Transition Metals in Control of Gene Expression

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Metalloproteins play structural and catalytic roles in gene expression. The metalloregulatory proteins are a subclass that exerts metal-responsive control of genes involved in respiration, metabolism, and metal-specific homeostasis or stress-response systems, such as iron uptake and storage, copper efflux, and mercury detoxification. Two allosteric mechanisms for control of gene expression were first discovered in metalloregulatory systems: an iron-responsive translational control mechanism for ferritin production and a mercury-responsive DNA-distortion mechanism for transcriptional control of detoxification genes. These otherwise unrelated mechanisms give rise to a rapid physiological response when metal ion concentrations exceed a dangerous threshold. Molecular recognition in these allosteric metal ion receptors is achieved through atypical coordination geometries, cluster formation, or complexes with prosthetic groups, such as sulfide and heme. Thus, many of the inorganic assemblies that otherwise buttress the structure of biopolymers or catalyze substrate transformation in active sites of enzymes have also been adapted to serve sensor functions in the metalloregulatory proteins. Mechanistic studies of these metal-sensor protein interactions are providing new insights into fundamental aspects of inorganic chemistry, molecular biology, and cellular physiology.

Inorganic substances are primary components of several intracellular communication networks. For instance, in many signal transduction circuits, kinase enzymes alter the action of regulatory proteins by attachment of phosphate groups in what is known as a covalent modification. In such cases, the main group element phosphorous, found on the far right side of the periodic table, forms bonds with a high degree of covalent character to oxygen or nitrogen atoms in protein side chains. Alkaline earth metal ions, such as calcium, which are on the far left side of the periodic table, have been adopted for use as diffusable signals. Calcium ions reversibly bind in specific electrostatic cavities of regulatory proteins such as calmodulin through what are principally nondirectional ionic interactions.

The transition metals are found between these extremes of the periodic table and form coordinate covalent bonds that spatially organize between two and nine different chemical moieties in a more or less rigid array depending on the metal and oxidation state. Studies of these transition metal centers in biological systems have provided new insights into catalysis and coordination chemistry (*1*), but recent breakthroughs are now revealing a rich chemistry of these elements in the regulation of gene expression. In several cases, the coordination chemistry of these metal ions is an important aspect of complex regulatory circuits within cells. One way to understand such circuits is to establish the structure, function, mechanism, metal specificity, and sensitivity of specific switching devices. To this end, I present a few representative gene-regulatory metalloproteins for which some aspects of the coordination chemistry, structure, and biological function are known.

Viruses, microbes, and nucleated cells all integrate the diverse substitution and redox chemistry of the transition metals into a variety of regulatory functions (Table 1). The expression of a wide range of genes is controlled by metalloproteins. These include genes encoding metal ion detoxification, uptake, storage, and homeostasis systems on one hand and primary or secondary metabolic pathways on the other. Proteins that transduce transition metal signals into changes in gene expression are said to have a metalloregulatory function. Transition metal signals can involve a change in the intra- or extracellular concentration of a metal ion or metal-ligand complex, such as heme. Metalloregulatory proteins are then a subset of regulatory proteins that act, at the physiological level, as components of metal-responsive genetic switches (*2*). In the broadest sense, a metalloregulatory protein can serve a sensory or regulatory role in a switching mechanism. In some cases, a single protein plays both roles.

The metal-specific receptor and allosteric switching functions of several metalloregulatory proteins have captured the attention of chemists and biologists as puzzles that contain clues about the molecular basis of metal ion recognition and the intracellular pool of low molecular weight (MW) metal complexes. These regulatory proteins can be simple switches or integral components of complex signal transduction circuits. Descriptions of their functions at a molecular level are providing a basis for models in cell biology and metal ion toxicology. Studies of these receptors also provide benchmarks for the design of inorganic pharmaceuticals and other agents that target specific biopolymer sites or metal-controlled pathways.

Metal-containing regulatory proteins can capitalize on the rich variety of coordination geometries and ligand exchange rates of metal centers. The latter can serve as binding sites for small diffusable ligands, such as dioxygen, nitric oxide, superoxide, and ammonia, that constitute a biological signal. The SoxR, Fnr, and NifA bacterial proteins may be metalloproteins that sense changes in O_2 partial pressure (or O_2^- concentration) and subsequently activate the superoxide stress-response regulon (*sox*) (*3*), anaerobic-metabolism pathways (*4*, *5*),

Table 1. Functions of metal coordination sites in regulatory proteins.* Parentheses indicate that aspects of the coordination chemistry have yet to be established.

Class	Function of metal center	Protein	Metal
1	Catalytic	IRE-BP (c-aconitase)	Fe
2	Metalloregulatory		
	Metal ion receptors	ACE1	Cu
		ArsR	(As)
		CopR-CopS	(Cu)
		DtxR	(Fe)
		Fur	(Fe)
		IRE-BP	Fe
		MerR	Hg
		PcoR-PcoS	(Cu)
	Metal-ligand receptors	HAP1	(heme)
3	Exogenous-ligand sensor }	SoxR	(Fe?)
4	Redox sensor }	Fnr	(Fe?)
		NifA	?
5	Light or magnetic-field sensor	—	—
6	Structural	Zn transcription factors (?)	Zn

*See text for references.

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and nitrogen-fixation genes (6), respectively. Transition metal centers anchored to the hypothetical class 4 and 5 metalloproteins (Table 1) are postulated to sense changes in cellular redox state or light intensity (7).

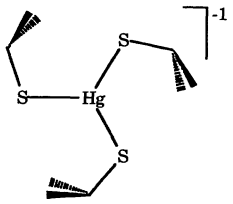
A majority of metalloproteins involved in gene expression may not have functional roles in transducing inorganic signals or monitoring change in metal ion concentrations. For instance, the function of zinc finger proteins may not involve changes in Zn(II) occupancy. In such cases, the metal center may simply be a ubiquitous and available cofactor whose only function is to maintain, under all physiological states, a constant three-dimensional structure for the protein. In such proteins, the coordination environment may only perform functions similar to a disulfide cross-link or a hydrophobic packing interaction. On the other hand, metal ion coordination may be a well-controlled posttranslational modification that is part of a functional switch in cellular control mechanisms and thus analogous to phosphorylation or Ca^{2+}-responsive networks. It is not clear how to categorize most zinc-containing transcription factors. Although the metal-responsive regulation of important metalloproteins such as human metallothionein, heme oxygenase, and plastocyanin (8, 9) is well established, the relevant metalloregulatory proteins have yet to be characterized.

MerR and Mercury Resistance

Extensive genetic, enzymatic, structural, biophysical, and inorganic studies of bacterial mercury resistance proteins (encoded by the *mer* genes) provide the first comprehensive picture of a tightly regulated metal-detoxification mechanism [for recent reviews see (10–14)]. The central enzyme is mercuric ion reductase, a structurally characterized flavoenzyme that reduces Hg(II) to the volatile Hg^0 form (10, 11). The energy-dependent uptake system can deliver other heavy metal ions to the reductase, but only the mercuric ion can be detoxified by reduction to a volatile state; Ag(I), Au(I), and Cd(II) inhibit the enzyme.

The transcriptional switching mechanism centers on MerR, a protein evolved to discriminate readily between Hg(II) and other metal ions with similar coordination properties. As a receptor, MerR is sensitive to nanomolar concentrations of Hg(II) and exhibits a high degree of selectivity. Gratuitous inducers, such as Cd(II) and Zn(II), can activate transcription through MerR only when their concentrations exceed micromolar levels (15).

Physical studies of stoichiometric Hg-MerR, Zn-MerR, and Cd-MerR complexes provide evidence for the molecular basis of both the sensitivity and selectivity of the metal binding site (14, 16). These data are consistent with a trigonal (but not linear) mercuric thiolate environment in Hg-MerR (17), similar to that in several model complexes, such as the one shown below (18).



Genetic data support a role for at least three cysteines in this receptor site (Table 2) (19). A mercuric ion binding site at the dimer interface has been proposed on the basis of the metal affinity of mutant heterodimer proteins (20). None of the above studies has unequivocally ruled out a role for coordination of a His side chain to the mercuric ion center. From an inorganic perspective, the molecular recognition underlying this combination of sensitivity and selectivity is essentially derived from coordinate-covalent interactions of the metal ion with at least three cysteinyl thiolates. Yet, MerR is more than an Hg(II)-specific chelating agent: It must transduce the signal by inducing conformational changes in the transcriptional apparatus.

