PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Zinc and Gene Expression [and Discussion]

B. L. Vallee, K. H. Falchuk and J. K. Chesters

Phil. Trans. R. Soc. Lond. B 1981 **294**, 185-197
doi: 10.1098/rstb.1981.0098

Phil. Trans. R. Soc. Lond. B **294**, 185–197 (1981) [185]
Printed in Great Britain

Zinc and gene expression

By B. L. Vallee and K. H. Falchuk
Center for Biochemical and Biophysical Sciences and Medicine,
Harvard Medical School, 250 *Longwood Avenue,*
Boston, Massachusetts 02115, *U.S.A.*

During the last two decades, zinc has been shown to be a functionally *essential* component of more than 120 enzymes and concurrently has been recognized as indispensible to normal cellular growth, development and differentiation of all species. However, the manner in which it exerts its effects are still unknown. Studies of the phytid *E. gracilis* have demonstrated that zinc is critical for transcription and translation, affecting the metabolism of RNA polymerases, mRNA and proteins, among which are histones and arginine-containing peptides. These and other data lead to the suggestion that this element has a fundamental role in gene activation and/or repression, accounting for its pervasive effects on cell metabolism.

Introduction

Although it has been suspected since ancient times that metals participate in biological processes, details of the manner in which they might exert a function have remained quite unknown until very recently. It is now appreciated that metals play roles in catalysis, in the control of hormonal and other regulatory functions, in the synthesis and stabilization of the structure of macromolecules, in muscle contraction, in nerve conduction and in transport. The alkali and alkaline earth metals as well as cobalt, copper, iron, manganese, molybdenum, nickel, zinc and others are known to be essential, many of them in the mechanism of action of specific enzymes.

'Trace' metals and biochemistry

Most of these elements occur in biological matter in very low concentrations, a fact that dominated the thinking about the subject until recently and presented not so much intellectual as analytical challenges. The presence or absence of a particular 'trace' element in a given biological matrix was the crucial experimental question preoccupying many investigators, and its answer became almost an end in itself; as a consequence, little thought needed to be given then as to how an affirmative answer would be pursued. Recent advances in methodology and instrumentation have solved most of the analytical problems that impeded progress. Nevertheless, it is important to realize that the difficulties and frustrations of the past have left an imprint on the field and are only slowly giving way to the new realities.

The collective designation 'Trace Element' has been employed variously to describe tissue content, the total knowledge of the subject or – by implication – even its potential importance. Whatever the connotation or viewpoint, this history-conditioned nomenclature has categorized pertinent metals, based entirely on the once poor detection limits and signal/noise ratios of analytical methods which are now so excellent that in this field the very definition of a 'Trace†' has become almost meaningless.

† Webster's *New World Dictionary*, 1962 edition: '"Trace", a very small amount, usually one quantitatively immeasurable.'

Of necessity, this classification of metals, based on their tissue concentrations, implies a now obsolete and, in fact, non-existent equivalence and ignores their unique chemical properties, characteristic biological occurrences and individual functions. Distinct biological roles for most essential metals are now well recognized, and approaches to the exploration of these functions have become standardized to the point where they have merged with those employed universally in biochemistry. As a consequence the metabolic implications of 'inorganic' biochemistry have become incorporated into the mainstream of biochemical knowledge and thought. With the benefit of hindsight, it is now apparent that for the past decade or two, progress in inorganic, organic and physical biochemistry has gone in parallel, all supporting one another. Thus, for example, the recognition, purification and characterization of metalloenzymes depended as much on progress in the physical chemistry of proteins and the methodology for their isolation and characterization, as on advances in spectroscopic, electrochemical, isotopic and other methods for the recognition of metals and their binding sites. Now the mainstream of biochemical understanding can best give the most effective direction to the study of both metal-dependent and all other metabolic processes.

Zinc and enzymology

The events that resulted in the recognition of zinc as an essential coenzyme-like component of many enzymes are reminiscent of the evolution of 'vitaminology' and constitute a similarly interesting chapter in the development of biochemistry (Vallee 1977).

