Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9984–9988, November 1991
Biochemistry

Zinc transfer from transcription factor IIIA fingers to thionein clusters

(metallothionein/zinc fingers/zinc clusters)

JIN ZENG*, BERT L. VALLEE†‡, AND JEREMIAS H. R. KÄGI*‡

*Biochemisches Institut der Universität Zürich, Winterthurerstrasse 190, CH-8057, Zürich, Switzerland; and †Center for Biochemical and Biophysical Sciences and Medicine, Harvard Medical School, 250 Longwood Avenue, Boston, MA 02115

Contributed by Bert L. Vallee, August 12, 1991

ABSTRACT **The rapid induction of thionein (apometallothionein) by many endogenous stimuli such as steroid hormones, cytokines, and second messengers suggests that this cysteine-rich, metal binding protein participates in an as yet undefined role in cellular regulatory processes. This study demonstrates with DNA and RNA binding assays and *in vitro* transcription measurements that thionein suppresses the binding of the *Xenopus laevis* zinc finger transcription factor IIIA (TFIIIA) to 5S RNA and to the 5S RNA gene and abrogates the capacity of TFIIIA to initiate the RNA polymerase III-catalyzed synthesis of 5S RNA. The effect is reversed by the addition of zinc and is not observed in the TFIIIA-independent transcription of a tRNA gene by the same RNA polymerase. In view of the strong tendency of thionein to complex posttransition metals such as zinc, one effect of its enhanced synthesis *in vivo* could be to reduce the intracellular disposability of zinc and thus modulate the actions of zinc-dependent enzymes and proteins, most notably those of the zinc finger transcription factors.**

Zinc is a constituent of many proteins and enzymes and as such is indispensable to catalysis, gene expression, and intracellular signaling. This functional variability has been attributed to its versatile chemistry, which, though seemingly prosaic, turns out to be ideally suited for deployment in biomacromolecular systems to subserve widely different biological processes. One of these properties is the stability of the Zn(II) oxidation state in a range of biological media, another is the multiplicity of its coordination number, and a third is its several and adaptable coordination geometries. Together these combine to generate a remarkably flexible spectrum of chemical affinities and reactivities (1). At present four major classes of zinc proteins can be distinguished: the >300 enzymes whose catalytic activity or structural integrity requires the presence of zinc, the seemingly innumerable zinc-dependent transcription factors that regulate long- and short-term gene expression, the glucocorticosteroid and other receptors, and the zinc cluster-bearing metallothioneins (1, 2).

The metallothioneins are ubiquitous proteins that were discovered almost contemporaneously with the first zinc enzymes, but a precise understanding of their biological role(s) has yet to be achieved. The mammalian forms contain 61 amino acid residues, among them 20 cysteines that bind a total of seven zinc (or cadmium) ions in two thiolate clusters—i.e., Zn_3Cys_9 and Zn_4Cys_{11}—located within the amino- and carboxyl-terminal domains, respectively (2). These structures, for which there are no appropriate inorganic models, combine high thermodynamic stability of metal binding with high kinetic lability of metal exchange, which may be characteristic. Thus, although their binding constants for zinc are comparable in magnitude to those of zinc metalloproteins, the rates at which they transfer metals upon exposure to chelating agents (4) and exchange metals among metallothionein isoforms (5, 6) are much higher. This kinetic lability of the zinc thiolate clusters allows them to play a dynamic metabolic role in which thionein and its conjugate zinc metallothionein serve as intracellular distributors and mediators of zinc wherever and whenever needed for whatever purpose (7). Thus, the thionein/metallothionein couple may be viewed as a collation/allocation system, regulating the availability of zinc pivotal to multiple and diverse functions on demand (8). Based on its unusual composition and structure, the participation of metallothionein in embryonic development, cellular differentiation, and the transmission of genetic information and cell proliferation was anticipated and predicted long before the relevant biochemistry became known and understood (9).

A number of studies have demonstrated that some apoenzymes can acquire zinc from metallothionein under *in vitro* conditions (10), but the *in vivo* significance of this phenomenon has not been confirmed. It is of interest that although metallothionein does not donate its zinc to the zinc-depleted form of the vertebrate zinc finger transcription factor Sp1 (11), an excess of the apoprotein thionein can abstract zinc from the native, zinc-containing form of Sp1, which consequently loses its capacity to bind to its cognate DNA and to activate downstream gene transcription by RNA polymerase II (12).