The steep response of the transcription rate (15) (Fig. 1A) to increases in Hg(II) concentration is an ultrasensitive, or threshold, phenomenon and an intrinsic attribute of the biological switching mechanism. When metal ions are titrated into bacterial strains harboring a fusion of the *mer* promoter to a luciferase reporter gene, a similar threshold effect, with a Hill coefficient of 2, is observed (Fig. 1B). The earlier in vitro transcription assays directly reflect the in vivo response (Fig. 1A) (15). Only one Hg(II) binds per dimer, suggesting that allosteric models for the O_2 affinity of hemoglobin do not apply. The cooperation in this biological response (15) may be a consequence of the association of both RNA polymerase (RNA pol) and MerR with the promoter in the absence of Hg(II) (21, 22). This closed, or transcriptionally inactive, complex constitutes the "off" position of the Hg(II)-responsive switch.

An unusual mechanism for transcriptional activation has been described for MerR (21). The Hg-MerR–DNA complex apparently stimulates RNA pol activity at the mercury-responsive promoter through an allosteric modulation of DNA structure (21–24). Both MerR and Hg-MerR bend DNA; however, the latter stabilizes a localized distortion of the DNA that is underwound by at least 30° more than in the repressed state (23). This signal-responsive conformation change makes the DNA a better template for RNA pol (Fig. 2). Other DNA binding proteins, such as the cyclic–adenosine monophosphate receptor protein (CAP), bend or twist DNA, but these distortions do not directly stimulate transcription.

Biological transistors. Before the more complex copper and iron regulatory systems are discussed, it is useful to compare this simple genetic switch with a component of an electronic device. In simple circuits, small changes in potential (chemical or electrical) can be detected by a device that responds by amplifying current flow in a separate circuit. In solid-state or microelectrochemical cells, such a device is known as a transistor. The

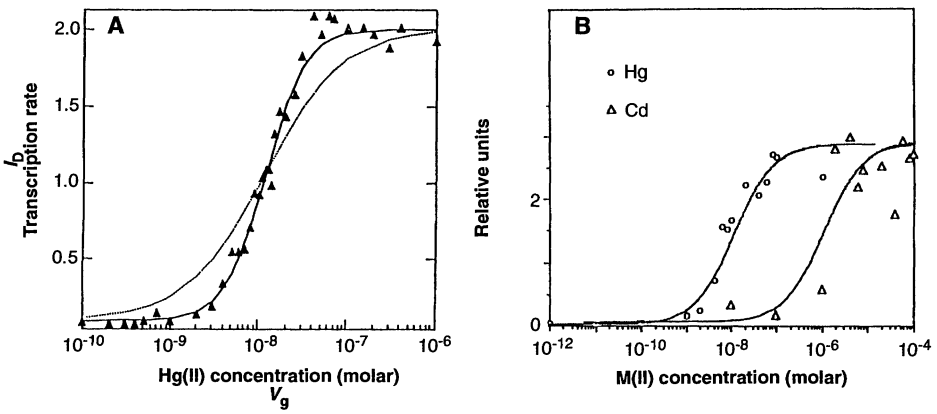


Fig. 1. Metal ion–dependent transcription of mercury-resistance promoters is mediated by the MerR metalloregulatory protein, as shown in both (**A**) in vitro (14) and (**B**) in vivo transcription assays (104). The transcription rate is shown as hundreds of moles of mRNA per mole of DNA template per hour. The steepness of the response to nanomolar concentration of Hg(II) indicates a threshold or ultrasensitive response, with a Hill coefficient of about 2 for all three solid curves. A response curve for a Hill coefficient restrained to 1 is shown as a dotted line in (A). Also apparent is the specificity of the MerR protein for Hg(II). As seen in (B), where the magnitude of the total activity for Cd has been normalized to that of Hg, the cadmium concentration must be at least two orders of magnitude higher than mercury before transcription is observed. See text for definition of I_D and V_g.

nucleoprotein complex of MerR and RNA pol bound to the promoter functions like a transistor, which can translate a change in the gate voltage (V_g), or input signal, into a change in drain current (I_D), or the transcription rate. The gate voltage corresponds to the chemical potential (concentration) of Hg(II), and the drain current corresponds to the increase in messenger RNA (mRNA) concentration as a function of time (Fig. 1A). Descriptions of more complex genetic circuits will require further definition of additional biochemical devices.

Iron Sensors

Molecular recognition of iron in three known metalloregulatory proteins can be achieved through divalent ion receptor sites, assembly of iron clusters containing inorganic sulfide, or binding of the iron porphyrin prosthetic group known as heme.

Microbial iron-responsive switches. Although the best understood iron-responsive metalloregulatory protein in microbial systems is Fur (ferric iron uptake regulation) (*25*), little is directly known about the metal ion coordination environment in the active form of the protein. Fur-like regulatory systems are ubiquitous in Gram-negative bacteria and have been described as metal-responsive repressors of iron uptake genes and as positive activators of acid-induced stress proteins (*26*). When iron concentrations in media or cytosol are elevated, intracellular iron [presumably in the Fe(II) form] is thought to act as a corepressor, inducing a specific DNA binding activity in the Fur protein and inhibiting transcription at promoters containing a Fur

Table 2. Potential metal-binding sequences in regulatory proteins. The number of metal ions per domain are given where known. Bold indicates metal ligation sites that have been directly established with the use of physical methods. C, Cys; D, Asp; H, His; I, Ile; K, Lys; L, Leu; M, Met; O, hydrophobic side chain; P, Pro; R, Arg; and V, Val.

Protein	Metal ions	Sequence
		Mercury- and copper-responsive transcription factors
MerR (*109*)	1 Hg/dimer	HC——X_{32}——CXCH . . . X_7 . . C
Cup2 (ACE1) (*66, 68*)	Cu cluster	CXXC . X_3H . X_4 . CXXH——X_{17}——CXHC——X_{14}——CXC——X_{16}——CXC . . X_5 . CXCH
AMT-I (*69*)	Cu cluster	CXXC . X_3H . X_4 . CXXH——X_{17}——CXHC——X_{14}——CXC——X_{26}——CXC . . X_5 . CXCH
		Iron coordination sites
HAP1 (*39*)	Heme	$K_{CP}I_{DH}$ R V
ALAS (*42*)	Heme	K_{CPO}M R L
FNR* (*4, 5*)		CXXHCXXC
SoxR* (*3*)		CXXCXC . . X_5 . C
Ferredoxin I† (*62*)	Fe_4S_4	**C**XX**C**XX**C** . X_3**C**
	Fe_3S_4	**C**XX**C** . X_4 . **C** . X_3**C**
Cytochrome c_3† (*110*)	2 heme	CXXCHH . . . X_{10} . . . . C . X_4 . CH
Endonuclease III (*111*)	Fe_4S_4	**C** . . . . . X_{16} . . . . **C**XX**C** . . X_5 . **C**
Rubredoxin† (*112*)	1 Fe	**C**XX**C** . . . . . X_{23} . . . . . **C**XX**C**
IRE-BP (*57*)	Aconitase form Fe_3S_4 or Fe_4S_4	**C**——X_{65}——**C**XX**C**
Fur (*112*)		HH——X_{37}——H——X_{14}——HHHXHXXCXXC——X_{21}——H . . X_6 . . H . . X_6 . . HC . X_4 . C . X_4 . HXH
		Single-stranded nucleic acid–binding proteins
gp32 (*79*)	1 Zn	**C**XXX**H** . . X_5 . **C**XX**C**
Retroviral fingers (*100*)	1 Zn	**C**XX**C** . X_4 . **H** . X_4 . **C**
		Double-stranded DNA binding proteins
Zif268 (*85*) SP1 (*92*) TFIIIA (*82*)	1 Zn	**C**XX**C** . . . . X_{12} . . . . . **H** . X_3 . **H**
ADR1 (*114*) Xfin (*115*)	1 Zn	**C** . X_4 . **C** . . . . X_{12} . . . . . **H** . X_3 . **H**
GAL4 (*88, 107*)	2 Zn cluster	**C**XX**C** . . X_6 . . **C** . . X_6 . . **C**XX**C** . . X_6 . . **C**
GATA-1 NH_2 (*99, 116*)		CXXC . . . . . X_{14} . . . . . HXXCXXC (X_3 . H)
GATA-1 COOH (*99*)	1 Zn, Fe, etc.	CXXC . . . . . X_{14} . . . . . DXXCXXC (. . X_6 . . H)
dsx (*117*)		CXXCXXH . . . X_8 . . . HXXH . X_4 . CXCXXC
RING 1 (*118*)		CXXC . . . . X_{12} . . . . . CXHXXCXXC . . . . . X_{11} . . . CXXC
LIM (*97, 98*)		CXXC . . . . . X_{16-23} . . HXXCXXCXXC . . . . . X_{16-21} . . CXX(C,H,D)
CRP (*98*)	2 Zn	CXXC . . . . . X_{17} . . . . . HXXCXXCXXC . . . . . X_{17} CXXC
Glucocorticoid receptors (*108*)	2 Zn	**C**XX**C** . . X_7 . . . H . . X_5 . **C**XX**C** . . . . . X_{11} . . . H . X_3**C** . . X_5 . **C** . . . X_9 . . . . **C**XX**C** . X_4 . C
		Nuclear proteins
Nup475 (*119*)		C . . . X_8 . . . C . . X_5 . C . X_3C . . . . . X_{18} . . . . . C . . . X_8 . . . C . . X_5 . CXXXC
nup153 (*120*)		CXXC . . . . X_{10} . . . CXXC
		Protein-protein interactions
E1a (289R) (*121*)		CXXCHXH . . . X_7 . . DXXCXXC
E6 (*122*)		CXXC . . . . . X_{30} CXXC
E7 (*122*)		CXXC . . . . . X_{29} CXXC
Protein kinase C (*123*)		H . . . . . X_{12} . . . . CXHC . . . . . X_{13} . . . . . CXXC . X_4HXXC . . . X_7 . . C

