View Article Online / Journal Homepage / Table of Contents for this issue

REVIEW

JAAS
www.rsc.org/jaas

Exploring the zinc proteome†

Wolfgang Maret‡

Center for Biochemical and Biophysical Sciences and Medicine, Department of Pathology, Harvard Medical School, Cambridge MA 02139, USA

Received 3rd July 2003, Accepted 8th October 2003
First published as an Advance Article on the web 24th October 2004

The advent of the complete sequences of several genomes offers unprecedented opportunities to identify all metalloproteins and to establish a comprehensive database for the role of essential metals in health and disease. This summary examines new aspects of zinc proteins, which are a major fraction of metalloproteins. Their coordination environments have typical spacings between the amino acids that provide the metal ligands. The recognition of such "signatures" spawned interest in searching sequence databases for possible zinc binding sites in other proteins, thus, in essence, performing genomic metal "analyses". In this manner, hundreds of catalytic zinc sites and thousands of structural zinc sites, many of which are generically referred to as zinc fingers, are predicted in the human proteome. However, dynamic zinc functions are not readily defined in these terms, suggesting that the number of zinc proteins (the zinc proteome) is significantly larger than the snapshot taken from currently known signatures. Protein interface sites, where zinc bridges proteins or their subunits, and inhibitory or regulatory sites, where zinc interacts with proteins transiently, are not all accounted for, nor are those for zinc in storage vesicles, transmembrane transporters, sensors, and proteins that participate in its cellular traffic. More structural work, experimental verification by analytical atomic spectroscopy, and speciation by hyphenated techniques, are needed before all signatures are defined and the complete zinc proteome can be functionally annotated.

Published on 24 October 2003. Downloaded on 22/01/2016 20:52:24.

From uncertain beginnings to a pervasive biological theme

Molecular zinc biology began just over 60 years ago with the discovery of carbonic anhydrase as a zinc enzyme.[1] Proof that a protein is a zinc protein rests upon purifying a sufficient amount to homogeneity and determining whether or not it contains a stoichiometric amount of zinc. Ineffective methods of protein purification and insensitive metal analyses impeded progress. In 1970, a count yielded about a dozen zinc enzymes.[2] Thereafter, rapidly improving analytical techniques accelerated the path of discovery so that by 1984 already more than 100 zinc enzymes were known.[3] During this period crystal structures of zinc enzymes became available. These structures served as standards of reference for "signatures" of the coordination environment of zinc in proteins.[4] Signatures are based on the types and number of metal ligands and the spacings between them in the sequence. Catalytic zinc sites generally contain three protein ligands, in addition to at least one water molecule, although additional protein ligands may sometimes be employed. A short spacer of amino acids separates the first two ligands, followed by a longer spacer to the third ligand.[5] With known signatures as references, sequence databases can be searched for signatures in related enzymes, for which structures are not yet available. If the signature is found, then the protein is *predicted* to contain zinc. This procedure is clearly not an analytical chemical method and calls for validation of specific results. In other words, one can predict that a site may bind zinc, but one can never be certain that it actually does until zinc has been measured directly. Yet, this heuristic procedure is exquisitely sensitive and specific, and moreover has proved to be very successful. It reaches the sensitivity of single molecule detection, as a gene can be amplified by PCR, sequenced, and its sequence analyzed. The underpinning of specificity is the rather bold assumption that biology evolved mechanisms to insert zinc and not any other metal ion into these sites. By and large, this assumption has stood the test of time for mammalian proteins, though it remains unknown where, when and how zinc proteins obtain their zinc. Prokaryotes appear to be more promiscuous in this regard. In some cases, they can either use different metal ions or have selected a metal ion other than zinc. Thus, selectivity is not necessarily embodied only in the signature itself, but rather influenced by the metalloregulatory system of the organism.

Significantly, it became possible to predict zinc sites and associated catalytic activities in completely different enzymes that have the same or similar signatures. A case in point is the recognition that the signature $HExxHx_nE$ (H = histidine, E = glutamate) of thermolysin is present in leukotriene A_4 (LTA_4) hydrolase.[4] Neither the zinc content nor the proteolytic function of this enzyme was known. When the prediction was tested, leukotriene A_4 hydrolase was indeed found to be a zinc-containing peptidase/esterase.[6,7] For predictions, the presence of ligands is as important as their absence. Thus, when the signature of thermolysin was compared with that in matrix metalloproteinases (MMPs), the absence of glutamic acid suggested that MMPs employ a different third ligand.[4] The structure of *Astacus* protease,[8,9] which became the founding member of an entire superfamily including MMPs, revealed that histidine in a novel signature $HExxHx_5H$ serves as the third ligand in MMPs.