The present study demonstrates that thionein also removes zinc from the *Xenopus laevis* zinc finger protein transcription factor IIIA (TFIIIA) in an analogous manner, thereby interfering with its functional properties. TFIIIA has been reported to contain from two to nine zinc atoms per molecule (13, 14), a variation that could be physiological or analytical. The following data show that increased thionein biosynthesis could suppress the binding of TFIIIA to its cognate DNA and to 5S RNA as well as its activating effect on 5S RNA gene transcription by RNA polymerase III.

MATERIALS AND METHODS

Cell Line. HeLa cells were maintained in Joklik modified minimal essential culture medium at 37°C.

Plasmids. The construction of 5S RNA and tRNA genes has been described (15, 16).

Purification of 7S Ribonucleoprotein (7S RNP) and TFIIIA. The 7S RNP was prepared from immature *X. laevis* oocytes by glycerol gradient centrifugation and DEAE-cellulose chromatography (17, 18). TFIIIA (M_r 38,600) was separated from 5S RNA by ammonium sulfate precipitation and quantified by the Bradford method with bovine serum albumin as

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TFIIIA, transcription factor IIIA; 7S RNP, 7S ribonucleoprotein; ICR, internal control region of the 5S RNA gene.

‡To whom reprint requests should be addressed.

a standard (19). TFIIIA is assumed to bind nine zinc ions maximally.

Preparation of Metallothionein and Thionein. $(Cd,Zn)_7$-metallothionein was purified from the livers of rabbits injected with $CdCl_2$; Zn_7-thionein was isolated from human liver (20). Thionein (M_r 6100) was prepared from metallothionein by acidification with HCl and removal of the metal by gel filtration in 10 mM HCl (21). Immediately before use, thionein was neutralized with 0.1 M Tris·HCl (pH 8.0).

Preparation of 5S RNA Gene Deoxyoligonucleotide Probe. A 50-base-pair (bp) deoxyoligonucleotide with the sequence of the internal control region of the 5S RNA gene (ICR) (22) was synthesized with an Applied Biosystems DNA synthesizer. After deprotection in ammonium hydroxide and lyophilization the DNA segment was purified by electrophoresis on a 12% sequencing gel.

Gel Retardation Assays for Binding of TFIIIA to DNA. The ^{32}P end-labeled ICR deoxyoligonucleotide probe was incubated with ≈200 ng of TFIIIA for 20 min at room temperature in an 18-μl volume containing 30 mM Tris·HCl (pH 7.5), 70 mM KCl, 2 mM $MgCl_2$, 0.5 mM dithiothreitol, 6% glycerol, 0.1% Nonidet P-40, 2 μg of poly(dI-dC), and 9 μM $ZnCl_2$, the last added to activate TFIIIA as previously suggested (14). Complex formation with TFIIIA was monitored by the retardation of the movement of the DNA probe on electrophoresis in a 6% native polyacrylamide gel run in 89 mM Tris borate buffer (pH 8.3) without EDTA. Gels were dried and exposed to x-ray film. To study the effects of thionein or of Zn_7-thionein on TFIIIA–DNA complex formation prior to incubation with the ICR probe, TFIIIA (final concentration, around 300 nM) was preincubated with thionein or Zn_7-thionein at room temperature for 20 min. To reverse the effect of thionein by zinc, appropriate amounts of $ZnCl_2$ were added together with the ICR probe and complex formation was allowed to proceed for another 20 min.

Gel Retardation Assays for Binding of TFIIIA to 5S RNA. Approximately 200 ng of TFIIIA was incubated on ice with 5S RNA, 3′ labeled with 5′-[^{32}P]pCp, with T4 RNA ligase (23) for 5 min in a 19-μl volume containing 30 mM Tris·HCl (pH 7.5), 70 mM KCl, 2 mM $MgCl_2$, 0.5 mM dithiothreitol, 6% glycerol, 0.1% Nonidet P-40, 2 μg of poly(dI-dC), 25 units of RNase inhibitor, and 9 μM $ZnCl_2$. To test if thionein or Zn_7-thionein inhibits complex formation, TFIIIA (final concentration, around 300 nM) was mixed with these agents on ice for 15 min before 5S RNA was added. Electrophoresis was performed with a 6% native polyacrylamide gel in 89 mM Tris borate buffer (pH 8.3) without EDTA. Gels were dried and exposed to x-ray film. To restore TFIIIA binding to 5S RNA in the presence of thionein by addition of zinc, appropriate amounts of $ZnCl_2$ were added together with 5S RNA and the mixture was incubated on ice for another 5 min.