*Iron content not established. †No role established in nucleic acid interaction.

binding site. Fur binding sites, or "iron boxes," are found upstream of many unrelated genes and implicate Fur as a global regulator of iron-responsive genes. Mutations in Fur affect functions as diverse as dicarboxylic acid synthesis, manganese resistance, and expression of the manganese form of superoxide dismutase, SodA (*25*). Transcription-translation and equilibrium dialysis assays suggest that the Fur protein does not discriminate well between Fe(II), Mn(II), Co(II), and Cd(II) (*27, 28*). Furthermore, the concentration of Fe(II) or Mn(II) required for full DNA binding activity (75 μM) in these in vitro assays is much higher than the apparent concentration required for half-maximal Fur-dependent repression in vivo (1 μM) (*29, 30*). Although the physiological roles of Fur may not require a high degree of selectivity, some component of the physiological switch may be absent in the in vitro assays.

Of the 12 His, 4 Cys, and 26 acidic side chains available for metal coordination in this 148–amino acid protein, paramagnetic nuclear magnetic resonance (NMR) studies implicate Mn(II) coordination to two or three His residues (31, 32, and 131) as well as carboxylate groups (*31*). These results must be considered preliminary in the absence of a firm correlation between metal content and biochemical activity for the NMR samples. In contrast, the alkylation studies and proteolysis-activity correlations of Neilands and co-workers (*25, 32*) have shown that the Cys thiol groups are essential for Fur activity and have led them to favor a COOH-terminal metal binding domain.

Virulence and iron: DtxR. Serum iron is a key resource on the battleground between a host and an infectious microorganism. It is not surprising that Fur and iron also modulate the expression of genes involved in bacterial virulence. A variety of pathogenic microorganisms express genes encoding diphtheria, Shiga, and cholera toxins when iron becomes the limiting nutrient in the growth medium (*33*). The DtxR protein of *Corynebacterium diphtheriae* is responsible for iron-dependent repression of the phage-encoded diphtheria toxin (*tox*) gene and may function as a global regulator similar to Fur (*34, 35*). Half-maximal DNA binding activity of DxtR in vitro is induced in the order [Ni(II)] < [Fe(II)] < [Mn(II)] < [Co(II)] (where brackets denote concentration) but only weakly by Zn(II) (*36*). All of these metals inhibit toxin production in vivo; however, the most potent inhibitor is Fe(II). As with Fur, the physiological and biochemical ranking of metal responses differ. Nothing is known about the metal ion coordination environment or repression mechanism, and there is little sequence similarity between potential metal binding sites in DtxR and Fur.

FecR-FecI. An unusual two-component metalloregulatory system has been proposed for the *Escherichia coli* Fe(III) citrate uptake (*fec*) genes (*37*). The *fec* genes are under Fur control and are derepressed in iron-limiting conditions; however, additional regulation has been observed when citrate concentrations in the medium are high (1 mM). The periplasmic FecR protein is proposed to be a ferric dicitrate receptor that communicates with membrane-bound FecI protein. Although the latter is proposed to be a DNA binding protein, neither protein is similar in sequence to the well-characterized family of two-component signaling proteins, nor has a metal receptor site been identified.

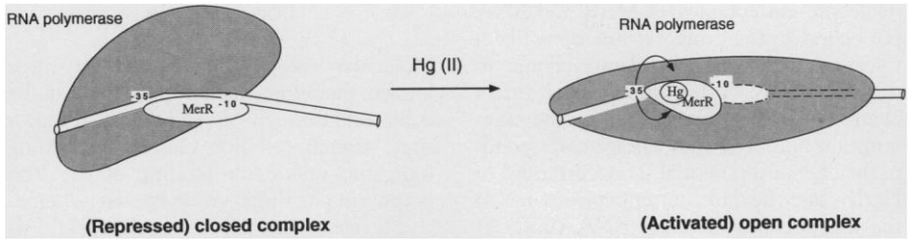


Fig. 2. Mechanism for Hg(II)-responsive transcriptional activation. The Hg-MerR protein distorts the DNA in a key step of the activation mechanism. Local underwinding of DNA at the center of the MerR binding site optimizes RNA polymerase contacts with, and strand separation of, the DNA duplex in the downstream portion of an otherwise weak promoter.

Eukaryotic Iron Metalloregulatory Proteins

HAP1: A heme receptor. A variety of nuclear genes encoding respiratory proteins in yeast are controlled at the transcriptional level by a heme sensor protein, HAP1 (*38*). As with most yeast transcription factors, HAP1 is a large protein (1389 residues) that binds to upstream activation sites in a variety of promoters, including that of the apo–cytochrome c gene. The HAP1 protein has been dissected into various functional domains, including an NH_2-terminal DNA binding domain analogous to that found in GAL4, an adjacent domain for specific heme binding, and a COOH-terminal acid-activation domain that establishes contacts with the transcriptional apparatus required for transcription initiation (*39*). This iron sensor has been localized to the nucleus and thus monitors changes in the nuclear heme pool (*40*).

The heme-sensing domain of HAP1 contains seven repeats of the Cys- and His-containing motif shown in Table 2 (*39*). In the absence of heme, this region of the protein is thought to interact with the adjacent DNA binding domain in a manner that precludes dimerization and sequence-specific DNA binding. Recent results of Guarente and co-workers (*38–41*) indicate that heme stimulates DNA binding by promoting dimer formation or by altering the interaction of other accessory proteins with the DNA binding domain. Intriguingly similar Cys-Pro-X-Asp-His motifs (where X is any amino acid) have recently been reported in the presequences of the precursors of erythroid δ-aminolevulinate synthases (ALASs) (*42*). This sequence confers heme-responsive regulation of the transport of preproteins into mitochondria and has been dubbed a heme regulatory motif (HRM). The ALAS catalyzes the synthesis of the first intermediate in heme biosynthetic pathways. These studies thus provide molecular insight into the well-known phenomenon of feedback inhibition: As cytosolic heme concentrations increase, preproteins such as ALAS or chimeras with a single HRM apparently bind heme and are inhibited in transport into the mitochondria. The similarity in the apparent roles of these motifs suggests that they may be tight binding sites for heme; however, direct physical evidence for such binding is lacking.

The enzyme ALAS is involved in iron traffic between the cytosol and mitochondria. In differentiated erythroid tissues, expression of ALASs is developmentally regulated, and translation of its mRNA is coordinated with cytosolic iron concentration through an iron-responsive element (IRE) in the 5′ untranslated region of the mRNA (*43, 44*). Regulation of these genes therefore is expected to parallel the regulation of ferritin; translation of ALAS should be derepressed as the cytosolic pool of low MW iron complexes increases (as described below) (*42*). As suggested by Tzagoloff and co-workers (*45*), heme-responsive regulation is apparently not confined to genes whose products are heme proteins. Rather, it is a general process for altering the synthesis of a broad spectrum of nuclear genes, the products of which will be targeted to the mitochondria and are required for respiration.