The above remarks regarding metals in general are applicable to zinc in particular, of course. Precise and simple methods for its identification and quantitative measurement and the successful control of contamination have become feasible during the last two decades. Progress with the isolation and characterization of zinc metalloenzymes, correlation of their structure with function and the delineation of the role of the metal in enzyme action and metabolism was almost explosive and particularly productive. As a consequence, viewpoints on the biological roles of zinc changed drastically: first thought to be mostly harmful, then of questionable importance, it is now known to be essential for the growth, development and survival of all living matter (Vallee 1976).

It is now 110 years since zinc was first recognized to be metabolically important in a mould. The first zinc metalloenzyme, carbonic anhydrase, was characterized only 40 years ago. Another 15 years elapsed before the second zinc enzyme, carboxypeptidase A, was identified, but during the subsequent 10 years, many additional ones were found (Vallee & Wacker 1970). The total now exceeds 120. They are present in all phyla, and are quite diverse both in the nature of the reactions that they catalyse and, presumably, with respect to the role that even a single metal such as zinc can play (Vallee 1976). They participate in a wide variety of metabolic processes including synthesis and/or degradation of carbohydrates, lipids, proteins and nucleic acids, and encompass all known types of enzymes, i.e. oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases.

Zinc and molecular biology

It is perhaps remarkable that zinc was recognized to be essential for growth and development of *all* living matter only within the last decade. Even though much experimental evidence had

already pointed in this direction this had not been generalized. This came to be appreciated through a series of major, closely interrelated biochemical advances in nucleic acid chemistry.

The earliest indication that zinc had a role in nucleic acid metabolism derived from Fujii's histochemical observation (1954) that this element is present in the cell nucleus, nucleolus and chromosomes. Subsequently it was demonstrated that substantial quantities of firmly bound zinc in RNA stabilize its structure (Wacker & Vallee 1959; Fuwa *et al.* 1960). Somewhat later, zinc was shown to be associated with, and to stabilize, DNA (Shin & Eichorn 1968) and ribosomes (Prask & Plocke 1971). Intimations of an even more extensive involvement of zinc in the metabolism of nucleic acids were derived from the inhibition of DNA polymerase activity by EDTA in cell cultures (Lieberman & Ove 1962) and the effect of zinc deficiency on the activity of both DNA and RNA polymerases in various tissues and organisms (Swenerton *et al.* 1969; Sandsted & Rinaldi 1969; Falchuk *et al.* 1975*a*).

TABLE 1. ZINC IN NUCLEIC ACID AND PROTEIN SYNTHESIS

	zinc (g at/mol)	source
DNA polymerases	1.0	*E. coli*
	4.2	sea urchin
	1.0	T_4 phage
		oncogenic viruses
	1.8–2.0	avian
	1.4	murine
	†	feline
	1.0	simian
	†	RD-144
RNA polymerases	2.0	*E. coli*
	2.4	T_7
	2.4	yeast (I)
	1.0	yeast (II)
	2.0	yeast (III)
	2.1	*E. gracilis* (I)
	2.2	*E. gracilis* (II)
	2.2	*E. gracilis* (III)
	2.0	*B. subtilis*
	7.0	*wheat germ* (II)
	5.5	N4 virion
tRNA synthetases	2.0	*E. coli*
deoxynucleotidyl transferases	1.0	calf thymus
elongation factor I	1.3	rat liver

† Molecular mass unknown.

Inhibition of the highly purified terminal deoxynucleotidyl transferase from calf-thymus by chelating agents was found to be reversed by zinc ions (Chang & Bollum 1970), leading to the suggestion that it is a zinc metalloenzyme. This conclusion was extended rapidly to other nucleotidyl polymerases on the basis of similar data. Metal analyses soon confirmed the presence of zinc in all classes of nucleotidyl polymerases from prokaryotes, eukaryotes and viruses. Thus, within a year of Bollum's observations, the RNA polymerase (Scrutton *et al.* 1971) and DNA polymerase (Slater *et al.* 1971) from *Escherichia coli* were shown to contain 2 and 1 g atom of zinc per mole, respectively. At present, nearly 20 nucleotidyl polymerases have been shown to contain zinc, and the available data are summarized in table 1.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

The role of zinc in these enzymes has been studied by approaches that are now standard. For example, both the activity and zinc content of the *E. coli* DNA and RNA polymerases can be abolished and removed respectively by 1,10-phenanthroline, and both are restored concomitantly by addition of zinc. These and other nucleotidyl polymerases are also inhibited by chelating agents other than 1,10-phenanthroline, e.g. EDTA, dipicolinic acid, 2,2′-bipyridine, 8-hydroxyquinoline-5-sulphonic acid. Non-chelating analogues of 1,10-phenanthroline, e.g. 1,7- or 4,7-phenanthroline, neither inhibit the enzyme nor remove its zinc atoms, demonstrating that this inhibition is metal-dependent and is not due to non-specific interactions. Zinc also participates in the action of tRNA synthetases and elongation factor I (figure 1).