Based on variations of a parent signature, enzymes are classified into superfamilies and families. The most comprehensive evolutionary tree has been constructed for metalloproteinases. Incidentally, their name does not draw attention to the fact that they are all zinc enzymes. In the human genome, the neutral zinc metallopeptidases have 87 members and rank

† Abbreviations: ZFP, zinc finger protein; PCR, polymerase chain reaction; MMP, matrix metalloproteinase; many of the acronyms used to describe proteins or protein domains such as the LIM or FYVE domain refer to three or more proteins in which the corresponding features were observed originally.

‡ Present address: Division of Human Nutrition, Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, 700 Harborside Drive, Galveston, TX 77555-1109, USA. Tel.: +1-(409) 772-4661. Fax: +1-(409) 772-6287.

DOI: 10.1039/b307540k

View Article Online

Published on 24 October 2003. Downloaded on 22/01/2016 20:52:24.

Table 1 Examples of signatures of catalytic zinc sites in mammalian enzymes[a]

Class I (Oxidoreductases)	
$Cx_{20}Hx_{106}C$	Alcohol dehdyrogenases
Class II (Transferases)	
$DxCx_{49-62}H$	Farnesyl and geranylgeranyl transferases
Class III (Hydrolases)	
$Hx_2Ex_{123}H$	Carboxypeptidases
Hx_3Hx_5H	Matrix metalloproteinases
$Hx_3H\cdots E$	Gluzincins such as LTA_4 hydrolase, neprilysin, angiotensin-converting enzyme
Others	Sonic hedgehog, GTP cyclohydrolase, adenosine deaminase
Class IV (Lyases)	
$Hx_1Hx_{22}H$	Carbonic anhydrases
Others	6-Pyruvoyltetrahydropterin synthase, glyoxalase I

[a] From ref. 11,12.

33rd among the most common protein families.[10] These proteinases and peptidases are the largest family of zinc enzymes in class III "hydrolases", one of the six classes of enzymes established in the enzyme nomenclature of the International Union of Biochemistry and Molecular Biology (IUBMB). Affirmed by the remarkable catalytic potential of zinc, zinc enzymes occur in other classes as well (Table 1). Catalytic zinc sites can contain two, or even three, zinc ions or zinc in combination with a different metal ion, *e.g.* copper, iron, magnesium. These additional metals have been called cocatalytic.[13] Cocatalytic sites often employ a bridging ligand such as side chains of aspartate/glutamate, histidine, or a carbamoylated lysine. Of course, this posttranscriptional modification of lysine cannot be detected in protein sequences. Despite the fact that ligands of cocatalytic sites are spread over a sizable portion of the entire protein, signatures of families are demarcated clearly in the primary structure. Superoxide dismutase, alkaline and acid phospatases, aminopeptidases and glyoxalase II are all mammalian zinc enzymes with cocatalytic metal sites.[11,12]

The discovery of zinc finger proteins (ZFPs) was a major step in defining the zinc proteome as it led to the recognition of a large number of zinc-dependent proteins. The term zinc finger was first used for the cysteine- and histidine-rich repeats in the sequence of *Xenopus laevis* transcription factor IIIA (TFIIIA).[14] In this classical zinc finger, zinc is bound in a C_2H_2 (C = cysteine, H = histidine) coordination motif and stabilizes a small protein domain that can "grip" DNA.[15] Over time, "zinc finger" became a generic term for structurally diverse zinc-binding motifs with functions in biological recognition of nucleic acids, other proteins, or lipids.[16] If one broadens the definition further "to include any compact domain stabilized by a zinc ion",[17] then the term also encompasses zinc-binding loops such as those in alcohol dehydrogenase and aspartate transcarbamoylase, which were known before the classical zinc finger motif was discovered, and one wonders whether or not this definition also includes other structural zinc sites that do not contain cysteine as a ligand, *e.g.* the structural zinc site in MMPs with a $HxDx_{12}Hx_{12}H$ (D = aspartate) signature. Since zinc does not stabilize a small domain in the zinc snap motif, which is a C_2H_2 motif, but "snaps" together the N- and C-terminal ends of *E. coli* 3-methyladenine DNA glycosylase I,[18] it appears that we are at a stage where exceptions test the rule. Therefore, in this article, we shall use the term zinc finger for zinc sites with four ligands, at least two of which are cysteines, *i.e.*, for C_2H_2, C_3H and C_4 motifs.

