THE INORGANIC CHEMISTRY/ MOLECULAR BIOLOGY INTERFACE

Organizers: Jeremy Berg, Thomas O'Halloran and Thomas Tullius

February 24-March 1, 1990

Plenary Sessions Page

Poster Sessions

Tutorial on Inorganic Chemistry

CJ 001 SOFT X-RAY ABSORPTION and K FLUORESCENCE of 3-d TRANSITION METAL COMPLEXES and PROTEINS

S. Cramer, P. Siddons, G. Smith, BNL, Upton, NY 11973; R. Prince, G. George, ER&E, Annandale, NJ 08801; C.-T. Chen, F. Sette, Y.-J. Ma, AT&T Bell Labs, Murray Hill, NJ 07974

Transition metal L-edges are sensitive to redox state, spin state, and ligand field. New spherical grating monochromator beam lines at the NSLS (Brookhaven) and SSRL (Stanford) enable for the first time high resolution spectroscopy in the 200-1000 eV range. To evaluate potential biochemical applications of this region, where 3d transition metal L-edges occur, electron-yield absorption spectra for a variety of V, Mn, Fe, Ni, and Cu complexes have been recorded. Using a special Ge detector with thin film diamond windows, we have also obtained preliminary L-edge fluorescence excitation spectra for a number of small metalloenzymes such as cytochrome c. The data have been analyzed in collaboration with Prof. John Fuggle (Nijmegen) using Hartree-Fock type atomic calculations combined with a ligand field splitting.

High resolution K fluorescence emission spectroscopy is an alternate means of probing metal d-levels. Using a spherically bent crystal spectrograph and position sensitive detector, data can be obtained that complements the L-edge analysis. The potential applications of these new technologies for bioinorganic problems will be discussed.

CJ 002 THE ELECTRONIC SPECTRA OF METALLOPROTEINS, David R. McMillin, Department of Chemistry, Purdue University, West Lafayette, IN 47907. Biological macromolecules serve as ligands for a variety of essential metal ions and metal-containing drugs. In fact, about one third of all known proteins depend upon metal ions for structure or function. Metal ions tend to have vacancies in the valence shell, hence the electronic spectrum provides a useful probe of the system. Spectral data can relate information about static molecular structure or the dynamic properties of the chromophore.

A brief overview of electronic structure will be provided including a discussion of the types of electronic transitions usually encountered as well as an indication of their energies and intensities. Several examples of mononuclear and polynuclear systems will be considered. As we shall see, additional information can be obtained when chemical methods can be linked to spectral studies. Metal replacement techniques and difference methods will be described. Finally, the relationships between electronic spectroscopy and other spectroscopic methods including luminescence techniques will be considered.

Tutorial on Molecular Biology

CJ 003 CHARACTERIZATION OF DNA-PROTEIN COMPLEXES INDUCED BY CHROMATE AND OTHER CARCINOGENS, Max Costa, Charles A. Miller III and Mitchell Cohen, Deptartment of Environmental Medicine, New York University Medical Center, New York, NY 10016.
DNA-protein complexes (DPCs) induced by chromate in intact cells have been isolated and the protein components analyzed. Chromate crosslinks three major proteins to the DNA at relatively non-cytotoxic levels, as detected by silver staining, but at higher concentrations many additional proteins are crosslinked. One protein had the identical molecular weight (45 Kd) and isoelectric point (5.4) as actin. We have recently identified this protein as actin by V8 protease mapping. Actin has also been been found to be crosslinked to the DNA by other agents, such as cis-platinum. The DPCs induced by chromate in the intact cells are disrupted in the presence of EDTA, suggesting that Cr(III) is involved in the complex. This was further investigated by reacting nuclei with Cr(III) and demonstrating the induction of DPCs in vitro. Additionally, purified DNA was reacted with actin in the presence of Cr(III) and DPCs were detected. The formation of these complexes in vitro proceeds slowly (> 8 h) since Cr(III) is kinetically inert. An antibody was raised to DPCs induced by chromate in intact cells. This antibody reacted primarily with a 97 Kd protein which, because it did not stain with silver, was not one of the proteins originally identified as comprising the chromate-induced DPC. This 97 Kd protein, as well as actin and several of the other proteins, were crosslinked to the DNA by a variety of other crosslinking agents, including formaldehyde and UV-light, as well as cis-platinum. These results demonstrate that DNA-protein crosslinking agents have remarkably common reactivity in crosslinking similar nuclear proteins to DNA in intact cells.

CJ 004 Metallothionein Gene Regulation in Man and Yeast

Michael Karin, Carla Buchman and Petra Skroch
Department of Pharmacology, M-036, School of Medicine
UCSD, La Jolla, CA 92093

Expression of metallothionein (MT) genes in human cells is regulated at the transcriptional level. In addition to metal ions belonging to groups Ib and IIb which bind to MT and induce transcription of their genes other potent inducers include glucocorticoid and progesterone hormones, phorbol ester tumor promoters, UV, various polypeptide growth factors and cytokines. This complex regulation is due to the mosaic structure of MT gene promoter which allows them to be recognized by multiple distinct transcription factors. Among these factors a central role is occupied by AP-1 which is a complex composed of the cJun and cFos protooncoproteins. This complex couples MT synthesis to stimulation of cellular proliferation.

In contrast transcription of the yeast MT encoding gene, CUP1, is induced only by group Ib ions (Cu^+, Ag^+). This induction is mediated by metal specific activation of the yeast MT transcription factor, the CUP2 protein. Both wild type and mutant forms of this protein were produced in E-coli and their biochemical properties analyzed in detail. The CUP2 protein contains a metal-regulated DNA binding domain composed of two subdomains which can be separated from each other by mutations.

CJ 005 *IN VIVO* WITH EASE: A BRIEF TUTORIAL ON CURRENT GENETIC METHODS RELEVANT TO THE ANALYSIS OF BIO-INORGANIC INTERACTIONS. Anne O. Summers, Department of Microbiology, University of Georgia, Athens, GA 30602

Biological information processing (i.e. genetics) is what distinguishes biology from chemistry. Both the initial cloning of the gene encoding an interesting protein as well as the subsequent biochemical and biophysical analysis of that protein are facilitated by using the cell's ability to manipulate and express information. Moreover, to change a single basepair of an organism's genetic code and subsequently to examine the activity of the altered gene product in the cytoplasm in which it has evolved over millenia is a strong test of any structure/function hypothesis. While "classical" genetics relied largely on *in vivo* manipulations, effective modern molecular genetics is a marriage of *in vitro* (including cloning) and *in vivo* techniques. Building on the basic concept of a "phenotype" (the manifestation of a mutation by a living organism), I'll describe key strategies in mutagenesis (*in vitro* and *in vivo*); selection and screening of mutations (including complementation and gene fusions); gene replacement techniques; *in vivo* footprinting of DNA binding proteins; and the powerful technique of pseudoreversion for identifying interacting domains of one or more proteins/nucleic acids. The use of microbial systems (both prokaryotes and lower eukaryotes) will be highlighted because of the relative ease with which they can be manipulated genetically and because of the great diversity of their interactions with metals.

Metal Ion Regulated Systems

CJ 006 REGULATION OF *Escherichia coli* SUPEROXIDE DISMUTASES: EVIDENCE FOR THE INVOLVEMENT OF THE *fur* LOCUS, James A. Fee, Eric C. Niederhoffer, Cleo M. Naranjo, and Katherine L. Bradley, Isotope and Structural Chemistry Group (INC-4), Los Alamos National Laboratory, Los Alamos NM 87545

The regulation of bacterial superoxide dismutase activities has been studied since the early '70s. This pioneering work revealed that the two *sod* loci of *E. coli* were subject to very different environmental stimuli. Thus, *sodA*, coding for MnSOD, appeared to be induced by dioxygen, perhaps as O_2^-, and by iron limitation; by contrast, *sodB*, coding for FeSOD, appeared to be induced by increased levels of Fe in the culture medium. In our talk, we will give an overview of the bacterial superoxide dismutases, review the early literature on SOD regulation, and present new data from our laboratory which shows that the product of the *fur* locus plays an important role in the regulation of the two *sod* genes. Our evidence shows that metallated Fur, acts as a transcriptional repressor of *sodA* while metallated Fur activates expression of *sodB*, probably in both transcriptional and post-transcriptional capacities. A model will be presented which explains most features of *sod* regulation in *E. coli*. Carried out under the auspices of the USDOE and supported by NIH grant GM35189.

CJ 007 THE COPPER FIST: REGULATION OF METALLOTHIONEIN GENE TRANSCRIPTION BY A METAL AND DNA BINDING PROTEIN: Dean Hamer, Valeria Culotta, Peter Fürst, Rebecca Hackett, Tsao Hsu and Stella Hu. National Institutes of Health, National Cancer Institute, Bethesda, MD 20892.

The induction of yeast metallothionein gene transcription by copper ions is directly mediated by the ACE1 (also known as CUP2) regulatory protein. Cu(I) ions cooperatively bind to the amino-terminal domain of ACE1 and alter its conformation such that it can recognize the metallothionein gene control sequences. Once bound to DNA, the acidic carboxy-terminal domain of ACE1 activates transcription by reversibly stimulating the formation of a committed transcription complex. We hypothesize that Cu(I) binds to the multiple cysteine residues of ACE1 to form a polynuclear, metallothionein-like "copper fist", which in turn forms the core for specific DNA binding "knuckles". Recent site-directed mutagenisis expermiments, together with the preliminary characterization of purified apo-ACE1, Cu-ACE1 and Ag-ACE1, support this model.

CJ 008 IRON REGULATION OF FERRITIN GENE EXPRESSION, H.N. Munro,[1,2] R.S. Eisenstein[1,2], A.J.E. Bettany[2] and H.A. Barton[2], [1]U.S.D.A. Human Nutrition Research Center on Aging at Tufts, Boston, MA 02111 and [2]Massachusetts Institute of Technology, Division of Toxicology, Cambridge, MA 02139.

In order to maintain intracellular iron at levels required for cellular metabolism, and to protect the cell from the oxidative stress of excess ionic iron, cells synthesize the protein ferritin to store iron in a safe and available form. Ferritin consists of 24 subunits forming a hollow shell which can store up to 4500 iron atoms as ferric oxyhydroxide. The protein shell is composed of two types of subunits, H (M_r ~ 21,000) and L (M_r ~ 19,000 to 20,000). Ferritin gene expression is sensitive to cellular iron status at the transcriptional and translational levels, thereby providing multiple mechanisms for maintaining iron homeostasis.

During the past ten years, determination of the structure of the genes, and mRNAs, encoding H- and L-ferritin has furthered our understanding of the mechanisms by which ferritin gene expression is controlled. Ferritin genes from the rat, mouse, chicken and human are composed of four similarly sized exons and three introns. The mRNAs for H- and L-ferritin are all about 1 kilobase in length and contain an unusually long 5' untranslated region (UTR), where a highly conserved 28 nt region, the Iron Responsive Element (IRE) is found. The IRE forms a stem-loop structure, and plays a key role in the iron-dependent translational regulation of ferritin synthesis.

In rat liver, we have shown that iron regulates ferritin mRNA accumulation by affecting the transcription rates of H- and especially the L-ferritin gene. In this tissue, the L-ferritin gene is transcribed at a higher rate than that observed for the H-ferritin gene. Administration of ferric ammonium citrate to rats results in a 2 to 3-fold increase in transcription of the L-ferritin gene and a smaller (0.5-fold) increase in H-gene transcription. These observations are consistent with the preferential accumulation of, and ability to store iron by, L-rich isoferritins.

Translational regulation of ferritin synthesis provides for a more rapid and quantitatively more important mechanism for regulating ferritin gene expression. Iron can induce as much as a 50-fold increase in the synthesis of ferritin protein with little change in ferritin mRNA levels through a shift of ferritin mRNAs from a non-translated pool of mRNA to an actively translated, polysomal pool. The identification of the highly conserved 28 nt sequence within the 5' UTR of ferritin mRNAs led to studies by our laboratory and others which elucidated the role of this cis-acting element in the translational regulation of ferritin synthesis. We and others found, that when linked to a heterologous mRNA (CAT), the H- or L-ferritin 5' UTR conferred iron-dependent regulation of CAT synthesis. Deletion of the first 70 nt of the ferritin 5' UTR, which included the IRE, resulted in derepression of translation of the fusion mRNA. Subsequently, we and others observed that rat, human and rabbit liver cytosol contained a repressor protein or proteins which bound to the IRE. Binding of the repressor to the IRE is affected by iron status in rat or human cells. Furthermore, translation of mRNAs containing mutated IREs, to which the protein does not bind, is not repressed.

The structural aspects of the IRE necessary for binding of the repressor protein are being investigated. Single nucleotide substitutions of the loop generally have little effect on repressor binding whereas deletion of one or more nucleotides from the IRE greatly diminish binding. Using ribonuclease mapping procedures, we have characterized the secondary structure of the IRE and are examining how specific mutations affect this. The forms of iron which affect repressor action are also being examined. Finally, we are using in vitro translation to probe the mechanism of repressor action.