***In Vitro* Transcription Assays.** The transcription of 5S RNA and $tRNA^{arg}$ genes and the isolation of the transcripts for analysis were carried out as described with minor modifications (24). HeLa cell nuclear extract prepared according to Dignam *et al.* (24) was used in the reaction. For 5S RNA gene transcription the reaction mixtures were supplemented with 0.8 μM purified TFIIIA. The plasmids containing the 5S RNA gene and the $tRNA^{arg}$ gene served as transcription templates at final concentrations of 5 μg/ml and 20 μg/ml, respectively. To study the effects of thionein and Zn_7-thionein, TFIIIA and nuclear extract were incubated with these agents on ice for 30 min before starting the transcription reaction by the addition of the plasmids and nucleotides.

RESULTS AND DISCUSSION

Thionein Inhibits Binding of TFIIIA to 5S RNA Gene. The chelating agents 1,10-phenanthroline and EDTA are known to reduce binding of *X. laevis* TFIIIA to the 5S RNA gene and to its transcription product, 5S RNA (13, 14). Thionein is one of the most potent naturally occurring metal binding agents and, hence, its effect on the interaction of this zinc finger protein with its cognate nucleic acids was investigated by means of a gel retardation assay. The 50-bp ICR DNA segment of the 5S RNA gene (22) was synthesized chemically and used as a TFIIIA binding site subsequent to ^{32}P end-labeling. The TFIIIA–DNA complex is found as a retarded band in polyacrylamide gel electrophoresis (Fig. 1*A*, lanes 1 and 2). To detect whether or not thionein inhibits the binding of TFIIIA to DNA, TFIIIA and thionein were mixed in binding buffer supplemented with 9 μM $ZnCl_2$ and incubated for 20 min before the addition of labeled ICR. Fig. 1*A* (lane 7) shows that as little as 4 μM thionein nearly completely prevents 300 nM TFIIIA from binding to the DNA probe. Moreover, the addition of Zn(II) reverses the interference of thionein with TFIIIA binding to ICR (Fig. 1*B*, lane 4).

Thionein Suppresses Interaction of TFIIIA with 5S RNA to Form a 7S RNP Complex. TFIIIA is known to bind to 5S RNA in a zinc-dependent manner (25) to form 7S RNP. Fig. 2*A* (lane 6) shows that in a binding buffer supplemented with 9 μM $ZnCl_2$, 4 μM thionein also abolishes the binding of 300 nM TFIIIA to the RNA. Further, addition of zinc reverses this effect (Fig. 2*B*, lane 4). Significantly, incubation of Zn_7-thionein with TFIIIA does not affect its binding to either DNA or RNA (Fig. 1*A*, lane 10; Fig. 2*A*, lane 10).

Thionein competition with TFIIIA for zinc is energetically feasible; the average apparent association constant for zinc in Zn_7-thionein, at pH 7, is about 2×10^{12} M^{-1} (21) and coincides with the minimum value of 10^{12} M^{-1} estimated for TFIIIA (24) and that of 2×10^{12} M^{-1} for a consensus zinc finger peptide of the Cys_2His_2 type (26). In the present experiments an ≈1.5-fold excess of metal binding equivalents of thionein over the total amount of free and TFIIIA-bound zinc in the reaction mixture was sufficient to abolish binding of TFIIIA to the 5S RNA gene and to 5S RNA (Fig. 1*A*, lane 7; Fig. 2*A*, lane 6). One might infer that under the conditions of these experiments zinc binding in the zinc finger protein is slightly stronger than in zinc-thionein. It is possible, of course, that upon neutralization part of the added thionein is lost due to oxidation. The similarity in the affinity of the metal for these proteins also accounts for the observation that in the zinc reversal experiments the competence of TFIIIA for nucleic acid binding is already restored when about half of the

