been identified since that time. GGH-TFP-Ni tethers a reactive Ni^{2+} chelate to TFP and cleaves calmodulin at a single, identifiable site in the presence of a peracid. TFP-DPA-Cu cleaves calmodulin in a reaction that is pH dependent, but O_2 and reducing agent independent. Concurrently, we have synthesized reagents that permit EDTA to be tethered to any position in a peptide or any thiol in a protein. The application of these reagents to the study of protein-induced and denaturant-induced conformational changes will be discussed.



CD 010 ASSEMBLY OF THE BINUCLEAR IRON CENTER OF *E. COLI* RIBONUCLEOTIDE REDUCTASE, *J. B. Bollinger, Jr.*[1],*D. E. Edmondson*[2], *B. H. Huynh*[2], *J. Filley*[3],*J. R. Norton*[3], *J. Stubbe*[1], [1]Massachusetts Institute of Technology, Cambridge, MA 01239; [2]Emory Univ., Atlanta, GA 30322, [3]Colorado State Univ., Fort Collins, CO 80523.

Physical biochemical methods have been used to investigate the mechanism of assembly of the unusual catalytically essential tyrosyl radical-dinuclear iron cluster cofactor of *E. coli* ribonucleotide reductase. Incubation of the apoB_2 subunit of reductase with Fe^{2+} and O_2 produces native B_2. This process has been monitored by using stopped-flow absorption spectroscopy, rapid-freeze quench EPR spectroscopy and rapid-freeze quench Mössbauer spectroscopy. The latter method has proven particularly informative as all of the iron-containing species including intermediates, starting material and product can be monitored simultaneously. A novel kinetically competent iron species with a sharp isotropic EPR signal with g=2.00 has been observed. This intermediate has been shown to be capable of oxidizing Y122 of B_2 to a Y· (tyrosyl radical). Isotope labeling studies using $^{57}Fe^{2+}$, $^{17}O_2$ and $H_2{}^{17}O$ have been undertaken to provide structural information about this intermediate. A second intermediate that exhibits an absorption feature at 565 nm has also been observed. Correlation of the stopped flow kinetic data with the rapid-freeze quench EPR and Mössbauer data has allowed a tentative proposal for the structure of this second intermediate to be put forth. This proposed structure differs from that previously suggested (*Science* 253, 292 (1991)). A mechanistic model that accommodates all available information will be presented.

DNA Structure and Chemistry

CD 011 MAKING IMAGES OF DNA IN SOLUTION USING THE CHEMISTRY OF THE HYDROXYL RADICAL, Thomas D. Tullius, Department of Chemistry, The Johns Hopkins University, Baltimore, MD 21218.

While recent X-ray crystal structures of oligonucleotides have made clear the structural polymorphism available to duplex DNA, more unusual structures (such as the four-stranded Holliday junction) have yet to be crystallized. Even unusual *duplex* DNA structures, such as bent DNA, remain incompletely understood. A further limitation of present knowledge is that the structures of only short lengths of DNA, fewer than two turns of the helix, have been determined at high resolution. Many of the more intriguing structural possibilities for DNA require for their expression long lengths of DNA or the presence of supercoiling. To study such systems we have developed an experimental approach, based on chemistry, for determining structural details of DNA in solution. The method makes use of the hydroxyl radical to cleave DNA. We generate the hydroxyl radical by the reaction of iron(II) EDTA with hydrogen peroxide. Since the EDTA ligand imparts a negative charge to the iron complex, it has little affinity for binding to DNA. The structural probe, then, is the neutral hydroxyl radical, which by its cleavage of the deoxyribose residues of DNA provides a high-resolution *image* of the shape of the DNA molecule in solution. We have applied this method to a variety of DNA molecules of unusual structure. I will describe in particular recent work on the structure of bent DNA. The kinetoplast DNA of trypanosomatids was the first widely-appreciated example of DNA that could, solely as the consequence of its sequence, adopt a curved trajectory. It was found early on that bending of kinetoplast DNA was the result of the occurrence, once per helical turn, of several short runs of adenines on one strand. This discovery stimulated the detailed investigation of the structural properties of adenine tract-containing DNA, natural and synthetic. We observed that the hydroxyl radical cleavage pattern of kinetoplast DNA was different from that of mixed sequence DNA, and suggested that narrowing of the minor groove along an adenine tract was related to bending. Subsequent work showed that not all adenine tracts led to DNA curvature. Hagerman demonstrated that the sequence 5'-$(CGT_4A_4)_n$-3' appears not to be bent by the criterion of anomalous gel mobility. We showed that the cleavage pattern of this sequence resembled more that of mixed-sequence, and not bent, DNA. Crothers found that other sequences of the type 5'-$(N_mT_nA_n)_x$-3' do, however, bend. We have performed quantitative hydroxyl radical cleavage experiments on a number of sequences of this type. Our work has led to a model for the influence of the 5'-TA-3' base step on the structure of DNA, and the influence of such a step on the ability of DNA to bend. We conclude that 5'-T_3A_3-3' resembles normal B-form DNA, while longer runs of 5'-T_nA_n-3' can still adopt the structural characteristics of bent DNA, although the central TA step influences the structure for two to three base pairs to either side.

CD 012 MONOVALENT CATIONS AND TELOMERIC DNA, James R. Williamson, John L. Battiste, Eric K. Dias, and Maki Inada, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139.

Telomeric DNAs from a variety of organisms form structures containing the G-quartet as the basic structural unit. The G-quartet is a square planar hydrogen bonded array of four guanine bases. The hallmark of the G-quartet structure is the selective binding of monovalent cations by a pocket at the center of the structure. Potassium is usually the preferred ion, with sodium bound somewhat less preferentially over the other monovalent ions. We are investigating the role of monovalent ions in stabilizing telomeric DNA structures using a combination of NMR spectroscopy and UV thermal denaturation studies.

We are investigating the ion binding properties of several related telomeric DNA sequences: d(TTTTGGGG)$_4$, d(TTGGGG)$_4$, which are the telomere sequences from Oxytricha and Tetrahymena, respectively, and a model system d(TTTGG)$_4$. We have observed that the potassium form of telomeric DNAs are extremely stable, and that in the case of d(TTTTGGGG)4, slowly unfolding species exist with lifetimes of minutes.

The formation of the slowly unfolding G-quartet structures appears to require the presence of at least four adjacent G-residues that permits formation of four stacked G-quartets. The formation of slowly unfolding structures in d(TTTTGGGG)$_4$ is correlated to the presence of unfolding intermediates.

Studies of the denaturation of telomeric DNAs as a function of monovalent ion concentration provides evidence that multiple ions bind cooperatively to the G-quartet structures formed by telomeric DNAs. The relative affinities of different monovalent ions can be measured by competition experiments.

Banquet Address

CD 013 THE STRUCTURE AND FUNCTION OF LIM MOTIFS, CHRISTOPHER T. WALSH, PETER MARK LI, JANICE REICHERT, AND THEODORE R. HOLMAN, *Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02215*

The cysteine-rich LIM motif is highly conserved between invertebrates and mammals. This motif shows similarity to the metal binding motifs of both zinc-binding proteins and the ferredoxins, which contain iron-sulfur clusters. Tandem copies of the LIM motif are found in a number of presumptive transcription factors, including the protein product of the *Caenorhabditis elegans* cell-lineage gene *lin-11*(1). To investigate the possible metal-binding properties of the LIM region of the Lin-11 protein, we expressed and purified a 151 amino acid fragment containing the tandem LIM motifs (2). The purified peptide was found to bind zinc (two atoms per protein molecule) and iron (as a redox-active iron-sulfur cluster, with four atoms of iron and four atoms of inorganic sulfide per protein molecule). The LIM motif has been postulated to play a role in either protein-protein interactions or protein-nucleic acid interactions. Tandem LIM motifs have been found in three proteins (*lin-11*, Isl-1(3) and *mec-3* (4))that each contain a homeodomain, a region which is known to be involved in DNA binding. In addition, tandem LIM motifs are encoded by two genes that do not also encode a homeodomain: Rhombotin (also known as Ttg-1) (5, 6) and *Rhom-2*(7). All three proteins which contain tandem LIM motifs as well as homeodomains have been implicated in differentiation, suggesting that LIM proteins might be responsible for the regulation of a distinct group of transcriptional events important in development. One possible function of the LIM domain might be to increase the DNA sequence specificity and affinity of the homeodomain. We used purified recombinant Ttg-1 protein, which does not contain a homeodomain but does contain iron and zinc, to select DNA sequences from random-sequence oligonucleotides. We find that Ttg-1 is capable of binding to G-C rich oligonucleotide sequences. It is incapable of binding to an oligonucleotide containing the Isl-1 homeodomain consensus sequence, TAAT. By comparison, Lin-11 is capable of binding both the TAAT consensus sequence as well as the G-C rich oligonucleotides derived from the Ttg-1 affinity selection. These preliminary results suggest that the LIM region of LIM proteins might interact with DNA independently of other DNA binding motifs.

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Photosynthesis

CD 014 PROBING THE COORDINATION SITES AND ASSEMBLY OF THE PHOTOSYSTEM II OXYGEN-EVOLVING COMPLEX USING SITE-DIRECTED MUTAGENESIS. Peter J. Nixon and Bruce A. Diner, Central Research and Development Dept., E. I. duPont de Nemours & Co., Wilmington, DE 19880-0173

The oxygen-evolving complex of Photosystem II (PSII) is composed of a tetranuclear Mn cluster coordinated by oxygen and nitrogen ligands. We have located four amino acid residues of the D1 polypeptide of the PSII reaction center of *Synechocystis* 6803 at which site-directed mutations specifically block water oxidation. One of these sites, D1-Asp170 is located within 9 residues of Yz, the redox-active tyrosine that oxidizes the Mn cluster. Eleven mutations have been constructed at this site and show the following order of light-saturated rates of O_2-evolution: Asp (100%)> Glu (≥60%)> His, Cys (20-40%)> Tyr, Arg, Met (10-20%)> Trp (~5%)> Asn (~1%)> Ser, Ala, Phe (0%). The values in parentheses are the rates relative to wild-type (WT). Reduced oxygen activity correlates in whole cells with an inability to reduce Yz^+ by a tertiary electron donor, indicating the loss of a functional oxygen-evolving complex (OEC). A similar ranking is shown in measurements of the Km of electron donation by Mn^{2+} to Yz^+ in isolated PSII core complexes with the lowest Km (1μM in WT) corresponding to the highest rate of O_2-evolution. Both rankings approximately follow the pKa of the replacement residue - the lower the pKa, the higher the rate of O_2-evolution and the lower the Km for Mn^{2+} donation to Yz^+. We propose that D1-asp170 is part of a high-affinity Mn^{2+} binding site which participates in the first step of assembly of the Mn cluster of the OEC.

The D1 polypeptide is synthesized in precursor form. Carboxy-terminal proteolytic processing at Ala344 to the mature form is required for assembly of the Mn cluster. Deletion of the 3' end of the *psbA* gene encoding the carboxy-terminal extension (16 residues) results, however, in a mutant that grows photoautotrophically. Thus the presence of the carboxy-terminal extension is required neither for the assembly of the reaction center nor for any step in the assembly of the Mn cluster.

We find, however, that the deletion of one more residue, Ala344, results in the total loss of O_2-evolving activity. This mutant shows a loss in tertiary electron donation associated with oxidation of the OEC, but retains the high affinity binding site for Mn^{2+} involved in the reduction of Yz^+ and in the first steps in assembly of the OEC. It is likely that carboxy-terminal D1-Ala344 is involved in later steps in the assembly of the OEC and may be a ligand to the fully assembled manganese cluster through its free carboxyl group. Such a role is one explanation for why processing of the carboxy-terminal extension is required for assembly of the OEC.

Two other sites that are crucial to oxygen evolution are also located near the carboxy-terminus of polypeptide D1. These are D1-His332 and D1-Asp342. Replacement of these residues by amino acids unable to act as metal ligands results in the complete loss of O_2-evolution despite retention of the high affinity Mn^{2+} binding site.

We conclude then that polypeptide D1 is directly involved in ligation of the Mn cluster of the OEC. D1-Asp170 near secondary donor, Yz, forms part of a high-affinity Mn^{2+} binding site and is probably involved in the early stages of assembly of the Mn cluster. D1-His332 and D1-Asp342 near the carboxy terminus and the free carboxyl group of C-terminal D1-Ala344, are all three involved in later steps in the assembly of the cluster and are good candidates for ligation of Mn in the final cluster.