CJ 009 REGULATION AND FUNCTION OF PLASMID RESISTANCE SYSTEMS FOR TOXIC HEAVY METALS, Simon Silver, Department of Microbiology and Immunology, University of Illinois College of Medicine, Chicago, IL 60680 Bacterial plasmids determine highly-specific resistances to a wide range of toxic heavy metal ions including Ag^{+}, AsO_2^{-}, AsO_4^{3-}, CrO_4^{2-}, Bi^{3+}, BO_3^{3-}, Cd^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+} (and organomercurials), Ni^{2+}, Pb^{2+}, SbO_2^{-}, TeO_3^{2}, Tl^{+}, and Zn^{2+}. In most cases, these systems are under tight regulation by trans-acting regulatory proteins and cis-acting operator regions. Recombinant DNA analysis has been applied to the mercury, cadmium, zinc, cobalt, arsenic, chromate, tellurium and copper resistance systems. Seven mercury resistance operons have been sequenced. The merR regulatory gene for the activator/repressor protein has been identified in 6 systems(the 7th lacks merR and is constitutively expressed). The MerR proteins are related in sequence and their functions are being analyzed with mutations. For arsenic resistance, the Gram negative plasmid R773 system determines a three-polypeptide ATPase that effluxes arsenic and antimony(III) and thus maintains low intracellular As(III), As(V), and Sb(III) levels. A fourth (arsR) regulatory gene determines a trans-acting polypeptide, whose function has not been thoroughly characterized. The arsR gene (and regulation) has been identified in a second (this time Gram positive) arsenic resistance determinant that shares only 3 of the 4 genes with the system from the Gram negative source. The first sequenced Cd^{2+} resistance determinant governs another membrane efflux ATPase that assures a low level of cellular Cd^{2+}. Reporter-gene fusion experiments show that cadmium specifically induces this system. The second sequenced cadmium resistance system also confers resistances to zinc and cobalt, via a complex efflux pump consisting of four polypeptides. It is not related at the sequence level to the cadmium ATPase. For chromate resistance, the first two sequenced systems are responsible for reduced cellular uptake. One functions inducibly and the other constitutively.

General reference: S. Silver and T.K. Misra (1988) Plasmid-mediated heavy metal resistances. Annu. Rev. Microbiol. 42: 717-743.

Nitrogenase

CJ 010 ALTERNATIVE NITROGENASE SYSTEMS, Paul E. Bishop, Agricultural Research Service, U.S. Department of Agriculture and Department of Microbiology, North Carolina State University, Raleigh, North Carolina 27695-7615. The diazotroph Azotobacter vinelandii possesses 3 genetically distinct nitrogenases. Nitrogenase-1 is the well characterized Mo-containing nitrogenase. Nitrogenase-2 is a V-containing enzyme complex and nitrogenase-3 does not appear to contain either Mo or V. Each of the 3 enzyme complexes is composed of 2 components; dinitrogenase reductase (also called component II or Fe protein) and dinitrogenase (also called component I or MoFe protein). The nucleotide sequence is known for the structural genes encoding the subunits of each of the 3 nitrogenases. nifH, vnfH and anfH encode the subunits making up dinitrogenase reductases-1,-2 and -3, respectively while nifDK, vnfDK, and anfDK encode the α and β subunits of dinitrogenases-1, -2, and -3. Dinitrogenases-2 and -3 each contain a newly discovered subunit (δ) encoded by vnfG and anfG. The organization of the structural genes is; promoter-nifHDK, promoter-vnfH, promoter-vnfDGK, and promoter-anfHDGK. The predicted amino acid sequences for the nifH, vnfH and anfH gene products exhibit more similarity amongst each other (63-91% identity) than those for the α and β subunits of the 3 different dinitrogenases (31-57% identity). Transcription of each operon appears to initiate at promoter sites that conform to the sigma factor-54 recognition sequence. Transcription responds to the presence or absence of Mo or V in the growth medium as well as fixed N. The nifHDKTY operon is expressed only in the presence of Mo while transcription of the vnfH-Fd operon occurs in the absence of Mo and the vnfDGK operon is expressed in the presence of V and the absence of Mo. The anfHDGK,ORF1,ORF2 operon is only expressed in the absence of Mo and V. Transcription of this operon also requires the vnfH gene product. Finally, expression of nitrogenases-2 and -3 requires genes (vnfA and anfA) encoding proteins similar to NifA (a DNA-binding protein required for activation of all nif operons).

CJ 011 RESISTANCE TO AND TRANSPORT OF MERCURY AND COPPER IN *ESCHERICHIA COLI*."

N.L. Brown, B.T.O. Lee*, J. Camakaris*, D.A. Rouch, and A.P. Morby

School of Biological Sciences, University of Birmingham,
PO Box 363, Birmingham B15 2TT, UK and
*Department of Genetics, University of Melbourne, Parkville 3052, Australia

Mercury and copper are toxic heavy metals. Whereas mercury has no known beneficial function *in vivo*, copper is an essential micronutrient. We have studied, by a combination of molecular and classical genetic techniques, the mechanisms of resistance to mercury and copper salts in *Escherichia coli* containing the determinant of resistance to the appropriate heavy metal. We have proposed models for the mechanisms of detoxification of mercury and excess copper by *Escherichia coli*. Both metal resistances involve transport of the heavy metal into the bacterial cell. In the case of mercury resistance mercuric ions are detoxified by the cytoplasmic enzyme mercuric reductase. In the case of copper resistance, the normal pathway for copper metabolism in *E. coli*, which involves uptake, storage and efflux of copper, is altered such that excess copper is modified and exported from the cell. Experiments will be described in which the transport of copper or mercury into bacterial cells carrying the appropriate resistance determinants is examined, and in which the detailed model for transport of mercury across the bacterial membrane is tested.

CJ 012 CRYSTALLOGRAPHIC STUDIES OF NITROGENASE IRON PROTEIN, Millie M. Georgiadis, Pinak Chakrabarti, Debbie Woo, John J. Kornuc and Douglas C. Rees, Division of Chemistry 147-75CH, California Institute of Technology, Pasadena, CA 91125.

Nitrogenase iron protein (Fe-protein) couples the hydrolysis of ATP to reduction of substrates (dinitrogen, protons, etc.) by the nitrogenase enzyme complex. In addition to this catalytic function, Fe-protein is involved in the synthesis and/or assembly of the iron-molybdenum cofactor of nitrogenase, and is subject to inactivation by ADP-ribosylation. Fe-protein is a dimer of two identical subunits that contains one 4 Fe:4S cluster per dimer. This cluster exhibits an equilibrium between S=1/2 and S=3/2 spin states, and has a different pattern of cysteine ligation from that observed in smaller bacterial ferredoxins. We have crystallized Fe-protein from *Azotobacter vinelandii* and *Clostridium pasteurianum*. Based on x-ray diffraction analyses at 2.8Å and 5Å of the *Azotobacter* and *Clostridium* proteins, respectively, several structural characteristics of Fe-protein are evident:

1. As expected from sequence homology, the *Azotobacter* and *Clostridium* Fe-proteins have a similar structure.

2. The Fe-protein subunits are related by a two fold symmetry axis. The cluster is located on this axis at one end of the molecule.

3. A prominent cleft straddling the two fold axis separates the two subunits.

4. The overall folding pattern of the Fe-protein polypeptide chain is of the mixed α/β class.

The current status of the tracing of the polypeptide chain will be presented at the meeting.

Zinc Proteins

CJ 013 BIFUNCTIONAL HYDROLYTIC ENZYME MIMICS BASED ON METALS

Ronald Breslow, Dan Berger, Ding-Li Huang, Jim Light, and Hans Thiem
Department of Chemistry, Columbia University, New York, NY 10027

Many hydrolytic enzymes use Zn^{2+} as a catalytic group, usually in cooperation with another catalytic group such as a basic carboxylate ion. The essential requirement is that the acidic metal and the basic cocatalyst be held in such a way that they cannot directly "shortcircuit", but must interact through the bridging atoms of the transition state for the reaction catalyzed.

We have studied the catalyzed hydrolysis of phosphate triesters and diesters by the combined action of a bound Zn^{2+} and a suitable base, and the hydrolysis of amides by the combined action of Co^{3+} and various bases. We have also examined the use of bimetallic catalysts for the combined acid-base mechanism. The results point the way to more effective enzyme mimics, and in some cases they suggest novel mechanistic interpretations of the action of the corresponding enzymes themselves.

1. S. Gellman, R. Petter and R. Breslow, "Catalytic Hydrolysis of a Phosphate Triester by Tetracoordinated Zinc Complexes", J. Am. Chem. Soc., **108**, 2388 (1986).
2. A. Schepartz and R. Breslow, "Hydrolysis of an Amide in a Carboxypeptidase Model Using Co(III) and Bifunctional Catalysts", J. Am. Chem. Soc., **109**, 1814-1826 (1987).
3. R. Breslow and S. Singh, "Phosphate Ester Cleavage Catalyzed by Bifunctional Zinc Complexes: Comments on the 'p-Nitrophenyl Ester Syndrome'", Bioorg. Chem. **16**, 408-417 (1988).
4. R. Breslow, Deeng-Lih Huang, and E. Anslyn,"On the Mechanism of Action of Ribonucleases: Dinucleotide Cleavage Catalyzed by Imidazole and Zn^{2+}", Proc. Natl. Acad. Sci. **86**, 1746-1750 (1989).
5. R. Breslow and J. Light, "The Hydrolysis of a Co(III) Chelated Amide Catalyzed by an Internal Phosphonic Acid Group", Bioorg. Chem. in press.

CJ 014 STRUCTURE AND FUNCTION OF MUTANT AND WILDTYPE ZINC FINGER PEPTIDES FROM YEAST TRANSCRIPTION FACTOR ADR1: IMPLICATIONS FOR DNA BINDING.

Grace Parraga, Jon R. Herriott, Suzanne J. Horvath* and Rachel E. Klevit. Dept. of Biochemistry SJ-70, University of Washington. Seattle WA 98195 and *Division of Biology, California Institute of Technology, Pasadena CA 91125.

We have been utilizing 2DNMR spectroscopy and distance geometry to determine the structure of chemically synthesised zinc finger peptides. The wildtype single zinc finger structure was shown to consist of a C-terminal amphiphilic α-helix, a hydrophobic core defined by the interaction of conserved leucine and phenylalanine residues, as well as a N-terminal turn or lasso structure and a flexible finger tip. These structural elements are linked thermodynamically and kinetically to tetrahedral zinc liganding as evidenced by temperature and pH titration studies of the zinc-bound domain.

ADR1 mutants containing single missense mutations in the zinc finger domains have been isolated and these are either completely deficient in DNA binding or bind very weakly. It was also shown for ADR1 that point mutations in either of the two zinc finger sequences affect the DNA binding properties of the protein indicating that both fingers are required for specificity of binding. Given these results, we have chemically synthesized the wildtype double finger domain from ADR1 (ADR1c) as well as single finger point mutants (ADR1b114V and ADR1b118Y). Both of the mutant zinc finger peptides are capable of tetrahedral zinc binding and folding into a structure similar to that determined for the wildtype peptide. Temperature studies showed that the point mutations did not destabilize the domain structure and 2DNMR studies indicated that the amphiphilic helix proposed to be involved in DNA-binding is still intact in both ADR1b114V and ADR1b118Y. The high resolution structures of the mutants will be discussed as well as resulting implications for DNA binding. The double zinc finger peptide (ADR1c) is also being studied by NMR spectroscopy. This 59 residue peptide contains both finger domains and the "hinge region" connecting both finger motifs.

In the absence of a high resolution structure of a zinc finger - DNA complex, the structure of DNA-binding mutants and of multiple finger domains will aid in increasing our understanding of how zinc finger domains bind to DNA with sequence specificity.

Metal Ion Probes; Calcium

CJ 015 PROBING NUCLEIC ACIDS WITH TRANSITION METAL COMPLEXES BY SHAPE-SELECTION, Jacqueline K. Barton, Division of Chemistry and Chemical Engineering, California Institute of Technology, Pasadena, CA 91125

Transition metal complexes have been designed which recognize and react at specific DNA sites based upon *shape-selection*, where the complexes are matched in terms of shape and symmetry to local conformations along the DNA strand. Most recently, novel rhodium complexes containing the pheanthrenequinone diimine (phi) ligand have been prepared which bind DNA avidly by intercalation in the major groove and which cleave DNA efficiently upon photoactivation. The analogue $Rh(phi)_2bpy^{3+}$ serves as an efficient, sequence-neutral photofootprinting reagent, while $Rh(phen)_2phi^{3+}$, because of its shape, targets nucleic acid sites which are open in the major groove. Chiral discrimination accompanies this recognition and, based upon the enantioselectivity, sites which are open owing to base tilt or propeller twisting may be discerned. These results demonstrate that such subtleties in nucleic acid structure as local propeller twisting may serve as elements of recognition by chiral molecules. Comparisons of cleavage positions, mapped based on shape-selection, with well-characterized nucleic acid structures are being drawn to develop schemes to describe sequence-dependent variations in structure along the helical polymer. Shape-selective cleavage of RNA is also efficient and specific. In particular $Rh(phen)_2phi^{3+}$ appears unique in targeting selectively the sites of triple base interaction in tRNA, where the major groove is open and accessible. Thus these transition metal probes may become useful also in deducing secondary and tertiary structures in folded RNA molecules.