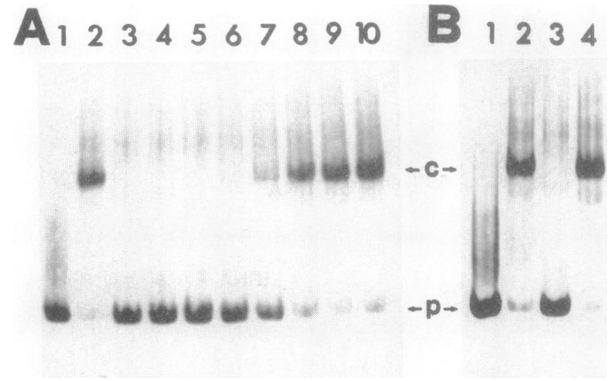

FIG. 1. Gel retardation assay for TFIIIA binding to the ICR of the 5S RNA gene using a 6% nondenaturing polyacrylamide gel. (*A*) Thionein inhibition of TFIIIA binding to ICR. Lane 1, no TFIIIA; lane 2, with TFIIIA (final concentration, 300 nM); lanes 3–9, addition of 64, 32, 16, 8, 4, 2, and 1 μM thionein, respectively; lane 10, addition of 68 μM Zn_7-thionein. (*B*) Reversal of thionein-induced blocking of TFIIIA binding to the ICR by the addition of zinc. Lane 1, no TFIIIA; lane 2, with TFIIIA (300 nM); lane 3, addition of 32 μM thionein; lane 4, addition of 32 μM thionein and then 130 μM zinc. c, TFIIIA–ICR complex; p, ^{32}P end-labeled ICR probe.

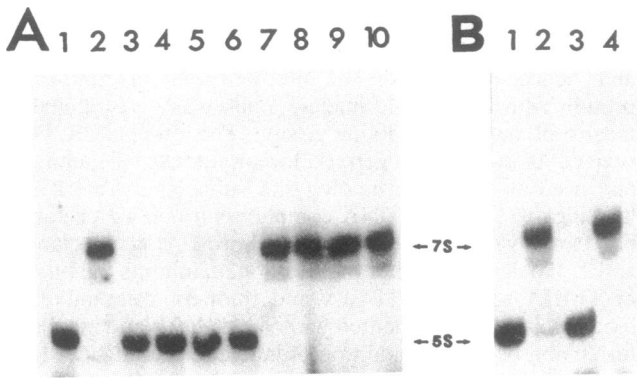

FIG. 2. Gel retardation assay for TFIIIA binding to 5S RNA using a 6% native polyacrylamide gel. (*A*) Thionein inhibition of TFIIIA binding to 5S RNA. Lane 1, no TFIIIA; lane 2, with TFIIIA (final concentration, 300 nM); lanes 3–9, addition of 32, 16, 8, 4, 2, 1, and 0.5 μM thionein, respectively; lane 10, 32 μM Zn_7-thionein. (*B*) Restoration of TFIIIA binding to 5S RNA in the presence of thionein by addition of zinc. Lane 1, no TFIIIA; lane 2, with TFIIIA; lane 3, addition of 32 μM thionein; lane 4, addition of 32 μM thionein and then 130 μM zinc. 5S, 5S RNA; 7S, TFIIIA–5S RNA complex.

excess thionein present is complexed by the added zinc (Fig. 1*B*, lane 4 and Fig. 2*B*, lane 4).

Thionein Inhibits the *in Vitro* Transcription of the 5S RNA Gene but not of the tRNA Gene. The functional consequences of the suppression of TFIIIA binding to its cognate DNA by thionein in a TFIIIA-dependent *in vitro* transcription system are documented in Fig. 3. A plasmid containing the 5S RNA gene served as transcription template (15). Another plasmid containing a $tRNA^{arg}$ gene that is known to be transcribed independently of TFIIIA was used as a control (16). Both templates were transcribed by RNA polymerase III of HeLa cell nuclear extract (24). The extract was supplemented with 600 ng of exogenous TFIIIA to activate 5S RNA gene transcription. The RNA products 5S RNA and tRNA were detected by autoradiography (24). Fig. 3*A* (lane 3) illustrates that the transcription of 5S RNA gene is reduced to a very low

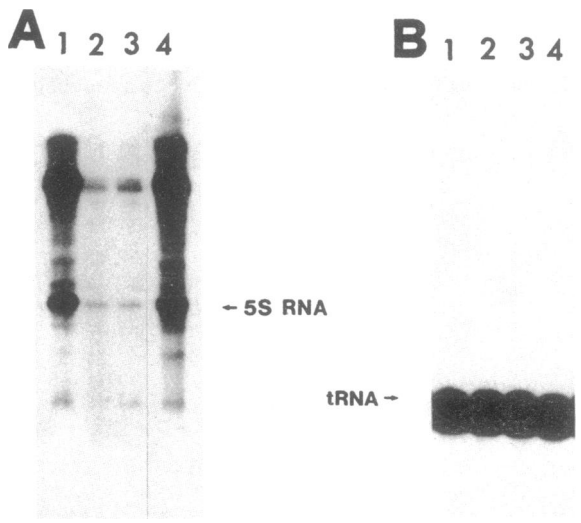

FIG. 3. *In vitro* transcription of 5S RNA and $tRNA^{arg}$ genes. (*A*) Inhibition of 5S RNA gene transcription by thionein. Lane 1, no thionein; lanes 2 and 3, addition of 45 and 4 μM thionien, respectively; lane 4, addition of 45 μM Zn_7-thionein. Note that the bands above and below 5S RNA reflect abortive transcripts due to inefficient initiation and termination (27). (*B*) No inhibition of $tRNA^{arg}$ gene transcription with thionein. Lane 1, no thionein; lanes 2 and 3, addition of 8 and 4 μM thionein, respectively; lane 4, addition of 8 μM Zn_7-thionein.