While most zinc enzymes were identified by direct analysis of zinc, database mining gained center stage for ZFPs. The success rate of finding zinc finger signatures in sequences is high, because the four ligands are relatively closely spaced and hence readily traced. Virtually any permutation of ligands is known (Table 2), and the ZFPs also can contain one, two or even three zinc ions. Further classification is based on three-dimensional structures. Seven protein folds in SCOP (structural classification of proteins)[32] are lumped together as one motif termed the treble clef finger.[33] It includes RING (really interesting new gene) fingers, protein kinase cysteine-rich domains, nuclear receptor-like fingers, LIM and FYVE domains, His-Me finger endonucleases and other domains that do not contain zinc. Some proteins do not reveal any sequence homology, perhaps as a result of the possibility that the motif has arisen independently multiple times.[34] The treble clef finger consists of a zinc knuckle, which is a conserved Cx_2C motif that provides two zinc ligands, a loop, a β-hairpin and an α-helix. The second pair of ligands is variable. Further topological classification puts zinc fingers into eight fold groups, among which the treble clef, the classical zinc finger, and the zinc ribbon account for the majority of all zinc fingers.[17] The remaining five groups are the Gag knuckle, the Zn_2C_6 motif, the TAZ2 domain, zinc-binding loops, and metallothionein.

Table 2 Examples of signatures of zinc finger proteins

Single zinc fingers—

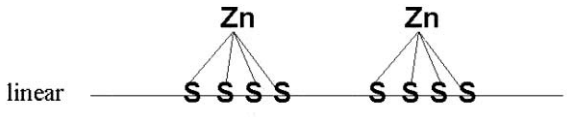
Motif	Example	Reference
C_2H_2		
CCHH	Classical zinc finger	19
HHCC	HIV-1 Integrase	20
CHHC	Zinc snap motif	18
C_3H		
CCCH	Smad3 MH1 domain	21
CCHC	Bir domain	22
CHCC	Ribosomal protein L44 (zinc ribbon)	16
HCCC	TAZ2 domain[a]	23
C_4		
CCCC	Zinc-bundle	24

Double zinc fingers—

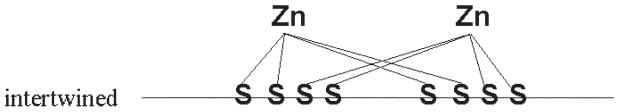
Motif	Example (domain) and consensus sequence		Coordination		Reference
CCCCHCCC	PHD	$Cx_2Cx_{9-21}Cx_{2-4}Cx_{4-5}Hx_2Cx_{12-46}Cx_2C$	C_4	C_3H	25
CCCHCCCC	RING	$Cx_2Cx_{9-39}Cx_{1-3}Hx_{2-3}Cx_2Cx_{4-48}Cx_2C$	C_3H	C_4	26
CCHCCCCC/H	LIM	$Cx_2Cx_{17-19}Hx_2Cx_2Cx_2Cx_{16-20}Cx_2C/H$	C_3H	C_4/C_3H	27
CCHCHCCC	DM	$Cx_2Cx_2Hx_8Hx_{3-4}Cx_4CxCx_{2-3}C$	C_3H	C_3H	28
HCCCCHCC	PKC	$Hx_{12}Cx_2Cx_{13}Cx_2Cx_4Hx_2Cx_7C$	C_3H	C_3H	29
CCCCCCCC	FYVE	$Cx_2Cx_{12}Cx_2Cx_4Cx_2Cx_{16}Cx_2C$	C_4	C_4	30
CCCCCCCC	DnaJ	$Cx_2C\cdots Cx_2C\cdots Cx_2C\cdots Cx_2C$	C_4	C_4	31

[a] Contains three zinc ions in the domain

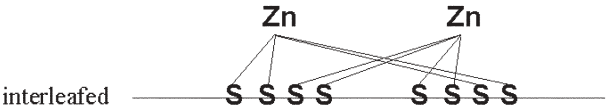
View Article Online



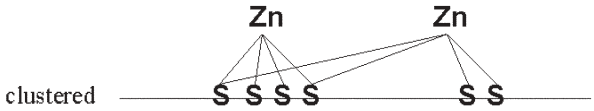
Scheme 1 Scheme 1.