CJ 016 STRUCTURES, FUNCTIONS, AND EVOLUTION OF CALCIUM-MODULATED PROTEINS Robert H. Kretsinger, Anthony Persechini, and Nancy D. Moncrief, Department of Biology, University of Virginia, Charlottesville, VA 22901

We have constructed a dendrogram relating 153 EF-hand proteins of known amino acid sequence. The EF-hand, or calmodulin fold, consists of 29 amino acids -- 1 - 10 in helix E; calcium coordinating side chains 10 X, 12 Y, 14 Z, 18 -X, 21 -Z; and 21 - 29 in helix F. This homolog domain is present in from two to eight tandem repeats in (nearly) all calcium-modulated proteins in the cytosol. The intradomain regions are much more highly conserved than are the interdomain regions. We identified twelve distinct subfamilies as well as eight unique proteins, perhaps the sole representatives of other subfamilies:

Name Ca-binding in Domains	1	2	3	4	5	6	7	8
Calmodulin	+	+	+/?	+/?				
Troponin C	+/-	+	+/-	+				
Essential Light Chain of Myosin	+/-	_	+/-	+/-				
Regulatory Light Chain of Myosin	+	-	-	-				
Sarcoplasm Ca-binding Protein	+	+/-	+/-	+/-				
Calpain	+	+	-	-				
Aequorin	+	-	+	+				
Strongylocentrotus ectodermal protein	+	+	+	+/-				
Calbindin	+	-	+	+	+	-		
Parvalbumin		-	+	+				
α-Actinin	+/-	+/-						
S100	+/-	+/-						
Calcineurin B	+	+	+	+				
Troponin C (Astacus)	-	+	-	+				
Calcium Vector Protein	-	-	+	+				
Caltractin	+	+	+	+				
CDC31	+	-	-	+				
25 kDa Protein (Tetrahymena)	-	+	+	+				
Ca-binding Protein (Lytechinus)	+	+	+	+	+	+	+	-
Ca-binding Protein (Streptomyces)	+	+	+	+				

Pairs of domains appear to be the stable functioning unit. In CaM and in TnC the second helix of domain 2, the interdomain linker, and the first domain of domain 3 form a central helix of eight or nine turns. In CaM the linker region of this central helix functions as a flexible tether permitting the two pairs of domains to enfold a helical portion of a target enzyme. The binding of Ca^{2+} ions facilitates this binding of target and bending of the central helix.

CJ 017 NUCLEASE ACTIVITY OF 1,10-PHENANTHROLINE-COPPER, David S. Sigman, Molecular Biology Institute, UCLA, Los Angeles, CA 90024. Recent studies of the chemical nuclease activity of 1,10-phenanthroline-copper have focused on 1) its reaction with RNA and 2) the targeting of its DNase activity using the lambda cro protein as a carrier ligand.

1) In its reaction with RNA, OP-Cu has shown unexpected specificity for single-stranded loop regions relative to double-stranded stem structures. If a stem structure is disrupted by site directed mutagenesis, it is possible to detect the single stranded regions as sites of heightened reactivity. When used as a footprinting reagent to study protein binding to RNA, enhanced sites of reactivity rather than protection are frequently observed.

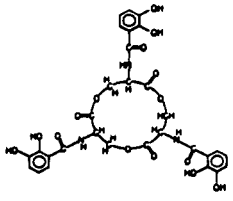
2) The lambda phage cro repressor protein has been transformed into a site-specific nuclease by converting the C-terminal alanine into a cysteine residue and then alkylating it with 5-iodoacetyl-1,10-phenanthroline. The OP-modified cro protein operator (OR-3) complex has been isolated by gel retardation, and scission activated by addition of cupric ion and thiol. Specific and efficient double stranded scission of OR-3 operator has been achieved. These results support the model of DNA binding by cro in which the C-terminus is constrained to the minor groove of the operator.

Metal Ion Physiology

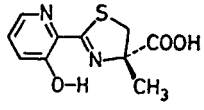
CJ 018 BIOLOGICAL PROCESSING OF PLATINUM-DNA ADDUCTS, Stephen J. Lippard, Department of Chemistry, Massachusetts Institute of Technology, Cambridge, MA 02139

The simple inorganic complex *cis*-diamminedichloroplatinum(II), *cis*-DDP or cisplatin, is currently a leading anticancer drug, being especially effective for the treatment of genitourinary tumors. It binds to DNA, forming primarily 1,2-intrastrand d(GpG) and d(ApG) crosslinks that bend the double helix toward the major groove, unwind the duplex, and inhibit replication. The stereoisomer, *trans*-DDP, which forms a different spectrum of DNA adducts, is chemotherapeutically inactive. These *cis*- and *trans*-DDP adducts have been incorporated site-specifically into DNA viral genomes and their effects on replication, transcription, and in vivo survival investigated. The results of these studies will be presented. The antitumor properties of *cis*-DDP may arise from differential processing of its DNA adducts. In pursuit of this hypothesis, we have isolated and cloned a cellular factor that binds specifically to DNA modified by cisplatin and related anticancer drugs. Studies with oligonucleotides containing specific adducts reveal that the 1,2-intrastrand *cis*-diammineplatinum(II) d(GpG) and d(ApG) crosslinks signal recognition by the factor. Details about the size of the factor, its homology with proteins of known sequence, and its biodistribution will be presented. The presence of such a damage-specific DNA binding factor in human cells suggests that it may be involved in the initial stages of repair, for example, recognition of the platinum lesion. Interestingly, the factor does not recognize DNA modified by *trans*-DDP or other biologically inactive platinum compounds. Hypotheses that link factor binding to cisplatin-damaged DNA with the anticancer properties of the drug will be discussed. This work was supported by grants from the National Cancer Institute and The Bristol-Myers Company. I thank my many talented co-workers and collaborators for their contributions.

CJ 019 NEW COORDINATION CHEMISTRY OF THE SIDEROPHORES, Kenneth N. Raymond, Department of Chemistry, University of California, Berkeley, CA 94720. For over thirty years it has been known that microorganisms produce low-molecular-weight complexing agents which have a very high affinity for ferric ion and facilitate its transport into the microbial cell. It has been found that these compounds, called siderophores, display a range of chemical and biological behavior in the actual transport process. In several cases it is now known that stereochemistry at the metal center plays a critical role in recognition of the metal complex by protein receptors at the cell surface. In a sense this represents a novel type of chiral recognition, since it is not based on chirality at carbon centers. Recent examples of this recognition in the siderophore enterobactin (shown below) and the structural and conformational behavior of enterobactin and synthetic analogs will be presented. Also to be described will be the coordination and structural properties of the unusual siderophore ferrithiocin (shown below).



Enterobactin



Ferrithiocin

CJ 020 MOLECULAR MECHANISMS OF CELLULAR ADAPTATION TO METAL STRESS
Dennis R. Winge, Rajesh Mehra and Charles Dameron, University of Utah Medical Center, Salt Lake City, UT 84132
Cells have evolved a variety of mechanisms to regulate the free intracellular metal ion concentration. Sequestration is a major mechanism by which eukaryotic cells resist the cytotoxic effects of heavy metal ions such as copper, cadmium, lead and mercury. The yeast *Candida glabrata* is novel in the variety of resistance mechanisms elaborated and the specific metalloregulation of these pathways. Copper or silver salts regulate the transcriptional activation of a family of metallothionein genes via a metal-specific trans factor. Cadmium ions can bind to the transcriptional activator protein but the complex appears incompetent in activating gene expression. Cells trained for high copper resistance by copper selection respond by increasing the gene copy number of one of the multiple MT genes by either disomy or increased tandem amplification. This facile amplification can also be achieved with ethane methane sulfonate in the absence of metal selection. The enhanced expression of MT increases the quantity of Cu(I) ions that can be sequestered. In contrast, cadmium salts regulate the enzymatic synthesis of a family of $(\gamma EC)_nG$ peptides varying in the number of dipeptide repeats. The initial Cd(II) complexes formed are Cd(II):glutathione clusters, but with the synthesis of $(\gamma EC)_nG$ peptides the metal complexes become exclusively Cd:$(\gamma EC)_nG$ peptide clusters. These clusters readily incorporate sulfide ions to form nanometer-sized cadmium:sulfide quantum crystallites. The crystallites stably sequester intracellular Cd(II) ions. Extracellular and cell-associated non-peptide coated CdS particles are also observed in wild-type cells. *C. glabrata* cells trained for a high cadmium resistance phenotype exhibit a marked diminution in the uptake rate.

Methods in Inorganic Chemistry; Methods in Molecular Biology; Other Topics

CJ 100 ACETYLENE (C_2H_2) AS A PROBE OF METAL SITES IN PROTEINS, Daniel J. Arp and Michael R. Hyman, Department of Biochemistry, University of California, Riverside, CA 92521. Although the interaction of C_2H_2 with several metalloenzymes has been documented, the widespread use of C_2H_2 as a probe of metal sites in proteins has not been adopted. Our research with three metalloenzymes illustrates the usefulness of C_2H_2 as a probe of metal sites in proteins. HYDROGENASE of Azotobacter vinelandii contains Ni and Fe. C_2H_2 is a slow-binding, reversible inhibitor which is competitive vs. H_2. Catalytically competent enzyme is required and spectroscopic studies (EPR and UV/Vis) reveal that C_2H_2 and H_2 interact similarly with the enzyme. C_2H_2 can be used to discriminate between "Fe only" hydrogenases, which are not inhibited by C_2H_2, and NiFe hydrogenases. AMMONIA MONOOXYGENASE catalyzes the oxidation of ammonia to hydroxylamine and putatively contains copper. C_2H_2 is a mechanism-based inactivator of this enzyme which becomes covalently attached to the enzyme during inactivation. 28 kD polypeptides in Nitrosomonas europaea and Nitrosococcus oceanus were labeled with ^{14}C-C_2H_2. XANTHINE OXIDASE contains flavin, 2 Fe_2S_2 centers and a molybdopterin prosthetic group. Acetylene inactivates only the reduced form of the enzyme and in a manner that is consistent with its binding to Mo. C_2H_2 may prove to be a universal inhibitor of Mo enzymes, as nitrogenase and nitrate reductase are also inhibited by C_2H_2.

CJ 101 CLONING OF A YEAST GENE CONTAINING A PUTATIVE ZINC-FINGER. Lawrence W. Bergman, Barbara K. Timblin and Doris R. Powell, Department of Chemistry, Clippinger Labs, Ohio University, Athens, OH 45701

We have isolated a single copy gene by screening a yeast λgt11 library with a labeled DNA fragment from the upstream activator sequences of the yeast copper metallothionein gene. Genetic analysis of this gene indicates that the gene is non-essential and maps to chromosome XII. DNA sequence analysis of the gene reveals an open reading frame of 432 amino acids, containing two regions homologous to the zinc-finger motif of the Xenopus TFIII A protein. Analysis of the promoter region reveals the presence of a repeated sequence element. Studies concerning the expression and function of the gene will be discussed.

CJ 102 SITE-SPECIFIC MUTAGENESIS STUDIES ON HUMAN FERREDOXIN
Vincent M. Coghlan and Larry E. Vickery, Dept. of Biological Chemistry, University of California, Irvine, CA 92717.

The animal ferredoxins are small, ~14 kDa, [2Fe-2S]-proteins which participate in electron transfer to mitochondrial P450 enzymes involved in steroid metabolism. We have recently succeeded in obtaining high level expression of human ferredoxin in *E. coli* (V.M. Coghlan and L.E. Vickery (1989) *Proc. Natl. Acad. Sci. USA* **86**, 835-839). The iron sulfur center is assembled and incorporated into the recombinant protein *in vivo*, and the purified holoprotein exhibits spectral and enzymatic properties similar to naturally occurring human ferredoxin. Oligonucleotide-directed mutagenesis is being used to investigate the roles of specific amino acid residues in the stability and folding of the protein, in iron coordination, and in binding and electron transfer to NADPH:ferredoxin oxidoreductase and cytochromes P450.

CJ 103 EFFECT OF LEAD ON GLOBIN mRNA IN VIVO. W.R. Farkas and C.A. Aethranis. Dept. of Environmental Practice and Program in Environmental Toxicology, Univ. of Tenn., Knoxville, TN 37916.
Plumbous ion has been shown to be a potent catalyst for the depolymerization of RNA in vitro Farkas, W.R., BBA. 155, 401 (1968) but the question of whether or not Lead-catalyzed RNA degradation also occurs in vivo has never been addressed. Our experimental design, to answer this question, was to transfuse rabbit reticulocytes into normal rabbits and rabbits that had been injected with different doses of lead acetate. After 24 hours the mRNA was isolated from reticulocytes of each rabbits by phenol extraction and affinity chromatography on oligo dT cellulose. The amount of mRNA per ml of packed reticulocytes was determined. The integrity of the mRNA was then determined with a cell-free reticulocyte translation system that was dependent on exogenous mRNA. The results showed that there was little difference in the amount of mRNA recovered from control and treated rabbits, but the ability of the mRNA to support globin synthesis was decreased by as much as 86% in the lead-treated rabbits. These data suggest that not only is mRNA attacked by lead in vivo but that the lead attacks the mRNA at just one or at least very few sites. Therefore, globin mRNA may have certain phosphodiester bonds that are especially susceptible to lead as is true for $tRNA^{Phe}$. Brown, R.S. et al Nature 303, 543 (1983).