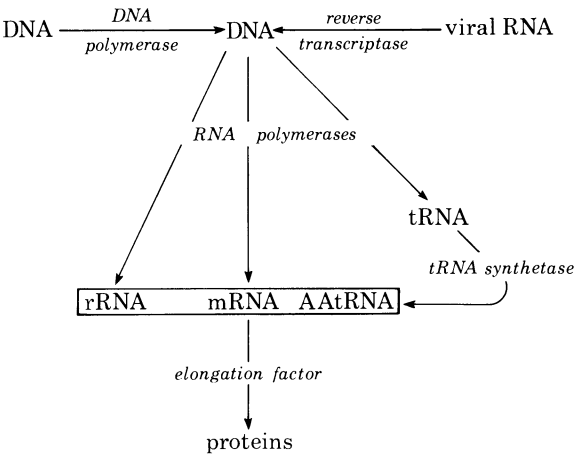


FIGURE 1. The role of zinc in transcription and translation. Italics indicate steps dependent on zinc enzymes or zinc proteins.

Zinc is thus indispensable to the enzymes that are critical both to DNA, RNA and protein synthesis and to their degradation. However, little or nothing was known until a short time ago about its role in cellular metabolism.

ZINC AND CELL BIOLOGY

Experimental zinc deficiency always results in arrest of growth, development and differentiation, frequently induces teratological abnormalities, these complex manifestations varying with the species.

An increasing number of spontaneous diseases of animals and man have proved to be related to abnormal zinc metabolism and/or deficiency (Li & Vallee 1980). Acrodermatitis enteropathica, a hitherto fatal congenital disease, deserves particular attention. It is now treated successfully and cured with zinc, though virtually nothing is known as yet either about the biochemical abnormalities underlying its manifestations or the mechanisms by which zinc reverses them. An acrodermatitis-like syndrome has also been observed after surgical removal of large segments of the intestine, when this was followed by prolonged intravenous therapy. Zinc supplements reversed the lesions.

As pointed out earlier, a number of observations suggested a role for zinc in cell metabolism. Though some of these can now be rationalized in terms of the known role of zinc in enzymes,

others would seem to require additional information in terms of the action of zinc at so far unspecified loci. Since overall growth, development and differentiation have been found to be affected by zinc deficiency in all species so far studied, it seemed necessary to investigate those subcellular steps that impart information through gene action or its environmental control, that participate in cell division and influence its consequences, and that concern all those subcellular processes involved in differentiation.

For such studies we set out more than 20 years ago to identify and select a suitable organism. A eukaryote was required that could readily be made zinc deficient and whose enzymes and subcellular organelles involved in nucleic acid and protein synthesis could be studied.

The unicellular protist, *Euglena gracilis* (strain Z) proved to be appropriate. This organism had earlier become a favourite of cell biologists and was the subject of a great deal of histological, morphological, functional and biochemical investigation. It is a heterotroph that grows readily in either the light or the dark, developing chloroplasts in the light but not in the dark, and with metabolic patterns characteristic of each of these conditions. It can be grown in a synthetic medium whose content of zinc and other metals can easily be defined and controlled (Price & Vallee 1962). All studies to be described here were made on organisms grown in the dark. In zinc-sufficient (+Zn) media the growth reaches a plateau after about 7 days, while it takes about 14 days in a zinc-deficient (−Zn) culture, which then contains only one-twentieth of the number of cells in the (+Zn) culture at plateau. The inhibition is readily and completely reversed by raising the Zn^{2+} content of the medium

The final zinc content of (−Zn) cells is greatly decreased, but, somewhat unexpectedly, the content of a number of metals other than zinc is increased in response to zinc deficiency.