Scheme 2 Scheme 2.



Scheme 3 Scheme 3.



Scheme 4 Scheme 4.

In mono-zinc enzymes, the ligands bind in the order in which they occur in the linear sequence. ZFPs and other proteins with multiple zinc sites, however, can harbor unexpected features that are clearly not evident from inspection of their linear sequences. In addition to utilizing the sulfur ligands of cysteine as they occur in the linear sequence (Scheme 1, example: LIM domain), the ligands can be intertwined/cross-braced (Scheme 2, example: FYVE domain) or interleafed (Scheme 3, example: DnaJ cysteine-rich domain). Another level of organization in so-called zinc/thiolate clusters has the ligands not only intertwined but also utilized twice (Scheme 4, example: Zn_2C_6 cluster motif). This binding mode is possible because a thiolate can serve as a bridging ligand. In metallothionein, the ligand-binding pattern is so complicated that there is no rational way of predicting how twenty ligands bind seven metal ions. Each zinc ion has four sulfur ligands from cysteines. In principle, 28 ligands would be required to supply seven metal ions with four ligands. Because only twenty ligands are available, eight of them are bridging ligands. There are two zinc/thiolate clusters in metallothionein.[35,36] In the β-domain of mammalian metallothioneins, nine thiolates bind three metals with three bridges. Such 3-zinc/thiolate clusters have now also been found in one domain of a family of lysine methyltransferases.[37] In the α-domain of metallothionein, eleven thiolates bind four metals with five bridges. So far, this 4-zinc/thiolate cluster is found only in metallothionein. With increase in the number of zinc atoms in these clusters the number of ligands per zinc is reduced, while maintaining tetracoordination of each metal, *i.e.*, ZnC_4 (1 : 4), Zn_2C_6 and Zn_3C_9 (1 : 3), and Zn_4C_{11} (1 : 2.9). Such binding is thought to establish thermodynamically stable, but kinetically labile, sites that are poised for metal transfer.[38]

Once the first genome sequences became available one could use this information to estimate the number of ZFPs in a given proteome[39] and to search for individual zinc enzyme families. The summation from different genomes in Table 3 is for the four most abundant ZFPs only. The frequency of ZFPs increases with the complexity of the organism, likely a reflection of the function of these proteins in development and regulation of multicellular organisms.[39] Some domains have been amplified specifically in certain organisms. In humans, the classical zinc finger is the most abundant SCOP superfamily.[40] Of these particular signatures (Table 3), none are found in *E. coli*. By no means does this lack imply that *E. coli* has no zinc proteins. Many zinc enzymes and also proteins with ZnC_4 motifs are present in bacteria. The Zn_2C_6 cluster occurs only in yeast and fungi. These observations emphasize that the zinc proteome of a given organism is a selection of all possible coordination motifs that have been realized in biology.

Table 3 Number of the most abundant zinc finger proteins in different genomes (InterPro Domains)[a]

Domain	*E. coli*	Yeast	Worm	Mustard weed	Mouse	Human
Finger	0	48	151	115	535[b]	706[c]
RING	0	35	126	379	112	210
LIM	0	4	36	8	74	81
PHD	0	16	24	71	70	84
Sum	0	103	337	573	791	1081

[a] The data for *E. coli* and mouse are from ref. 39 and from the ensembl database (http://www.ensembl.org), respectively. The data for the other genomes are from ref. 10. [b] Broken down in the ensembl database into different groups on 4/30/03. [c] The total number of classical zinc finger domains in humans is 5092.[40] This number and the one given in the Table differ, because on average each ZFP contains seven fingers, with a maximum of 36 fingers.