CJ 104 SITE-DIRECTED MUTAGENESIS OF AZOTOBACTER VINELANDII FERREDOXIN I. Siiri E. Iismaa, Ana E. Martin, C. Dave Stout, Gerard Jensen, Philip J. Stephens and Barbara K. Burgess, Department of Molecular Biology and Biochemistry, University of California, Irvine, CA 92717.
We are using the 7Fe ferredoxin from Azotobacter vinelandii (AvFdI) to study the relationships between protein primary structure and [Fe-S] cluster structure and reactivity. AvFdI contains one [4Fe-4S] cluster coordinated by Cys residues at positions 20, 39, 42 and 45, one [3Fe-4S] cluster coordinated by Cys residues 8, 16 and 49 and two free Cys residues, 11 and 24. Previous characterization, by x-ray crystallographic and spectroscopic methods, of a site-directed mutant version of AvFdI (C20AFdI) in which the Cys at position 20 has been altered to Ala, demonstrates that the [4Fe-4S] cluster is retained, but that the protein structure is altered to allow Cys residue 24 to serve as a cluster ligand (Martin et al., PNAS, in press). Two additional mutants (C24AFdI and C20A/C24AFdI) have now been constructed. C24AFdI has been overexpressed in A. vinelandii approximately 50-fold relative to wild-type. The results show that unlike C20AFdI, the C24AFdI protein is as stable as the wild type protein in vivo. The C24AFdI protein has been purified in large quantities (450 mg/kg cells), diffraction-quality crystals have been obtained and its characterization will be presented. Overexpression of C20A/C24AFdI is also in progress. The functionality and redox properties of these mutant AvFdIs will be discussed.

CJ 105 PHOTOFOOTPRINTING OF PROTEIN-DNA BACKBONE CONTACTS AND DNA CONFORMATIONS BY URANYL SALTS, Claus Jeppesen and Peter E. Nielsen, Department of Biochemistry B, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N. The excited state of the uranyl(VI) ion (UO_2^{2+}) is strongly oxidizing and uranyl also forms stable complexes with inorganic phosphate. We have exploited these properties of the uranyl ion and shown that irradiation (300nm < λ < 420nm) of uranyl DNA complexes induces single strand nicks in the DNA. The uranyl mediated photocleavage of DNA is sequence neutral and presumably arises by photooxidation of the deoxyriboses of the DNA backbone by uranyl ions complexed to proximal phosphates. Using the λ-repressor/operator O_R1 system, we have, furthermore, shown that uranyl photofootprinting of protein-DNA complexes reports direct contacts between the protein and the DNA (phosphate) backbone. Uranyl photofootprinting of E.coli RNA polymerase bound to deoP1 promoter DNA revealed both DNA cleavage inhibition and enhancement, which can be interpreted in terms of the structure of the RNA polymerase-promoter open complex and the mechanism of its formation. Finally, uranyl photofootprinting results on CRP/operator and TFIIIA/5S-ICR complexes will be presented.

Ref: Nielsen et al. (1988) FEBS Lett. 235, 122-124.
Jeppesen & Nielsen (1989) Nucleic Acids Res. 17, 4947-4956.

CJ 106 A PROTEIN CONTAINING A DESULFOREDOXIN-LIKE CENTER AND A NOVEL MONONUCLEAR IRON CENTER FROM THE SULFATE-REDUCING BACTERIUM Desulfovibrio desulfuricans, I. Moura, J.J.G. Moura, CTQB, Oeiras, Portugal; B.H. Huynh, N. Ravi, K.C. Chen, Emory University, Atlanta, GA 30322; M.-Y. Liu and J. LeGall, University of Georgia, Athens, GA 30602.
Desulforedoxin is a small protein which has been found in Desulfovibrio (D.) gigas; it is isolated as a dimer of M.W. 7,000 containing 2 rubredoxin-like centers. We have found in extracts of D. dedsulfuricans a protein of larger molecular weight (16 KDaltons by SDS-gel electrophoresis). Iron analysis detects the presence of two iron atoms per minimal molecular weight. In the native state, one of the iron sites is very similar to the desulforedoxin center as revealed by its EPR spectral characteristics (S=5/2, E/D ~0.08). The other iron was shown to be in the ferrous state by Mossbauer spectroscopic studies. N-terminal amino-acid sequence analysis has shown the protein to be very homologous to the product of the so-called RBO gene recently discovered in D. vulgaris by M.J. Brumlik and G. Voordrow (J. of Bacteriol., 1989, 171 (9), 4996-5004).

CJ 107 Hg(II) THIOLATE CHEMISTRY: SOLID-STATE ^{199}Hg NMR AS A PROBE OF COORDINATION NUMBER AND GEOMETRY, Michael J. Natan, Clark F. Millikan, Jeffrey G. Wright, and Thomas V. O'Halloran, Department of Chemistry, Northwestern University, Evanston, IL 60208.

The first application of solid-state ^{199}Hg CPMAS NMR spectroscopy to determination of structure and bonding in a series of Hg(II) thiolates is reported. From differences in chemical shift anisotropy ($\Delta\sigma$) and asymmetry (η) for three- and four-coordinate Hg(II) thiolates, unambiguous identification of coordination number can be made. In addition, ^{199}Hg CPMAS NMR spectroscopy is shown to be sensitive to subtle differences in geometry within a given coordination number. The technique can also provide a measure of secondary bonding interaction, an important aspect of Hg(II) coordination chemistry. Solid-state spectra of structurally characterized Hg(II) thiolate compounds provides a structure/chemical shift correlation against which solution NMR data, often complicated by rapid ligand exchange and dissociation processes, can be compared. The data described here augur an understanding of the function of the protein MerR, a metalloregulatory protein thought to bind Hg(II) with three cysteines.

CJ 108 STRUCTURAL CHARACTERIZATION OF THE HG SITES IN *MerR* J.E. Penner-Hahn and H.T. Tsang, Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109-1055 and T.V. O'Halloran and J.G. Wright , Department of Chemistry and Department of Cellular and Molecular Biology, Northwestern University, Evanston, Illinois 60201

X-ray absorption spectroscopy has been used to investigate the Hg environment in the metallo-regulatory merR gene product. The EXAFS data are well modelled using a single shell of 3±1 sulfur nearest neighbors with an average Hg-S distance of 2.43±0.01 Å. The EXAFS amplitude alone does not permit precise determination of the Hg-S coordination number, however the Hg-S bond length is too long for a HgS_2 structure and too short for a HgS_4 structure. The presence of an additional low-Z ligand (e.g. N) could be accommodated within these data, however there is no need to include such a scatterer in order to model the data. There is no evidence for ligands at longer distance (≥ 2.6 Å). Consideration of the EXAFS data and the known structural chemistry of Hg suggest that the Hg is only coordinated to three of the available cysteine ligands. The XANES data are consistent with this interpretation and show a strong similarity between MerR and trigonal-planar $[Hg(SR)_3]^-$ models.

CJ 109 DNA TARGETED ACTIVATION OF DIOXYGEN BY METAL COMPLEXES IN CELLS, D.H. Petering, R.W. Byrnes, W.E. Antholine, M. Mohan, R.X. Xu, P. Fulmer, and P. Davis, Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, WI 53201.
Mechanisms of oxidative cleavage of DNA by four metal complexes have been compared by EPR, chemical and biological methods. At low DNA/drug ratios, FeBleomycin (Blm) causes site-specific damage because 2 electron activation of O_2 occurs while O_2Fe(II)Blm is bound to DNA. The analogous chemistry of O_2-Co(II)Blm involves adoption of a specific conformation of the complex when bound to DNA, which then reacts through a peroxo-bridged intermediate. This is also evident from the reaction of DNA-bound O_2-Co(II)Blm and with Fe(II)Blm. In contrast, although pyridoxalthiosemicarbazonato Cu(II) and glutathione (GSH) activate O_2 to ·OH through a catalytic redox cycle for Cu, cellular single strand cleavage of DNA occurs via non-specifically generated ·OH. This is inferred from the effects of superoxide dismutase, catalase, and DMSO on DNA damage as measured by alkaline elution. Using the same tools, an intermediate case is Cu^{+} + 1,10-phenanthroline, which enter cells independently as shown by the predominance of a Cu-histidine like ESR spectrum in cells. In cells they catalyze non-site specific activation of O_2 to H_2O_2 but apparently carry out the Fenton reaction to form ·OH and damage DNA when bound to the structure. Finally, Cu^{2+} + 2,9-dimethyl-1,10-phenanthroline (NC) damages DNA even though it cannot catalyze ·OH formation from O_2. This may be related to the demonstrated reduction of Cu(II)$(NC)_2$ by DNA that occurs because of the high redox potential of the complex. Supported by American Cancer Society grant CH-466, NIH grant CA-22184, and the International Union Against Cancer.

CJ 110 *XENOPUS* ZINC FINGER PROTEIN TFIIIA HAS DIFFERENT MODES OF BINDING TO SPECIFIC SITES IN 5S RNA AND 5S DNA, Paul J. Romaniuk, Qimin You and Nik Veldhoen, Department of Biochemistry and Microbiology, University of Victoria, Victoria, BC Canada, V8W 2Y2

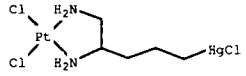
TFIIIA has a dual biological role in the *Xenopus* oocyte. It acts as a positive transcription factor, binding to an internal control region and modulating the expression of the *Xenopus* 5S RNA genes during oogenesis. The protein also forms a specific 7S RNP complex with newly synthesized 5S RNA, stabilizing the RNA for storage in the cytoplasm of immature oocytes. As a result of these functions, TFIIIA binds specifically to DNA and RNA, two nucleic acids which have distinctly different conformations.

TFIIIA has nine zinc fingers. Speculation on how this protein binds to specific targets in both DNA and RNA has centered most recently on the idea that the TFIIIA binding site on the 5S RNA gene adopts an RNA-like A type double helical structure, and that the two distinct domains on the 5S RNA involved in TFIIIA binding co-axially stack to form a continuous helical structure more reminiscent of DNA.

Our laboratory has created a series of site directed mutants of synthetic *Xenopus* oocyte 5S RNA genes. We have measured the binding affinity of TFIIIA for both the genes, and *in vitro* 5S RNA transcripts of the genes, using a nitrocellulose filter binding assay. Binding studies with over 60 mutant 5S RNAs have shown that TFIIIA forms a specific interaction with 5S RNA through a series of weak structure-specific or sequence-specific protein-RNA contacts. The strongest down mutation occured in the hinge region (loop A) between the three helical domains of the 5S RNA, suggesting that a particular co-axial stacking arrangement of these domains is the most critical element in determining TFIIIA binding affinity to 5S RNA. By measuring the equilibrium binding of TFIIIA to the parent 5S DNA mutants, we have found that there are strong sequence-specific contacts formed between TFIIIA and 5S DNA. These map to the previously described Box A, Intermediate Element, and Box C areas of the internal control region. Our results suggest that TFIIIA has different modes of binding to specific sites in 5S RNA and 5S DNA.

CJ 111 INDUCED CALCIUM DEFICIENCY SYNDROME IN FORESTS AFFECTED BY ACID DEPOSITION, Walter C. Shortle, USDA Forest Service, Northeastern Forest Experiment Station, P.O. Box 640, Durham, NH 03824. A high calcium requirement for adult trees has long been known as a major difference between trees and crop plants. Unlike other major essential elements (N,P,K), calcium is not recovered fron mature living sapwood as it is converted to a non-living heartwood core in the stem of forest trees. Calcium remains sequestered in wood until it decays. It is from the decaying organic layer of the forest floor that most calcium and magnesium is made available to forest trees. Small inputs from the atmosphere and from mineral soil make up leaching losses in a balanced ecosystem. However, evidence from several sources indicates that an accelerated loss of calcium has accompanied increased pulses of strong acid anions entering the forest ecosystem as the result of acid deposition, an undesirable by-product of 20th century economic development. During early stages of acid deposition, exchangeable calcium and magnesium are mobilized producing a "fertilizer effect", as well as accelerated leaching. However, this positive effect is followed by "induced deficiency" as essential divalent cations, calcium, and magnesium, are brought into the same range of concentration as trivalent cations, aluminum and iron, which have also been mobilized by increasing acidity and ionic strength. Suppressed uptake of essential cations is followed by suppressed growth of tree meristems. Prolonged growth suppression leads to loss of tree defenses against endemic abiotic factors and biotic agents. Thus, widespread death of spruce and fir in northern forest ecosystems can be linked to acid deposition through interactions with exchangeable base metals in the rooting zone of adult trees.

CJ 112 A NOVEL HETEROBIFUNCTIONAL INORGANIC-BASED REVERSIBLE DNA-PROTEIN CROSSLINKER Stephen P. Watton, Cynthia Kelsey and Thomas V. O'Halloran, Department of Chemistry, Northwestern University, Evanston, IL 60208 USA. The ability to reversibly crosslink nucleic acid-binding proteins to DNA *in vivo* would greatly facilitate the isolation and study of regulatory factors. The heterobimetallic compound *cis*-dichloro-(5-chloromercuripentane-1,2-diamine)-platinum(II) (**1**), designed to act as a selective and reversible DNA-protein crosslinker,



(**1**)

has been prepared and characterized. Design of the crosslinker is based on the known DNA-binding activity of derivatives of the antitumor agent, cisplatin, and the high affinity of organomercurials for amino acid side-chains containing soft donor atoms in proteins. The synthesis of **1**, and preliminary results indicating that the molecule exhibits reversible crosslinking of proteins to DNA, will be discussed, as well as potential applications for this novel reagent.