Large quantities of cells of known metal content can thus be obtained at various stages of growth to establish the effects of zinc deficiency on the growth, morphology, cell cycle, mitotic apparatus and organelles of the organism (Wacker 1962; Prask & Plocke 1971; Falchuk *et al.* 1975*a*, *b*).

The nature of the cell cycle in (+Zn) and (−Zn) cells has been detailed (Falchuk *et al.* 1975*b*). The DNA content of (−Zn) cells is twice normal, growth is arrested and hence there is no division. The rate of incorporation of [^{3}H]uridine into RNA and [^{14}C]leucine into proteins of (−Zn) *E. gracilis* is greatly reduced, while nucleotides, peptides and amino acids accumulate. Though there is no change in the amount of each class of RNA synthesized, the composition, but not the function, of mRNA is altered (Falchuk *et al.* 1978). Conversely, since the stability of the ribosomes which rRNA generates is decreased markedly, the function, but not the composition, of rRNA is modified. Further, there is a dramatic accumulation of paramylon granules containing β-1,3-glucan (Falchuk *et al.* 1975*a*).

Analysis of peptides and proteins produced by (+Zn) and (−Zn) cells reveals that the synthesis of some of these ceases in zinc deficiency, while others newly appear. In particular, zinc deficient cells synthesize a family of arginine- and asparagine-rich polypeptides, which disappear completely when zinc sufficiency is restored (see below).

With an organism such as *E. gracilis* the biochemical consequences of zinc deficiency can be studied by isolation of its presumptive target systems. When the zinc dependence of the nucleotide polymerases of bacterial systems was first described (Scrutton *et al.* 1971), it seemed that the characterization of these enzymes in *E. gracilis* could be a promising approach. However, at that time, the purification and hence the characterization of the corresponding enzymes from

eukaryotes in general was still technically difficult. It was, therefore, not a foregone conclusion that the enzymes from eukaryotes would also be zinc enzymes.†

Prokaryotes depend on one RNA polymerase, while eukaryotes require three polymerases. Thus, the experimental confirmation of the proposition that they are zinc dependent seemed necessary if future efforts were to be soundly based.

TABLE 2. PROPERTIES OF DNA-DEPENDENT RNA POLYMERASES FROM ZINC-SUFFICIENT AND ZINC-DEFICIENT *E. GRACILIS*

RNA polymerases	$10^{-3}M_r$	$100\ V_i/V_c$†	OP* inhib.	Zn g at./mol
		zinc-sufficient		
I	630	100	+	2.1
II	700	0	+	2.2
III	650	25	+	2.2
		zinc-deficient		
'X'‡	650§	40	+	1.8

* 1,10-phenanthroline.
† In the presence of α-amanitin at 200 μg/ml.
‡ The only polymerase found in (−Zn) cells.
§ Estimate.

TABLE 3. RNA CONTENT OF (+Zn) AND (−Zn) *E. GRACILIS*

	total RNA per cell/pg	percentage ribosomal	percentage transfer	percentage messenger
(+Zn)	20 ± 5	79 ± 5	15 ± 3	6 ± 2
(−Zn)	19 ± 5	74 ± 4	15 ± 3	11 ± 3

We were able to isolate RNA polymerases I, II and III, which are typical of those of eukaryotes. Table 2 summarizes their characteristics, which resemble those of the analogous enzymes obtained from other sources. The establishment of their metal content would have presented difficulties in view of the paucity of material then available, and their high molecular masses combined with the low metal stoichiometry would all have created a difficult analytical problem. Fortunately, we had designed a microwave emission spectrometer capable of measuring quantitatively picogram amounts of metals, including zinc (Kawaguchi & Vallee 1975). The procedure was most effective for detecting nanogram to picogram quantities of functional zinc atoms in the reverse transcriptases from oncogenic viruses. It was equally effective in the characterization of the enzyme obtained at the same time from (−Zn) *E. gracilis*.

In contrast with the *three* polymerases isolated from (+Zn) cells, (−Zn) cells were found to contain only *one* RNA polymerase, as in prokaryotes. The properties of the enzyme from (−Zn) cells differ from all three in (+Zn) organisms, but it does contain a full complement of zinc atoms and generates all three, i.e. ribosomal, transfer and messenger, RNAs. Exposure of (−Zn) organisms to a zinc-sufficient growth medium restores the activity to all three polymerases normally present.