Toward a functional annotation of the zinc proteome

While already impressive in scope, the zinc proteome as just described is incomplete because it accounts mainly for the catalytic and structural functions of zinc with known signatures. As such it represents only a fraction of all functions of zinc.

There are many examples where zinc ions bridge subunits of the same or different proteins. In some of these protein interface zinc sites[11,12] three ligands are on one chain and constitute a signature that resembles that of catalytic zinc sites. This coordination leaves one coordination position for the docking of a ligand of another protein. In most cases, predictions are virtually impossible because the ligands are on different proteins and because bidentate motifs such as Cx_nC have functions other than metal-binding. Protein interface zinc sites are important elements both in establishing permanent and in modulating transient protein–protein interactions.[41] Their functions include catalysis, inhibition of enzymatic or other activity, assembly and disassembly of multisubunit macromolecular complexes, and formation of protein/receptor complexes. The dependence of the interaction on zinc ions, whose availability is controlled tightly, suggests new ways of regulating protein supramolecular assembly and quaternary structure. In addition, zinc sites with cysteine ligands may be susceptible to oxidation, because reactions with the redox-active sulfur ligand control the binding of the redox-inert zinc ion.[42] An example pertains to nitric oxide synthase, where oxidation of the thiolate ligands of the zinc interface site uncouples enzymatic activity.[43] In the so-called zinc hook motif,[44] a zinc dimerization motif with a Cx_2C signature on both the nuclease Mre11 and on the ATPase Rad50 establishes an architecture that is the basis for the biological function of these proteins in homologous recombination and non-homologous end-joining of DNA. A ZnC_4 dimerization motif has also been observed in a zinc clasp structure that tethers the protein tyrosine phosphatase Lck to the T-cell co-receptors CD4 and CD8.[45] Whether or not this interaction is also redox-modulated is unknown. Other protein interface zinc sites occur in integral membrane proteins such as potassium channels[46]

Published on 24 October 2003. Downloaded on 22/01/2016 20:52:24.

View Article Online

Published on 24 October 2003. Downloaded on 22/01/2016 20:52:24.

and in the γ-aminobutyric acid (GABA) receptor, where zinc inhibits.[47]

Some enzymes are reversibly inhibited by low nanomolar concentrations of zinc.[48] Since fluctuations of cellular zinc occur in this concentration range,[49,50] modulation of these sites by zinc is thought to be physiologically significant. These zinc-inhibited proteins, the signatures of which are also unknown, have not been recognized as zinc proteins, because zinc ions dissociate during their isolation. Such regulatory functions of zinc affect energy metabolism, mitochondrial function, and cellular signal transduction. In the context of discussions that zinc is a signalling ion,[51] there is the potential for a relatively large number of zinc-regulated proteins that would all be part of the zinc proteome. Finally, mediated by zinc-dependent transcription factors, zinc affects gene expression. cDNA microarray and quantitative PCR technologies identified many proteins that are either positively or negatively regulated by dietary zinc levels in mice[52] or by changing zinc levels in growth media of cultured cells.[53]

A third recent development is the discovery of a cellular zinc homeostatic system. It consists of families of membrane transporter proteins, which regulate cellular and vesicular influx and efflux of zinc, sensors, and cytosolic proteins such as metallothionein/thionein, which participate in zinc traffic between organelles.[54] These proteins employ new mechanisms of zinc binding and release, which are realized in yet additional coordination environments with characteristic signatures and chemical properties that make it possible to perform these uniquely biological functions. Membrane transporters have histidine-rich motifs ($(HX)_n$ repeats) that are thought to be potential zinc-binding domains,[55] and the *E. coli* zinc transport protein ZntA has a novel signature with two cysteine and one aspartate ligand.[56]

Classification on the basis of signatures and topologies, *i.e.*, structure alone, is unsatisfactory. In parallel to the speciation of zinc proteins in terms of signatures, we need to understand the function(s) of each signature. A functional annotation of the zinc proteome will include these new functions as well as others yet to be defined. Classifying all zinc sites that have no apparent function as "structural", is misleading. Therefore, even known sites are annotated incompletely. Apart from the fact that zinc can have a structural function in catalytic sites, zinc turns out to have catalytic or other functions in sites that were once believed to be solely structural. Some zinc finger sites can be redox-active under physiological conditions[57] or catalytically active, *e.g.* in the *E. coli* ADA protein.[58] When 207 zinc fingers from 92 experimental protein structures were analyzed with regard to how the proteins shield their zinc cores electrostatically, the ADA protein, human immunodeficiency virus nucleocapsid protein P7, and the C-terminal finger of the estrogen receptor were identified as labile and reactive ZFPs.[59] It appears that we are just beginning to explore the functional potential of zinc in biology.