Iron-Responsive Translational Regulation

Ferritin and transferrin receptor. Metal-responsive control of the concentrations of both ferritin and transferrin receptors in mammalian cells is achieved by a single metal sensor that regulates the initiation of translation of mRNA into protein. Indeed,

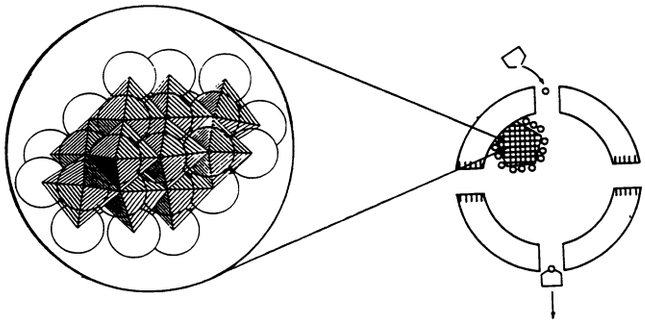
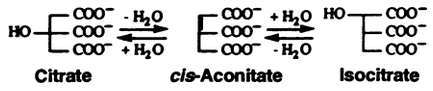


Fig. 3. Diagram of ferritin core (iron, circles; low MW chelating ligand, open pentagon entering circle) showing ligand-assisted loading or removal of iron. (**Inset**) Model compound for the mixed-valent iron hydrox-oxo core. The oxygen atoms are shown as open spheres and the 12 iron atoms as edge-shared octahedra. [Adapted from (*48*)]

the first example of translational regulation was found in the ferritin system by Munro and co-workers (*46*).

Ferritin isolated from bacterial, plant, or mammalian sources is a large 24-subunit protein that can internalize up to 4500 atoms of iron in an iron oxo-hydroxo mineral lattice (*47*). A structural and spectroscopic model compound for the ferritin core is shown in the Fig. 3 (*47, 48*). As the chief intracellular reservoir for iron, ferritin is loaded or disgorged as cellular conditions require (*49*). Iron uptake from the serum involves at least two other proteins. Transferrin receptor (TfR), when localized on the cell surface, binds the serum protein transferrin (Tf), a species capable of coordinating two Fe(III) ions in a pH- and carbonate-dependent manner. Upon binding of Fe-Tf to TfR, the entire complex is internalized, and following the acidification of vesicles, iron is released from Tf. Diffusion or transport of iron to cytosolic and mitochondrial proteins then occurs by unknown mechanisms. While in transit, these metal ions are considered to be part of a poorly characterized pool of low MW iron complexes that are ultimately funneled into heme cofactors, into nonheme sites in ferritin and other iron proteins, and into iron-sulfur centers.

Concentrations of ferritin and TfR are tightly controlled by an RNA binding metalloregulatory protein, IRE–binding protein (IRE-BP), that moonlights as the iron sulfide–containing enzyme cytosolic aconitase (c-aconitase) when iron concentration is elevated. Aconitase interconverts citrate and isocitrate (Scheme 1). The mitochondrial enzyme, m-aconitase, is the entry point for glycolysis products into the tricarboxylic acid (TCA) cycle; however, a physiological role for c-aconitase activity is not obvious.



Scheme 1. Interconversion of citrate and isocitrate by aconitase.

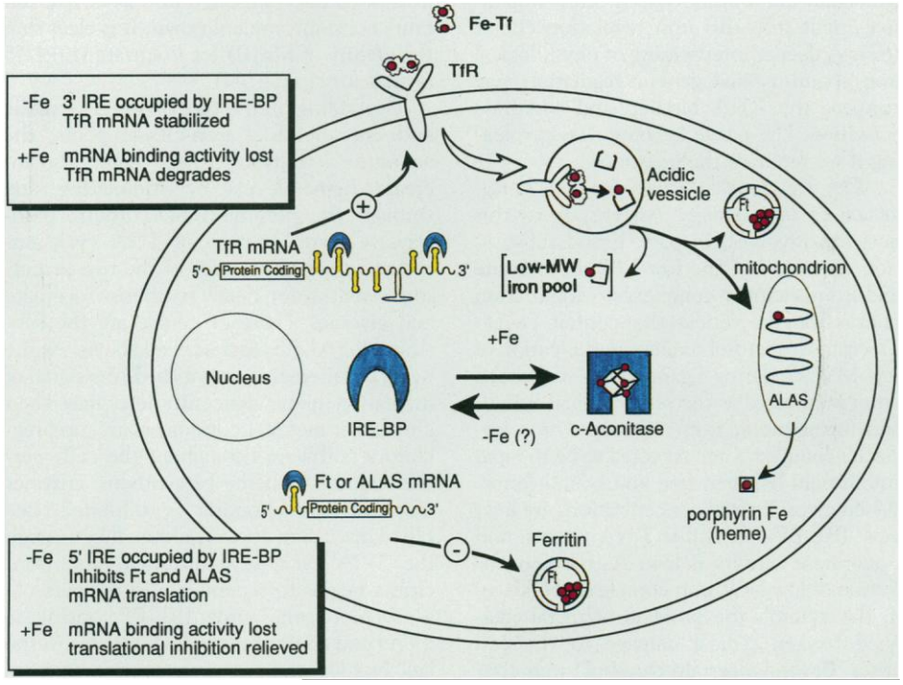


Fig. 4. Aspects of iron homeostasis involving IRE-BP regulation of ferritin and TfR mRNA translation. Iron, red; stem-loop RNA sequence known as IRE, green; and IRE-BP (c-aconitase), blue. [Adapted from (*105*)]

Posttranscriptional regulation. In the absence of iron, the IRE-BP controls gene expression by interacting with an IRE: a small, stable stem-loop RNA structure containing about 30 nucleotides (*50*). The IREs are found in the 5′ untranslated region of the ferritin and ALAS mRNA or the 3′ untranslated region of the TfR mRNA (*49*). The IRE-BP binds to a stem-loop IRE (Fig. 4) if iron concentration is below a threshold value (*51–53*). When IRE-BP is bound to the 5′ IRE of ferritin or ALAS (*42*), it acts as a repressor of the ferritin translation of that mRNA. Under the same conditions, and apparently with similar affinity, the IRE-BP binds to the 3′ end of the TfR mRNA. This prevents the otherwise rapid degradation of this mRNA and thus increases the degree of TfR expression. Consequently, more TfRs are synthesized, and the cell increases the uptake of iron from serum. When excess iron is available to the cell (or to the protein in vitro), the IRE-BP loses its RNA binding activity and can be isolated as a protein with a cluster of four iron atoms and four sulfur atoms ([4Fe4S]) (*54, 55*). Thus, IRE-BP differentially regulates the rate of translation of one mRNA and the stability of the other in an economical mechanism. Transferrin expression itself may be regulated in a similar manner (*56*).

Isolation and characterization of the IRE-BP allowed a rapid advance of our understanding of this mechanism: Its sequence is identical to that of the known peptide fragments of beef liver c-aconitase (*55*). The latter is similar in sequence (30% identity) to pig heart m-aconitase, whose x-ray crystal structure is known (*57*). The fully loaded iron form of IRE-BP demonstrates aconitase activity but no RNA binding activity (*58*).

Iron-sulfur cluster as sensor. One of the more tantalizing observations of this system concerns the interconversion of IRE-BP by iron-chelating agents in vitro. The IRE-BP purified from cells grown under iron starvation conditions exhibits high RNA binding activity but low aconitase activity (*54, 59*). Kennedy and Beinert (*60*) have shown that conversion of the apo form to the [4Fe4S] enzyme can be accomplished in vitro by simple addition of Fe(II) and reductant: Apoaconitase retains S^0 perhaps in the form of a persulfide after iron removal. The exact Fe:protein stoichiometry of the iron-saturated form of IRE-BP has yet to be determined. As first demonstrated by Holm and coworkers (*61*) for synthetic models, the clus-

ter can "self-assemble" upon addition of iron to the protein. The reverse reaction, from aconitase to RNA binding activity, has not been accomplished under physiological conditions. Complete removal of the iron from in vitro IRE-BP requires high pH and high concentrations of reducing agents, detergents, or oxidizing agents.