CD 015 ELECTRON TRANSFER IN PHOTOSYSTEM I, John H. Golbeck, Department of Biochemistry, University of Nebraska, Lincoln, NE 68593-0718

The Photosystem I reaction center is a light-driven plastocyanin:ferredoxin oxidoreductase. Its function is to transform a photon into chemical free energy, culminating in the reduction of $NADP^+$ to NADPH. The process can be divided into three parts: light capture, charge separation, and charge stabilization. The initial absorption of a photon by an antenna chlorophyll is followed by the generation of a charge-separated state within the photochemical trapping center. The charge-separated state is relatively short-lived, and must be stabilized by the delocalization of the electron down a chain of bound electron acceptors. This allows the initial photochemical reaction to be separated in space and lengthened in time, a necessary pre-condition for the oxidation and reduction of diffusible electron carriers on the stromal and lumenal sides of the thylakoid membrane. The electron transfer components in Photosystem I include a chlorophyll primary electron donor, P700, a chlorophyll primary electron acceptor, A_0, a quinone intermediate electron acceptor, A_1, and three iron-sulfur centers, F_A, F_B, and F_X. After absorption of a photon, the singlet exciton migrates from an excited antenna chlorophyll to the reaction center trap, bringing about charge separation between P700 and A_0. The electron is then passed through the intermediate acceptors A_1 and F_X to the terminal electron acceptors F_A/F_B. This strong reductant then reduces the soluble iron-sulfur protein, ferredoxin. Ferredoxin is located in the stromal phase of the chloroplast and interacts with ferredoxin:$NADP^+$ oxidoreductase to reduce $NADP^+$ to NADPH. It is now clear that the Photosystem I reaction center core contains a heterodimer of the *psaA* and *psaB* polypeptides which are chloroplast-encoded in higher plants. These proteins bind 100 chlorophyll *a* molecules and 10-15 β-carotene molecules along with most of the other electron-transport components necessary for light absorption, charge separation, and long-lived charge stabilization. The components include a chlorophyll electron donor, a chlorophyll electron acceptor, and two quinone molecules, which are known to be phylloquinones (vitamin K_1). A common feature with the bacterial reaction center and Photosystem II is the probable existence of a 'special pair' structure for the primaryelectron donor P700. As found in Photosystem II and bacterial reaction centers, there may be a structural element - in Photosystem I, the [4Fe-4S] cluster F_X, in Photosystem II and bacterial reaction centers the non-heme iron - which joins the heterodimer along the presumed, pseudo-C_2 symmetry axis near the stromal surface of the thylakoid. The Photosystem I reaction center also contains a number of low molecular mass polypeptides, labeled *psaC* through *psaJ* as of this writing. With the exception of *psaC*, *psaD* and *psaF* (the F_A/F_B polypeptide, the ferredoxin 'docking protein' and the plastocyanin 'docking protein', respectively) the functions of these low molecular mass polypeptides are unknown. Most of the low molecular mass polypeptides, except for *psaC*, *psaI*, and *psaJ* are encoded in the nuclear DNA in eucaryotes. We already suspect that most of the 'catalytic' components of both Photosystem I and II are encoded in the chloroplast genome of higher plants. In this context, it may be convenient to think of the *psaC* polypeptide as an 'add-on' feature, where the gene was copied from a soluble bacterial 2[4Fe-4S] ferredoxin and modified slightly to permit binding of the polypeptide to the *psaA* and *psaB* proteins.

Molecular Biology

CD 100 A NOVEL CALCIUM-BINDING POLYPEPTIDE FROM CHICKEN EMBRYO. *James A. Bassuk and Richard A. Berg, Dept. of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854. (908)-463-4595.*

We have isolated from chicken embryos a novel 53-kDa polypeptide. This 53-kDa polypeptide (p53) possesses an amino acid terminus which is highly similar to calreticulin, the major calcium-binding protein of the endoplasmic reticulum. Like calreticulin, p53 avidly binds calcium. p53 cross-reacts with antibodies raised against rabbit muscle calreticulin. However, p53 displays several properties dissimilar to calreticulin but similar to the 55 kDa protein disulphide isomerase (PDI) polypeptide. p53 is capable of cleaving the disulphide bonds of insulin under conditions where PDI is active. p53 copurifies with PDI and calreticulin, but is separated by ion-exchange chromatography. p53 cross-reacts strongly with antibodies specific for bovine PDI and cross-reacts to varying degrees with six different preparations of antibodies specific for chicken PDI. PDI is thought to be identical to the β-subunit of prolyl 4-hydroxylase, an enzyme which requires iron for activity. Anti-bovine PDI immunoglobulins selected by purified p53 react with bovine PDI but not with the β-subunit of prolyl 4-hydroxylase, suggesting that p53 shares epitopes with bovine PDI but not with the chicken prolyl 4-hydroxylase β-subunit. Amino acid compositional analysis of the HPLC-purified p53 yielded unique data when compared to calreticulin, PDI and other PDI-like polypeptides. A 20 residue sequence of an internal cyanogen bromide fragment of HPLC-purified p53 gives a nearly identical match with β-endorphin from a variety of species. We hypothesize that p53 is a hybrid protein which has acquired the ability to bind calcium and to cleave disulphide bonds through exon shuffling during evolution. The sequestration of calcium within cells is a fundamental process of the regulation of cellular metabolism. Supported in part by NIH AM31839.

CD 101 EFFECTS OF LEAD ON KIDNEY CALBINDIN CONCENTRATIONS John D. Bogden, Sylvia Christakos, Sheldon Gertner, Francis Kemp, Zhengang Yang, and Ching Chu, UMDNJ-New Jersey Medical School, Newark, NJ 07103-2714

Binding of heavy metals by proteins can be important in metal detoxification. The 28,000 dalton vitamin D dependent calcium binding protein calbindin - D28K can bind four lead atoms per molecule with greater affinity than its binding of calcium at the same sites. A recent study found that lead administration can influence calbindin - D28K levels in the chick intestine. We studied the effects of chronic adminstration of lead and diets of modified calcium content on kidney lead and calbindin concentrations. Weanling male Sprague-Dawley rats were randomly assigned to one of 9 treatment groups and were fed diets containing 0.1%, 0.5%, or 2.5% Ca. Lead was administered in the drinking water at concentrations of 0, 50, or 100 micrograms/ml (ppm) for 52 weeks. Kidney lead concentrations were determined by flameless atomic absorption spectrophotometry and calbindin levels by radioimmunoassay after radioiodination of the protein with I-125. SDS polyacrylamide gel electrophoresis was used to isolate renal calbindin. Kidney lead and renal calbindin levels differed significantly ($P<0.01$) among the 9 treatment groups. Kidney lead concentrations were about 20-fold higher in rats fed 0.1% Ca and given 50 or 100 ppm lead than in their counterparts fed 0.5% Ca in their diets. The lowest kidney lead concentrations were found in rats fed 2.5% Ca diets. Renal calbindin levels were highest (mean $\pm$ SE = 5.89 $\pm$ 0.69 ug/mg protein) in rats fed 0.1% Ca and no lead, and were lowest (2.18 $\pm$ 0.35 ug/mg protein) in animals fed 2.5% Ca and no lead. Lead inhibited the expected rise in renal calbindin levels in animals fed 0.1% Ca, but it caused increased levels in rats fed 2.5% Ca. The decrease in kidney calbindin in rats fed 0.1% Ca and given lead may potentiate the nephrotoxicity of the very high kidney lead concentrations found in these animals. Conversely, the combination of much lower kidney lead concentrations and relatively high calbindin levels in rats fed 2.5% Ca may act together to reduce lead toxicity.

CD 102 ENGINEERING ALLOSTERIC FUNCTION: AN ALLOSTERIC METAL BINDING SITE. Michelle Browner, David Hackos, Barry Haymore* and Robert Fletterick. Department of Biochemistry, Box 0448, University of California, San Francisco, CA 94141, *Monsanto Company, St Louis, MO 63167.

The regulatory switch of glycogen phosphorylase is necessarily allosteric because the enzyme is always bound to its substrate in the glycogen particle. Both intra- and intercellular signals provide information about the metabolic state of the cell or organism and thereby control the level of enzymatic activity. The activation of phosphorylase either by phosphorylation of serine 14 or AMP binding causes a conformational change that is transmitted to the active site, whereby substrates are bound more readily. A reductionist view of the structural changes associated with activation would focus on the movement of the two phosphorylase dimers toward each other and the strengthening of contacts at the dimer interface either by phosphoserine or AMP binding. Given this basic notion of an allosteric switch, can another mode of activation be added using the existing machinery and a new ligand binding site? The amino acid residues, valine 45 and tyrosine 75, which are within van der Waals and hydrogen bonding distance of AMP in the activated conformation, were replaced with histidine. Thus, a new allosteric site for phosphorylase was engineered. Modelling predicted that in the activated conformation, this pair of histidine side chains could coordinate a metal atom. Zinc was shown to bind tightly (K_a=6μM) and cooperatively to the engineered phosphorylase. Structure determinations of the mutant phosphorylase in the absence and presence of zinc are in progress.

CD 103 DEFECTIVE ZINC UTILIZATION PATHWAY DECREASES GENE EXPRESSION. Wen-Gang Chou, Marina Kriajevskaia and Wei Zhu, Cancer Center and Department of Biochemistry, University of Rochester Medical Center, Rochester, NY 14642

A wild type Chinese hamster ovary cell line and its DNA repair deficient mutant (xrs) were used to study the regulation of gene expression. Eleven species of mRNA were underexpressed in the xrs mutants. Nucleotide sequence analyses of the cloned cDNA identified 6 of those mRNAs coded for ferritin heavy chain, a retroviral sequence, ribosomal protein S17, L7 with B2 repetitive sequences and myosin light chain 3nm, respectively. The other 5 mRNAs coded for yet-unidentified proteins since no sequence homology was found in the NIH Gene Bank data base. Several lines of evidence suggested that the underexpression was not due to a defect in the gene itself. First, it is unlikely that all of those 11 genes are mutated in the xrs mutants. Second, the transcription of ferritin gene in the mutant is stimulated by iron to the same level as in wild type cells. Third, the methylation pattern of LTR of the retroviral sequence is the same in the wild type and the mutant cells. The mechanism of underexpression is most likely due to a defect of a cellular factor(s). This conclusion was supported by the following experimental results. The cloned 3' LTR was linked to a reporter gene, CAT. The expression of CAT was 5 to 10 fold lower in the xrs mutant than in the wild type cells, using the transient expression system by DNA transfection assay. In addition, the level of mRNAs in the mutant was restored to the same level as wild type cells by zinc-pyrithione. This effect was not detected when zinc chloride was used. Our results suggest that the xrs mutants are defective in normal pathway of zinc utilization. The decreased utilization of zinc ion leads to the decreased level of expression of certain genes. In accordance with this hypothesis, it is interesting to know that the control region of ferritin gene and LTR of the retroviral sequence contains several motifs which transcription factor Sp1 binds to. This system will be further characterized in detail in order to study the pathway of zinc utilization with special reference to regulation of gene expression.

CD 104 Zn_2Cys_6, Zn_1Cys_4 and Cd_2Cys_6 COMPLEXES OF THE DNA BINDING DOMAIN OF THE GAL4 TRANSCRIPTION FACTOR, Joseph E. Coleman, Kevin H. Gardner, Tao Pan, Lena Basile and Edwin Rivera, Department of Molecular Biophysics & Biochemistry, Yale University, New Haven, CT 06510

The GAL4 transcription factor from yeast contains within its N-terminal DNA binding domain a binuclear metal binding site made up of 6 cysteine ligands, C^{11}-X_2-C^{14}-X_6-C^{21}-X_6-C^{28}-X_2-C^{31}-X_6-C^{38}. GAL4 is one of 14 fungal transcription factors which conserve this sequence in their N-terminal DNA binding domains. Two of the $-S^-$ donors, contributed by C11 & C28, form bridging ligands between the two metal ions. Binding of Zn(II) or Cd(II) to this site is essential to induce the conformation of GAL4 required for the protein to recognize the specific DNA sequence, UAS_G, to which GAL4 binds. The DNA binding domain of GAL4 consisting of the N-terminal 62 residues has been cloned and overproduced. ^1H-^1H COSY difference spectra using single and double ^{113}Cd filters confirms not only that the six C residues are the sole ligands to the two ^{113}Cd ions, but that Cys 11 & 28 are the bridging ligands. Two dimensional ^1H-^{113}Cd double quantum correlation spectra show the six C ligands to fall into two groups; C 11, 14 & 21 with primary coordination to the ^{113}Cd with a NMR signal at 669 ppm and C 28, 31 & 38 with primary coordination to the ^{113}Cd with a NMR signal at 707 ppm. Both C11 & 28 show weak, ~5 Hz, coupling to the second ^{113}Cd nucleus in the 2D heteronuclear ^1H-^{113}Cd COSY spectrum. When Cd(II) is exchanged for Zn(II), the cluster appears to expand to accommodate the larger Cd(II) ion as suggested by changes of 2 to 4 Hz in the $^3J_{HN\alpha}$ coupling constants for the amino acid residues which form the polypeptide loops enclosing the cluster, residues 10 to 40. A metal-ligand structure derived from the ^1H-^{113}Cd heteronuclear NMR as well as the polypeptide backbone connectivity around the cluster as determined from short range ^1H-^1H NOE's is presented. While Cd cooperatively forms a 2Cd cluster under all conditions, Zn will form both Zn_2 and Zn_1 forms of the GAL4 DNA binding domain, both of which bind to the UAS_G DNA sequence. Regulation of the conformation of a transcription factor by the number of metal ions incorporated will be discussed. Supported by NIH Grants DK09070 and GM21919.

CD 105 **ISOLATING COPPER TRANSPORT GENES FROM S. CEREVISIAE : Valeria Culotta, Craig Berchtold and Xiu Fen Liu. Department of Environmental Health Sciences, Johns Hopkins University School of Hygiene and Public Health. Baltimore, Md. 21205**

The yeast S. cerevisiae represents an ideal organism in which to identify the key cellular factors controlling uptake and accumulation of heavy metals. Our general approach to identifying metal transport factors has been to isolate mutants of yeast defective for uptake or efflux of copper (Cu) ions, then to utilize these mutants to clone the corresponding Cu transport gene through complementation. Thus far we have isolated two Cu-resistant complementation groups of yeast (designated "*cur1*" and "*cur2*"), that are defective for Cu transport. Both mutants are associated with decreases in initial rate of Cu uptake, yet distinct components of Cu transport are affected in two strains. Mutant *cur2* is defective for saturable Cu uptake, while *cur1* is affected in an apparent non-saturable component of transport. The putative *CUR1* transport gene has been cloned by complementation and consists of gene sequences that are highly toxic to bacteria. Work is currently underway to characterize the *CUR1* gene and to clone the *CUR2* gene by complementation. Overall the utility of the Cu transport mutants described here should prove valuable for genetically dissecting the multiple components of Cu ion transport.