The zinc proteome as part of the zinc metallome

Even if predicted to contain zinc, proteins might not be isolated as zinc metalloproteins, because their zinc sites are kinetically labile and hence do not contain zinc permanently. At present, we simply do not know how many additional signatures of zinc sites exist. Estimates are that we have structural information for about 40% of the human proteome.[40] Genome analyses with the tools of bioinformatics alone will not suffice to define the zinc proteome. Identification of the interaction of zinc with proteins, structural analyses of binding sites, and metal analyses by atomic spectroscopy continue to lay the foundation for the discovery of new signatures that are the basis for future searches. Above and beyond, zinc proteomics has a quantitative dimension. In any event all these zinc species have to add up to the total of about 3 g of zinc in a human.[60] This could mean that not only do we have to account for all zinc proteins, but also for the zinc metallome,[61] *i.e.*, all possible zinc functions that may be related to biomolecules other than zinc proteins.

In biology, zinc has only one valence state (+2) and for the most part only three protein ligands: cysteine, histidine, and glutamate/aspartate. Yet, based on these few partners, biology developed an extraordinarily rich zinc coordination chemistry and variability where all possibilities of coordination are realized, *i.e.*, three modes of binding for histidine (with either one of the imidazole nitrogens or with both in bridging metals), three for glutamate/aspartate (with one oxygen, both oxygens or both in bridging metals) and two for cysteine (as ligand for one zinc or bridging two zinc ions), with participation of outer-sphere ligands or occasional recruitment of other ligands. From the way biology has utilized zinc and found solutions for its specific requirements, the analytical chemist can learn a lot about selectivity and the biomimetic chemist about function—a burgeoning field, indeed.

Tools

Unlike other transition elements, divalent zinc has a filled d-shell of electrons and therefore is spectroscopically "silent" for most techniques that are commonly employed for studying the structure and function of metal ions in biology. However, cobalt(II) can be used as a spectroscopic probe for zinc proteins.[62] In protein sites, cobalt(II) is virtually isostructural and isofunctional with zinc, but differences in the electronic properties of the two ions allow the role of the metal in the protein to be probed. Thus, substitution of zinc by cobalt can affect pK_a values of bound ligands and specific rates of ligand binding and dissociation. Cobalt cannot be employed for *in vivo* studies of the functions of zinc, however. In order to study the dynamics of zinc in the cell or in the intact organism, zinc isotopes, both radioisotopes and stable isotopes,[63] are useful, but more significantly, fluorescent probes now allow determination of fluctuations of cellular zinc, study of zinc transfer between proteins, and imaging zinc *in vivo*.[64] These new tools in zinc research clearly reflect the shift of focus toward studies of the dynamic aspects of zinc biology.