level by mixing thionein (4 μM) with TFIIIA prior to the initiation of the transcription reaction, whereas the same concentration of Zn_7-thionein did not inhibit (Fig. 3*A*, lane 4). The transcription of the tRNA gene was not affected by thionein (Fig. 3*B*, lanes 2 and 3), which indicates that in this system, thionein specifically inhibits TFIIIA-dependent transcription activation. This also denotes that thionein does not inactivate the zinc metalloenzyme RNA polymerase III and thus implies a relatively stronger binding of zinc to this enzyme (but see below). In accord with this view the slight inhibition of transcriptional activity is comparable to that observed in the presence of high concentrations of 1,10-phenanthroline (13), albeit at a much higher concentration of thionein—i.e., >45 μM under the present conditions.

The inhibition of TFIIIA-activated transcription by thionein shown by the *in vitro* studies is also observed *in vivo* (28). Injection of appropriate amounts of thionein into *X. laevis* oocytes blocks TFIIIA-dependent 5S RNA synthesis (26). Jointly these results and those obtained earlier with Sp1 support the suggestion (12) that, by withdrawal of zinc, thionein restricts the supply of zinc to zinc finger-containing proteins *in vivo*. The present data demonstrate unequivocally that thionein competes effectively for the zinc atoms of TFIIIA *in vitro* and *in vivo*. This results in a loss in the capacity of TFIIIA to activate transcription of the 5S RNA message, but this can be restored by addition of zinc. These experimental findings together with those reported for Sp1 (12) recall earlier suggestions that thionein itself may be physiologically essential (9).

Properties of Metallothionein. Two principal features of metallothionein were responsible for stimulating the initial interest in its chemical characteristics and potential biological role: its highly unusual amino acid composition, 20 cysteines out of 61 amino acids, and its extraordinary metal content, seven gram-atoms per mol. The subsequent discovery of the inducibility of metallothionein (or thionein?) by cadmium and other metals generated even greater interest in the protein by suggesting its participation in the detoxification of heavy metals. Still later observations that it is also induced by nonmetal species, including various hormones (e.g., glucagon, glucocorticoids, catecholamines), interleukins 1 and 6, and interferon, as well as ethanol and yet other metabolites and toxins, suggested a role in the cellular response to nutrition and stress. Although most attention has been directed solely toward metallothionein and its relationship to metal toxicity, this aspect may have been overemphasized, and it now seems appropriate to reconsider the earlier suggestion (9) that thionein itself might have a cardinal metabolic role.

Induction of Metallothionein (Thionein). Cadmium and zinc are among the most potent stimulators of metallothionein biosynthesis (3). These metals, when present in excessive amounts, bind to specific trans-acting proteins that in turn activate metal-responsive elements in the 5′ region of the metallothionein gene to enhance transcription of the thionein message (28). Expression of the apoprotein allows it to bind the excess metals, diminish their concentration, and, hence, control by feed-back its own biosynthesis. This mechanism may define one of the principal biological roles of thionein, to protect the cell from heavy metal toxicity, but this may not necessarily be its only function.

The many other known stimulators of metallothionein biosynthesis that are not metals (3) may operate through metal-independent mechanisms. Typically the cellular response to such stimulators is determined by measuring either the increase in thionein mRNA or the formation of metallothionein, the latter most often by indirect methods. One question that arises in connection with this induction process is the source of metal for metallothionein accretion. Although metallothionein has been thought to be a storage form of zinc,

as may well be the case under certain circumstances, it would seem to be an unlikely source of zinc for newly synthesized thionein. Several extracellular sources can be considered. A small but labile fraction of the total body zinc circulates in blood plasma primarily bound to two proteins, α_2-macroglobulin and a 68-kDa protein believed to be either albumin or perhaps transferrin (29). The amount of zinc in the 68-kDa fraction has been shown to change markedly and rapidly in response to certain hormonal signals. Moreover, treatment of rats with interleukin 1 induces a transient decrease in plasma zinc prior to an increase in liver metallothionein (30). The plasma zinc could well shift to the hepatocyte where it would be incorporated into metallothionein. On a quantitative basis the fall in plasma zinc cannot account for all of the metallothionein that is produced, and other sources of zinc such as bone, skin, and intestine may contribute, though over a longer time scale. The mechanisms for zinc mobilization from these tissues are not known but they may not be sufficiently rapid to serve as an adequate response to a hormonal signal.