CD 106 METALLOTHIONEIN STRUCTURAL MOTIF INVOLVED IN GENE EXPRESSION, C.T. Dameron, P.J. Arnold, D.R. Winge, Departments of Medicine and Biochemistry, University of Utah Medical Center, Salt Lake City, UT 84132

Metal ions regulate the expression of metallothionein (MT) genes in yeast through intracellular metal ion sensor proteins. The sensor molecules, ACE1 and AMT1 in Saccharomyces cerevisiae and *Candida glabrata* respectively, mediate the transcriptional activation of metallothionein genes. The initial event in transcriptional activation is the specific interaction of the sensor proteins with DNA promoter sequences upstream of the MT structural genes. Our studies with the N-terminal DNA-binding domain of ACE1 show that Cu(I) or Ag(I) ions bind to ACE1 and induce a protein conformation enabling high affinity association with the promoter sequences. ACE1 and AMT1 exhibit structural features analogous to MTs. The chemistry of the metal clusters in MT provides insight into the basis of the metal ion activation and specificity seen in the protein:DNA interactions. Metallothionein and the Cu(I)-metallo-regulatory protein families contain multiple cysteinyl residues within CXC and CXXC sequence motifs in domains containing a paucity of apolar residues. The lack of appreciable regular secondary structure implies that metal binding in ACE1 as in MT contributes substantial energy of stabilization. The entropy effect arising from packing hydrophobic groups away from the solvent seen in typical soluble proteins is of less importance. This permits metal-thiolate coordination chemistry to dictate the folding pathway. The Cu(I):thiolate polynuclear cluster in the active CuACE1 resembles the Cu(I):thiolate cluster (Cu_7S_{10}) found in *S. cerevisiae* MT in exhibiting trigonal Cu(I) coordination, an identical Cu-S mean distance of 2.24 Å and Cu-Cu distances of 2.7 Å. In contrast to the all-or-nothing cluster formation in *S. cerevisiae* CuMT, CuACE1 exhibits a biphasic nature in the formation of the CuS center. A family of fungal sensor proteins exemplified by ACE1 and AMT1 appear to have evolved with a metallothionein structural motif as the basis for its function in metalloregulation.

CD 107 CELL SPECIFIC EXPRESSION OF METALLOTHIONEIN GENES IN *C.ELEGANS* J.H. Freedman*, D. Dixon+, A. Fire+, and C.S. Rubin*, *Albert Einstein College of Medicine, Bronx, NY, 10461 and +Carnegie Institution of Washington, Baltimore, MD, 21210. *Caenorhabditis elegans* provides a model system for investigating cellular and molecular mechanisms underlying adaptation to heavy metal stress. *C. elegans* exposed to 0.1 mM $CdCl_2$ expressed high levels of two iso-MTs. *C. elegans* MTs are 65% identical, but have no significant overall sequence homology with conserved eukaryotic MTs. The gene encoding MT-2 (*mt1-2*) comprises 330 bp and contains a single intron. The DNA flanking the 5'-end of *mt1-2* has a putative consensus sequence for a metal regulatory element (MRE). The *mt1-1* gene is organized similarly, but apparently lacks an MRE. In the absence of Cd, *C. elegans* contains a low level of *mt1-1* mRNA and no *mt1-2* mRNA. Within 30 min after exposure to Cd *mt1-2* mRNA accumulates *de novo*. The content of *mt1-2* mRNA increases rapidly and substantially over a 2 h period. In contrast the amount of *mt1-1* mRNA is stable for 2 h and then increases precipitously. Thus *mt1-1* gene expression may be a secondary response to Cd exposure. To determine the individual cells and molecular mechanisms responsible for basal and Cd-induced MT expression, lines of stable, transgenic *C. elegans* were created. These nematodes contained the *mt1-1* promoter/enhancer (P/E) region (1.7 kb) upstream from a β-galactosidase (β-gal) reporter gene (*mt1-1*::β-gal); the *mt1-2* P/E (2.3 kb) ligated to β-gal (*mt1-2*::β-gal); or a shorter *mt1-2* P/E (0.35 kb) driving β-gal transcription. Both *mt1-2*::β-gal constructs were silent in untreated *C. elegans*, but yielded high level expression of β-gal in all of the intestinal cells of Cd-treated animals at various developmental stages. Thus, the 350 bp DNA sequence that precedes the *mt1-2* structural gene confers Cd-inducible, cell type-specific activation of *mt1-2* gene transcription. The *mt1-1*::β-gal reporter gene was selectively and constitutively expressed in 3 pharyngeal cells in the absence of Cd. When transgenic *C. elegans* harboring this construct were stimulated with Cd, β-gal expression was observed in all intestinal cells and the pharyngeal cells noted above. Thus, the *mt1-1* P/E confers either constitutive or inducible transcriptional control of *mt1-1* expression in cells with different developmental histories and physiological functions.

CD 108 YEAST CuZnSOD MUTANTS.

Edith B. Gralla, Janet A. Graden, Yi Lu, and Joan S. Valentine. Dept. of Chem. and Biochem., UCLA, Los Angeles, CA 90024-1569.

Copper, zinc superoxide dismutase (CuZnSOD) is being studied in our laboratory with respect to its structure/function relationships, spectroscopic characteristics, and biological properties. The biological studies are focused on yeast mutant strains lacking either or both SODs (CuZnSOD and/or MnSOD).

For the physical studies, we used site directed mutagenesis to produce a series of yeast CuZnSODs with altered metal binding ligands. Purified proteins and metal substituted derivatives have been characterized by electronic absorption and EPR. Five His to Cys single mutations, spanning both the copper and the zinc site, were shown to retain wild type geometry. Zinc site mutants exhibited new sulfur to copper or sulfur to cobalt charge transfer bands similar to type I (blue) copper proteins, whose characteristics the mutants were designed to mimic. (CuZnSOD is a type II copper protein.) Wild type CuZnSOD reacts slowly with ascorbate while these mutants react rapidly, indicating changes in electron transfer properties. Mutants with the copper to zinc bridging ligand (His 63) changed to Glu, Tyr or Ala, have also been generated, in order to explore the role of the bridging His as well as to mimic other types of metalloprotein active sites. The Tyr derivative shows a new absorbance band (probably phenolate to copper charge transfer), as well as new pH dependence of spectroscopic properties. The Glu mutant has a different EPR spectrum from wild type, indicating a change in the geometry of the copper site. Detailed characterization will be presented, and the biological activity will be discussed.

CD 109 DNA BINDING BY TRANSCRIPTION FACTOR AP-1 IS MODULATED BY METAL IONS, PARTICULARLY SELENIUM AND GOLD SALTS, Malcolm L. Handel, Colin K.W. Watts, Richard O. Day and Robert L. Sutherland, Cancer Biology Division, Garvan Institute of Medical Research & Dept. Clinical Pharmacology, St Vincent's Hospital, Darlinghurst, Sydney, Australia.

We have previously shown that metal ions with high affinity for thiols inhibit the DNA binding and transcriptional activity of progesterone receptor. The DNA binding domains of Jun and Fos, which form the AP-1 heterodimer, both contain a thiol on a cysteine residue which can be oxidised or alkylated with consequent inhibition of binding to the AP-1 response element. We tested whether metal ions with high affinity for thiols might also inhibit AP-1 binding its response element.
Nuclear extracts of T-47D breast cancer cells were incubated with metal ions followed by incubation with radiolabelled AP-1 response element prior to gel retardation assay. Bands, identified by anti-Jun and anti-Fos antibodies and by unlabelled competitor as representing AP-1 bound to its response element, were excised and quantified by Cerenkov counting.
Metal ions inhibited binding in a dose dependent manner in a rank order which correlates with their thiol binding affinity. Approximate IC_{50} concentrations (μM) are shown:

selenite > Au^{+} > Cu^{2+} > Cd^{2+} > Co^{2+} > Zn^{2+} > Fe^{2+} > Ni^{2+} >> Mn^{2+}
1.5 3 5 25 50 55 100 150 >1000

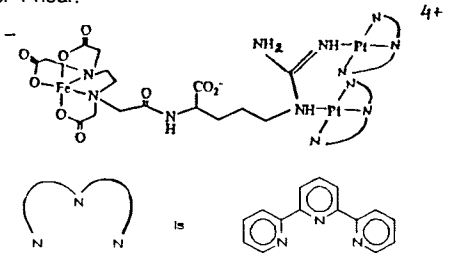
Au^{+} was added as aurothiomalate. Sodium thiomalate and the antirheumatic thiol drug penicillamine had no effect.
Jun and Fos are proto-oncogene products which transcriptionally activate many genes involved in inflammation and cell proliferation. We suggest that inhibition of AP-1 and other transcription factors with thiols in their basic DNA binding domains may play an important role in the therapeutic mechanism of some metals. Possible examples include the anticancer and anti-inflammatory effects of Se in animal models and the antirheumatic effects of gold salts. In most instances of transcriptional regulation by metal ions the gene product is a metalloprotein and/or is involved in homeostasis of that metal. In contrast the effect of metals described here is pharmacological rather than physiological.

CD 110 CONSTRUCTION OF NEW LIGAND BINDING SITES IN PROTEINS OF KNOWN STRUCTURE, Homme W. Hellinga, Fred M. Richards, Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511. We have devised a molecular model building program (DEZYMER) which builds new ligand binding sites into a protein of known three-dimensional structure, leaving the backbone fold intact. The program searches for a constellation of backbone positions arranged such that if appropriate side-chains were placed there, they would bind the ligand according to pre-defined geometry of interaction specified by the experimentalist. These binding sites are introduced by the program by taking into account simple rules such as steric hindrance, atomic close-packing and hydrogen bonds to a first approximation. A test case is presented where the copper binding site found in blue-copper proteins is introduced into *E. coli* thrioredoxin.

CD 111 **REGULATION AND EXPRESSION OF THE ARSENIC RESISTANCE OPERON FROM Staphylococcus aureus PLASMID pI258**, Guangyong Ji and Simon Silver, Department of Microbiology and Immunology, University of Illinois, Chicago, IL 60680
The arsenic resistance operon from Staphylococcus aureus plasmid pI258 was cloned and sequenced. The DNA sequence contains three genes in the following order: arsR, arsB and arsC. The predicted amino acid sequences of the genes are homologous with those of the ars operons of plasmids pSX267 from Staphylococcus xylosus and R773 from Escherichia coli. The cloned staphylococcal ars operon confers resistances to arsenate, arsenite and antimonite in S. aureus and Bacillus subtilis. The same operon was also expressed in E. coli and conferred resistance to arsenite but little resistance to arsenate and antimonite. Regulation of the pI258 ars operon was studied by using a translational arsB-blaZ fusion in S. aureus, and a transcriptional arsB-luxAB fusion in E. coli. The ars operon was induced by all three oxyanions (for which resistances occur) plus Bi(III) in S. aureus, but only by arsenate and arsenite in E. coli. Northern blot DNA/RNA hybridization analysis showed inducible synthesis of a full-length ars mRNA, about 2.1 kilobases in size both in S. aureus and in E. coli. S. aureus ars proteins were expressed in E. coli from the T7 phage 10 promoter under the control of the T7 RNA polymerase. Primer extension (reverse transcriptase) analysis showed that the ars mRNA started at the same position (nt 17 and 18 upstream from the arsR ATG) both in S. aureus and in E. coli. An internal deletion mutation in arsB resulted in decreased resistance to arsenate and total loss of arsenite and antimonite resistances. Partial deletion of 56 bp from the 3' end of the arsC gene resulted in loss of resistance to arsenate; the determinant retained arsenite and antimonite resistances.

CD 112 EFFICIENT CLEAVAGE OF PLASMID DNA BY A NEW INORGANIC REAGENT, Nenad M. Kostić and Edwin L. M. Lempers Department of Chemistry, Iowa State University, Gilman Hall, Ames, IA 50011

We report the complex [{Pt(trpy)}$_2$Arg-edta]. The structure of its bis(terpyridineplatinum) moiety was previously determined in our laboratory by X-ray crystallography. Binding of this moiety to calf-thymus DNA and to two duplex oligonucleotides was previously examined in our laboratory by many biophysical and biochemical methods. This diplatinum complex binds to the minor groove and does not intercalate. The structure and composition of the present complex are determined by elemental analysis and by UV-vis, mass, and ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopic methods. In the presence of Fe^{2+} and dithiothreitol the new complex cleaves the pBR 322 plasmid DNA nearly as efficiently as the well-known reagent methidiumpropyl-edta, which is often used for footprinting. The new reagent, too, is sequence-neutral. Optimal conditions for single-strand cleavage are as follows: 10 μM basepairs, 0.10 μM [{Pt(trpy)}$_2$Arg-edta], 0.10 μM Fe^{2+}, 0.10 mM dithiothreitol, and 5 mM NaCl in a Tris-HCl buffer at pH 7.4 and 25°C for 1 hour.



CD 113 PRODUCTION OF HUMAN METALLOTHIONEIN IIA SPECIFIC ANTISERUM USING A METALLOTHIONEIN ISOFORM-SPECIFIC SYNTHETIC PEPTIDE. Shiu-Ming Kuo, Joseph M. DeFilippo and John S. Lazo, Department of Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261

We synthesized a 13 mer peptide based on the unique amino acid sequence of human metallothionein (MT)-IIA and conjugated to keyhole limpet hemocyanin. After three intradermal injections in rabbits, we found immunoreactivity toward the peptide based on competitive ELISA. We further characterized the specificity of antiserum from one rabbit toward different MTs purified from human liver. Detectable ELISA immunoreactivity was seen with 1:300 primary antiserum dilution when the well was coated with as little as 5 ng human MT-IIA. The color intensity was found to directly related to the coating amount of human MT-IIA. In contrast, no ELISA reactivity was seen with up to 100 ng per well of human MT I. The reactivity toward MT-II at 5 ng per well was also titer-dependent whereas there was no reactivity to human MT-I even at 1:20 dilution of the antiserum. The antiserum also showed reactivity toward commercially available rabbit liver MT-II but not to rabbit liver MT-I and horse kidney MT . The reactivity to rabbit MT-II was found to be coating concentration- and titer-dependent as well. These results demonstrate for the first time that human MT isoform specific antibodies can be generated using a synthetic peptide. This antiserum should be useful in ELISA to quantify MT-II in samples of human origin as well as possibly for immunohistochemical studies.