References

1 D. Keilin and T. Mann, *Biochem. J.*, 1940, **34**, 1163.
2 B. L. Vallee and W. Wacker, *The Proteins*, ed. H. Neurath, Academic Press, New York, 2nd edn., 1970, vol. 5.
3 B. L. Vallee and A. Galdes, *Adv. Enzymol. Relat. Areas Mol. Biol.*, 1984, **56**, 283.
4 B. L. Vallee and D. S. Auld, *Biochemistry*, 1990, **29**, 5647.
5 B. L. Vallee and D. S. Auld, *FEBS Lett.*, 1989, **257**, 138.
6 J. Z. Haeggström, A. Wetterholm, R. Shapiro, B. L. Vallee and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, 1990, **172**, 965.
7 J. Z. Haeggström, A. Wetterholm, B. L. Vallee and B. Samuelsson, *Biochem. Biophys. Res. Commun.*, 1990, **173**, 431.
8 W. Stöcker, R. L. Wolz, R. Zwilling, D. J. Strydom and D. S. Auld, *Biochemistry*, 1988, **27**, 5026.
9 W. Bode, F. X. Gomis-Ruth, R. Huber, R. Zwilling and W. Stöcker, *Nature*, 1992, **358**, 164.
10 International Human Genome Sequencing Consortium, *Nature*, 2001, **409**, 860.
11 D. S. Auld, in *Handbook on Metalloproteins*, eds. I. Bertini, A. Sigel and H. Sigel, Marcel Dekker, Basel, Switzerland, 2001, 881.
12 D. S. Auld, *BioMetals*, 2001, **14**, 271.
13 B. L. Vallee and D. S. Auld, *Biochemistry*, 1993, **32**, 6493.
14 J. Miller, A. D. McLachlan and A. Klug, *EMBO J.*, 1985, **4**, 1609.
15 A. Klug and J. W. R. Schwabe, *FASEB J.*, 1995, **9**, 597.
16 J. H. Laity, B. M. Lee and P. E. Wright, *Curr. Opin. Struct. Biol.*, 2001, **11**, 39.
17 S. S. Krishna, I. Majumdar and N. V. Grishin, *Nucl. Acids Res.*, 2003, **31**, 532.
18 K. Kwon, C. Cao and J. T. Stivers, *J. Biol. Chem.*, 2003, **278**, 19442.