Although the chemical reactivity of the c- and m-aconitase iron-sulfur clusters are subtly different, there are several important features that distinguish them as a class separate from classical [4Fe4S] clusters found in hydrolytic and electron transfer proteins, such as the endonuclease III and the putative DNA binding protein ferredoxin I (Table 2) (*62*). First, the cluster of m-aconitase is anchored to the protein by three instead of four Cys-Fe bonds. The fourth iron (Fig. 5) is chemically and spectroscopically unique. This iron is also the binding site for the substrate citrate, the product isocitrate, and a water molecule that plays a key mechanistic role. Abstraction of this iron to give the [3Fe4S] form of the protein eliminates aconitase activity. In early studies of m-aconitase, the fourth iron was lost upon purification, and full activity of m-aconitase could only be obtained by addition of excess Fe(II) and reductant. This inconvenience proved to be an adventure misconceived: The lability of this family of clusters may be an important feature of the unexpected regulatory function of c-aconitase.

As expected from studies of the m-aconitase, the iron-loaded form of IRE-BP can be stripped of the fourth iron with concomitant loss of aconitase activity; however, this alone is not sufficient to restore RNA binding activity. Complete disassembly of the cluster is apparently required for the latter activity in vitro (*59*). Furthermore, the substrate citrate can stabilize the [4Fe4S] form of protein against chelating agents and act as an antagonist for conversion to the RNA binding form. Both of these attributes have important implications for gene expression, cytoplasmic citrate metabolism, and iron homeostasis.

Why iron-sulfur? IRE-BP is the first example of a nucleic acid binding regulatory protein that has a completely independent enzymatic activity. Is the aconitase activity of IRE-BP a coincidence, an evolutionary relic, a negligible component of total c-aconitase activity, or a physiologically important activity in iron transactions? The aconitase activity of IRE-BP may be an essential aspect of iron homeostasis that links the low MW iron pool in the cytosol and the "crossroad" for secondary metabolite (heme) interconversion and energy utilization: the TCA cycle.

First, it is unlikely that the only physiological role of an iron-sulfur cluster in the IRE-BP is metal sensing because the coordination chemistry of the sensor protein is different from that of the regulated proteins. Ferritin loads iron in an iron oxo-hydroxo lattice, but IRE-BP requires the formation of an iron sulfide–containing cluster in the molecular recognition event. In contrast, the metal ion receptor sites in other metalloregulatory proteins discussed here are very similar to the metal coordination environment in the regulated protein. Why does ferritin break this admittedly small trend? One possibility involves the versatile reaction chemistry of iron-sulfur clusters. The well-known sensitivity of the iron-sulfur cluster of aconitase to reductants and oxidants may allow other types of stress-responsive input into this iron regulation circuit (*63*). A deeper intertwining of physiological iron chemistry and genetic regulation may connect the RNA binding and aconitase activities. The puzzle becomes less perplexing if we rephrase the question.

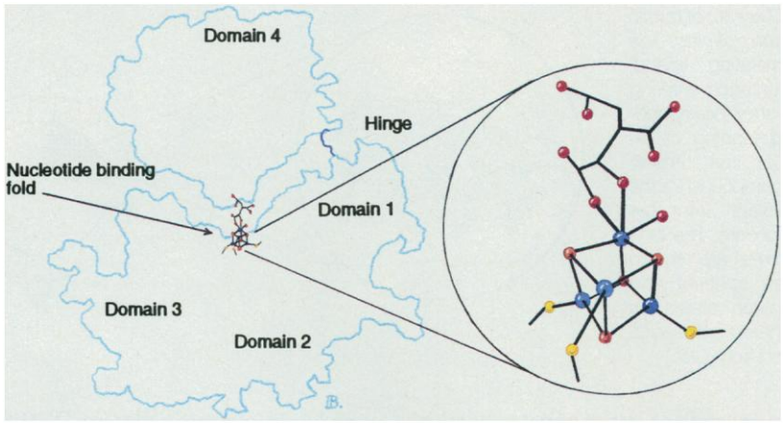


Fig. 5. Structural analogy of IRE-BP and pig heart m-aconitase. (**Inset**) The [4Fe4S] cluster of m-aconitase with water and isocitrate coordinated to the unique iron (*106*). Orange spheres, inorganic sulfide; yellow spheres, sulfur atoms of Cys; red and black denote oxygen and carbon atoms, respectively. A region of aconitase similar to a nucleotide binding fold is indicated as a potential RNA binding site.

Why is c-aconitase activity involved in regulation of iron storage? An answer to this question involves, in part, ligand substitution chemistry of the low MW iron-citrate and iron-isocitrate complexes. Mobilization of iron from the vesicles that contain Fe-Tf–TfR into the cytosol requires participation of low MW chelating ligands (*64*). Citrate is often implicated as the physiological ligand, and furthermore, iron-citrate and iron-isocitrate complexes are expected to be in rapid equilibrium between free and bound forms. When cytosolic iron concentrations are low, most IRE-BP is bound to RNA targets, and c-aconitase activity is low. As the concentration of low MW iron complexes builds up in the cytosol, the problem of metal-catalyzed oxygen radical damage to the cell arises. Beyond a certain threshold iron concentration, ferritin is required to absorb the excess iron. The affinity of IRE-BP for iron is likely poised at this threshold.

Upon assembly of the [4Fe4S] form of the protein, three events could cooperatively stabilize the iron flux, beginning with repression of TfR synthesis. Ferritin levels rise as rapidly as the ribosome translates mRNA, and a burst of c-aconitase activity comes on line. Third, the isocitrate concentration increases at the expense of the citrate pool in the cytosol. This simple shifting of the citrate:isocitrate ratio by the iron-sulfur form of IRE-BP could directly facilitate iron loading of ferritin by converting a portion of the low MW iron-citrate pool into a more thermodynamically and kinetically labile form: iron-isocitrate. Although iron-isocitrate stability constants are unknown, it is clear that the affinity of Mn(II) for isocitrate is ~1/15 of that for citrate (*64*).

Depending on the flux between cytosolic and mitochondrial iron-citrate pools, the aconitase activity of IRE-BP could also increase heme levels by stimulating flux through the porphyrin biosynthetic pathways. Citrate, by way of TCA cycle enzymes, serves as a source of the two precursors essential for heme synthesis: succinate and glycine. These of course are the substrates for ALAS, as described above. As the heme requirements are satisfied, the signal of increasing heme concentrations may then alter other metabolic, homeostatic, and regulatory pathways throughout the cell. Further import of heme biosynthetic enzymes into the mitochondria is inhibited (see HRM discussion above), citrate flux through the TCA cycle is diminished, and iron-citrate begins to accumulate in the cytosol.

As more genes under IRE-BP control are found and analytical methods emerge in the cell biology of iron, more precise connections between cytosolic citrate, low MW intracellular iron pools, and the iron-dependent respiratory chains may emerge.

Copper Sensors

Copper-responsive regulation of the *Saccharomyces cerevisiae* copper metallothionein (MT) and CuZn–superoxide dimutase genes is mediated by the transcriptional activator protein ACE1 (also known as Cup2) (*9*, 66–68). A homologous protein, AMT1, has been identified in *Candita glabrata* (Table 2) (*69*). The mechanism of transcriptional regulation by this protein, like HAP1, involves metal-induced binding of the factor to a specific upstream activation sequence in the 5′ end of the MT promoter (*68*). Below a threshold concentration of copper, the protein does not bind DNA; however, as copper concentrations rise in the cell, a Cu(I)–cysteinyl thiolate cluster forms in a cooperative manner with a parallel increase in the specific DNA binding activity (*70*). The 122 NH_2-terminal amino acids of this protein mediate metal-specific binding and contain a series of Cys-X-Cys and Cys-X_2-Cys sequences (Table 2) typical of all known MT proteins. As with MTs, the sequence exhibits a dearth of hydrophobic residues, and thus, the metal cysteinyl-thiolate cluster provides the free energy of stabilization for the tertiary fold.