While this work was in progress, McLennon & Kier (1975) isolated the DNA polymerases of

† It should be recalled that the aldolases from microorganisms are, but those from higher systems are not, metalloenzymes. Similarly, the superoxide dismutase of microorganisms contains manganese or iron, while that of higher organisms contains copper and zinc or – in the mitochondrial systems – manganese as the metals critical for enzymic activity (Fridovich 1974).

E. gracilis and reported indirect, but convincing, evidence that these enzymes, too, contain functionally essential zinc atoms, thereby completing the information needed for an investigation of the consequences of zinc deficiency on the polymerases of this organism, the RNA products and the eventual proteins.

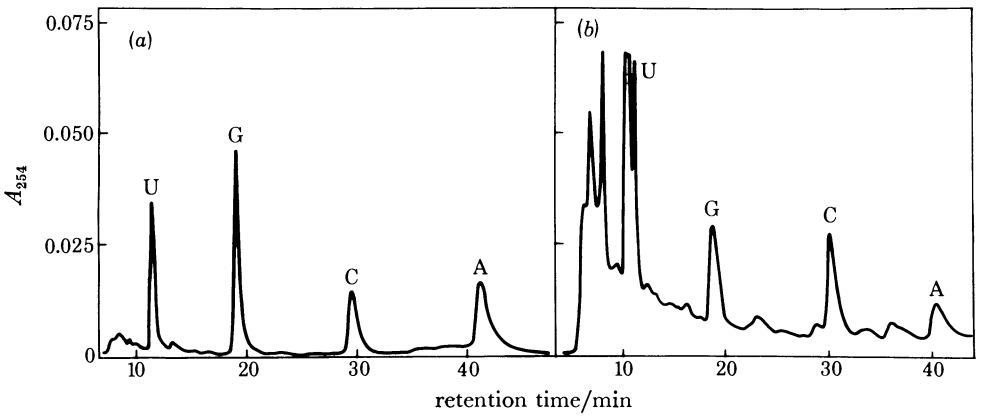


FIGURE 2. The bases of *E. gracilis* mRNA from (*a*) (+Zn) cells and (*b*) (−Zn) cells were analysed by high-pressure liquid chromatography. The retention time and ultraviolet spectra differ for each base, permitting base separation and identification. The mRNA from (+Zn) cells contains only the four major bases, uracil, guanine, cytosine and adenine, while the mRNA from (−Zn) cells contains additional, unknown bases. Moreover, the ratios of (G+C)/(A+U) differ between the two: for (+Zn) it is 1.6 and for (−Zn) it is 2.9 (Falchuk *et al.* 1978).