CD 114 METAL-THIOL COORDINATION IN THE E7 ONCOGENIC PROTEIN OF PAPILLOMA VIRUS, B.A. Kurz, C.T. Dameron, D. Smotkin, D.R. Winge, Departments of Biochemistry, Medicine, Obstetrics and Gynecology, University of Utah Medical Center, Salt Lake City, UT 84132

E7 proteins from the papillovirus isolated from humans (HPV16) and rabbits (CRPV) have been expressed in *E.coli* from a plasmid encoding a chimera of E7 open reading frame DNA fused to the 3' end of the ubiquitin gene with a factor X cleavage site as a linker. Overexpression of the chimeric E7 allowed simple purification of E7 with up to 98 % purity. The carboxyl-terminal half of the E7 molecule contains multiple cysteinyl residues and resembles Cys_4-type motifs known to coordinate Zn(II) ions. Human and rabbit E7 molecules are predicted to contain 7 and 8 cysteines respectively and all are present in the reduced thiolate state in the *E. coli* produced proteins. *E. coli* produced human E7 contained one Zn(II) ion bound per molecule. The Zn(II) atom is readily displaced by Cd(II) in a 1:1 stoichiometry or Cu(I) in a 2 Cu:1 Zn stoichiometry. Purified rabbit E7 contained between 1-2 Zn(II) ions, one of which was readily removed by Chelex 100. Titrations of rabbit E7 with Cd(II) or Cu(I) in metal exchange studies demonstrated that 2 mol eq. Cd(II) or 3 mol eq. Cu(I) would bind per mol protein. The ultraviolet absorption spectra of metal-exchanged E7 molecules revealed transitions consistent with metal:thiolate charge transfer bands. Zn(II) displacement with Cu(I) resulted in luminescent molecules with emission centered near 580 nm at room temperature similar to the emission seen in other Cu-thiolate complexes such as Cu-metallothionein. The emission is indicative of Cu(I) coordination in an environment shielded from water implying a compact protein structure. The Zn(II) atoms can be removed from E7 at neutral pH without precipitation of the protein.

CD 115PURIFICATION AND CHARACTERIZATION OF *O*-ACETYLSERINE SULFHYDRYLASE ISOENZYMES FROM CADMIUM RESISTANT *DATURA INNOXIA*, Cheryl R. Kuske[1], Lawrence O. Ticknor[2], Mary E. Rice[1], and Paul J. Jackson[1], [1]Genomics and Structural Biology Group, Life Sciences Division, and [2]Statistics Group, Analysis and Assessment Division, Los Alamos National Laboratory, Los Alamos, NM 87545.

In the presence of cadmium and certain other trace metal ions, metal tolerant cell suspension cultures of *Datura innoxia* synthesize large amounts of poly(γ-glutamylcysteinyl)glycines [(γ-EC)$_n$Gs, where n=2-5], polypeptides which specifically bind certain toxic metal ions. We are currently purifying enzymes involved in biosynthesis of (γ-EC)$_n$Gs and their precursors, to study regulation of the pathway and for peptide sequencing. Three isoenzyme forms (A, B, and C) of *O*-acetylserine sulfhydrylase, which catalyze the synthesis of cysteine from *O*-acetylserine and sulfide, have been purified. Isoenzymes A and C are both 62 kD MW. Isoenzyme B is 86 kD MW. Each isoenzyme is composed of two identical subunits and contains pyridoxal-5'-phosphate as a cofactor. All isoenzymes are active over a broad temperature range, with highest activity between 42 and 54°C. They are active only between pH 7 and 8. *O*-acetylserine sulfhydrylase isoenzymes are inhibited by higher concentrations of both substrates. Preliminary kinetic analysis suggests that isoenzyme A is activated in the presence of cadmium. Further kinetic analysis of these isoenzymes will be presented.

CD 116 METAL INDUCED STRESS RESPONSE IN A HELA DERIVED CADMIUM RESISTANT CELL VARIANT, Arturo Leone, Liliana Minichiello, Stefania Cigliano, Cinzia Verde and Paolo Remondelli, Department of Biochemistry and Medical Biotechnology, University of Naples, Italy, I - 80131

We have investigated the effect of the exposure to cadmium, zinc and copper on the expression of MTs and two Heath Shock genes, Hsp70 and GRP78, in normal and in a HeLa derived cadmium resistant cell variant, named H454. No amplification of functional MT genes was detected in the genomic DNA through Southern blot technique.In the resistant cells differently from the original HeLa, exposure to zinc and copper after twenty four hours growth in absence of metals did not produce the accumulation of both Hsp70 and GRP78 proteins and mRNAs, which was still stimulated by cadmium. Conversely, MT mRNAs were synthesized following exposure to each of the three metals and were overexpressed after cadmium induction.Mobility shift assays revealed that zinc addition to EDTA treated H454 superinduced nuclear extracts was able to reconstitute a complex of proteins bound to the MRE3/4 region of the HMTIIa promoter. Such phenomena was not observed using normal or induced HeLa nuclear extracts.

CD 117 MODULATION OF MERCURY RESISTANCE OPERON EXPRESSION IN GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA, Tapan K. Misra, Debabrata Mukhopadhyay, Hongri Yu, Lien Chu, Mitch Horwitz and Kun Soo Kim, Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL 60680

The expression of mercury resistance (*mer*) operons isolated from Gram-negative bacteria is modulated by the products of two *mer* genes. One of the regulatory proteins, MerR, functions as a negative regulator in the absence of Hg(II), and as an activator in the presence of inducing concentrations of Hg(II). Experimental data shows that another regulatory protein, MerD, down-regulates the expression of the *mer* operon in the presence or the absence of Hg(II). Purified MerR and MerD proteins bind with a common operator sequence. Recent genetic and biochemical studies suggest that in the Gram-positive bacterium *Staphylococcus aureus*, only one regulatory protein (encoded by the gene, previously identified as ORF2 by Laddaga *et al.*, Proc. Natl. Acad. Sci., USA, vol. 84, 5016-5110, 1987) negatively regulates the expression of the pI258-encoded *mer* operon in the absence of Hg(II) and induces the expression of the operon in the presence of Hg(II).

CD 118 EXPRESSION OF A SYNTHETIC GENE FOR THE AMINO ACID SEQUENCE OF *CLOSTRIDIUM PASTEURIANUM* RUBREDOXIN, Sonia E. O'Dell, Marly K. Eidsness, Donald M. Kurtz, Jr., Robert L. Robson and Robert A. Scott, Departments of Chemistry and Biochemistry and Center for Metalloenzyme Studies, University of Georgia, Athens, GA 30602

A synthetic gene based on the amino acid sequence of *Clostridium pasteurianum* rubredoxin was constructed, cloned and overexpressed in *E. coli*. The gene was cloned in *Ec* 71/18 and expressed in the T7 RNA polymerase/promoter system, using strain *Ec* HMS262. In this system the control plasmid lacking the inserted rubredoxin gene does not express rubredoxin, indicating that *Ec* HMS262 does not have a native rubredoxin. The expression system has been scaled up to a 100 liter fermenter, and approximately 350 mg of the cloned holo- (i.e., iron-containing) rubredoxin can be obtained from 220 g wet weight of *E. coli*. After purification of the cloned rubredoxin by anion-exchange chromatography, UV/vis spectroscopy and metal analyses indicated that the as-isolated synthetic gene product is a mixture of holo- and zinc-substituted rubredoxins. The molecular weights of the holo- and zinc-substituted cloned rubredoxins were, within error, the same as that of authentic *Clostridium pasteurianum* rubredoxin, as determined by urea/SDS polyacrylamide gel electrophoresis. The UV/visible absorption and resonance Raman spectra of the cloned holorubredoxin are indistinguishable from those of the oxidized form of native rubredoxin. N-terminal amino acid sequencing of HPLC-purified fractions shows that the gene product consists of four polypeptides with the initial N-terminal sequences of nMet-Met, Met-Met, nMet-Lys and Met-Lys (where nMet stands for a blocked N-terminus). Site-directed mutagenesis of the synthetic rubredoxin gene is planned in order to test the effects of protein structure on redox and electron transfer properties of the iron site.

CD 119 BACTERIAL RESISTANCES TO TOXIC HEAVY METALS: NEWER SYSTEMS, Michael Rhodes, Susan Rhodes, Guangyong Ji, and Simon Silver, Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL

Bacterial plasmids contain specific genetically-determined resistances to a wide range of toxic heavy metals including Hg^{2+}, Cd^{2+}, Zn^{2+}, AsO_2^-, AsO_4^{3-}, CrO_4^{2-}, Cu^{2+}, Co^{2+}, Pb^{2+}, and other metals. Recent progress on less-familiar systems such as cadmium (and zinc in Gram positive bacteria), cadmium (and zinc and cobalt in Gram negative bacteria), arsenic (and antimony in both Gram positive and Gram negative bacteria) will be presented. A new cyanobacterial metallothionein system produces a metal-binding polypeptide containing 9 out of 58 amino acids as cysteine, analogous to metallothioneins of animals, eukaryotic microbes, plants and animals.

This system is being studied by molecular biology techniques, including regulation in response to heavy metals, cloning and sequencing and over-production and functioning of the metallothionein protein.

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N.J. Robinson, A. Gupta, A. P. Fordham-Skelton, R. R. D. Croy, B.A. Whitton, and J. W. Huckle (1990). Prokaryotic metallothionein gene characterization and expression: chromosome crawling by ligation-mediated PCR. Proc. Roy. Soc. Lond. series B 242, 241-247.
S. Silver and M. Walderhaug (1992). Regulation of chromosomal and plasmid cation and anion transport systems. Microbiol. Rev.**56**, March issue, in press.

CD 120 CLONING AND EXPRESSION OF A cDNA ENCODING AMPHIBIAN (*XENOPUS LAEVIS*) METALLOTHIONEIN.
Elise Saint-Jacques and Carl Séguin. Centre de recherche en cancérologie de l'Université Laval, l'Hôtel-Dieu de Québec, Québec, Canada, G1R 2J6.

Metallothioneins (MTs) are cystein-rich proteins which bind to heavy metals such as copper, zinc and cadmium. These proteins are thought to play an important role in zinc and copper homeostasis as well as in heavy metal detoxification. Amphibians are of interest for studying the biological roles of MTs because of their phylogenetic position among vertebrates. Using PCR techniques, degenerate oligodeoxynucleotide primers and DNA prepared from a *Xenopus* liver cDNA library, we successfully amplified a fragment hybridizing to mouse MT-I cDNA. DNA sequence analyses revealed that the fragment was 62% identical to the mouse MT-I sequence. We use this amplified PCR fragment as a probe to screen the cDNA library and found two different classes of clones. Of 15 recombinant phages whose DNA hybridized with the PCR fragment, 14 contained an insert of approximately 700 bp and one had an insert of 900 bp. While the latter was not analysed further, the insert of one of the 14 phages was subcloned into a plasmid and sequenced. The cDNA is 730 nucleotides long, contains a single open reading frame of 62 amino acids, of which 20 are cysteine residues, and a 3' non-coding region of 522 bp. The position of the cystein residues is perfectly conserved with respect to MT sequences of other species and the amino acid composition of purified *Xenopus* MT protein agreed with the amino acid composition and sequence deduced from the cloned cDNA. *Xenopus* MT shows 66% amino acid identity with mouse and chicken MTs, 61% identity with trout MTs but is no more similar in sequence to MT-I than it is to MT-II isoforms from various vertebrates. Levels of *Xenopus* MT mRNA in the liver were rapidly induced up to 30 fold by metals (Cd^{2+}, Zn^{2+} and Cu^{2+}). Supported in part by the C.R.S.N.G.

CD 121 Metal Ion Regulation of Cell Adhesion Receptors: Elucidation of Mechanism by the *in situ* generation of Co(III):Receptor Complexes.
Jeffrey W. Smith, Edward F. Plow and Randy Piotrowicz., Committee on Vascular Biology, The Scripps Research Institute. 10666 N. Torrey Pines Road, La Jolla, CA. 92037.

Cell adhesion to the extracellular matrix is mediated by cell surface receptors termed integrins. The integrins consist of an α and ß subunit, and are directly involved in many important biological phenomena including bone resorption, platelet aggregation, neurite extension, tumor invasion and atherosclerosis. Many integrins bind to the arg-gly-asp (RGD) cell adhesion sequence originally identified in fibronectin and now known to exist in most extracellular matrix proteins. Importantly, the binding of integrins to their ligands in the extracellular matrix via the RGD sequence is divalent metal ion-dependent. The structure and location of the metal binding sites within the integrin primary structure is unknown. The α subunits contain four repeats with limited homology to the helix-loop-helix Ca^{2+}-binding proteins like calmodulin and troponin C. However, the integrin repeats lack two key features critical to the function of the HLH proteins; 1) an acidic residue in the 12th position of the metal binding loop, and 2) sequences flanking the loop which enable α-helix formation. Thus, the structure and function of these domains within the integrins remains an enigma, as does the mechanism of metal-dependent ligand binding.

The relatively low affinity of divalent metals for integrins previously hampered elucidation of this mechanism. However, we have made progress in this regard by probing the metal ion binding sites of integrins with cobalt, which can be converted from Co(II) to Co(III) by mild oxidation *in situ*. This conversion generates a complex which is inert to exchange, locking cobalt(III) into the receptor. Upon oxidation, between 3 and 5 Co(III) ions are incorporated per receptor molecule. This mole ratio correlates well with the presence of 4 putative metal binding sites on the integrin α subunits. Coincident with the conversion of Co(II) to Co(III) is the loss of ligand binding ability, suggesting significant cross-talk between the metal ion and ligand binding domains, and raising the possibility of a ternary complex between ligand receptor and metal ion. Furthermore, inclusion of ligand during the oxidative conversion of Co(II) to Co(III) protects the receptor from inactivation, bolstering the hypothesis that extracellular matrix proteins interact with integrins via a metal-bridged ternary complex. Other results demonstrate that the tight link between the metal and ligand binding domains provides a level of regulation equivalent to an On-Off switch and regulates the ligand binding specificity of the integrins.