Between six and eight copper ions are coordinated for every 11 Cys side chains of ACE1 in one or more polynuclear clusters (*71*). The local geometry around each copper ion appears to be trigonal with distortions from planarity. Although the three-dimensional structures of Cu-MT and Cu-ACE1 are not known, luminescent, ultraviolet absorption and circular dichroism spectroscopies suggest that the copper is coordinated with cysteinyl thiolates in a trigonal geometry (*71*, *72*). Features of the x-ray absorption edge of $Cu_{6.8}$-ACE1 are also consistent with distortion of copper coordination from a trigonal planar geometry and are similar to those found for the Cu(I) coordination environment of Cu-MT (*71*). These results are interpreted in terms of a single cluster containing about seven coppers for every 11 Cys side chains.

The specificity of the ACE1 protein for Cu(I) is achieved through the formation of a metal cluster with ligation geometry similar to that observed for the protein that it regulates, namely Cu-MT. In contrast to the IRE-BP cluster, no inorganic sulfide is incorporated. Finally, cluster formation is cooperative, which should lead to an ultrasensitive transcriptional response similar to that observed for the MerR protein (*70*). In contrast to MerR, allosteric modulation of ACE1 binding may well involve cooperation between multiple binding sites, as observed with the affinity of hemoglobin for O_2. In the case of MerR, it appears that only a single mercury is bound by the receptor protein.

Bacterial copper regulation. A variety of microbial metal resistance genes exhibit metal-responsive transcriptional regulation and make use of specific metalloregulatory proteins (*13*). The ArsR protein mediates the arsenic-responsive regulation of the arsenate and arsenite resistance operons (*73*), and CzcR regulates the transcription of cobalt, cadmium, and zinc resistance determinants (*13*, *74*). The SmtB protein exhibits limited sequence similarity to ArsR and MerR and mediates Zn-responsive regulation of a bacterial MT gene involved in Zn-Cd tolerance (*75*). On the other hand, copper-responsive transcriptional regulation of genes that protect bacterial cells from the adverse effect of elevated copper concentrations departs from the systems above and appears to use a phosphorylation cycle in the signal transduction pathway. The copper resistance operons have been sequenced from plant pathogens (*Pseudomonas syringae*) and enteric bacteria (*E. coli*) that were routinely exposed to copper-containing antimicrobial agents. These microbes can encounter concentrations of copper (>20 mM in rich media) that would otherwise be toxic to the wild-type organism.

The *E. coli* resistance mechanism apparently involves Cu efflux, and the *P. syringae* mechanism involves sequestration; however, the resistance genes show a high degree of similarity to each other. Both systems require two proteins for copper-responsive transcriptional activation of the resistance genes. The *copR* and *copS* regulatory genes of the *P. syringae* systems, characterized by Cooksey and co-workers (*76*), are homologous with the *E. coli pcoR* and *pcoS* genes sequenced by Lee, Brown, and co-workers (*77*). They, in turn, share key regions of similarity with the family of proteins involved in two-component signal transduction pathways. This class of regulators is involved in the communication of changes in environmental status into changes of expression of specific genes. The *pcoS* and *copS* genes are similar to the sensor components of this family and apparently encode membrane-spanning proteins that monitor changes in copper concentrations in the periplasm. Once stimulated by copper binding, the sensors are envisioned to phosphorylate CopR or PcoR, which then activate transcription of the copper-resistance promoters. If borne out by biochemical studies, this may be the first example of the metal-responsive transcription event being mediated through a classical phospho-relay signal transduction pathway. No data are available on the copper binding site.

Zinc Proteins in Gene Expression

Zinc is a ubiquitous component of chromatin, enzymes involved in transcription (*78*), and accessory transcription factors that relay a variety of intra- and intercellular signals to the nucleus and the transcriptional apparatus (*79*). Physiological and biochemical studies have suggested an equally wide array of metalloprotein functions (*79*). The three-dimensional structures of several Cys-rich zinc binding domains involved in DNA recognition (*80*) are known; these metal binding domains can also mediate protein-protein interactions, as discussed by Berg (*81*). Because the gross structural features of domains within a protein can be readily achieved through peptide secondary structures without resort to cofactors such as zinc, a question concerning the physiological function of zinc occupancy arises.

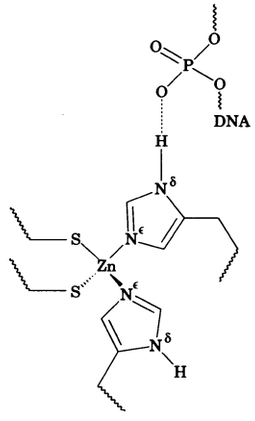
The importance of small metal-organized protein domains that chelate zinc ions through two Cys and two His side chains was first suggested for the *Xenopus* transcription factor IIIA (TFIIIA) (*82*, *83*). The structures of several of these zinc-organized domains have been elucidated with two-dimensional NMR methods and x-ray crystallography [for a recent review, see (*80*)]. Zinc plays a key structural role in three of Harrison's seven structurally characterized classes of DNA binding domains (*84*). In contrast to the MTs, which lack aromatic side chains, these zinc-stabilized domains use hydrophobic interactions in concert with the coordinate covalent Zn-S and Zn-N bonds to stabilize local protein structure. Recent x-ray crystallographic studies of the Zif268 protein (*85*) reveal that three TFIIIA-like zinc fingers together can specifically recognize a 9–base pair (bp) sequence of duplex DNA with each zinc finger domain contacting 2 bp within each 3-bp subsite (Fig. 6). A similar mode of recognition is likely for SP1 and TFIIIA interaction with DNA (*86*). The zinc coordination sphere in these finger domains has a net electrostatic charge of zero, and one of the histidines bound to zinc in the central finger forms a hydrogen bond to a phosphate oxygen in the DNA backbone (Scheme 2). The shortest distance between a zinc center and a phosphate oxygen is about 5 Å. Of the three classes of zinc motifs discussed here, the zinc centers of Zif268 make the closest approach to the DNA backbone.

Two other structural classes of metal-organized DNA binding domains are represented by the binuclear zinc center in GAL4 and a loop-helix domain of the glucocorticoid receptor. In both cases, the stereochemistry at the zinc centers is approximately tetrahedral. As with the Zif268 crystal structure, these two zinc peptide–DNA complexes represent a large class of homologous domains found in other transcription factors. Several other motifs consisting of some repetition of Cys and His residues within a short stretch of peptide have been shown to mediate sequence-specific binding to DNA. Although a large number of putative zinc-binding motifs have been proposed on the basis of sequence similarity, only those proteins that

have been demonstrated to bind metals as independent peptides or require metal in activity of the protein are shown in Table 2. Regulatory proteins in the steroid or nuclear receptor superfamily adopt similar zinc-dependent DNA binding motifs. As with the structurally established motifs, each of the sequences listed in Table 2 represents a large class of proteins that have functionally related DNA binding domains. It has been estimated that genes encoding "zinc finger" proteins could amount to 1% of the human genome and as much as 8% of chromosome 19 (*87*). Zinc may not be the operative metal in some of these cases.

The GAL4 structure (Fig. 7) (*88*) exhibits a common feature of metal-protein chemistry, namely cluster formation through a bridging cysteinyl thiolate. As with the TFIIIA-like fingers, the zinc anchors the loop between conserved cysteines and stabilizes a specific conformation. Several of these side chains then directly contact the DNA in a sequence-specific pattern. Unlike the TFIIIA-like zinc fingers, the metal ions are more buried in GAL4. The net electrostatic charge on each zinc ion has increased to -1, and the closest metal phosphate contact has increased to about 7 Å relative to the Zif268 structure.

As in the case of GAL4, the glucocorticoid receptor involves exclusively Cys coordination of Zn(II), but in contrast, none of the Cys residues bridge the two metal centers. Each zinc ion in the monomer plays a different role (Fig. 8): One positions the DNA recognition helix, and the other positions a loop involved in protein-protein interactions. Both zinc ions are more buried relative to the Zif268 centers, and four thiolates are coordinated to the divalent ion. The net electrostatic charge per zinc has increased to -2, and the closest approach of a zinc center to a phosphate oxygen has increased to about 8 Å. This distance is found for the NH_2-terminal zinc, which anchors the DNA recognition helix (Fig. 8).



Scheme 2. Hydrogen bonding between Zn-coordinated histidine and DNA backbone.