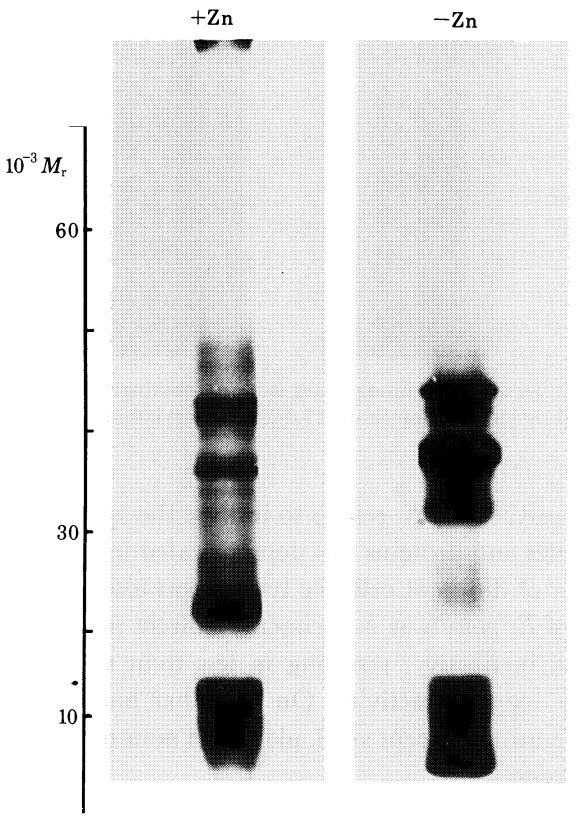


FIGURE 3. Cell-free translation products of mRNAs from (+Zn) and (−Zn) *E. gracilis* in a reticulocyte cell S_{30} lysate. Autoradiographs of the [^{35}S]methionine-labelled polypeptides were made after their separation by electrophoresis in 12.5% polyacrylamide gels.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Zinc deficiency does not alter the total RNA content of *E. gracilis* cells (table 3). The amounts of both total and tRNA from (−Zn) and (+Zn) cells are similar to those found in eukaryotic organisms, including *E. gracilis*. In (−Zn) cells, however, the amount of rRNA is slightly less while that of the mRNA, 11% of the total, is almost twice that of (+Zn) cells. The purine and pyrimidine contents of rRNA from both types of cells are identical. The guanine content of tRNA decreases from 34 to 24% in (−Zn) cells and cytosine increases slightly from 27 to 38%, but the adenine and uracil contents are identical. The mRNA of (+Zn) cells contains the usual four bases, i.e. uracil (U), guanine (G), cytosine (C), and adenine (A). In contrast, that of (−Zn) cells, while also composed of these four bases, contains three major and several minor additional bases as yet unidentified, but which are not present in mRNA from normal cells. The (G+C)/(A+U) ratios of the mRNA samples, 1.6 for (+Zn) and 2.9 for (−Zn) cells, differ strikingly (figure 2) (Falchuk *et al.* 1978). While zinc deficiency changes the composition of mRNA, it does not alter its functional capacity as expressed by translation.

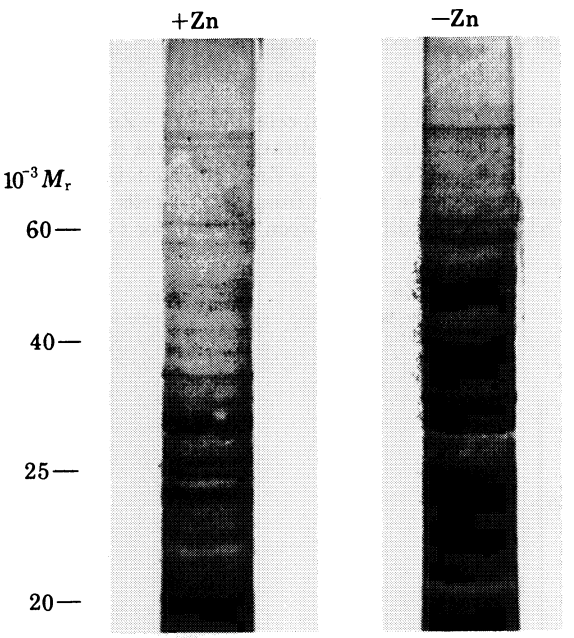


FIGURE 4. (+Zn) and (−Zn) *E. gracilis* cell homogenates were chromatographed on CM-Sephadex A-50. The polypeptides were separated by electrophoresis on 12.5% polyacrylamide gels. Sections of the resultant gels are compared.

The capacity of purified mRNAs of *E. gracilis* to initiate the synthesis of both large numbers and different types of peptides and proteins was demonstrated in a cell-free translation system. The suitability of a number of different cell-free lysates was also tested for their capability to accept the mRNA species of *E. gracilis* as messengers to direct protein synthesis. An *E. gracilis* S_{30} lysate, however, proved ineffective, resulting in less than two fold activation of protein synthesis compared with endogenous activity. On the other hand, both the reticulocyte and wheat germ S_{30} lysates are known to accept viral, plant and mammalian mRNAs, and both have proved to be suitable and effective.

A number of polypeptides of relative molecular mass $M_r > 10\,000$ are produced in the (+Zn) mRNA system, but are absent from that using mRNA of (−Zn) cells. Conversely, the (−Zn) system contains proteins that are either absent or present in amounts that differ from those of the (+Zn) system (figure 3).

By using intact cells the types and/or quantities of particular proteins obtained from (+Zn) and (−Zn) *E. gracilis* also differ. Gel electrophoresis of proteins, separated on CM-Sephadex A-50, demonstrates that a number of bands in fractions from (+Zn) are absent from (−Zn) cells, but there are also examples of the converse. Importantly, both (+Zn) and (−Zn) cells share many other polypeptides (figure 4).