CD 122 YEAST METALLOTHIONEIN SUPPRESSES A REQUIREMENT FOR COPPER-ZINC SUPEROXIDE DISMUTASE UNDER CONDITIONS OF OXIDATIVE STRESS,
Katherine T. Tamai*, Edith B. Gralla#, Joan S. Valentine# and Dennis J. Thiele*, *Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI, 48109-0606; and #Department of Chemistry and Biochemistry, University of California at Los Angeles, Los Angeles, CA 90024-1569.

Metallothioneins (MTs) are low molecular weight, cysteine-rich metal binding proteins found in most eukaryotic organisms. Although the major function for MTs is thought to be in metal homeostasis and detoxification, a number of other functions have been proposed such as protection from DNA damaging agents and free radical detoxification. The fact that mammalian MT gene transcription is inducible by metals, interferon, interleukin, glucocorticoid hormone and a wide variety of other stress-related effector molecules is consistent with MTs having multiple roles.

The *S. cerevisiae* (baker's yeast) metallothionein gene (*CUP1*) is the only case in which genetic evidence clearly demonstrates a role for this protein in metal detoxification. Transcription of *CUP1* is induced by the metal copper, through the action of a copper-dependent DNA-binding *trans*-activator known as ACE1. The rapid induction of MT biosynthesis protects cells from the toxic effects of copper, a metal known to elicit the formation of damaging free radicals. In our studies aimed at identifying other cellular conditions which activate *CUP1* transcription, we have found that growth on non-fermentable carbon sources or in the presence of oxygen gives rise to elevated levels of *CUP1* mRNA. Because these same conditions facilitate the formation of oxygen radicals *in vivo*, we investigated whether *CUP1* may play a role in free radical detoxification.

We have found that activation of *CUP1* transcription during growth on non-fermentable carbon sources requires a specific *CUP1* promoter region and that this induction is independent of the other known *CUP1* *trans*-activators ACE1, ACE2 or HSF (Heat Shock Transcription Factor), previously identified in our laboratory. In addition, we have shown that expression of the *CUP1* gene can suppress the lactate-negative phenotype or the paraquat-sensitivity of a strain bearing a deletion of the copper, zinc superoxide dismutase gene (*SOD1*) which encodes a highly efficient free radical detoxifying enzyme. These studies are consistent with the yeast metallothionein protein and perhaps all MTs, playing an important role in protecting cells from oxidative damage.

CD 123 ANALYSIS OF METALLOTHIONEIN GENES IN *Candida glabrata*,
J.L. Thorvaldsen, R.K, Mehra and D.R. Winge, Departments of Medicine and Biochemistry, University of Utah Medical Center, Salt Lake City, UT 84132

Candida glabrata, an imperfect fungus, harbors multiple genes encoding metallothionein (MT): MT-I, MT-II_a and MT-II_b. We have disrupted MT-II_a an amplified locus, and the single copy gene MT-II_b to determine the roles of various MT genes in resistance to copper ions in *C. glabrata*. The concentration of copper sulfate required to inhibit by 50% the growth (IC_{50}) of the *C. glabrata* strain harboring the amplified MT-II_a locus and the single copy MT-II_b and MT-I genes was 7 mM in a synthetic complete medium. The IC_{50} decreased to ~1 mM when the amplified MT-II_a locus was deleted. The disruption of the MT-II_b gene decreased the IC_{50} further to 0.1 mM. The resistance to copper in the strain lacking the amplified MT-II_a locus was primarily attributable to MT-II_b since we were able to purify MT-II protein from this strain. The resistance to copper in the strain lacking both the MT-II genes was attributable to MT-I, no evidence was found for the production of $(\gamma EC)_nG$ isopeptides. Northern analysis of the total RNA from varied *C. glabrata* strains indicated no significant changes in the expression of the MT-I gene in the presence or absence of the MT-II genes. The comparison of the nucleotide sequence of the MT-II_b gene to that of the MT-II_a revealed the same coding sequence with some changes in the 5' region and substantial differences in the 3' region. The 3' region of the MT-II_a gene confers autonomous replication in a host-vector system we have developed for *C. glabrata*. Neither MT-I sequence nor MT-II_b sequence confer autonoumous replication. The presence of an autonomously replicating sequence may be important for the amplification of the MT-II_a locus.

CD 124 HUMAN METALLOTHIONEIN ISOFORM GENE EXPRESSION AND DNA METHYLATION STATUS IN CISPLATIN SENSITIVE AND RESISTANT CELLS. Ya-Yun Yang and John S. Lazo, Dept. of Pharmacology, Univ. of Pittsburgh , Pittsburgh, PA 15261

Cis-diamminedichlorplatinum (CDDP) is an important antitumor agent but native and acquired resistance limits its usefulness. Overexpression of metallothioneins (MT) has been proposed as a mechanism of CDDP resistance. In humans, there are six characterized MT isoforms, but it is not known whether they are functionally equivalent. In this study, we have developed and used human MT (hMT) isoform-specific oligonucleotide probes to assay hMT expression in human SCC25 and SCC25.CP^R cells, which are 7 fold CDDP resistant. SCC25.CP^R cells overexpressed hMT-II$_A$ 8 fold and hMT-I$_E$ 5 fold compared with SCC25 cells. We found no significant expression of hMT- I$_B$, and I$_G$ mRNA in either cell type. DNA methylation is thought to regulate the cell-specific expression of genes. 5'-azacytidine (AZC) is a potent demethylating agent, which can be used to reveal the DNA methylation status of constitutively expressed and inducible genes. To further understand what factors are responsible for the overexpression of MT, we have examined the effects of DNA hypomethylation by AZC in SCC25 and SCC25.CP^R cells. We pretreated these cells with 16 μM AZC for 72 hr, and then treated them with either Zn (100μM), Cd (10μM), or saline for another 9 hr. Total RNA was extracted and hMT isoform expression was determined. We found that AZC pretreatment increased basal hMT-II$_A$ and I$_E$ mRNA levels in SCC25 but not SCC25.CP^R cells, suggesting hypomethylation of the basal regulatory element in SCC25.CP^R cells. We speculate that this may be a factor in the higher steady-state mRNA levels for hMT-II$_A$ and I$_E$ in this CDDP resistant cells.

CD 125 HETEROLOGOUS EXPRESSION OF METALLOTHIONEIN GENES FROM *Candida glabrata* IN *Saccharomyces cerevisiae*, Wei Yu, V. Santhanagopalan, D.R. Winge, University of Utah Medical Center, Salt Lake City, UT 84132

Transformation of a Cd-sensitive strain of *Saccharomyces cerevisiae* with a 2μ-based yeast shuttle vector containing multiple copies of the metallothionein II$_a$ gene of *Candida glabrata* conferred enhanced cellular resistance to cadmium salts. The resistance was plasmid-borne and dependent on multiple copies of the MTII$_a$ gene. Surprisingly, the presence of multiple MTII$_a$ genes did not result in formation of Cd-metallothionein as the sequestration form of Cd(II). Rather, a mixture of Cd-glutathione (γECG) and sulfide-containing Cd-(γEC)$_2$G peptide complexes were observed. Cd-glutathione and CdS-(γEC)$_2$G peptide complexes were also observed in extracts from non-transformed cultures containing cadmium salts. This is the first observation of phytochelatin-like (γEC)$_n$G peptides in *S. cerevisiae*. The lack of Cd-metallothionein in the transformed cells prompted the construction of a shuttle vector containing the gene for the MTII$_a$ coding region under the ADH1 constitutive promoter (designated pAMII). As a control a vector containing the *CUP1* MT gene from *S. cerevisiae* was also prepared with the same ADH1 promoter (designated pAC). Transformation of a *cup1-* strain of *S. cerevisiae* with these vectors resulted in the expected Cu^r phenotype but a Cd^r phenotype only in the pAC tranformants. Cells containing the pAMII transformants yielded only limited resistance to cadmium salts. Cells constitutively expressing the *CUP1* MT gene gave the expected CdMT and CuMT molecules in cell extracts when cells were challenged with cadmium or copper salts respectively. The pAMII-containing cells yielded the expected CuMTII when cultured in medium containing copper sulfate, but no appreciable quantity of CdMTII was observed. The pAMII vector was mutagenized with hydroxylamine *in vitro* and transformed back into *S. cerevisiae*. Cadmium-resistant clones have been selected and are being evaluated for the presence of CdMTII.

CD 126 METAL-REGULATED GENE TRANSCRIPTION IN THE YEAST Candida glabrata , Pengbo Zhou, Mark Szczypka,Tomasz Sosinowski and Dennis J. Thiele, Department of Biological Chemistry, 4311 Med. Sci. I, University of Michigan, Ann Arbor, MI 48109-0606

Metallothioneins (MTs) are low molecular weight, cysteine rich metal binding proteins that play a major role in metal homeostasis of many eukaryotic cells. MT biosynthesis is transcriptionally regulated by high environmental metal concentrations. The yeast Candida glabrata provides an interesting model system to study metal-activated MT gene transcription since this yeast contains a family of MTs; a unique MT-I gene and an amplified MT-II gene. mRNA levels for MT-I and MT-II are induced by copper and silver.

We have set up a positive genetic selection in a copper sensitive host strain of S. cerevisiae and cloned a gene from C. glabrata, denoted AMT1, which encodes a metal-activated transcription factor. AMT1 gene encodes a polypeptide of 265 amino acids in length and bears several features similar to the metal-activated DNA binding protein, ACE1, we previously isolated from S. cerevisiae. AMT1 protein, synthesized in E. coli, binds as a monomer to multiple sites in the MT-I and MT-II promoters in response to copper or silver ions, the same metals that activate transcription of these genes in vivo. As for ACE1, AMT1 is essential for metal-activated expression of the C. glabrata MT-I and MT-II genes.

When the AMT1 locus is disrupted, MT-I and MT-II metal-activated gene transcription is abolished, thereby rendering the cells sensitive to copper poisoning. In addition, MT-II basal level transcription is decreased. Even though the AMT1 disruption strain displays a copper sensitive phenotype, there is little or no effect on its resistance to cadmium. This observation provides evidence that C. glabrata utilizes distinct pathways to confer resistance to different metals. These experiments should greatly facilitate the studies of structure-function relationships important for metal-activated DNA binding proteins and the role these proteins play in the metal homeostatic processes of eukaryotic cells.

Synthetic/Biophysical Methods; Photosynthesis

CD 200 CLONING, SEQUENCE, PROPERTIES AND POSSIBLE FUNCTION OF A YEAST PROTEIN THAT RECOGNIZES *cis*-DDP-MODIFIED DNA

Steven J. Brown, Patti J. Kellett and Stephen J. Lippard. Department of Chemistry, Massachusetts Institute of Technology, Cambridge MA 02139

The generally accepted target for the antitumor drug *cis*-diamminedichloroplatinum(II) (*cis*-DDP) is DNA. Covalent Pt-DNA adducts have well characterized structural features that may be important in their biological recognition and processing. Recently we have cloned and sequenced a human protein structure-specific recognition protein, or SSRP, that binds to DNA containing intrastrand cross-links formed by *cis*-DDP.[1] This protein and others like it may be important in mediating the anticancer properties of the drug. In an effort to determine the natural function of these proteins, we have isolated a related gene from the yeast *Saccharomyces cerevisiae* by screening a λgt11 yeast expression library. DNA sequencing reveals an open reading frame of a partial clone which translates to yield a novel protein which we term the yeast structure-specific recognition protein (ySSRP). Like the human SSRP, the ySSRP contains a HMG-box domain which is believed to be important for *cis*-DDP-modified DNA binding. Sequence similarities of the ySSRP to the human SSRP and other proteins will be discussed. Genetic analysis of the function of the ySSRP will be presented in relation to the proposed mechanisms of *cis*-DDP.

[1]Bruhn, S. L.; Pil, P. M.; Essigmann, J. M.; Housman, D. E.; Lippard, S. J., in press.

CD 201 STUDIES OF THE ROLE OF HEME IN THE REGULATION OF SOLUBLE GUANYLYL CYCLASE

Judith N. Burstyn, Anita E. Yu, Thomas D. Kim and Elizabeth A. Dierks, Dept. of Chemistry, University of Wisconsin, Madison, WI 53706

Guanylyl cyclase was isolated from bovine lung by preparation of a cytosolic extract followed by ion exchange chromatography, dye-ligand chromatography and gel filtration chromatography. When isolated, the enzyme contains a mixture of ferrous and ferric heme. Titration with dithionite causes the Soret maximum to shift to 428 nm, indicative of reduction of the heme center. Oxidation, by ferricyanide or by exposure to air, causes a shift in the Soret to 420 nm followed by loss of the heme moiety from the enzyme. Heme depleted enzyme can be reconstituted by the addition of Fe(II)PPIX. The visible spectral characteristics of the oxidized and reduced heme and the CO and NO heme adducts of guanylyl cyclase will be presented. The position of the Soret band in the CO adduct at 420 nm clearly indicates that the axial ligand is not a cysteine thiolate. The extent of activation of guanylyl cyclase by sodium nitroprusside, a source of NO, increases linearly with increasing heme content until the heme binding site is saturated. This observation is consistent with the role of the heme as the site of NO interaction with the protein. Carbon monoxide does not activate the enzyme regardless of the heme content although clear evidence of direct interaction with the heme is observed in the visible spectrum. The binding constant for NO to the heme is at least six orders of magnitude greater than that of CO. The ESR spectrum of the NO adduct of the protein shows splitting of the g_z signal into three sharp peaks. The signal resembles that of other five coordinate nitrosyl hemes and shows no evidence of coupling to another nitrogen nucleus.