In each of these three classes, we find different interplay between the cationic metal center and the anionic phosphate backbone. The metal-phosphate distance increases as the number of cysteines coordinated to the zinc centers (and thus, the net negative charge on the coordination sphere) increases. This small trend is consistent with, but not decisive evidence for, an electrostatic role of the metal in the structure of the nucleoprotein complex. However, it presents a paradox of sorts. From electrostatic considerations alone, an increase in the number of Cys residues coordinated to a zinc ion beyond two should result in a weakly repulsive interaction of the DNA backbone with the metal center and thus provide an unfavorable free energy term in the protein-DNA binding interaction. Why then are the 3- and 4-Cys-containing motifs of zinc proteins, such as the nuclear receptor superfamily, so frequently used for binding to polyanions such as nucleic acids? Perhaps consideration of the biological functions of these proteins and the substitution chemistry of zinc can shed light on this issue. For instance, consider both the DNA and the apo forms of these proteins as ligands competing for zinc cations. Transition metals are known to bind to phosphate backbone and nucleobase moieties of nucleic acids in a manner that can alter the local structure (*89*). Specifically, micromolar Zn(II) can induce a 55° bend in DNA fragments containing the binding site for the prototypical zinc finger protein TFIIIA. Other cations, such as Mn(II), Ni(II), Co(II), Mg(II), and spermidine, have little or no effect (*90*). The competition between proteins and nu-

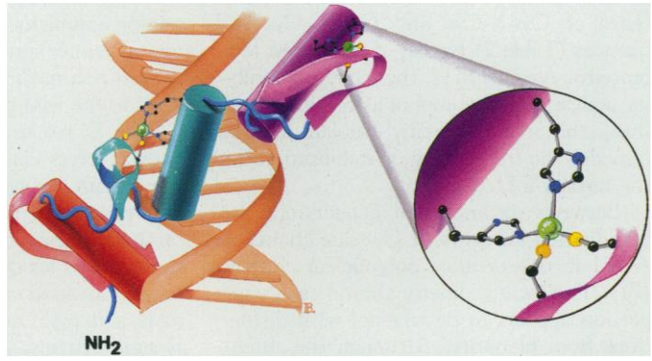


Fig. 6. Structure of the 90–amino acid DNA binding domain of Zif268 bound to an 11-bp DNA fragment. Each of three zinc binding domains is shown in a different color. (**Inset**) Zinc atoms, green; sulfur atoms of Cys, yellow; and carbon atoms, black. Note that zinc coordination to two cysteinyl thiolates and two histidines leaves an overall neutral charge at the zinc center. An Nε hydrogen atom of one histidine coordinated to Zn(II) in each domain also forms a hydrogen bond contact with a oxygen atom in phosphate backbone (Scheme 2). [Redrawn from (*85*)]

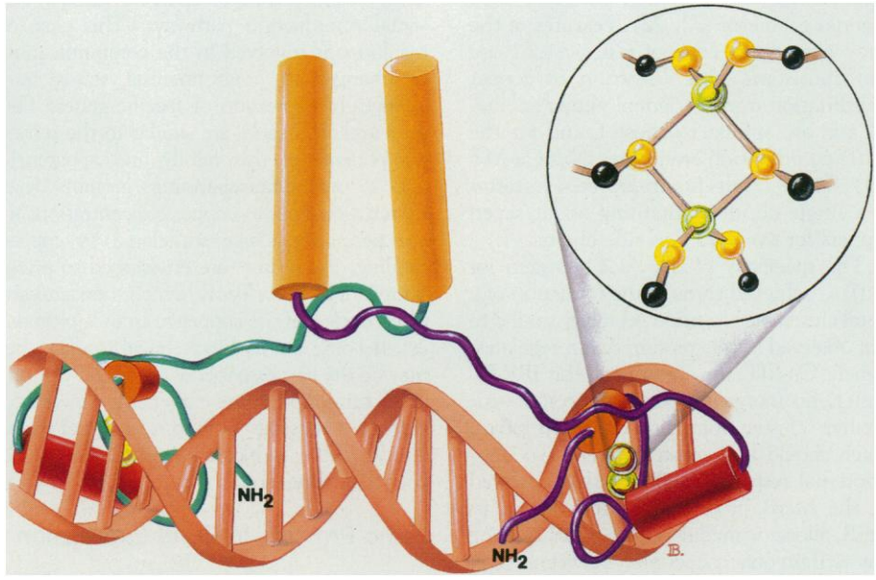


Fig. 7. Structure of the 65–amino acid DNA binding domain of GAL4 bound to a 17-bp DNA fragment. Each monomer of the dimeric DNA binding domain binds two zinc ions in a binuclear cluster arrangement. As originally shown in NMR studies by Pan and Coleman (*107*), two Cys residues bridge the zinc ions. The net charge per zinc center is -1, and the closest approach of zinc to the oxygen atoms on the DNA backbone is ~7 Å. [Redrawn from (*88*)]

cleic acids is of physiological relevance if one of the functions of these metalloproteins in their apo form is to remove tightly bound zinc ions from a condensed chromatin site in order to expose the binding sites for the sequence-specific regulatory proteins.

Structural Economy and Metalloregulatory Roles for Zinc

Concerning the role of zinc in DNA binding domains of transcription factors, the structural economy of this metal center in protein folding has been noted. In each of the categories of DNA binding modules described above, zinc ions cap either the NH_2- or COOH-terminus of what is termed the recognition helix. This α helix protrudes into the major groove of DNA and establishes sequence-specific contacts with bases and the backbone. Alpha helices are also the key recognition elements in other, but not all, DNA binding domains, such as the α-helical coiled coil, also known as a leucine zipper. In most of the above proteins, the recognition helix is buttressed by a core of supporting hydrophobic residues that essentially form a structural brace. As Luisi (*91*) elegantly framed it, "In the zinc modules, metal coordination, supplemented by hydrophobic interactions, carries out the corresponding architectural function with structural economy."

The question arises whether the spatial and thermodynamic advantages of using a transition metal simply to organize protein and nucleic acid structure are balanced by the necessity of supplying a cellular infrastructure that facilitates a posttranslational modification of the protein, namely Zn(II) insertion. If zinc is a ubiquitous and diffusable component of cells that can saturate the correct high affinity binding site of a metalloprotein upon its emergence from the ribosome, there is no problem. On the other hand, if zinc homeostasis is a tightly controlled process in which cellular machinery maintains a differential balance of zinc in organelles and the cytoplasm, then one might consider the cofactor requirement a physiological expense instead of an advantage. Although a good deal is known about the biochemistry and physiology of zinc, less is known about the cell biology of zinc (*78*), and we must look to calcium and iron for comparisons. Clearly in those cases, extensive cellular machinery is in place to maintain appropriate intracellular metal concentrations as a function of cell cycle or subcellular localization. Zinc may be an economical atom from a structural point of view; but, as with iron, low MW zinc complexes can catalyze biopolymer hydrolysis. As such, the concentration of the free ion may be kept very low through a network of Zn-specific storage and mobilization proteins including MT. The hidden cost associated with this homeostasis suggests a more complex role for the ubiquitous presence of metal-binding motifs among gene-regulatory proteins.

In zinc metalloregulation scenarios, the activity of subclasses of transcription factors could be controlled by changes in metal ion occupancy, which, in turn, are modulated in response to a variety of cellular signals. Does nature use the zinc occupancy of these proteins as a covalent, posttranslational modification that provides a pathway for intracellular information transfer? By analogy to phosphorylation, can accessory regulatory factors insert or remove zinc? Or could control of Zn(II) occupancy in regulatory proteins be achieved in a manner analogous to Ca(II)-calmodulin pathways with regulatory events that "gate" a pool of diffusable low MW zinc complexes? Although EDTA or physiological zinc chelators such as MT can remove zinc from SP1 or TFIIIA and inhibit their activities in vitro (*82*, *92–94*), these experiments do not establish a physiological role for zinc occupancy in regulation.

Alternatively, upon translocation to nuclear compartments, metal-binding regulatory proteins, such as protein kinase C, or nuclear receptors, such as the glucocorticoid receptor, may facilitate access of transcription factors to DNA targets by displacing metal ions from condensed chromatin. Metal ions, polyamines, and histone proteins are required to achieve extensive condensation of the anionic DNA into chromatin (*95*). Isolation and study of nuclear proteins of the scaffold, nuclear pores, and chromatin consistently reveals a high metal content (*95*). Interestingly, nuclear-pore complex proteins (Table 2) have been shown to contain Cys-X_2-Cys motifs.