Metal Sites in Proteins

CJ 200 NICKEL AND HYDROGENASE BIOSYNTHESIS IN Escherichia coli, David H. Boxer, Clive J. Edmonds and Susan E. Holt, Department of Biochemistry, Dundee University, Dundee Scotland, U.K.

Three hydrogenase activities have been identified in E. coli. Two of the enzymes responsible have been characterised as nickel-containing metalloenzymes. Mutants defective at hydE have been isolated in which all three activities are absent. When the mutants are grown in media supplemented with nickel salts all hydrogenase activity is restored. The mutants are not defective in nickel uptake and are able to synthesise active nickel-containing urease from a plasmid carrying the K. aerogenes urease structural gene. The hydE locus has been cloned and its DNA sequence established. A single open reading frame encoding a M_R 25,000 polypeptide with a putative metal binding site (MCTTCGCG) at the N-terminal was found. The role of the hydE product in the internal metabolism of nickel and hydrogenase expression is being explored.

CJ 201 **REGULATION OF HUMAN GLUTATHIONE PEROXIDASE GENE EXPRESSION BY SELENIUM.** **SUNIL CHADA[1], CONSTANCE WHITNEY AND PETER NEWBURGER.** University of Massachusetts Medical School, Worcester, MA 01655, [1]Viagene Inc., San Diego, CA 92121.

Selenium is toxic at high doses, yet metabolically essential in trace amounts. The only eukaryotic selenoprotein of known function is glutathione peroxidase (GPx). This enzyme is expressed ubiquitously and is responsible for detoxifying peroxides and hydroperoxides, which if left unchecked, may damage important biomolecules such as DNA and membrane fatty acids. Selenium is incorporated into this protein as a selenocysteine residue in the active site of the enzyme.

We have isolated the cDNA corresponding to human GPx. Sequence analysis indicated that the selenocysteine residue in the active site is incorporated at a UGA opal terminator codon. Thus the GPx mRNA constitutes the first example of natural suppression of a terminator codon in human cells. We have examined regulation of the human GPx gene by selenium. GPx enzymatic activity and immunoreactive GPx protein fall by 30-fold upon selenium depletion, however GPx mRNA levels and rate of transcription of the GPx gene do not change with selenium depletion. The mechanism of this post-transcriptional regulation was investigated.

CJ 202 THE DNA BINDING DOMAIN OF GAL4 FORMS A BINUCLEAR METAL ION COMPLEX, Joseph E. Coleman and Tao Pan, Yale Univ., New Haven, CT 06510

The transcription factor GAL4 from Saccharomyces cerevisiae requires Zn(II) or Cd(II) for specific recognition of the UAS_G sequence (Pan & Coleman, PNAS, 86, 3145, 1989). An N-terminal fragment consisting of the first 63 amino acid residues of GAL4 (GAL4(63)) has been obtained by partial tryptic proteolysis of a cloned and overproduced N-terminal domain of 149 residues, GAL(149*). We show that GAL4(63) contains the minimal GAL4 DNA binding domain. GAL4(63) binds tightly 1-2 gram atoms of Zn(II) or 2 gram atoms of Cd(II). ^{113}Cd NMR of ^{113}Cd(II)-substituted GAL4(63) reveals structural identity between the metal binding domains of GAL4(63) and that of the larger precursor GAL4(149*). ^{113}Cd(II) can be substituted for the Zn(II) in GAL4(63) and two ^{113}Cd NMR signals are observed at δ = 706 ppm and 669 ppm, both suggesting coordination of ^{113}Cd(II) to 3 or 4 $-S^-$ ligands. With the exception of the N-terminal methionine, the only sulfur containing residues are the six highly conserved cysteines. ^{1}H(^{113}Cd) heteronuclear double quantum NMR shows that the β-protons of all 6 Cys are coupled to ^{113}Cd. High resolution ^{1}H NMR of Zn(II)GAL4(63) and Cd(II)GAL4(63) show the two proteins to have almost identical conformations and are present as monomers in solutions up to mM concentration. This leads us to postulate that GAL4 does not possess a TFIIIA-like "Zn-finger", but forms a binuclear metal cluster involving all six cysteines in a "cloverleaf"-like array. GAL4(63) contains about 60% α-helix estimated from circular dichroism. Removal of the native Zn(II) causes substantial unfolding of the secondary structure.

CJ 203 STRUCTURAL AND FUNCTIONAL STUDIES OF THE GLUCOCORTICOID RECEPTOR ZINC FINGER REGION, Leonard P. Freedman, Ben Luisi, Weixin Xu and Paul B. Sigler, Program in Cell Biology and Genetics, Memorial Sloan-Kettering Cancer Center, New York, NY 10021 and Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT 06511.

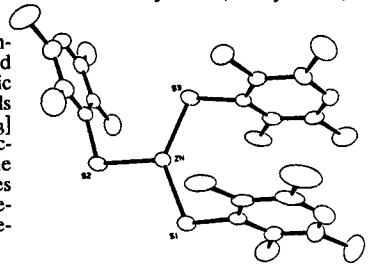
We have expressed and purified a derivative of the DNA binding domain of the rat glucocorticoid receptor. This 86 amino acid protein coordinates two Zn^{2+} ions, each by four cysteine sulphur atoms, in a tetrahedral configuration, and metal coordination is essential for specific DNA binding and protein folding. The receptor finger region has a 100-fold higher affinity for Cd^{2+} than for Zn^{2+}, and the Cd^{2+} reconstituted protein can also bind specifically to its cognate DNA (glucocorticoid response element, GRE). A GRE generally contains two hexameric half-sites separated by three base pairs. Reflecting the approximate dyad symmetry of a GRE, two protein molecules bind to one GRE, as seen by gel mobility shift analysis. However, the DNA binding domain does not dimerize in solution. We have also observed that the spacing between the conserved half-sites can be changed without affecting DNA binding. We have recently succeeded in obtaining crystals of the protein/DNA complex, which we are presently characterizing.

CJ 204 INTERACTION OF CA(II) AND EU(III) WITH SYNTHETIC PEPTIDES WHICH MIMIC THE SEQUENCE OF PUTATIVE CA(II)-BINDING SITES OF THE SKELTAL AND CARDIAC MUSCLE SARCOPLASMIC RETICULUM CA(II) PUMPS, Tom R. Herrmann and Adil E. Shamoo, Physics Dept., Eastern Oregon State College, La Grande, OR 97850, and Dept. of Biological Chemistry, School of Medicine, University of Maryland at Baltimore, 660 W. Redwood St., Baltimore, MD 21201. One of us (AES) has proposed that one of the high-affinity Ca(II) sites of the sarcoplasmic reticulum Ca(II) pump protein consists in part of the following sequences, present in skeletal and cardiac Ca(II) pumps: Thr-Glu-Pro-Val-Pro-Asp-Pro-Arg (skeletal) and Thr-Asp-Pro-Val-Pro-Asp-Pro-Arg (cardiac). We have characterized these and several analogs with modified residues by two means: (1) conventional measurement of the Ca(II) binding constant, and (2) using Eu(III) as a laser-excited luminescent probe to gain information about coordination number and charge neutralization. These measurements will be presented and comparisons made between the sequences above and their analogs, as well as to similar measurements which have already been done with the native enzyme.

CJ 205 THE CALCIUM-BINDING SITE IN THE GALACTOSE CHEMORECEPTOR PROTEIN: CRYSTALLOGRAPHIC AND METAL BINDING STUDIES, Bruce L. Jacobson, Meenakshi N. Vyas, and Florante A. Quiocho, Department of Biochemistry, Rice University, Houston, Texas 77251, Department of Biochemistry, Baylor College of Medicine, Houston, Texas 77030, and the Howard Hughes Medical Institute,One Baylor Plaza, Houston, Texas 77030 We have determined the relative affinities for various metals which bind to the calcium-binding site of the D-galactose-binding protein in solution. In order of affinity the metals are: $Ca^{2+} \sim Tb^{3+} \sim Pb^{2+} > Cd^{2+} > Sr^{2+} > Mg^{2+} >> Ba^{2+}$. The binding affinity for calcium (K_d = 2 x 10^{-6}M) , the slow off-rate determined for terbium (1 x 10^{-3} sec^{-1}) and that the metal-binding site is unperturbed by sugar binding argue for a structural role. Furthermore, we have crystallographically refined the structure of the binding protein with the calcium substituted by cadmium, compared it with the calcium-bound structure, and found them to be identical. The results of these structural and solution studies support the hypothesis that for a given metal-binding loop, cation hydration energy, size, and charge are major factors contributing to binding affinity.

CJ 206 THREE-COORDINATE [M(S-CYS)$_3$] CENTERS, A POSSIBLE NEW COORDINATION GEOMETRY FOR ZINC, CADMIUM AND MERCURY, Stephen A. Koch, Eric S. Gruff, and Donna Phanamus, Department of Chemistry, State University of New York at Stony Brook, Stony Brook, NY 11794

We have synthesized and structurally characterized the first examples of three-coordinate $[M(SR)_3]^{1-}$ complexes of Zn and Cd and the second example of a $[Hg(SR)_3]^{1-}$ complex. Spectroscopic properties of these complexes will be presented to serve as models for a biologically occurring [M(S-Cys)$_3$] center. [Zn(S-Cys)$_3$] coordination centers should be considered as a possibility in zinc-finger proteins. The agreement between the Hg-S distance in the Hg metalloregulatory protein MerR and in $[Hg(SR)_3]^{1-}$ complexes strongly suggests a [Hg(S-Cys)$_3$] coordination in MerR. We predict that the cadmium complex of MerR will also have a three-coordinate [M(S-Cys)$_3$] site.



CJ 207 BIOCHEMICAL AND STRUCTURAL STUDIES OF PEPTIDES CORRESPONDING TO THE ZINC FINGER OF THE YEAST GAL4 PROTEIN, Thomas Kodadek, Andrew Hansen, Michael Van Hoy and Lesley Brown, Department of Chemistry, University of Texas at Austin, Austin, TX 78712.

The yeast GAL4 protein, a transcriptional activator, recognizes a 17 base pair upstream activation site (UAS) by virtue of a "zinc finger" domain. Based on sequence comparisons, the structure of this metal and DNA binding region appears to be different than the better known class of zinc fingers represented found in TFIIIA and many other proteins. Genetic studies have suggested that only 40-50 amino acids compromise the single GAL4 zinc finger, making this system ideal for detailed studies of site-specific DNA-protein interactions.

We will discuss chemical and biochemical probes of the structure and function of peptides corresponding to the GAL4 zinc finger. In particular, site-directed mutagenesis studies that yield information concerning the role of various amino acid residues will be presented, as well as preliminary NMR studies of the structure of the metal-binding region.

CJ 208 CHARACTERISATION OF A DESIGNED METAL-BINDING SITE INTRODUCED INTO A MODEL PROTEIN, Lynne Regan, Laboratory of Molecular Biology, M.R.C., Cambridge, England, Neil Clarke and Carl Pabo, The Johns Hopkins University Medical School, Baltimore, MD, William DeGrado, E. I. du Pont de Nemours & Co., Wilmington, DE. Zn-binding sites in proteins are well characterized from several x-ray crystal structures. Therefore, we thought it might be possible to use this information to design a Zn-binding site into a protein which does not normally bind metals. Such an exercise provides a test of our understanding of the requirements for a metal-binding site and also, if successful, provides a simple model system for further study. The protein into which the metal-binding site was introduced is a simple, model, 4-helix bundle protein whose design and properties have been described (L. Regan and W. F. DeGrado, Science, (1988) 241, p976). The potential metal-binding site was identified using a computer program which tried Cys and His side chains, in all combinations of four, to find a possible tetrahedral binding site for Zn. One such site was identified, consisting of 2 Cys and 2 His ligands, and was introduced into the 4-helix bundle framework. The resulting protein has been shown to bind Zn, Co and Cd. The characterization of the binding of these metals and the properties of the protein will be discussed in detail.

CJ 209 Site Directed Replacement of a Zinc Coordinate Cysteine in Aspartate Transcarbamoylase Yields a Holoenzyme with Altered Allostery. CJ Strang, ME Wales, and JR Wild. Biochem. and Biophys., Texas A&M University. Aspartate transcarbamoylase has a holoenzyme structure of 6 catalytic and 6 regulatory subunits. The binding of effector molecules to the regulatory chain affect the enzymic activity within the catalytic chain, some 60A away. Within the regulatory chain, and near the chain:chain interface lies a zinc atom which is coordinated to the regulatory chain backbone by 4 cysteine γ-sulfhydryl linkages. The zinc coordinate Cys residues were changed to either His or Asp by oligonucleotide directed mutagenesis. The resultant mutant proteins had altered properties. For Cys 109, change to a His resulted in a holoenzyme which demonstrated no inhibition of activity by CTP or CTP and UTP in combination; neither did it demonstrate activation of activity with ATP. Replacement of the same Cys with Asp resulted in no formation of holoenzyme. At Cys 114 of the regulatory chain, replacement of this residue with either His or Asp resulted in lack of holoenzyme assembly. Structural and kinetic properties of the C109H mutant will be discussed.