View Article Online

19 M. S. Lee, G. P. Gippert, K. V. Soman, D. A. Case and P. E. Wright, *Science*, 1989, **245**, 635.
20 M. Cai, R. Zheng, M. Caffrey, R. Craigie, G. M. Clore and A. M. Gronenborn, *Nature Struct. Biol.*, 1997, **4**, 567.
21 J. Chai, J.-W. Wu, N. Yan, J. Massagué, N. P. Pavletich and Y. Shi, *J. Biol. Chem.*, 2003, **278**, 20327.
22 C. Sun, M. Cai, A. H. Gunasekera, R. P. Meadows, H. Wang, J. Chen, H. Zhang, W. Wu, N. Xu, S. C. Ng and S. W. Fesik, *Nature*, 1999, **401**, 818.
23 R. N. De Guzman, H. Y. Liu, M. Martinez-Yamout, H. J. Dyson and P. E. Wright, *J. Mol. Biol.*, 2000, **303**, 243.
24 C. D. Mackereth, C. H. Arrowsmith, A. M. Edwards and L. P. McIntosh, *Proc. Natl. Acad. Sci. USA*, 2000, **97**, 6316.
25 A. D. Capili, D. C. Schultz, F. J. Rauscher, III and K. L. B. Borden, *EMBO J.*, 2001, **20**, 165.
26 P. N. Barlow, B. Luisi, A. Milner, M. Elliott and R. Everett, *J. Mol. Biol.*, 1994, **237**, 201.
27 G. Pérez-Alvarado, C. Miles, J. W. Michelson, H. A. Louis, D. R. Winge, M. C. Beckerle and M. F. Summers, *Nature Struct. Biol.*, 1994, **1**, 388.
28 L. Zhu, J. Wilken, N. B. Phillips, U. Narendra, G. Chan, S. M. Stratton, S. B. Kent and M. A. Weiss, *Genes Dev.*, 2000, **14**, 1750.
29 U. Hommel, M. Zurini and M. Luyten, *Nature Struct. Biol.*, 1994, **1**, 383.
30 T. Kutateladze and M. Overduin, *Science*, 2001, **291**, 1793.
31 M. Martinez-Yamout, G. B. Legge, O. Zhang, P. E. Wright and H. J. Dyson, *J. Mol. Biol.*, 2000, **300**, 805.
32 L. LoConte, B. Ailey, T. J. Hubbard, S. E. Brenner, A. G. Murzin and C. Clothia, *Nucl. Acids Res.*, 2000, **28**, 257.
33 N. V. Grishin, *Nucl. Acids Res.*, 2001, **29**, 1703.
34 B. K. Sharpe, J. M. Matthews, A. H. Y. Kwan, A. Newton, D. A. Gell, M. Crossley and J. P. MacKay, *Structure*, 2002, **10**, 639.
35 A. Arseniev, P. Schultze, E. Wörgötter, W. Braun, G. Wagner, M. Vašák, J. H. R. Kägi and K. Wüthrich, *J. Mol. Biol.*, 1988, **201**, 637.
36 A. H. Robbins, D. E. McRee, M. Williamson, S. A. Collett, N. H. Xuong, W. F. Furey, B. C. Wang and C. D. Stout, *J. Mol. Biol.*, 1991, **221**, 1269.
37 X. Zhang, H. Tamaru, S. I. Khan, J. R. Horton, L. J. Keefe, E. U. Selker and X. Chen, *Cell*, 2002, **111**, 117.
38 W. Maret, K. S. Larsen and B. L. Vallee, *Proc. Natl. Acad. Sci. USA*, 1997, **94**, 2233.
39 N. D. Clarke and J. M. Berg, *Science*, 1998, **282**, 2018.
40 A. Müller, R. M. MacCallum and M. J. E. Sternberg, *Genome Res.*, 2002, **12**, 1625.
41 W. Maret, in *Handbook of Metalloproteins*, ed. A. Messerschmidt, Wiley, Chichester, UK, 2003, in the press.
42 W. Maret and B. L. Vallee, *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 3478.
43 M.-H. Zou, C. Shi and R. A. Cohen, *J. Clin. Invest.*, 2002, **109**, 817.
44 K.-P. Hopfner, L. Craig, G. Moncalian, R. A. Zinkel, T. Usui, B. A. L. Owen, A. Karcher, B. Henderson, J.-L. Bodmer, C. T. McMurray, J. P. Carney, J. H. J. Petrini and J. A. Tainer, *Nature*, 2002, **418**, 562.
45 P. W. Kim, Z.-Y. J. Sun, S. C. Blacklow, G. Wagner and M. J. Eck, *Science*, 2003, **301**, 1725.
46 K. A. Bixby, M. H. Nanao, N. V. Shen, A. Kreusch, H. Bellamy, P. J. Pfaffinger and S. Choe, *Nature Struct. Biol.*, 1999, **6**, 38.
47 A. M. Hosie, E. L. Dunne, R. J. Harvey and T. G. Smart, *Nature Neurosci.*, 2003, **6**, 362.
48 W. Maret, C. Jacob, B. L. Vallee and E. H. Fischer, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 1936.
49 D. Atar, P. H. Backx, M. M. Appel, W. D. Gao and E. Marban, *J. Biol. Chem.*, 1995, **270**, 2473.
50 B. Turan, H. Fliss and M. Désilets, *Am. J. Physiol.*, 1997, **272**, H2095.
51 C. Frederickson, *Sci. STKE*, 2003, pe18.
52 J. B. Moore, R. K. Blanchard and R. J. Cousins, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 3883.
53 R. J. Cousins, R. K. Blanchard, M. P. Popp, L. Liu, J. Cao, J. B. Moore and C. L. Green, *Proc. Natl. Acad. Sci. USA*, 2003, **100**, 6952.
54 W. Maret, *J. Nutr.*, 2003, **133**, 1460S.
55 L. A. Gaither and D. J. Eide, *BioMetals*, 2001, **14**, 251.
56 L. Banci, I. Bertini, S. Ciofi-Baffoni, L. A. Finney, C. E. Outten and T. V. O'Halloran, *J. Mol. Biol.*, 2002, **323**, 883.
57 S.-H. Lee and W. Maret, *Antiox. Redox Signal.*, 2001, **3**, 531.
58 L. C. Myers, G. L. Verdine and G. Wagner, *Biochemistry*, 1993, **32**, 14089.
59 A. T. Maynard and D. G. Covell, *J. Am. Chem. Soc.*, 2001, **123**, 1047.
60 R. A. McCance and E. W. Widdowson, *Biochem. J.*, 1942, **36**, 392.
61 R. J. P. Williams, *Coord. Chem. Rev.*, 2001, **216–217**, 583.
62 W. Maret and B. L. Vallee, *Methods Enzymol.*, 1993, **226**, 52.
63 N. F. Krebs and K. M. Hambidge, *BioMetals*, 2001, **14**, 397.
64 R. B. Thompson, D. Peterson, W. Mahoney, M. Cramer, B. P. Maliwal, S. W. Suh, C. Frederickson, C. Fierke and P. Herman, *J. Neurosci. Methods*, 2002, **118**, 63.

Published on 24 October 2003. Downloaded on 22/01/2016 20:52:24.