Finally, are some of the metal-dependent transcription factors and regulatory proteins listed in Table 2 really zinc proteins in vivo? Many metals can substitute for zinc and stabilize the peptide structure (*96*). Note, for instance, the resemblance between bonafide zinc finger motifs and the iron- or heme-binding motifs of rubredoxin and cytochrome c_3 (Table 2). In the latter case, the cysteines bind the porphyrin, not the metal. Although many may adopt metal-induced folds analogous to those described here, in most cases it is too early to say that the physiologically relevant metal ion is Zn(II). The LIM motif (a Cys-rich sequence listed in Table 2) of lin-11 has been isolated and shown to contain zinc and an iron-sulfur cluster when the peptide is purified from *E. coli* (*97*). Another LIM-domain protein (cCRP) isolated from its endogenous source has no iron but two zinc ions per LIM motif (*98*). Recent studies of peptides corresponding to the DNA recognition motifs of the GATA-1 protein (Table 2), an erythroid-restricted transcription factor, indicate that sequence-specific DNA binding is obtained when Fe(II), Co(II), or Cd(II) is substituted into the metal-binding site (*99*). Furthermore, the iron-substituted peptide exhibits the highest affinity for DNA, binding about twice as tightly to DNA as the Zn(II), Co(II), and

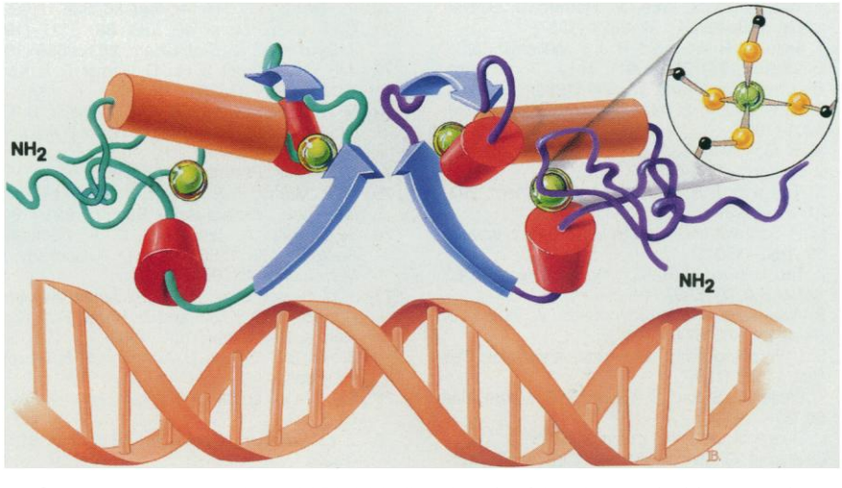


Fig. 8. Structure of the 86–amino acid DNA binding domain of the glucocorticoid receptor bound to a 19-bp DNA fragment. Note the presence of two zinc ions per monomer. Each zinc ion, including those closest to the DNA backbone, are coordinated through four Cys groups and the net charge on each zinc center is −2. The closest approach of the zinc center to oxygen atoms on the DNA backbone is ~8 Å. The latter are located at the NH_2-termini of the α helices involved in protein-DNA recognition (red). The two zinc ions furthest from the DNA stabilize the protein-protein interface and thus facilitate dimerization. [Redrawn from (*108*)]

Cd(II) peptides. As one muses about the nature of the metal that occupies these putative metal-containing transcription factors in vivo, it is necessary to consider the subcellular distribution of metals, transport mechanisms, storage sites, and insertion mechanisms (*31*). Unfortunately, these aspects of the cell biology of transition metals are still unclear.

Although most of the transcription factors with Cys and His motifs may well be zinc-binding proteins, skepticism is warranted in individual cases until metal content can be correlated with the physiological action or biochemical activity of the purified protein. Tests of zinc metalloregulatory models will require advances in our understanding of subcellular zinc localization and metal ion distribution as a function of the cell cycle. Another challenge is to gauge the metal occupancy of a variety of metalloproteins in the cytosol and nucleus in response to physiological stimuli.

Prospects

Many of the questions raised in the pursuit of metalloregulatory mechanisms depend on fundamental but unresolved issues of cell biology or aqueous coordination chemistry. Thus, they provide an impetus for development of stereochemistry, electronic structure, and substitution mechanisms of transition metals in distorted, low symmetry coordination environments typical of the biological milieu. Chemical studies of this broad class of specific metal ion receptors are providing new principles of molecular recognition for inorganic species and serve as a foundation for understanding the pharmacological and toxicological properties of metal ions in general. Summers and co-workers (*100–103*), for instance, have demonstrated that the zinc-binding Cys_3His domain of human immunodeficiency virus nuclear capsid protein (Table 2) is a target of an antiviral drug. As we grasp the complex regulatory circuitry of the cell and its interaction with the inorganic world as well as with other cells, the chemical and physical properties of the molecular switching components are providing new insights into both the inorganic chemistry and the biology of metals.

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Metal Compounds in Therapy and Diagnosis

Michael J. Abrams and Barry A. Murrer

There is increasing interest in the use of metal-containing compounds in medicine. This review describes several therapeutic applications, such as the use of platinum complexes in cancer chemotherapy, gold compounds in the treatment of arthritis, gallium in hypercalcemia, bismuth in anti-ulcer medication, and sodium nitroprusside in hypertension. The use of metal radionuclides in diagnosis and radiotherapy and the role of paramagnetic metal complexes as contrast agents in magnetic resonance imaging are also discussed.

Although most modern pharmaceuticals are purely organic compounds, the use of metal-containing agents for both therapy and diagnosis is of increasing interest. Perhaps it is not surprising that because of their unusual properties or value, or both, metals and metal compounds (such as preparations of iron, zinc, copper, gold, mercury, and bismuth) were used in medical practice from antiquity through the middle ages (*1*). In fact, the first modern chemotherapeutic agent was Erlich's arsphenamine, an organoarsenic compound. This drug, sold under the name Salvarsan, was introduced in 1910 and was the first effective treatment for syphilis (*2*).

The diverse therapeutic uses of metal compounds discussed in this article reflect the fact that most of these applications were discovered serendipitously. The discovery of diagnostic and radiotherapeutic agents containing metals has been the result of a somewhat more rational procedure in that specific chemistry was developed to take advantage of some physical characteristic of the metal atom such as radioactive decay or paramagnetism.

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Therapeutic Applications of Metal Compounds

Platinum antitumor drugs. The serendipitous aspect of inorganic drug research is nowhere better illustrated than in the discovery of platinum anticancer drugs. The observation in 1965 by Rosenberg (*3*) of the inhibition of bacterial division by cisplatin (Fig. 1) formed during electrolysis experiments with platinum electrodes in nutrient media led to the antiproliferative effects of the compound being applied to therapy of human tumors, and today platinum drugs are among the most active and widely used clinical agents for the treatment of advanced cancer. In testicular cancer, the addition of platinum drugs to treatment regimes has led to a dramatic increase in survival rate: Before cisplatin was available, only about 5% of patients were cured. Today 80 to 90% of patients can expect long-term disease-free survival. Platinum drugs also have clinical utility in the treatment of ovarian, bladder, head and neck, and small-cell lung cancer, and combination with other agents in these types of disease is still being explored. Proceedings of a recent international symposium summarize current developments (*4*).

The ultimate target for platinum antitumor drugs is DNA. The interactions of these drugs with DNA have been extensively reviewed (*5*), and it would appear that the Pt-GG intrastrand cross-link is the critical lesion that leads to cytotoxicity. Recently, proteins that bind to the Pt-DNA lesion have been identified that are homologous to the HMG1 protein, and indeed HMG1 itself can bind to the damaged area (*6*). Research continues to provide insight into the relevance of this discovery, which could lead to more specific and more active platinum drugs.

During early clinical trials it was clear that although cisplatin was an active antitumor agent, it was also extremely toxic; nephrotoxicity is now less of a problem, as intravenous hydration is usually given with a course of cisplatin. Nausea and vomiting are substantially controlled by the new generation of serotonin antagonists, leaving neurotoxicity as the major dose-limiting effect. An alternative approach has been to control the toxicities of cisplatin by design of a "second generation" drug. Antitumor testing of a series of analogs and kinetic studies on the loss of their leaving groups (chloride in the case of cisplatin) showed that antitumor activity was retained across a range of reactivity, but that the toxic side effects were directly related to the rate of ligand loss. This discovery led to preparation of a series of substituted malonate derivatives with reduced reactivity as compared with cisplatin, from which carbo-