CJ 210 ZINC-BINDING DOMAINS IN ESCHERICHIA COLI DNA TOPOISOMERASE I, Yuk-Ching Tse-Dinh, Marcia Berk, Andrew Danylchuk, Department of Biochemistry and Molecular Biology, New York Medical College, Valhalla, NY 10595.
Escherichia coli DNA topoisomerase I catalyzes the interconversion of DNA topoisomers by the concerted breaking and rejoining of a DNA phosphodiester bond. It has high affinity for single-stranded DNA. There are three intrinsic zinc bound in each enzyme molecule (Tse-Dinh & Beran-Steed, J. Biol. Chem. 263: 15857 (1988)). Each zinc is proposed to coordinate to four cysteine residues. We have replaced the zinc bound to the enzyme with cadmium with retention of catalytic activity. Protease-resistant fragments of topoisomerase I were purified by HPLC and characterized by N-terminal sequencing. The zinc binding motifs appear to form protease-resistant domains. The E. coli strain AS17 has a mutation in the chromosomal topoisomerase I gene. It requires complementation by topoisomerase I function encoded by a plasmid for growth at 42°C. This is utilized in a scheme for identification of topoisomerase I mutants that require increased concentration of zinc in vivo for enzyme activity. Three such mutants have been isolated following random mutagenesis. We are also carrying out site specific mutagenesis of the cysteines proposed to be zinc coordination site as well as other conserved residues in the zinc binding motifs.

CJ 211 RECOMBINANT HUMAN TRANSFERRIN N-TERMINAL HALF-MOLECULE: CHARACTERIZATION OF SELECTIVELY DEUTERATED AND MUTATED PROTEINS. Robert C. Woodworth and Anne B. Mason, Department of Biochemistry, University of Vermont, Burlington, VT 05405, and Ross T.A. MacGillivray and Walter Funk, Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada V6T 1W5.
Baby hamster kidney (BHK) cells transformed with a pNUT plasmid containing the cDNA for the N-terminal half-molecule of human transferrin (hTF/2N) express the recombinant protein in 0.1 g quantities when grown in roller bottles. When grown on a medium containing ring-deuterated His, Phe, Trp and ring-3, 5-[$^{2}H_{2}$]Tyr, these cells produce hTF/2N with only His C(2)H and Tyr 2,6-H resonances in the aromatic region of the NMR spectrum. The His C(2)H resonances move to higher field as the pH increases, as previously reported. On formation of the ternary complex, Ga(III)hTF/2N(C_2O_4), certain C(2)H resonances return to lower field and two Tyr 2,6-H resonances move to higher field, consistent with the ionization of these resides. Thus the selectively deuterated protein has made possible the partial dissection of the NMR spectrum of hTF/2N, a difficult task in the non-deuterated 40 kDa protein. A site-directed mutant (^{63}D->G)hTF/2N has also been produced by recombinant means. In this protein, one of the metal ligands (^{63}D) has been obliterated. The consequences of this change on metal binding have been evaluated. Supported by USPHS Grant DK21739 and MRC of Canada Grant MT10317.

CJ 212 COORDINATION CHEMISTRY OF THE MerR PROTEIN: PHYSICAL AND CHEMICAL EVIDENCE FOR A HG(CYS)$_3$ BINDING SITE,
Jeffrey G. Wright* and Thomas V. O'Halloran*§, *Department of Chemistry and §Department of Biochemistry, Molecular Biology, and Cellular Biology, Northwestern University, Evanston, IL 60208-3113.

The MerR metalloregulatory protein is an ultrasensitive, highly selective metal-responsive switch governing the expression of plasmid-borne Hg(II) resistance genes. Optical spectroscopic studies of Hg(II)-binding have implicated a single three- or four-coordinate Hg(II) binding site in the dimeric protein. The trigonal planar Hg(II)-thiolate complex, [Et_4N][Hg(S-Bu^t)$_3$], has recently been structurally characterized in our laboratory. Unlike the only other structurally characterized Hg(SR)$_3$ complexes having aromatic thiolate ligands, this model compound is not plagued by overlapping $\pi \rightarrow \pi^*$ transitions and is thus suitable for identifying S$\rightarrow$Hg charge transfer bands in the UV. The spectra obtained for the trigonal Hg(II)-thiolate complex closely correspond to those obtained for the Hg-MerR protein. Hg-edge EXAFS for Hg-MerR suggests that Hg(II) is bound to three cysteinyl thiolates at an average bond distance of 2.43(2) Å, consistent to the bond distance obtained by X-ray crystallography for the three-coordinate alkyl thiolate model compound. A variety of spectroscopic studies are in progress to further resolve the Hg(II) coordination geometry and probe the mechanism of this Hg(II)-biosensor.

CJ 213 APPROACHES TO STUDYING COPPER TRANSPORT SYSTEMS, J. Camakaris, S. Rogers, B.T.O. Lee, D. Rouch*, N. Brown* and R. Farrell, Department of Genetics, University of Melbourne, Parkville, Victoria 3052, Australia and *Biological Sciences, University of Birmingham B15 2TT, U.K.
Cellular requirements for copper are small, yet carefully regulated transport systems must exist to deliver Cu specifically to Cu-dependent enzymes without toxic effects ensuing. In order to elucidate Cu transport in Escherichia coli Cu-sensitive/Cu-dependent mutants were isolated and characterised physiologically. The properties of these mutants were consistent with a system which involved uptake, intracellular transport and storage, and efflux. A 2.2 kb cloned genomic DNA fragment partially complements the phenotype of a Cu uptake mutant whilst a distinct 2.4 kb fragment completely complements the phenotype of a putative Cu storage/carrier mutant. The latter mutant fails to produce a Cu inducible 11 kda copper-binding protein. Cloning procedures need to take into account that over-expression of Cu-binding proteins may be lethal. FPLC and Western blot systems have been developed to facilitate analysis of Cu-binding proteins. These studies have revealed that fractionation profiles may be distorted by redistribution of ^{64}Cu to high affinity ligands.

Metal Ion Regulation

CJ 300 INFLUENCE OF METALS ON FERROCHELATASE FROM AZOSPIRILLUM BRASILENSE, Larry L. Barton and Miles J. Price, Department of Biology, University of New Mexico, Albuquerque, NM 87131. Ferrochelatase (EC 4.99.1.1.) is the final enzyme in heme synthesis and it catically inserts Fe^{2+} into protoporphyrin IX to produce protoheme. Verification that this reaction was enzymic in nature was from the loss of activity with boiling, the dependency of the reaction on protein and an optimum pH of 6.0 - 6.5 was obtained. The ferrochelatase activity in this nitrogen-fixing bacterium, Azospirillum brasilense, is 10-fold greater than reported for other biological systems and displays a wide spectrum of activities with numerous metal cations. The formation of metalloporphyrin with Cu^{2+} by the membrane fraction was 5-10 times greater than with Fe^{2+} after corrections were made for nonenzymatic activity. The enzyme in A. brasilense was firmly bound into the plasma membrane and was stimulated with steric and oleic acids but not by pamalitic acid. The apparent K_m for Fe^{2+} was 21 uM while the apparent K_m with Cu^{2+} was 22 uM. We suggest that a single enzyme was used by both Fe^{2+} and Cu^{2+} ans consistent with this was the inhibition of radiolabeled Fe^{2+} incorporation by Cu^{2+}. Metalloporphyrin synthesis was enzymatically produced with Ni^{2+}, Zn^{2+} and Co^{2+} but not by Mg^{2+} and of these only Co^{2+} and Zn^{2+} inhibited synthesis of the protoheme. Protoporphyrin specificity was observed by this enzyme in that deuteroporphyrin was more active than protoporphyrin and mesoporphyrin was the lease active with activities of 0.51, 0.31 and 0.22 of heme produced per hr. per min per mg of protein, respectively. The addition of dithiothretol increased the activity of ferrochelatase by about a factor of 9 which suggests activation of sulfhydral groups on the enzyme.

CJ 301 SYNTHESIS AND DEGRADATION OF PLANT FERRITINS, Jean-François Briat, Anne-Marie Laboure, Jean-Pierre Laulhère, Anne-Marie Lescure, Stéphane Lobréaux, Olivier Massenet and Dominique Proudhon, Laboratoire de Biologie Moléculaire Végétale, CNRS URA 1178, Université Joseph Fourier, BP 53X, 38041 Grenoble cedex, France

In order to study environmental and developmental control of ferritin synthesis and degradation in plants we have developed two different physiological systems and investigated the effect of iron exchange by plant ferritin on their structure.

The environmental control of ferritin synthesis was studied using soyabean cell suspension cultures in which addition of 500 µM FeIII-citrate induced ferritin accumulation. This protein accumulation corresponded to an increase by 40 fold of *in vitro* translatable mRNA and was completely inhibited by 4 µg/ml actinomycin D. Therefore, iron induction of plant ferritin synthesis is more likely controlled at the transcriptional level rather than at the translational level.

The developmental control of ferritin synthesis and degradation was studied using pea hydroponic cultures under stable, non-starving iron feeding (100 µM FeIII-EDTA). Ferritins are synthesized in reproductive and storage organs of pea from a polyadenylated mRNA coding for a 32 kDa precursor of the 28 kDa pea ferritin subunit. Ferritins accumulate in seeds and are not detectable in vegetative organs throughout pea development. Iron stored in seed ferritins is used the first days of germination, driving the degradation of the protein shell. This degradation involves a shortening of the 28 kDa subunit by specific cleavages due to radical damages downstream Leu 21 in its NH_2 terminal part. The degradation pathway can be reproduced *in vitro* using reducers such as ascorbate or light; it is blocked by darkness or o-phenantroline. cDNA cloning experiments are in progress using both systems.

CJ 302 CUP2: A TRANSCRIPTIONAL ACTIVATOR RECOGNIZING ASYMMETRICAL SITES AND BINDING COOPERATIVELY. C. Buchman, P. Skroch, W. Dixon, T. Tullius and M. Karin, Department of Pharmacology, UCSD School of Medicine, San Diego, CA 92093 and Department of Chemistry, Johns Hopkins University, Baltimore, MD 21218. CUP2/ACE1 is a transcriptional activator of the yeast metallothionein gene (CUP1), whose DNA-binding activity is regulated by the binding of monovalent copper ions. CUP2 is constitutively expressed, but when the levels of copper are low, it is present as an inactive apoprotein. When the intracellular levels increase by exposure to extracellular copper, CUP2 rapidly binds Cu and undergoes a conformational change enabling it to bind to the UASc and activate transcription of the CUP1 gene. CUP2 contains twelve cysteines arranged in a configuration similar to that of metallothionein in which they have been shown to be responsible for coordinating copper. A cysteine to tyrosine point mutant, ace1, has a significantly reduced ability to recognize UASc, as does cup2, a mutant in which a glycine has been changed to a glutamic acid residue in a basic region which lies within the DNA binding domain. To further study the protein-DNA interactions of CUP2, wildtype and mutant proteins have been expressed as nonfusion proteins in bacteria and purified to near-homogeneity. A combination of mobility shift, footprinting, methylation interference and in vitro transcription assays show that two CUP2 molecules bind to the UASc and that the binding of the second one is cooperative, with the mutants showing less or no cooperativity. The two sites recognized are asymmetrical and each site can be further divided into two independent regions which are recognized by different portions of the protein. Although CUP2 represents a new class of metal-dependent DNA-binding proteins different from the zinc-finger family, it can serve as a paradigm illustrating the evolution of recognition specificity in this type of DNA-binding protein.

CJ 303 HIGH-RESOLUTION FOOTPRINTS OF THE CUP2 ACTIVATOR PROTEIN BOUND TO THE UASc REGION OF THE CUP1 PROMOTER, Wendy Dixon*, Carla Buchman#, Michael Karin# and Thomas Tullius§*, *Department of Biology and §Department of Chemistry, The Johns Hopkins University, Baltimore, Maryland 21218, and #Department of Pharmacology, School of Medicine, University of California, San Diego, La Jolla, CA 92093. The protein CUP2 acts as a transcriptional activator for the yeast copper metallothionein gene. It contains 12 cysteine residues and requires copper for DNA binding. We have performed hydroxyl radical footprinting, DNase I footprinting, and methylation interference experiments with the CUP2 protein and the *ace*1-1 mutant protein (Cys-11 to Tyr) to characterize in detail the interactions between these proteins and the UASc region of the CUP1 promoter. CUP2 protects three regions in the UASc from hydroxyl radical attack. These three protected regions are on one face of a DNA molecule with 10.5 bp/helical turn. The guanosines which interfere with CUP2 DNA binding when methylated are in the major grooves between or directly flanking these protected regions. The results for the *ace*1-1 mutant protein indicate that *ace*1-1 binds with a lower affinity and to a smaller region of the UASc than the wild type CUP2 protein. A model for the interaction of CUP2 with its DNA binding site will be presented.