CD 202 Fe EXAFS STUDIES OF NITROGENASE: ANALYSIS OF LONG Fe-Fe INTERACTIONS,

J. Chen (LBL, Berkeley, CA 94720), S.J. George, J. Christiansen, and S. P. Cramer (U. California, Davis, CA 95616), R. Tittsworth and B. Hales (LSU, Baton Rouge, LA 70803), N. Campobasso and J. Bolin (Purdue U., West Lafayette, IN 47907), G.N. George (EXXON R&D,Annandale, NJ 08801).

The bacterial enzyme nitrogenase is responsible for the fixation of atmospheric dinitrogen to biologically accessible ammonia. Its active center comprises an iron-sulfur cluster of unknown structure which contains one other metal, either Mo, V or Fe. Previous Mo EXAFS of both solutions and crystals of nitrogenase found characteristic Mo-S and Mo-Fe distances of 2.4 Å and 2.7 Å respectively (1). Here we report the Fe EXAFS of solutions and single crystals of MoFe protein. For both solutions and crystals, the Fe EXAFS reveals not only the expected 2.3 Å Fe-S and 2.6 Å Fe-Fe interactions, but longer 3.8 Å Fe-Fe and 4.3 Å Fe-S distances. Based on these results and the previous Mo EXAFS data, a Mo capped prismane model is proposed. The analysis of our single crystal Fe EXAFS is consistent with this model.

(1) Flank, A.M.; Weininger, M.; Mortenson, L.E.; and Cramer, S. P.; *J. Am. Chem. Soc.* **1986**, *108*. 1049-1055.

CD 203 DNA PROTEIN CROSSLINKS INDUCED BY CHROMATE IN INTACT CELLS,

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Hexavalent Chromium is actively transported into cells, and one of the primary lesions induced by this agent is the DNA-protein crosslink. During the intracellular reduction of hexavalent chromium various reactive intermediates are formed that produce strand breaks, and ultimately the trivalent form of chromium crosslinks proteins to DNA. The composition of DNA-protein crosslink formed in intact mammalian cells needs further study, although previous work from our laboratory has shown that the trivalent form of chromium is involved in complexing actin to DNA. What sites are bound on the DNA, and what amino acids are involved in this crosslinking in intact cell are unknown? Human osteosarcoma cells were incubated with ^{51}Cr sodium chromate for two hours. DNA was isolated and digested by DNase 1, nuclease P1 and alkaline phosphatase. Fractions of nucleotides monophosphate and nucleosides were separated using C18 reverse phase column. When the radioactivity of each fraction was determined, it was found that ^{51}Cr was most likely associated with the phosphate backbone. Using radioactively labelled amino acids, we have also been studying the amino acid component in the protein-DNA crosslinks. Several amino acids have been investigated including cysteine, methionine, tryptophane and glutamic acid. Only cysteine was found to be crosslinked to DNA by potassium chromate, and the response was dose dependent in intact cultured cells. The chromium induced cysteine DNA crosslink material was stable to both 2-mercaptoethanol and EDTA at pH 7.5, but was labile at pH 4.5. These results strongly suggest that chromium (III) is crosslinking protein to DNA through cysteine linkages in the protein and the phosphate backbone on the DNA. A modified method involving potassium-SDS precipitation of DNA-protein complexes is also being utilized to detect DNA-protein crosslinks in intact cells. This method is very rapid and a sensitive way to detect the formation of this lesion in intact cells.

CD 204 SEQUENCE-SELECTIVE BINDING OF TRANSITION METALS BY NUCLEIC ACIDS: AN NMR STUDY

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The interactions of two transition metals with a series of synthetic oligonucleotides has been investigated using NMR. Evidence is presented for the sequence-selective binding of the transition metals Mn^{+2} and Co^{+2} to the Eco RI restriction site d(CGTGAATTCACG) and related sequences. Paramagnetic relaxation of specific protons indicate that both Mn^{+2} and Co^{+2} selectively coordinate to G4 N7 in the major groove of the duplex, while Co^{+2} may have an additional binding domain in the restriction site's minor groove. Phosphorus NMR also indicates a preferential binding of the paramagnetic metals to the phosphates of the T3pG4 step and the G2pT3 step. A model invoking the chelation of metal to backbone phosphates and G4 N7 is invoked to explain the preferred coordination. The binding of Mn^{+2} and Co^{+2} to related DNA sequences has been examined by NMR in order to probe steric and electronic factors involved in the sequence-selective binding of DNA. NMR studies of the selective coordination of divalent metal ions to different nucleic acid structural motifs, specifically the DNA hairpin d(CGCGTTGTTCGCG) and the A-form RNA dodecamer r(CGUCAAUUCACG), are also described. Possible modes of metal coordination to these hairpin and double-stranded RNA structures are compared to the parent B-form DNA.

CD 205 EXAFS STUDIES OF THE NATIVE AND Zn(II)-SUBSTITUTED FORMS OF CYTOCHROME C_{551},

Gregg R. Dieckmann, Timothy L. Stemmler, David J. Detlefsen, Vincent L. Pecoraro and James E. Penner-Hahn, Department of Chemistry, University of Michigan, Ann Arbor, MI 48109-1055

Cytochrome C_{551} is a heme-based electron transfer protein involved in respiration in the prokaryotic organism *Pseudomonas aeruginosa*. The structure of this protein has been determined both crystallographically[1] and using 2-D NMR[2]. The Zn(II)-substituted analog was previously prepared to study the binding constants of the protein with its physiological partner azurin. EXAFS data has been used to compare metal coordination numbers and average metal-ligand bond lengths of the Fe(II) and Zn(II) forms of the protein. The average metal-ligand bond lengths for the six coordinate Fe(II) protein are: R_{ave}(5 N) = 1.99(2) Å and R(1 S) = 2.32(2) Å; those for the five coordinate Zn(II) form are: R_{ave}(5 N) = 2.14(2) Å.

[1] Alamassy, R.J., Dickerson, R.E., *PNAS, USA* (1978) 75(6), 2674.

[2] Detlefsen, D.J., Thanabal, V., Pecoraro, V.L. and Wagner, G., *Biochem.* (1991) *30*(37), 9040.

CD 206 ELECTRON TRANSFER ASSOCIATED WITH DIOXYGEN ACTIVATION IN THE B2 PROTEIN OF RIBONUCLEOTIDE REDUCTASE FROM *E. COLI*

Timothy E. Elgren, John B. Lynch, Carlos Juarez-Garcia, Eckard Münck, Britt-Marie Sjöberg and Lawrence Que, Jr.* Department of Chemistry, University of Minnesota, Minneapolis, MN 55455.

Each of the two β peptides which comprise the B2 protein of *E. coli* ribonucleotide reductase (RRB2) possesses a nonheme dinuclear iron cluster and a tyrosine residue at position 122. The oxidized form of the protein contains all high spin ferric iron and 1.0 - 1.4 tyrosyl radicals per RRB2 protein. The apparent inefficiency of radical production (<1/diiron cluster) has led us to further characterize the oxygen activation associated with this metalloprotein [1]. In order to define the stoichiometry of *in vitro* dioxygen reduction catalyzed by fully reduced RRB2 we have quantified the reactants and products in the aerobic addition of Fe(II) to metal-free $RRB2_{apo}$ utilizing an oxygraph to quantify oxygen consumption, EPR to measure tyrosine radical generation, and Mössbauer spectroscopy to determine the extent of iron oxidation. Our data indicate that 3.1 Fe(II) and 0.8 Tyr122 are oxidized per mol of O_2 reduced. Mössbauer experiments indicate that less than 8% of the iron is bound as mononuclear high spin Fe(III). Further, the aerobic addition of substoichiometric amounts of ^{57}Fe to $RRB2_{apo}$ consistently produces dinuclear clusters, rather than mononuclear Fe(III) species, providing the first direct spectroscopic evidence for the preferential formation of the dinuclear units at the active site. These stoichiometry studies were extended to include the phenylalanine mutant protein (Y122F)RRB2 and show that 3.9 mol eq of Fe(II) are oxidized per mol of O_2 consumed. Our stoichiometry data has led us to propose a model for dioxygen activation catalyzed by RRB2 which invokes electron transfer between iron clusters.

1. Elgren, *et al. J. Biol. Chem.* 266, 19265-19268 (1991).

CD 207 *Abstract Withdrawn*

CD 208 RNA DOUBLE HELIX UNWINDING BY RECOMBINANT HIV-1 NUCLEOCAPSID (NC) PROTEIN, David P. Giedroc and Raza Khan, Department of Biochemistry and Biophysics, Texas A&M University, College Station TX 77843-2128. The nucleocapsid protein (NC) of all animal retroviruses, encoded by the *gag* gene, is the major structural protein of the core ribonucleoprotein complex, bound to genomic RNA in mature virions. Mature NC ($p7^{NC}$) from human immunodeficiency virus type 1 (HIV-1) is a 71 amino acid, basic protein which contains two Cys_3His Zn(II) "zinc finger" domains. This region of the *gag* gene product is absolutely essential for genomic RNA encapsidation, retroviral replication and infectivity. These studies seek to address the structural role that one or both Zn(II) domains in RNA binding, which remains poorly defined. We describe the subcloning, expression and purification of HIV-1 NC, denoted NC71, from an inducible phage T7 RNA polymerase promoter in *E. coli*. Ultraviolet circular dichroism (UV-CD), fluorescence, and near UV absorbance spectroscopies have been used to characterize the complexes that $Zn(II)_2$ and metal-free NC71 form with poly(A) and poly[r(A-U)], as well as mixed *E. coli* transfer RNA ($tRNA^{mixed}$). We show that NC71 can efficiently collapse the positive UV-CD Cotton effect of $tRNA^{mixed}$ in a reaction not dependent on bound Zn(II). This unwinding of tRNA by NC71, when expressed on a *nucleotide* basis, is consistent with physicochemical parameters determined with poly(A). These spectral changes parallel the temperature-induced denaturation of $tRNA^{mixed}$. The prototype helix-destabilizing protein, T4 gene 32 protein, appears unable to unwind $tRNA^{mixed}$ under the same conditions. The mechanistic implications of these findings on RNA:RNA annealing and replication primer tRNA positioning by NC will be discussed.

CD 209 MINOR GROOVE BINDING OF $RU(PHEN)_3^{2+}$ TO $d(CGCGATCGCG)_2$ STUDIED BY 2D-NMR, Astrid Gräslund[1], Mikael Leijon[1], Magdalena Eriksson[1], Catharina Hiort[2] and Bengt Nordén[2], [1]Dept. Medical Biochemistry and Biophysics, University of Umeå, S-901 87 Umeå, Sweden, [2]Dept. Physical Chem., Chalmers University of Technology, S-412 96 Gothenburg, Sweden

The structure of the chiral transition metal complex $Ru(1,10\text{-phenantroline})_3^{2+}$ binding to the oligonucleotide $d(CGCGATCGCG)_2$ has been investigated by two-dimensional proton NMR spectroscopy. Both the Δ- and Λ-enantiomers of $Ru(phen)_3^{2+}$ are in rapid exchange with the oligonucleotide on the NMR timescale. Several nuclear Overhauser effects are observed between protons on the phenantroline chelates and the oligonucleotide. The DNA protons engaged in NOE interactions with the ligand are all located in or around the minor groove. An extensive change of the chemical shift of the aromatic proton H2 of adenine, located in the minor groove, is seen upon addition of the metal complex. These observations establish that both Δ- and Λ-$Ru(phen)_3^{2+}$ preferentially bind to the central AT-region in the minor groove of $d(CGCGATCGCG)_2$. The NMR-data also provide strong indications for a non-intercalative binding mode.

CD 210 ADDUCT IDENTIFICATION AND DNA REPLICATION INHIBITION BY AN ORALLY ACTIVE, UNSYMMETRICAL PLATINUM COMPLEX, John F. Hartwig and Stephen J. Lippard, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

Recently, the cisplatin analog *cis-trans-trans*-$[Pt(NH_3)(C_6H_{11}NH_2)(OC(O)C_3H_7)_2Cl_2]$ (**1**) has been found to be an effective anticancer drug when administered orally in experimental animals.[1] We have investigated the spectrum of DNA adducts formed by the Pt(II) reduction product of this complex, $[Pt(NH_3)(C_6H_{11}NH_2)Cl_2]$ (**2**), which is formed following ingestion. As with cisplatin, intrastrand d(GpG) cross-links are the major adducts. The presence of one cyclohexylamine ligand, however, gives rise to two Pt-d(GpG) isomers, one with the cyclohexyl group oriented cis to the 5' nucleoside and one with orientation cis to the 3' nucleoside. The cyclohexyl group of **2** has a significant effect on the secondary adducts formed by this complex with DNA.

We have incorporated a single isomer of the d(GpG) adducts into an M13 plasmid and studied the ability of the different geometries to inhibit DNA replication. The site at which DNA synthesis is blocked by the two isomers depends upon the orientation of the cyclohexylamine ligand.