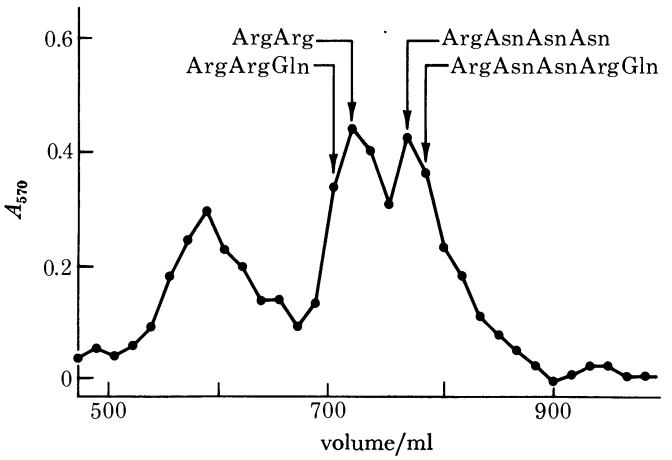


FIGURE 5. Arginine-rich peptides from (−Zn) *E. gracilis*. A fraction obtained from an acid extract of cells was chromatographed on Dowex 50 W-X2. The peptides of selected fractions (arrowed) were separated by high-voltage electrophoresis and sequenced.

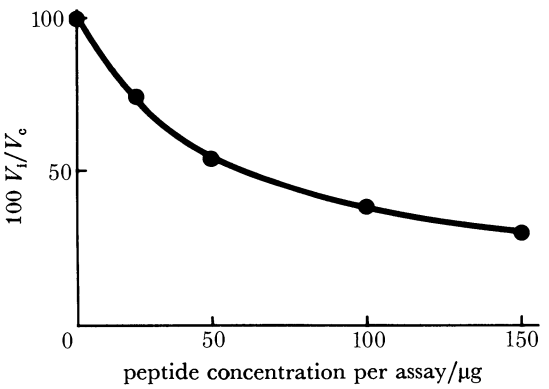


FIGURE 6. The effect of an arginine-rich fraction from (−Zn) *E. gracilis* on RNA polymerase II activity. Various amounts of the peptide fractions were incubated with RNA polymerase II and their activity assayed (Falchuk *et al.* 1978).

Some 15 years ago, when the role of arginine in enzymes was completely unknown, a number of arginine-rich polypeptides characteristic of (−Zn) cells only were discovered in our laboratory, though they were not characterized at the time. While it was an unusual finding, the preponderance of arginine did not have any functional implications. Since then, there has been a considerable increase in knowledge about functions of this amino acid residue. The subject has been reviewed, and its importance to biochemical reactions, particularly the binding of cationic substrates and coenzyme-bearing phosphate groups to arginine, has been pointed out (Riordan 1979).

The preponderance of arginine in some histones and in protamines, both known to bind to DNA, led us to isolate and characterize these arginine-rich peptides to elucidate the biochemical

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

basis of zinc deficiency and its consequences on transcription and translation. The positive charge of the arginine-rich peptides dictated the methods that had to be chosen for their purification. Chromatography of an acid extract results in a fraction containing more than ten arginine-rich peptides, four of which have now been sequenced (figure 5). Arginine constitutes nearly 50% of the unresolved material, with asparagine, glutamine and lysine accounting for the remainder. Zinc-deprived cells, on recovery from this depletion in a zinc-sufficient medium, do not contain any of this material.

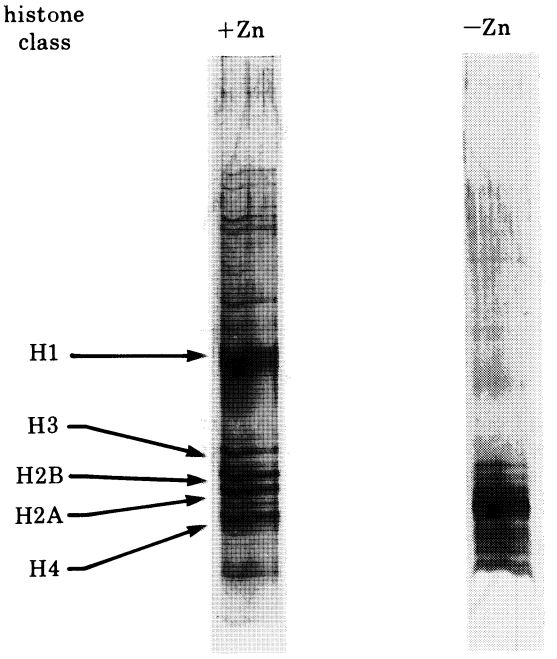


FIGURE 7. Histone fractions from (+Zn) and (−Zn) *E. gracilis* were electrophoresed on 15% polyacrylamide gels (0.1% sodium dodexyl sulphate, pH 8.8). Identification of histones was by the criteria of Panyim & Chalkley (1971) and Jardine & Leaver (1978).