CJ 304 MANGANESE REGULATES THE SYNTHESIS OF MANGANESE PEROXIDASE FROM PHANEROCHAETE CHRYSOSPORIUM, Michael H. Gold, Julie A. Brown and Jeffrey K. Glenn, Chemical & Biological Sciences, Oregon Graduate Institute of Science & Technology, Beaverton, OR 97006-1999.
Manganese peroxidase (MnP) is one of two heme peroxidases secreted by the lignin-degrading white rot basidiomycete Phanerochaete chrysosporium. Spectral and kinetic evidence indicates that the H_2O_2-oxidized states (compounds I and II) and the catalytic cycle of MnP are similar to those of horseradish peroxidase (1). MnP oxidizes Mn^{II} to Mn^{III} and the Mn^{III} in turn oxidizes the terminal phenolic substrates (1). The results described here demonstrate that Mn regulates the synthesis of MnP. The appearance of MnP activity in nitrogen-limited cultures of P. chrysosporium is dependent on the presence of Mn. Cultures grown in the absence of Mn^{II} develop normally and produce normal levels of the secondary metabolite veratryl alcohol, but have no MnP activity. Immunoblot analysis indicates that appearance of MnP protein in the extracellular medium is also dependent on the presence of Mn. Intracellular MnP activity and protein are also only detectable in cells grown in the presence of Mn. MnP mRNA is detected by Northern blot analysis only in cells grown in the presence of Mn. If Mn is added to 4-day-old nitrogen-limited, Mn-deficient cultures, extracellular MnP activity appears within 6 hours and reaches a maximum after 18 hours. Both actinomycin D and cycloheximide inhibit the induction of MnP activity by Mn. These results indicate that Mn, the substrate of the enzyme, is involved in the transcriptional regulation of the MnP gene.

(1) H. Wariishi, L. Akileswaran, M. H. Gold (1988) Biochemistry 27, 5365.

CJ 305 MUTATIONAL ANALYSIS OF ACE1: A COPPER BINDING TRANSCRIPTIONAL ACTIVATOR OF THE YEAST METALLOTHIONEIN GENE. Rebecca H. Hackett, Stella Hu, and Dean H. Hamer. National Institutes of Health, National Cancer Institute, Bethesda, MD 20892. Transcription of the yeast metallothionein (MT) gene is mediated by the ACE1 regulatory protein in the presence of copper ions. Recent work from our lab has shown that the interaction of Cu(I) ions with the amino terminal domain of ACE1 alters its conformation in a manner that allows it to recognize and bind to the MT gene promoter sequences. We have proposed that Cu(I) ions bind to the multiple cysteine residues of ACE1 to form a "copper fist" similar in structure to MT itself. Site directed mutagenesis of these cysteines and basic amino acids in the proposed DNA binding "knuckle" regions has allowed us to directly examine their roles in Cu and/or DNA binding. In particular, several cysteine to serine mutants fail to interact with Cu(I) as determined by a proteolytic protection assay. In contrast, at least two basic residues appear to be involved in DNA binding but not in the interaction with Cu(I). Recently, rabbit antisera has been raised against purified E.coli ACE1. The affinity purified serum can be used to monitor the levels of the mutant ACE1 proteins in crude yeast extracts by immunoblot analysis. In addition, the various mutant ACE1 proteins can be localized in the cell with this antiserum by immunofluorescence microscopy.

CJ 306 COPPER METABOLISM IN *SACCHAROMYCES cervisiae*, Daniel J. Kosman, Brian Crawford, and Chun-Ming Lin, Department of Biochemistry, SUNY, Buffalo, NY 14214. *Net* Cu uptake in *S. cerevisiae* is saturable, energy dependent and essentially specific for Cu: V_{max} = 2.1 nmol Cu/min/mg protein, K_m = 4.4 μM. Essentially no net uptake of Cu occurs at 4° C. Cu-naive cells exhibit only net uptake while cells grown in or preincubated with Cu exhibit both net uptake and exchange. The exchangeable site(s) is specific for Cu also but the energy independence of exchange and its normal temperature dependence indicate these sites are external to the plasma lemma. Inhibition of net Cu uptake and of the development of the exchangeable pool by cycloheximide is observed. This inhibition can be attributed to either a direct role of protein synthesis in Cu transport, or to the disassembly of polymerized actin (filaments) which cycloheximide causes. The energy dependence of net uptake noted could also be due to this depolymerization which occurs rapidly in glucose-starved cells. The fact that the exchangeable pool depends on prior *net* uptake indicates that this pool is metabolically downstream and may be considered part of a Cu excretory and/or storage pathway. Fractionation of whole cells and of protoplasts indicates that this exchangeable pool is associated with a component of the cell wall which appears as a void volume peak in a Superose 12 FPLC profile. Three other protein Cu-binding fractions are seen in this profile, two of which can be identified as Cu-metallothionein and Cu,Zn superoxide dismutase based on results from the respective deletion mutants. The third component, which elutes with mol mass 115 kDa, binds Cu relatively weakly since it appears only when extract [Cu] ≥ 10 μM when the extract contains Cu-metallothionein and at [Cu] ≥ 1 μM when the extract does not. It appears also in extracts from a mutant which exhibits Cu sensitivity despite an elevated Cu-thionein synthesis, indicating that the elevated thionein is a result and not the cause of the elevated cellular Cu associated with the sensitivity and the Cu binding to this third, weakly binding species.

CJ 307 METALLOTHIONEIN GENE EXPRESSION IN A CADMIUM RESISTENT HELA CELL VARIANT, Arturo Leone, Paolo Remondelli, Liliana Minichiello and Stefania Cigliano, Department of Biochemistry and Medical Biotechnology, University of Naples, II Medical School, Naples 80131, Italy. We have isolated a HeLa cell variant able to grow at 45 μM cadmium chloride, a non permessive metal concentration. Duplication time of the isolate is sixteen hours, approximately twice the normal HeLa counterpart, in medium containing both 45 μM $CdCl_2$ and 80 μM zinc chloride. Metallothionein gene expression was examined by Northern blot analysis after twenty four hours growth in normal medium and twelve hours induction with copper, zinc and cadmium; the latter element appeared the strongest inducer, although this effect is strictly dependent upon the growth state of the cells; full confluent cadmium induced cells showed much higher accumulation of MT RNA compared with rapidly growing cells; conversely, the basal level of MT RNA was considerably elevated in growing cells.
We also investigated by SDS-PAGE the effect of elevated concentrations of the three metals on protein biosynthesis of the Cd resistent variant and normal HeLa cells: in the variant cell isolate, MT accumulation in response to cadmium and copper paralleled the appearance of peptides of different molecular weights, some of them comigrating with proteins appearing in HeLa cells after exposure to heavy metals or heath.

CJ 308 REGULATION AND FUNCTION OF THE NICKEL-DEPENDENT CARBON MONOXIDE DEHYDROGENASE FROM *RHODOSPIRILLUM RUBRUM*, P. W. Ludden, S. E. Ensign, M. Campbell, L. Skjeldal, G. P. Roberts#, P. Stephens*, M-C McKenna*, Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, #Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, *Department of Chemistry, University of Southern California, Los Angeles, CA 90089.
Rhodospirillum rubrum is a non-sulfur, purple, photosynthetic bacterium which has the capability to grow with carbon monoxide as a carbon source. The enzyme which allows metabolism is the carbon monoxide dehydrogenase (CODH), a nickel- and iron-sulfur enzyme. Synthesis of CODH in *R. rubrum* is induced in the presence of carbon monoxide, and can be produced in a nickel deficient form by starving the cell for nickel. The apo-form of the enzyme (lacking nickel) can be purified and activated in vitro by addition of nickel to the purified enzyme. Isotopes of nickel can be substituted into the purified enzyme as can nickel analogs including iron and cobalt. Analysis of apo- and holo- enzyme by various spectroscopic techniques demonstrate the role of nickel in the enzyme mechanism and provide information as to the ligands for nickel in the enzyme. CODH can be obtained in an oxygen stable form. Reduction of the oxygen stable enzyme with dithionite or carbon monoxide converts the oxygen stable (but catalytically inactive) enzyme to a catalytically active form which is oxygen labile. Attempts to convert the catalytically competent form to the oxygen stabile form of CODH are continuing. Oxygen represses synthesis of CODH in photosynthetically grown cells but not in actively respiring cells. The role of oxygen in mediating the synthesis of CODH in aerobic and anaerobic conditions is being investigated.

CJ 309 NOVEL AROMATIC LIGAND EFFECTS ON THE IRON DEPENDENT CONTROL OF BACTERIAL SUPEROXIDE DISMUTASE GENE EXPRESSION, Eric C. Niederhoffer, Cleo M. Naranjo, Katherine L. Bradley and James A. Fee, Isotope and Structural Chemistry Group (INC-4), Los Alamos National Laboratory, Los Alamos, NM 87545

Bacterial superoxide dismutases (SOD) are presently classified by their ability to catalyze the disproportionation of superoxide anion to dioxygen and hydrogen peroxide. We have recently reported [Niederhoffer, Naranjo, Bradley, and Fee, Control of *Escherichia coli* superoxide dismutase (*sodA* and *sodB*) genes by the ferric uptake regulation (*fur*) locus, submitted to J. Bacteriol.] some molecular aspects of *sod* gene expression involving the ferric uptake regulation (*fur*) locus. During the course of our investigations using chromosomal Φ(*sodA'-'lacZ*) and low-copy plasmid-borne (*sodB'-'lacZ*) protein fusions, we observed some interesting effects involving aromatic metal chelators. The expected ~4-fold Fe dependent response of the fusion genes, in Fur^+ backgrounds grown under aerobic and anaerobic conditions, was found with the polyanionic iron chelator diethylenetriaminepentaacetic acid. By contrast, aromatic chelators such as 1,10-phenanthroline (PHEN) and 2,2'-bypyridine, were able to induce (*sodA'-'lacZ*), in Fur^+ and Fur^- strains, ~10 to 20 fold under anaerobic conditions. Similar treatment of aerobically growing cells containing (*sodB'-'lacZ*) gave ~10- to 15-fold repression. Treatment of cells with structural analogs incapable of binding metals, such as 1,7-PHEN and 4,7-PHEN, resulted in apparent aerobic induction of (*sodA'-'lacZ*). In addition,the role of respirative and fermentative growth in *sod* promoter control will be presented. These results support the notion of different control mechanisms involved in *sod* gene expression. We are currently employing DNA-protein footprinting and *in vitro* transcription assays to establish the necessary requirements for formation of competent RNA polymerase-*sod* promoter transcription complexes and the molecular basis for *sod* regulation by the *fur* system. This work was supported by U. S. P. H. S. grant GM35189.

CJ 310 ULTRASENSITIVE ACTIVATION OF TRANSCRIPTION BY MERR IN RESPONSE TO HEAVY METAL IONS, Diana M. Ralston*, Betsy Frantz§, and Thomas V. O'Halloran*§, §Department of Chemistry and *Department of Biochemistry, Molecular and Cellular Biology, Northwestern University, Evanston, IL 60208.

Activation of mercuric ion resistance (*mer*) genes is mediated by the protein MerR in response to heavy metal ions. Binding of a single atom of mercuric ion by MerR dimers is sufficient for a rapid induction of transcriptional initiation by RNA polymerase, since both proteins are associated with the *mer* promoter in the absence and presence of Hg(II). In addition to an increase in the rate of isomerization from closed to open transcription complexes and the induction of a distortion in the promoter DNA, we observe that the presence of Hg(II) also causes MerR to stimulate transcription with greater sensitivity (*ultrasenstivity*) than systems obeying Michaelis-Menton kinetics. This ultrasensitivity is also observed in response to Cd(II), Zn(II) and Au(I).

CJ 311 MANGANESE IONS AS A REGULATOR FACTOR OF GLUCOSE CATABOLISM AND ORGANIC ACIDS PRODUCTION BY HETEROTROPHIC BACTERIA.

Sebryanaya M.Z., Holodok L.P., Babenko J.S. State University, institute of Geology. 320027.Dniepropetrovsk, Fuchik str.15.

Heterotrophic bacteria g.Achromobacter leaching manganese catabolize glucose in present of increasing quantities of manganese ions. It leads to increase the enzymes activities of glucolytic and pentosophosphate pathways, such as fructoso-1,6-diphosphate aldolase, glucoso-6-phosphatedehydrogenase and transketolase. The main mechanism of manganese leaching is functioning of citric cycle with hyperproduction oxicarbonic acids (10 - 15 g/l), reducing manganese oxides. Mn^{++}increases activity of citricsyntase, isocitricdehydrogenase, malatdehydrogenase without changing activity cuccinatedehydrogenase. Organic acids production is grown in presence of manganese ions concentration from 0,01 up to 0,1% decreased when Mn^{++}concentration run up to 0,3-0,5%. Cell growth is not suppresed under 0,3-0,5% Mn^{++}.

CJ 312 KEY ELEMENTS IN THE RECOGNITION OF THE *merOP* REGULATORY SITE BY THE Hg(II) BIOSENSOR, MerR. A.O. Summers, S.J. Park, A. Heltzel, B.D. Gambill, P. A. Totis. Microbiology Department, University of Georgia, Athens, GA 30602

The 144 amino-acid protein, MerR, activates or represses transcription of the bacterial Hg(II) detoxification operon (*merTPCAD*) in response to the presence or absence (respectively) of Hg(II). MerR also represses its own transcription from a divergent promoter whose control region overlaps that of *merTPCAD*. *In vivo* DNA footprinting and oligonucleotide-directed mutagenesis of the dyadic *merOP* (operator-promoter) reveals a subset of bases in the dyad essential for the divergent activation and repression. Double mutants of *merOP* are markedly impaired in *in vitro* binding of MerR. However, the single-site *merOP* substitution mutants retain some MerR binding ability, suggesting that an operator half-site is sufficient for simple binding. Unlike its modest effect on the *in vitro* interaction between wild-type *merOP* and MerR, the addition of Hg(II) markedly diminishes *in vitro* MerR binding with all of the *merOP* dyad mutants, consistent with the idea that a Hg(II)-induced conformational change in MerR is involved in operon activation (the "Twist and Shout" model). Besides this complex regulation of transcriptional initiation, the *mer* operon is also subject to transcriptional attenuation during elongation. The occurrence of sites conserving essential elements of the *merOP* region ("OP-like" sites) at several other positions in the operon and the observation of MerR binding *in vitro* to DNA fragments containing these sites implicates *merR* in this attenuation process.