Intracellular proteins might very likely be the first source of metal for newly synthesized thionein. It is known that zinc coordination varies considerably among zinc proteins (1) and that this can manifest, in part, in markedly different stability constants. The nature of the zinc binding donor atoms can account for some of this difference, but even proteins with the same kind of ligands can differ in zinc binding properties. Hence, *de novo* biosynthesis of thionein could be accompanied by a redistribution of zinc among the various intracellular zinc proteins. Indeed, this may be a function for the induced synthesis of thionein—namely, to down-regulate the activity of zinc proteins within the cell. In this case it would be more appropriate to view the cellular response to hormones and cytokines, for example, as an induction of the biosynthesis of thionein rather than of metallothionein. Moreover, a series of observations make it reasonable to suppose that the zinc-dependent transcription factors would be prime targets for activity modulation.

Such a role for thionein is supported by the recent observations that in the promotion of a mouse epidermis tumor by phorbol 12-myristate 13-acetate there is a transient arrest of cell division that occurs concomitantly with the appearance of thionein mRNA (31). When the same agent was employed to study thymocytes, it was found to induce a movement of zinc from the nucleus to the cytosol (32). A similar tendency toward redistribution of zinc was seen in conjunction with the induction of thionein mRNA by estradiol at the time of ovulation in rainbow trout (33). It is also interesting that although the biosynthesis of TFIIIA and thionein in *X. laevis* oocytes is regulated in development, the former is expressed maximally in the early phase (34) and the latter is expressed in mid-to-late phase (35). In addition, the induction of immediately early zinc finger genes such as *krox* and *egr* by mitogens in mammalian cells (36) is followed by the emergence of thionein mRNA (35).

Considering the seemingly innumerable proteins exhibiting the zinc finger (37) and zinc twist (2) motifs, these findings are of more than casual interest and would seem to point toward a general role of thionein in the regulation of such zinc-dependent systems. The metallothionein/thionein couple has an intermediate half-life (38) and, as noted, the rate of thionein biosynthesis is influenced significantly by a large number of hormones and mediated by various signaling pathways (39). As a consequence, the supply of thionein varies widely as a function of the developmental state of tissues (40) and their cellular activity (27). This may change the availability of zinc to zinc-dependent systems and modulate their activities. Thus, the induction by such diverse substances and metabolites might denote a response to demands either in regard to metabolic processes in need of regulation by thionein, of its inhibition by metals—e.g., in particular cadmium—or the reversal of that inhibition through greater production of thionein and finally of regulation by this or other means of ongoing cellular functions, be they catalytic or gene expression. This would confer on the metallothionein/thionein couple the characteristics of a signal transducer.

The pathway of zinc from TFIIIA to thionein remains to be elucidated. The reaction may proceed via release of the metal into the free zinc ion pool from which it is taken up by thionein. It may also be transferred from TFIIIA to thionein by collision and direct exchange via an intermediate complex. Recent ^{111}Cd NMR saturation transfer experiments (5) and radioisotope redistribution studies with ^{65}Zn, ^{109}Cd, and ^{203}Hg (6) have demonstrated, in fact, that metal exchange between metallothionein isoforms proceeds readily and rapidly by such a direct intermolecular ligand interchange process (6). By analogy, similar temporary intermolecular contacts could provide the opportunity for and perhaps underlie the specificity of ligand exchange between the zinc finger domains of TFIIIA and thionein that accounts for the net transfer of zinc observed in this study. In such a transfer process, thionein might be viewed as a catalyst of the thermodynamically determined flow of zinc to and from its many sites of action.