[1]Giandomenico, C. M.; Abrams, M. J.; Murrer, B. A.; Vollano, J. F. **1991**, submitted for publication

CD 211 CHARACTERIZATION AND CLONING OF THE IRON-RESPONSIVE ELEMENT BINDING PROTEIN FROM RAT LIVER, Elizabeth A. Leibold and Bing Guo, Human Genetics and Molecular Biology and Department of Medicine, University of Utah Medical Center, Salt Lake City, Utah 84112. Iron-responsive-element binding proteins are RNA-binding proteins which regulated the expression of proteins involved in iron homoestasis. These include ferritin, transferrin receptor and 5-aminolevulinate acid synthase. The IRE-BPs bind to specific sequences, the iron-responsive elements (IREs) in the untranslated region of the mRNAs encoding these proteins. IREs located in the 5'-untranslated region mediate translational repression; IREs in the 3'-untranslated region mediate mRNA stabilization. The rat liver IRE-BP was purified to homogeneity by conventional chromatography. The amino terminus of rat liver IRE-BP was confirmed by direct sequence analysis and is 100 amino acids longer than reported human IRE-BP. A cDNA library was screened with a PCR fragment derived from two oligonucleotides corresponding to IRE-BP peptide sequence. Of the eight overlapping clones isolated, one clone contained the entire coding and 3'-untranslated regions and 20 nt of 5'-untranslated region. Primer extension analysis using rat liver poly(A)+ RNA and an oligonucleotide corresponding to the first 25 nt in the coding region, produced two reverse transcription products of 120 nt and 200 nt indicating that the cDNA clone is not full length. The mRNA encoding rat liver IRE-BP is 3700 nt in length and is expressed all rat tissues examined. IRE-BP binding activity was decreased in rats treated with iron over the course of 16 hr without a change in the levels of IRE-BP mRNA. These data suggested that the IRE-BP is regulated post-transcriptionally by iron. Comparison of the predicted amino acid sequences of rat and human liver IRE-BP indicated that they contain 92% homology. Both the human and rat IRE-BP showed homology with the mitochondrial Fe-S protein aconitase. The highest degree of homology between the IRE-BP and aconitase was observed for amino acids which form the active sites of aconitase. The structural similarities between the IRE-BP and mitochondrial aconitase suggests that the IRE-BP may have evolved from a common metal binding motif by the addition of a RNA binding site. Models for IRE-BP activation by iron are proposed.

CD 212 ONE-STEP PURIFICATION OF GLYCOGEN PHOSPHORYLASE BY METAL ION AFFINITY CHROMATOGRAPHY. Christine B.H. Luong, Michelle F. Browner, Barry Haymore* and Robert J. Fletterick, Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94143, and *Monsanto Company, St Louis, MO 63167.

The enzymatic activity of glycogen phosphorylase is required by virtually all organisms and cells. The reaction catalyzed by phosphorylase is the breakdown of glycogen leading to the rapid mobilization of glucose for production of chemical energy. Phosphorylase has been long studied from a wide variety of organisms and tissues because of its central role in glycogen degradation. Furthermore, the mammalian isozymes have been a classic system for investigating allosteric regulation. Many different procedures have been developed for purifying phosphorylase enzymes. In general, the purification methods have involved a differential precipitation step followed by one or more column chromatography procedures. Thus the purification is often time consuming and not directly applicable to other phosphorylase enzymes.

We describe a purification scheme for glycogen phosphorylase that can be used for both the rabbit muscle and the human liver enzymes. Starting from a crude bacterial extract, clarified by centrifugation, muscle phosphorylase was purified 100 fold in a single step by immobilized metal ion adsorption chromatography (IMAC). This procedure is simple, rapid, and very quantitative; 90% of the starting enzyme was recovered. We have also shown that the enzyme purified in this manner can be used to obtain protein crystals for X-ray diffraction experiments. The general utility of this method for purifying phosphorylase enzymes was demonstrated using the liver isozyme. The sequence alignment of the muscle and liver enzymes and assessment of the surface exposed histidines suggested that the liver enzyme would also bind the IMAC column, although not as tightly as the muscle protein. This prediction was confirmed by the purification of human liver phosphorylase.

CD 213 MULTIFUNCTIONAL ACTIVATION OF THE *TETRAHYMENA* RIBOZYME BY Mg^{2+}. Timothy S. McConnell, Daniel Herschlag and Thomas R. Cech, Howard Hughes Medical Institute, Department of Chemistry and Biochemistry, University of Colorado, Boulder, CO 80309

The *Tetrahymena* ribozyme (L-21 Scal ribozyme) requires Mg^{2+} to catalyze a site-specific endonuclease reaction: $G_2C_1U_0C_{-1}A_{-2}(S) + G \rightarrow G_2C_1U_0C_{-1}U_{-2}(P) + GA_{-3}$. Substrate binding involves helix formation between the RNA substrate, **S**, and the ribozyme, as well as tertiary interactions. To better understand how the active ribozyme utilizes Mg^{2+}, the Mg^{2+} dependence of individual rate constants has been examined at concentrations greater than those required for ribozyme folding (2-10 mM, 50°C, pH 7). The rate constant for association of **S** increases 12 fold with a $K_{1/2}^{Mg} = 1.5$ mM with the free ribozyme. The Mg^{2+} effect on the rate constant for association of **S** and the absence of a Mg^{2+} effect (< 2 fold) on the rate constant for dissociation of **S** show that the binding of **S** and Mg^{2+} are thermodynamically coupled. In contrast, the rate constants of association and of dissociation of **P** are unaffected by Mg^{2+}, suggesting that the metal interacts directly with the pA$_3$ part of **S**. At low Mg^{2+} concentrations, addition of Ca^{2+} or, to a lesser extent, Ba^{2+}, enhances the rate of binding for the RNA substrate; however, Sr^{2+} does not. This preference for certain divalent ions suggests that the effect on binding of **S** arises from a distinct binding site (**Site 1**) and not from ionic strength effects. The reverse reaction, $E{\cdot}P + GA \rightarrow E{\cdot}S' + G$ ($S = G_2C_1U_0C_{-1}A_{-2}$), requires the binding of a Mg^{2+} ion to reach the transition state. The value for binding of this Mg^{2+} to E·P is equal to that for **Site 1** and limits the interactions of this site to pA$_{-3}$. A model for **Site 1** is proposed in which the Mg^{2+} ion interacts with the reactive phosphate throughout the reaction. The following provides evidence for three sites in the E·S complex, one of which is proposed to be **Site 1**, seen above with the free enzyme. Initial experiments showed no effect of Mg^{2+} concentration on the rate of dissociation of **S**, or the rate of chemistry in the forward reaction. Therefore, competition studies with Ca^{2+} were performed to probe for Mg^{2+} ions tightly bound to the E·S complex (K_d < 2 mM). First, a Mg^{2+} decreases the rate constant for the dissociation of **S** (**Site 2**). **S** and **P** bind with similar affinity, dissociating slowly in Mg^{2+}, so that this Mg^{2+} site is presumably responsible for the tight binding of both. However, **Site 1** only affects the binding of **S**; thus, **Site 2** is physically distinct from **Site 1**. Finally, analysis of metal ion inhibition of the chemical step suggests that two Ca^{2+} ions compete with two Mg^{2+} ions, providing a model for ribozyme catalysis involving a two metal ion active site. Relative metal binding affinities in the E·S complex (Mg^{2+}: Ca^{2+}, 1:1 and 1:3) suggest that the former site involved in chemistry is **Site 1**; the latter site, in which Ca^{2+} binds 3 fold better, cannot be **Site 2** (Mg^{2+}: Ca^{2+} is greater than 3:1) and is defined as **Site 3**. Hence, these studies provide evidence for three metal ions involved in the reaction mechanism: one in binding, one in chemistry, one in both. Although some of these sites may also have a functional role in folding, this work suggests that the ribozyme uses Mg^{2+} in more than just stabilization of the folded polyanion tertiary structure.

CD 214 ALTERED NITROGENASE MoFe PROTEINS: PROBES FOR THE DISTRIBUTION AND CATALYTIC FUNCTION OF PROSTHETIC GROUPS, William E. Newton* and Dennis R. Dean#, Departments of *Biochemistry and #Anaerobic Microbiology, Virginia Tech, Blacksburg, VA 24061, USA

Nitrogenase is the biological catalyst for N_2 fixation. It consists of a smaller Fe protein, which acts as a ATP-binding, specific one-electron donor to the larger $\alpha_2\beta_2$ MoFe protein, which contains the substrate-binding site. The MoFe protein contains two types of prosthetic group. One type, the P clusters, are extruded as about four [4Fe-4S] clusters but their form within the protein is unclear. The other type is the M centers, which can be extruded as the FeMo-cofactors. Current dogma is that the P clusters operate as electron-storage and -delivery systems for the FeMo-cofactors, at which N_2 fixation occurs.

Our working model examines the spatial organization and functional properties of these two types of prosthetic group. Our major targets are: (i) the regions surrounding the strictly conserved α-subunit His-195 and Cys-275 residues, which we propose as two FeMo-cofactor-binding domains; and (ii) the strictly conserved Cys residues, #62, #88 and #154 in the α-subunit and #70, #95 and #153 in the β-subunit, which we propose as providing two sets of nearly identical P-cluster environments as demanded by Mössbauer spectroscopy.

Tests of this model, using site-directed mutagenesis-gene replacement techniques to substitute these likely prosthetic-group ligands, are underway. Correlated changes in EPR, which arises in the bound FeMo-cofactor, and catalytic properties occur on substitution in regions (i), confirming α-subunit-bound FeMo-cofactor as the substrate-reducing site. All substitutions in region (ii) at the proposed P-cluster-binding Cys residues, #62 and #154 in the α-subunit and at #70 and #95 in the β-subunit, result in complete loss of EPR and catalytic activities. However, some substitutions at either #88 in the α-subunit or #153 in the β-subunit result in full EPR activity but with a concomitant decrease in catalytic activity. This phenotype is consistent with a role for these residues in intramolecular electron transfer and as P-cluster ligands. These relevance of these results to the working model will be described.

CD 215 **STRUCTURE-SPECIFIC RECOGNITION OF CISPLATIN-DNA ADDUCTS BY HMG-1** Pieter Pil and Stephen J. Lippard. Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139. Cisplatin is an effective and highly tissue-specific antitumor drug, the cytotoxicity of which is believed to derive from its coordination to DNA. The structures of the intrastrand cross-links formed by cisplatin with DNA, characterized by crystallographic and gel electrophoretic methods, exhibit bending and unwinding of the DNA helix. Such altered DNA structures may serve as recognition motifs for structure-specific DNA binding proteins. We have identified HMG-1 as one such structure-specific binding factor. The protein HMG-1 binds with high affinity to *cis*-[Pt(NH$_3$)$_2${d(GpG)}] and *cis*-[Pt(NH$_3$)$_2${(dApG)}], but not to *cis*-[Pt(NH$_3$)$_2${d(GpTpG)}], intrastrand cross-links in DNA. By using a 100 bp DNA probe containing a single site-specific *cis*-[Pt(NH$_3$)$_2${d(GpG)}] intrastrand cross-link, the affinity of HMG-1 for the adduct has been measured to be ~ 10^8 M. The specificity and affinity of binding to HMG-1 suggests the possibility that HMG-1 and related proteins may play a role in the mechanism of action of cisplatin, several possibilities for which will be presented.

CD 216 SITE-SPECIFIC PROTEOLYSIS BY AN IRON CHELATE: A MECHANISTIC STUDY, Tariq M. Rana and Claude F. Meares, Department of Chemistry, University of California, Davis, CA 95616

Site-specific cleavage of proteins with metal chelates is a new approach for designing artificial proteolytic reagents which are directed by proximity to a peptide bond, rather than by an amino-acid residue type. In the presence of ascorbate and H_2O_2, an iron chelate attached to cysteine-212 of the enzyme human carbonic anhydrase I quickly cleaved the protein at a single site to produce two discrete fragments. These peptides were isolated and characterized by N-terminal and C-terminal sequencing to locate the site of cleavage between residues Leu-189 and Asp-190. The transfer of an ^{18}O atom from $[^{18}O]H_2O_2$ (or $[^{18}O]O_2$) to the carboxyl group of Leu-189 was demonstrated by mass spectrometry. Quantitative experiments revealed that one molecule of H_2O_2 and one molecule of ascorbate afforded the hydrolysis of one peptide bond (1:1:1 stoichiometry), and that the reaction required both ascorbate and H_2O_2. Measurements of the yield of new N-termini showed that most (if not all) of the HCAI molecules were cleaved by hydrolysis of a peptide bond, rather than some other reaction. The process is catalytic, since related experiments on the protein bovine serum albumin revealed two cleavage events for each polypeptide chain cleaved. The hydroxyl radical scavengers thiourea, mannitol, and t-butanol had no significant effect. These results may be explained by generation of a highly nucleophilic oxygen species, such as peroxide coordinated to the tethered iron chelate, which attacks a single carbonyl carbon nearby. To be effective, such a process would require rapid, efficient trapping of the reactive nucleophile by a nearby peptide group before it could decompose to form hydroxyl radical. Thus the extreme selectivity observed for the cleavage reaction could be explained by the geometric requirements of attack by a tethered nucleophile, rather than by a diffusible species such as hydroxyl radical. Further developments in this technology could allow the mapping of ligand binding sites on proteins, analogous to current "footprinting" methods for mapping binding sites on nucleic acids.

CD 217 IDENTIFICATION OF A SECOND PROTEIN THAT SPECIFICALLY BINDS THE IRON-RESPONSIVE ELEMENT. Felipe Samaniego, Tracey A. Rouault, Joe B. Harford and Richard D. Klausner. Cell Biology and Metabolism Branch, National Institute of Child Health and Human Development, National Institutes of Health. Bethesda, MD 20892.

The RNA iron-responsive element (IRE) motif mediates iron-dependent regulation of ferritin mRNA translation and transferrin receptor (TfR) mRNA survival. Iron deprivation induces high affinity binding of the IRE-binding protein (IRE-BP) to the IRE positioned in the 5' UTR of ferritin and the 3' UTR of TfR mRNA, respectively. This binding results in translational arrest of ferritin biosynthesis and stablization of TfR mRNA. The human, rabbit and mouse cDNAs encoding the IRE-BP have been molecularly cloned. These cDNAs encode proteins that are greater than 90% identical to each other. A second human cDNA clone (termed 18.2) was isolated that displays 61% identity to the IRE-BP. With antisera raised against an 18.2-specific peptide, the 18.2 protein was identified in cell lysates. The transfected 18.2 protein has been shown to bind the IRE stem loop. The previously cloned human IRE-BP has been demonstrated to have aconitase enzymatic activity. The 18.2 protein contains 16 of the 18 conserved active site residues believed to be required for aconitase activity.