CJ 313 DEREPRESSION OF FERRITIN mRNA TRANSLATION BY HEMIN IN VITRO, R.E. Thach[1], J.-J. Lin[1], S. Daniels-McQueen[1], M.M. Patino[2], L. Gaffield[2], and W.E. Walden[2]. [1]Department of Biology, Washington University, St. Louis, MO 63130; [2]Department of Microbiology and Immunology, University of Illinois at Chicago, Chicago, IL 60612.

The mechanism by which ferritin synthesis is induced in the presence of iron has been investigated. Pre-incubation of a 90 kDa ferritin repressor protein (FRP), either free or complexed with a L-ferritin transcript, with hemin or Co^{+++}-protoporphyrin IX prevents subsequent repression of ferritin synthesis in a wheat germ extract. This inactivation of FRP is time, temperature, and concentration dependent. Neither $FeCl_3$, nor Fe^{+++} or Fe^{++} chelated with EDTA, nor Zn^{++}-protoporphyrin IX, nor protoporphyrin IX caused inactivation of FRP under comparable conditions. Combinations of $FeCl_3$ with H_2O_2 produced only a slight inactivation of FRP. FRP that had been inactivated by hemin remained chemically intact, as revealed by SDS-PAGE. Inclusion of chelators of iron or free radical scavengers did not alter the inactivation produced by hemin. Under optimum conditions, neither hemin nor Co^{+++}-protoporphyrin IX significantly affected the translation of an apolipoprotein transcript, which was used as an internal standard. These and other results show that hemin is an inducer of ferritin synthesis in vitro.

CJ 314 REGULATION OF FERRITIN SYNTHESIS: CHARACTERIZATION OF AN IRON SENSITIVE REPRESSOR OF FERRITIN mRNA TRANSLATION, W.E. Walden[1], M.M. Patino[1], G.R. Swenson[1], M.M. Beck[1], I.V. Nowak[1], J.M. Ross[2] and L. Gaffield[1], [1]Department of Microbiology and Immunology, College of Medicine, University of Illinois at Chicago, Chicago, IL 60612 and [2]Department of Chemistry, Central State University, Wilberforce, OH 45384.

Exposure of cells to excess iron results in an increase in ferritin synthesis of as much as 50-100 fold. This regulation occurs at the level of translation as a result of the specific interaction of a protein repressor with 28 nucleotides within the 5' UTR of ferritin mRNA, the Iron Responsive Element (IRE). Binding of the repressor to the IRE is sensitive to the iron status of the cell. We have isolated such a repressor of ferritin mRNA translation from rabbit liver. This repressor, which we call "ferritin repressor protein" (FRP), is a 90 kDa protein which appears to be composed of a single polypeptide. It inhibits ferritin mRNA translation in a specific and dose dependent manner when added to a wheat germ in vitro translation system programmed with liver poly A^+ mRNA. Furthermore, translation of a heterologous mRNA in which the IRE has been placed in the 5' UTR is specifically inhibited by FRP, whereas translation of the same mRNA lacking the IRE is not. FRP activity is severely reduced in extracts of cells previously incubated in excess iron, demonstrating that FRP is sensitive to iron in vivo. FRP binds to the IRE with very high affinity, having a dissociation constant of approximately 10^{-10} M. This interaction is unaffected in vitro by iron salts, however it is disrupted by hemin. Further analysis of this repressor is in progress. (Supported by grants from the NSF and The Schweppe Foundation.)

Metal Ion Detoxification; Siderophores

CJ 400 PHLOEM METAL TRANSPORT: A POTENTIAL ROLE FOR GLUTATHIONE IN ZINC AND COPPER TRANSFER. L. Gene Albrigo and Kathyrn C. Taylor. Citrus Research and Education Center, University of Florida, Lake Alfred, FL 33850 Metal-binding components were examined in phloem exudate from mature Ricinus communis plants to determine what components function as metal carriers. Fractions were separated by a DEAE-Sephadex column (NaCl gradient) followed by HPLC on a Zorbax CN column (isocratic H2O:MEOH:TFA). Two major peaks, both containing Zn and Cu, were separated by HPLC. Amino acid analyses indicated that both HPLC peaks contained glutathione. The second peak eluted with a retention time similar to peak 1 when the pH of the sample was titrated from 8.2 to 6.5 or lower. This shift was reversible. These two metal-glutathione forms may represent different binding coordination, GSH and GSSG binding forms, different ratios of metal to glutathione, or combinations of these factors. This complexing activity in the phloem of a higher plant suggests that glutathione may function as an organic carrier for phloem transport of Zn and Cu.

CJ 401 EFFECTS OF HIGH DIETARY CALCIUM ON LEAD TOXICITY IN THE RAT, John D. Bogden, Sheldon B. Gertner, Francis W. Kemp, Robbie McLeod, Kay Bruening, and Haingsub R. Chung, UMDNJ, New Jersey Medical School, Newark, NJ 07103. Chronic Pb exposure can increase blood pressure and the incidence of renal neoplasms in rats. Prior studies suggest that Ca may protect against some of the toxic effects of Pb. We examined the potential of increased Ca in the diet to modify the effects of Pb on tissue Pb concentrations, blood pressure, and the incidence of renal tumors. Male Wistar rats (n=48, 5 weeks old) were randomly assigned to one of 6 treatment groups. They were fed a low (0.2%) or high (4.0%) Ca diet for 31 weeks and given 0,1 or 100 ppm Pb in drinking water. In the low Ca groups, increasing concentrations of Pb produced graded increases in mean blood pressure. Rats receiving 4.0% Ca had higher mean blood pressures than the animals on the 0.2% Ca diet. The 4.0% Ca diet also produced renal and urinary bladder stones in some rats. There were dose dependent increases in tissue Pb concentrations. The high calcium diet did not prevent tissue lead accumulation, but caused significant decreases in kidney copper, femur magnesium, and iron in kidney, liver, and testes. Precancerous and cancerous renal lesions occurred to the greatest extent in the rats receiving 100 ppm Pb and the high Ca diet. Very high dietary Ca does not protect against Pb-induced increases in blood pressure or Pb accumulation in tissues and may produce nephrocalcinosis. High dietary Ca may also increase the incidence of Pb-induced renal cancers and produce elevations in blood pressure in the rat. (Supported by the NSF Industry/University Cooperative Center for Research in Hazardous and Toxic Substances and the American Heart Association - New Jersey Affiliate)

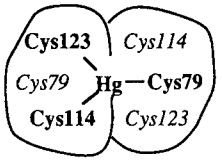
CJ 403 MEDIA-DEPENDENT PRODUCTION OF CUPRIC SULFIDE BY *E. coli* pRJ1004 COPPER RESISTANCE SYSTEM

James W. Bryson*, Thomas V. O'Halloran*, Jim Camakaris† and Barry T.O. Lee†, Department of Chemistry, Northwestern University, Evanston, IL 60208 USA (*), Department of Genetics, University of Melbourne, Parkville, Victoria, Australia 3052 (†).

It has been postulated from genetic studies that the *E. coli* plasmid pRJ1004 confers resistance to copper by enhanced efflux and modification of copper. In searching for physical evidence for the effluxed copper complex, we have found that *E. coli* expressing the pRJ1004 copper resistance system produce CuS in Luria-Bertani broth (LB) supplemented with high concentrations of $CuSO_4$, as confirmed by X-ray powder diffraction, energy dispersive spectroscopy and elemental analysis. However, since CuS is not precipitated from cultures in $CuSO_4$-supplemented minimal media M9 in which the resistance system is known to be operating, CuS may not be the primary effluxed Cu compound, but rather a decomposition product of that complex produced by the rich LB media. Chromatographic characterization of the putative detoxification complex will also be discussed.

CJ 404 GENOTOXICITY OF NICKEL COMPOUNDS, Nelwyn T. Christie and Donna M. Tummolo, Institute of Environmental Medicine, New York University Medical Center, Box 817, Tuxedo, NY 10987.
Nickel is well-established as a human and animal carcinogen and is capable of transforming cells in culture. The mechanism(s) of nickel genotoxicity are not well understood; in particular, the anomalously low mutagenic responses typically reported are inconsistent with the potent carcinogenic potential of nickel. Our laboratory and others have demonstrated nickel effects on chromosomal structure (gaps, breaks, deletions and rearrangements) and nickel inhibition of DNA replication, two endpoints of genotoxicity that suggested nickel should be mutagenic. Recently, we reported a strong mutational response (20-50 fold over background) to nickel as a particulate compound in a mutational assay consisting of the bacterial gpt gene inserted into the mammalian V-79 cell line. Studies of nickel mutagenesis at the HGPRT locus in the parental V-79 cells have indicated a very low mutational yield at this x-linked locus. The difference in the mutational response of the two genes to nickel is similar to that we have observed for x-rays at these two loci and is therefore, compatible with the hypothesis that nickel produces mutations by a deletion mechanism. This possibility is being examined in clones mutant in the gpt gene by polymerase amplification and DNA sequencing. This research was supported in part by USEPA Grant R184751 and in part by NIEHS Grant ES00260.

CJ 405 REGULATION OF TRANSCRIPTION BY MERCURIC ION IN A MARINE *BACILLUS*, John D. Helmann, Barry T. Ballard, and Christopher T. Walsh, Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA 02115
Resistance to mercuric ion and organomercurial compounds is mediated by an inducible *mer* resistance determinant under the control of the MerR metalloregulatory protein. This small, dimeric DNA binding protein is both an intracellular Hg(II) receptor and a transcriptional effector responsible for both positive and negative control of the *mer* operon. All known MerR proteins contain three evolutionarily conserved cysteine residues (positions 79,114, and 123 in the primary sequence of the *Bacillus* protein) and bind a maximum of one Hg(II) ion per dimer. We have used site-directed mutagenesis to alter all four of the cysteine residues in the *Bacillus* MerR protein to alanine, both individually and in combination. Biochemical analysis of the altered proteins and a series of *in vitro* generated heterodimeric proteins demonstrates that three cysteine residues per dimer are both necessary and sufficient for formation of a high affinity Hg(II) binding site. Therefore, we propose that the active form of the MerR metalloregulatory protein contains Hg(II) bound in a unique tricoordinate, subunit bridging complex as illustrated here:



CJ 406 LARGE SCALE GENERATION AND ANALYSIS OF METALLOTHIONEIN MUTANTS, F.A. Jacobs, T. Roemer, F. Romeyer, D. Semeniuk and R. Brousseau. Biotechnology Research Institute, NRCC. 6100 Royalmount Ave., Montreal, CANADA, H4P 2R2. Metallothionein (MT) is a small cysteine-rich protein of approximately 61 amino acids which specifically binds a small number of metal species (Hamer, Ann. Rev. Biochem., 55 (1986) 913-951). We have expressed the α-domain of human MT (α-HMT) in *E. coli* and have specifically replaced the metal-coordinating cysteine residues with a random selection of amino acids implicated in metal coordination. Large numbers of these clones producing the modified αHMT-containing fusion proteins were assayed by Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES) to simultaneously analyze 16 different metals for their specific bioaccumulation. Bacterial cultures producing the original αHMT-containing fusion proteins bioaccumulated Cd^{2+} and Cu^{+} similarly to full-length HMT-containing fusion proteins, even in the presence of 14 other heavy metal ion species. Analysis of mutant αHMT-containing fusion proteins revealed that approximately eight percent of the clones specifically bioaccumulate metals. Of this group of clones, all bioaccumulated barium, cadmium and zinc, but some demonstrated selectivity towards chromium, copper, indium and lead. Sequence and other analyses of these and other metal-bioaccumulating αHMT-containing clones are currently being performed. These data will form part of a database which may suggest general rules for the construction of metal-chelating peptides. These rules can then be tested by the direct synthesis of specific mutant metallothionein-like genes, production in *E. coli* and assessment by ICP-AES.

CJ 407 "REGULATION OF COPPER METABOLISM AND RESISTANCE IN *ESCHERICHIA COLI*."

B.T.O. Lee, N.L. Brown*, A.Bergemann, J. Camakaris, and D.A. Rouch

Department of Genetics, University of Melbourne, Parkville 3052, Australia
and *School of Biological Sciences, University of Birmingham,
PO Box 363, Birmingham B15 2TT, UK

Copper is an essential micronutrient, but is toxic in excess. In *Escherichia coli* a plasmid-borne determinant confers inducible resistance to copper. Mercury is a toxic heavy metal and has no known beneficial biological function. Inducible resistance to mercury is also found in *E. coli*. The regulation of induction of copper resistance and mercury resistance can be expected to differ due to the requirements for copper homeostasis in the bacterial cell. Mercuric ion resistance should be subject to simple one-way regulation, but that of copper should have two-way regulation. The regulation of mercury resistance genes has been studied in one of our laboratories and elsewhere. In this presentation, we describe experiments in *E. coli* that demonstrate the involvement of chromosomal (metabolic) genes in the regulation of the copper resistance genes of plasmid pRJ1004. The nucleotide sequence of the plasmid-borne copper regulatory gene has allowed us to propose a model of the regulation of copper resistance and metabolism in *E. coli*. Experiments to test this model will be discussed.