Thermodynamics and Kinetics of Metal Exchange. The ability of thionein to remove zinc from other zinc proteins and of metallothionein to donate zinc is constrained by thermodynamic and kinetic considerations that can be traced to the structure of the zinc cluster in metallothionein. The very first characterization of the protein indicated that sulfur is the soft base responsible for its remarkable avidity for metal atoms, especially zinc and cadmium (41, 42). Structural studies by x-ray diffraction analysis and one- and two-dimensional NMR have since confirmed that zinc and cadmium are bound to thionein in the form of thiolate clusters, a structure for which an appropriate inorganic model precedent did not exist. Moreover, the binding constants of these clusters are comparable in magnitude to those of other zinc metalloproteins. On the basis of thermodynamics, the removal of zinc from metallothionein by a biological competitor molecule could be accomplished on an equimolar basis if the competitor had an equal or greater stability constant. If it was a weaker binder, removal could only occur through mass action by large molar excesses. Conversely, the removal of zinc by thionein from other zinc proteins would generally be expected to be thermodynamically favorable but would require that the release of zinc from the donor protein be rapid. In this regard, the exchange lability of metallothionein is critical. The rates at which the clusters transfer metals upon exposure to chelating agents (4) and exchange metals among metallothionein isoforms (5, 6) are much higher than expected on the basis of conventional zinc mercaptide chemistry. It is this *kinetic lability* of the zinc thiolate clusters in conjunction with their thermodynamic stability that allows the thionein/metallothionein couple to have a responsive dynamic metabolic role, one that seemed to be precluded based on hitherto known zinc metalloprotein coordination chemistry. This simultaneous kinetic lability and thermodynamic stability of the thionein sulfur donor together with and enhanced by the discovery of previously unknown motifs of zinc ligands in DNA binding proteins (43) now extends innovative hypotheses regarding the biological role of zinc coordination.

In the other direction, and more relevant to the present results, thionein is a strong chelator that can efficiently and rapidly remove zinc from zinc finger proteins. Here it is the exchange lability of zinc from these fingers that is significant. In contrast, any kinetic inertness of zinc in slowly exchanging metalloenzyme sites would confer immunity to zinc removal

and inactivation as the result of transient increases in thionein concentration. This may explain why RNA polymerase is not inhibited on the time scale of the experiments described herein.

There remains a persistent difficulty to demonstrate the occurrence of substantial quantities of free thionein *in vivo*. The very affinity of the molecule for metal ions and the absence of spectral probe properties of the apoprotein based on aromatic amino acids or other distinctive physicochemical characteristics have presented formidable obstacles to the identification of the species either *in vivo* or *in vitro*. Moreover, the ready oxidation of the sulfur cluster under aerobic conditions further complicates the task of demonstrating the mechanism of donation of zinc to zinc catalytic, cocatalytic, structural, finger, cluster, and twist sites (43) and to define and measure the order and magnitude of relative stabilities and rates of zinc exchange and the role of cadmium in these processes, if any. It has clearly now proved possible to show by a number of means (6) that such exchanges do take place, particularly among isoforms of metallothioneins and among metallothioneins from different species (5, 6), but the detailed examination of the range of zinc proteins to whose formation the putative exchange with thionein might be essential remains to be defined.

We thank Dr. C.-W. Wu for the gift of the plasmid used in this study. This work was supported by the Canton of Zürich, the Swiss National Science Foundation (Grant 3.160-0.88), and the Endowment for Research in Human Biology, Inc.