CD 218 SITE SPECIFIC CLEAVAGE OF pBR322 PLASMID BY $Fe(HPTB)(OH)(NO_3)_4$ IN THE PRESENCE OF H_2O_2 Leah M.T.Schnaith, Richard S.Hanson and Lawrence Que Jr. Departments of Chemistry and Microbiology, University of Minnesota, Minneapolis, MN 55455

The supercoiled plasmid pBR322 is cleaved by treatment with $Fe_2(HPTB)(OH)(NO_3)_4/H_2O_2$ to afford its linear form. (HPTB = N,N,N',N',-tetrakis(2-benzimidazolyl methyl)-2-hydroxy-1,3-diaminopropane) The dinuclear complex has been shown to bind H_2O_2 to form a peroxide adduct that mimics some of the reactivity properties of methane monooxygenase. The DNA cleavage is not inhibited by HO· radical scavengers such as benzoate, formate and DMSO indicating that a diffuse hydroxyl radical cleavage mechanism, as in $Fe(EDTA)/H_2O_2$ does not operate in this case. When the isolated linearized plasmid was treated with restriction enzymes, discrete fragments of the plasmid were detected by autoradiography, indicating that the cleavage occurs at a site specific region. The site of cleavage was determined to be near the *PstI* restriction enzyme site based on the relative fragment lengths. This site is probably near the ampicillin encoding region of the pBR322 plasmid. The location of the cleavage is currently under investigation. The implications of these observations will be discussed.

CD 219 PROBING THE FUNCTIONAL DOMAINS OF NITRATE REDUCTASE, L.P. Solomonson, M.J. Barber and A.C. Cannons, Department of Biochemistry and Molecular Biology, USF College of Medicine, Tampa, FL 33612.

Chlorella assimilatory nitrate reductase (NR) is a homotetramer with each subunit (M_r 100 kDa) containing FAD, cytochrome b_{557} and Mo-pterin prosthetic groups. Reducing equivalents from NADH enter the enzyme at FAD and leave via Mo-pterin to NO_3^-. Limited proteolysis at a protease-sensitive hinge region using *S. aureus* V8 protease has enabled resolution of the protein into FAD- and heme/Mo-containing domains via cleavage at a specific E residue (#647) yielding a FAD domain (M_r 30 kDa, 265 residues) with N- and C-terminal sequences of AAP and EQT, respectively. The FAD domain retains NADH:ferricyanide reductase activity and yields midpoint potentials of -386 mV and -176 mV for the FAD/FAD^- and $FAD^-/FADH_2$ redox couples. In contrast the heme/Mo domain (M_r 70 kDa) retains reduced flavin:nitrate reductase and reduced methyl viologen:nitrate reductase activities and exhibits a midpoint potential of -166 mV for the $heme_o/heme_r$ redox couple. The portion of the heme/Mo domain containing the b_{557} prosthetic group has been cloned and expressed in *E. coli*. The heme midpoint potential for the recombinant domain was +16 mV suggesting a role for the Mo-pterin domain in maintaining the unusually low heme potential of NR. Electrochemical analysis suggested that the reaction of NO_3^- at the Mo-pterin center is the rate-limiting step in NR catalysis. Supported by NIH, NSF and USDA.

CD 220 IDENTIFICATION OF A RNA BINDING DOMAIN IN THE FERRITIN REPRESSOR PROTEIN

Greg R. Swenson, Livia Gaffield and William E. Walden, Department of Microbiology and Immunology, University of Illinois at Chicago, IL 60612

The Ferritin Repressor Protein(FRP) is a 97 kDa protein which regulates translation of ferritin mRNA in response to iron. This control results from the interaction of FRP with the iron responsive element (IRE), a conserved 28 nucleotide sequence located at the 5' end of all ferritin mRNAs. To gain insight into structure/function relationships in FRP, we utilized proteolysis of native FRP in combination with UV crosslinking to radiolabeled IRE. Digestion of native FRP with a variety of proteases generates initial fragments of 68 kDa and 30 kDa. Amino terminal sequence analysis of these peptides generated with chymotrypsin show that the 30 kDa fragment corresponds to the carboxy terminus of the protein; the 68 kDa fragment corresponds to the amino terminus. UV crosslinking of radiolabeled IRE transcripts to FRP followed by chymotryptic digestion places an RNA binding domain within the 68 kDa fragment. Further digestion of this fragment localizes this domain to a 15 kDa fragment. The significance of these results to the recently observed homology between FRP and aconitase will be discussed. (Supported by grants from the NSF and the ACS.)

CD 221 Heme Triggers Crosslinking and Degradation of the Ferritin Repressor Protein During Induction of Ferritin Synthesis *in vivo*. Robert E. Thach, Lisa Smith-Goessling, and Susan Daniels-McQueen, Biology Dept., Washington University, St. Louis, MO 63130

It was previously reported that heme specifically derepresses ferritin translation *in vitro* (*Science, 247*: 74-77), and spontaneously crosslinks to a specific site on the ferritin repressor protein (FRP) (*PNAS, 88*: 6068-6071). More recently, the stability of the FRP in cultured cells has been investigated under conditions which induce ferritin synthesis. In the absence of a supplemental source of iron it was found that FRP slowly turns over, with a half life of approximately 20 hours. If *de novo* synthesis of FRP is inhibited with actinomycin D under these conditions, a gradual derepression of ferritin synthesis results. Addition of iron to the cell culture medium greatly increases both the degradation rate of FRP and the derepression of ferritin synthesis. Of the various forms of iron tested, hemin was the most efficient by far. The effect of iron [in the form of ferric ammonium citrate (FAC) plus transferrin (Tf)] on FRP degradation is enhanced by porphyrin precursors, such as δ-aminolevulinic acid (ALA) and porphobilinogen (PBG), but is decreased by inhibitors of porphyrin synthesis, such as desferrioxamine mesylate (Desferal) and succinylacetone (SA). Similar effects of these same reagents on ferritin synthesis could be observed under appropriate conditions. These results suggest that heme is the most effective form of iron for potentiating FRP degradation. Intermediates in the degradative pathway were also investigated. These apparently include FRP covalently crosslinked either to itself or other proteins. No other resolvable cellular protein was similarly affected by any of these reagents. These observations suggest that ferritin synthesis can be induced *in vivo* through the action of heme on FRP: this results in FRP degradation and consequent derepression of ferritin mRNA translation. (Supported by grants from NSF and NIH.)

CD 222 MECHANISTIC STUDIES ON THE THERMAL, ELECTROCATALYTIC, AND PHOTOLYTIC CLEAVAGE OF DNA BY METAL COMPLEXES. H. Holden Thorp, Neena Grover, William A. Kalsbeck, Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695

We have shown recently that the complex $Ru^{IV}(tpy)(bpy)O^{2+}$ is an effective agent for cleavage of DNA [1]. This reaction can be performed either stoichiometrically by the addition of $Ru^{IV}(tpy)(bpy)O^{2+}$ or electrocatalytically by the electrolysis of $Ru^{II}(tpy)(bpy)OH_2^{2+}$ at 0.8 V. This system is unique in that rate constants for the cleavage of DNA by Ru(IV) can be determined directly from UV-vis kinetics, and further mechanistic studies by cyclic voltammetry are possible. The results of these studies will be presented.

Studies on the binding affinity of these complexes show that incorporation of known intercalating ligands such as phenanthroline and dipyrido[3,2:a-2',3':c]phenazine (dppz) imparts substantial binding affinity to the Ru(IV) agents. Further, chiral derivatives based on $Ru^{II}(bpy)_2(py)OH_2^{2+}$ show the expected stereoselectivities [2]. Structures and properties of a number of these derivatives will be described.

Other results on photolytic reactions involve the cleavage of DNA by $Pt_2(pop)_4^{4-}$ [3]. This complex shows unique reactivity in that it operates efficiently upon low-energy (450 nm) photolysis. Scavenger studies show conclusively that the cleavage reaction involves direct attack of the excited state on the nucleic acid and not a diffusable intermediate such as •OH. As a result of this and the high negative charge of $Pt_2(pop)_4^{4-}$, unusual effects of buffer ionic strength on the rate of photocleavage are observed.

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CD 223HUMAN PROTEINS THAT BIND TO DNA MODIFIED BY THE ANTICANCER DRUG CISPLATIN, D.K. Treiber, X.Q. Zhai, E.E. Trimmer, S.J. Lippard, and J.M. Essigmann, Massachusetts Institute of Technology, Cambridge, MA 02139

cis-Diamminedichloroplatinum(II) (cisplatin) is a highly effective antitumor agent that is believed to have DNA as its critical intracellular target. The mechanism(s) by which the DNA bound forms of cisplatin contribute to antitumor efficacy have yet to be established definitively, but several possibilities include inhibition of DNA and RNA polymerases, titration of transcription factors and other key cellular proteins away from their normal sites of action, and initiation of premature programmed cell death. Of possible importance to several of these models is the existence of recently discovered proteins that bind cisplatin-modified DNA. Such proteins could contribute directly to events proposed in these models or could serve to prolong the half-life of putatively toxic cisplatin lesions by masking them from DNA repair enzymes. A functional property of at least a subset of these proteins is suggested by the observation that they bind selectively to DNAs modified by platinum drugs proved to be effective in clinical evaluations. Modified western blotting analysis reveals the presence in various human cell lines of several cellular proteins that bind to DNA modified by cisplatin but not to DNA modified by the clinically inactive trans isomer. Two of these proteins are of ca. 27 kDa, one of which is the high mobility group protein HMG-1 (see abstract by Pil et al.). Larger species reported here include proteins of ca. 86, 93, 95 and 104 kDa. The relationship of these proteins to members of the HMG class will be discussed.

CD 224 MOLECULAR BIOLOGY OF THE FERRITIN REPRESSOR PROTEIN

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Synthesis of ferritin, the iron storage protein, is regulated in coordination with iron availability. This regulation occurs at the level of translation and is mediated by a sequence specific RNA binding protein, the Ferritin Repressor Protein (FRP). FRP binds to a conserved 28 nucleotide sequence (the Iron Responsive Element; IRE) located at the 5' end of all ferritin mRNAs. The IRE forms a moderately stable stem/loop structure to which FRP binds with high affinity (K_d = 20-60 pM). The interaction of FRP with the IRE is insensitive to iron in vitro under aerobic conditions. FRP activity is sensitive to hemin and to sulfhydryl oxidizing reagents in vitro, suggesting that the mechanism of regulating FRP activity by iron in vivo is complex. In order to gain more insight into this regulatory system, we have cloned a cDNA to rabbit liver FRP. The FRP cDNA is 3.5 Kbp long and has a long open reading frame of 889 codons. Translation of in vitro transcripts of this cDNA in reticulocyte and wheat germ extracts results in synthesis of the 98 kDa FRP protein. Mutagenesis of the cDNA in combination with RNA binding analysis of FRP synthesized from the cDNA is underway in order to identify functional domains within the protein. (Supported by grants from the NSF and ACS.)

CD 225 CHROMIUM(VI)-INDUCED DNA DAMAGE DIFFERENTIALLY AFFECTS GENE EXPRESSION,

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The effect of the carcinogen chromium(VI) on expression of genes inducible by porphyrinogenic drugs and by metals has been examined in chick embryo liver *in vivo*. Chromium(VI) suppressed the induction of 5-aminolevulinate synthase and cytochrome P-450 mRNA by porphyrinogenic drugs in 14-day chick embryo liver, although chromium(VI) increased the basal steady-state mRNA levels of these two inducible genes. The chromium(VI)-induced changes in the steady-state levels of the two genes were primarily due to changes in the transcription rate levels of these genes. The effect of chromium(VI) on the expression of these genes was bimodal, correlating with the presence of chromium-DNA adducts and chromium(VI)-induced DNA cross-links, suggesting that chromium(VI)-induced DNA damage led to changes in gene expression. Chromium(VI) suppressed the metal-induction of metallothionein in this system, without affecting its basal expression. Chromium(VI) also had no effect on the steady-state mRNA levels of housekeeping genes, such as conalbumin (avian transferrin), β-actin, and albumin. Hence, chromium(VI) appears to have multiple effects on gene expression of inducible *vs.* constitutive genes, without affecting the overall rate of RNA synthesis in chick embryo liver. The differential effect of chromium(VI) on gene expression may be due to different rates of initial DNA damage or DNA repair in constitutive *vs.* inducible genes or to different DNA conformational changes caused by chromium(VI)-induced DNA damage. Chromium(VI)-induced DNA damage may alter critical DNA-protein interactions that regulate transcription.

CD 226 REGULATED REDUCTION AND UPTAKE OF COPPER BY YEAST CELLS, Weimin Yang, Dean Hamer, National Cancer Institute, National Institutes of Health, Bethesda,MD.20892

Although the predominant form of copper in the environment is Cu(II), the overwhelming intracellular species is Cu(I). We show that intact yeast cells can enzymatically reduce external Cu(II) to Cu(I). At least two different proteins are involved in this unusual redox reaction: FRE1, a cell surface protein previously recognized by its role in ferric ion reduction, and UPC31, a cytoplasmic protein that contains an ADP-binding site and appears to be involved in energy transduction. Cells that lack copper reductase, due to a fre1-Δ1 mutation, are deficient for copper stimulated growth but resistant to high levels of copper. Conversely, cells that overproduce the reductase, due to the mutation upc31-1, exhibit increased copper uptake and growth stimulation but are hypersensitive to copper toxicity. These studies show that copper reductase plays an important role in copper uptake, utilization and detoxification. The synthesis of the copper reductase complex is repressed by copper ions at the level of FRE1 gene transcription. This homeostatic regulatory mechanism, which is independent of the copper stimulation of metallothionein gene transcription, requires several trans-acting factors and cis-acting FRE1 control sequences.