1. Vallee, B. L. & Auld, D. S. (1990) *Biochemistry* **29,** 5647–5659.
2. Vallee, B. L., Coleman, J. E. & Auld, D. S. (1991) *Proc. Natl. Acas. Sci. USA* **88,** 999–1003.
3. Kägi, J. H. R. & Schäffer, A. (1988) *Biochemistry* **27,** 8509–8515.
4. Li, T.-Y., Kramer, A. J., Shaw, C. F., III, & Petering, D. H. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 6334–6338.
5. Otvos, J., Chen, S. M. & Liu, X. (1989) in *Metal Ion Homeostasis: Molecular Biology and Chemistry*, eds. Hamer, D. H. & Winge, D. R. (Liss, New York), pp. 197–206.
6. Schmid, R. F. (1991) Diploma Thesis (Univ. of Zürich, Zürich, Switzerland).
7. Vallee, B. L. (1991) *Methods Enzymol.* **205**, 3–5.
8. Vallee, B. L. (1987) *Experientia Suppl.* **52,** 5–16.
9. Vallee, B. L. (1979) *Experientia Suppl.* **34,** 19–40.
10. Udom, A. O. & Brady, F. O. (1980) *Biochem. J.* **187,** 329–335.
11. Zeng, J. (1991) Ph.D. Thesis (Univ. of Zürich, Zürich, Switzerland).
12. Zeng, J., Heuchel, R., Schaffner, W. & Kägi, J. H. R. (1991) *FEBS Lett.* **279,** 310–312.
13. Hanas, J. S., Hazuda, D. J., Bogenhagen, D. F., Wu, F. Y.-H. & Wu, C.-W. (1985) *J. Biol. Chem.* **258,** 14120–14125.
14. Miller, J., McLachlan, A. D. & Klug, A. (1985) *EMBO J.* **4,** 1609–1614.
15. Sakonju, S., Bogenhagen, D. F. & Brown, D. D. (1980) *Cell* **19,** 13–25.
16. Silverman, S., Schmidt, O., Söll, D. & Hovemann, B. (1979) *J. Biol. Chem.* **254,** 10290–10294.
17. Picard, B. & Wegnez, M. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 241–245.
18. Hanas, J. S., Bogenhagen, D. F. & Wu, C.-W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2142–2145.
19. Shang, Z., Windsor, W. T., Liao, Y. D. & Wu, C.-W. (1988) *Anal. Biochem.* **168,** 156–163.
20. Bühler, R. H. O. & Kägi, J. H. R. (1974) *FEBS Lett.* **39,** 229–234.
21. Vasak, M. & Kägi, J. H. R. (1983) in *Metal Ions in Biological Systems*, ed. Sigel, H. (Dekker, New York), Vol. 15, pp. 213–273.
22. Fairall, L., Rhodes, D. & Klug, A. (1986) *J. Mol. Biol.* **192,** 577–591.
23. England, T. E. & Uhlenbeck, O. C. (1978) *Nature (London)* **275,** 560–561.
24. Dignam, J. D., Martin, P. L., Shastry, B. S. & Roeder, R. G. (1983) *Methods Enzymol.* **101,** 582–598.
25. Shang, Z. G., Liao, Y. D., Wu, F. Y.-H. & Wu, C.-W. (1989) *Biochemistry* **28,** 9790–9795.
26. Krizek, B. A., Amann, B. T., Kilfoil, V. J., Merkle, D. L. & Berg, J. M. (1991) *J. Am. Chem. Soc.* **113,** 4518–4523.
27. Peck, L. J., Millstein, L., Eversole-Cire, P., Gottesfeld, J. M. & Varshavsky, A. (1987) *Mol. Cell. Biol.* **7,** 3503–3510.
28. Zeng, J. & Kägi, J. H. R. (1991) *Experientia* **47,** A38 (abstr.).
29. Falchuk, K. H. (1977) *N. Engl. J. Med.* **296,** 1129–1134.
30. Cousins, R. J. & Leinart, A. S. (1988) *FASEB J.* **2,** 2884–2890.
31. Bohm, S., Berghard, A., Pereswetoff-Morath, C. & Toftgård, R. (1990) *Cancer Res.* **50,** 1626–1633.
32. Csermely, P., Szamel, M., Resch, K. & Somogyi, J. (1988) *J. Biol. Chem.* **263,** 6487–6490.
33. Olsson, P.-E., Zafarullah, M. & Gedamu, L. (1989) *Biochem. J.* **257,** 555–559.
34. Shastry, B. S., Honda, B. M. & Roeder, R. G. (1984) *J. Biol. Chem.* **259,** 11373–11382.
35. Olsson, P.-E., Zafarullah, M., Foster, R., Browder, L. W. & Gedamu, L. (1989) *J. Cell. Biochem. Suppl.* **13B,** 182.
36. Herschman, H. R. (1989) *Trends Biochem. Sci.* **14,** 455–458.
37. Berg, J. M. (1990) *Annu. Rev. Biophys. Biophys. Chem.* **19,** 405–421.
38. Laurin, D. E. & Klasing, K. C. (1990) *Biochem. J.* **268,** 459–463.
39. Hamer, D. H. (1986) *Annu. Rev. Biochem.* **55,** 913–951.
40. Karin, M. (1985) *Cell* **41,** 9.
41. Kägi, J. H. R. & Vallee, B. L. (1960) *J. Biol. Chem.* **235,** 3460–3465.
42. Kägi, J. H. R. & Vallee, B. L. (1961) *J. Biol. Chem.* **236,** 2435–2442.
43. Vallee, B. L. & Auld, D. S. (1991) in *The Role of Protein Structure in Biological Regulation*, 20th Linderstrøm-Lang Conference, eds. Sottrup-Jensen, L., Petersen, T. E., Clark, B. F. C. & Jörnvall, H. (L. Sattrup-Jensen, Århus, Denmark), pp. 19–22.