Since arginyl residues are known to bind phosphate groups with particular avidity (Riordan 1979), the effect of these arginyl peptides on the activity of the DNA-dependent RNA polymerases seemed to be an obvious avenue for further research. Indeed, this peptide fraction markedly inhibits the activity of RNA polymerase II from *E. gracilis* (figure 6).

The curtailed synthesis of the *E. gracilis* RNA polymerases in zinc deficiency implies that the absence of the metal both prevents the synthesis of a number of normal proteins (or peptides) and promotes the synthesis of others, thus suggesting that there are selective effects on gene expression.

Investigations of gene expression must examine proteins that bind to DNA and which facilitate or restrict the interaction of RNA polymerases with DNA to regulate transcription; among these proteins the histones are most prominent. Hence, the histones from (+Zn) *E. gracilis* were characterized by standard procedures (Jardine & Leaver 1978). They contain histone fractions H_1, H_3, H_2B, H_2A and H_4 as evidenced by their characteristic migration in 15% polyacrylamide gels (Panyim & Chalkley 1971). In the histone region the material from (−Zn) cells gives a pattern of bands migrating in a manner distinctly different from that seen with material from (+Zn) cells (figure 7). Moreover, staining with Sakaguchi reagent reveals an additional fraction in the most cathodic region, present in the (−Zn) material, but virtually

absent from the (+Zn) material. It comprises the arginine-rich peptide fraction also obtained by chromatography and described above as being composed of 50% arginine.

While the physical and functional characteristics of these materials need to be defined in greater detail, it is apparent that zinc deficiency in *E. gracilis* affects the metabolism of histones. The importance of histones to chromatin structure is well recognized. Thus, its ordered structure is presumed to be maintained, on the one hand, by histone H_2A, H_2B, H_3 and H_4 binding to DNA, resulting in a repeating globular complex, and on the other by H_1 binding to the intervening

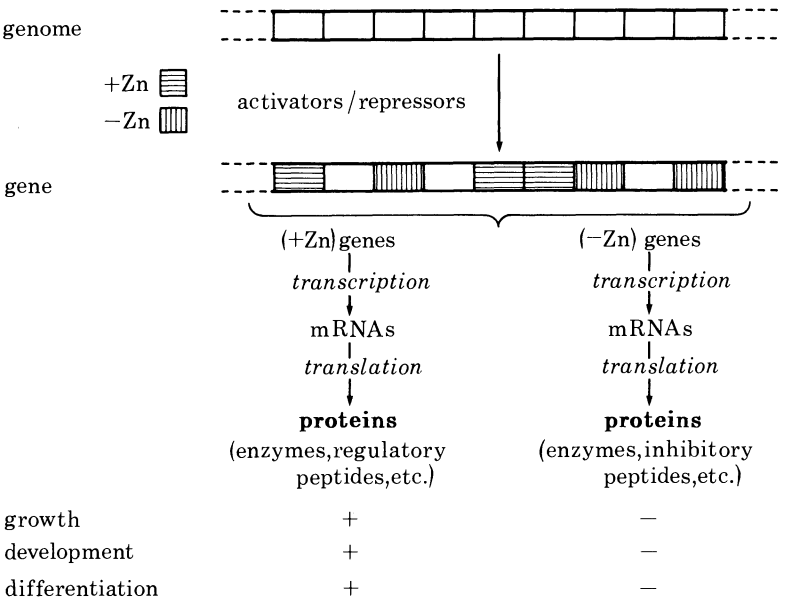


FIGURE 8. Expression of some genes is affected by binding of activators and/or repressors, and this process is affected by zinc. Activated (or repressed) genes are then transcribed (or not) into mRNA. This results in products (or their lack). The consequences of (−Zn) are manifested as alterations or cessation of growth, development and differentiation.

DNA segments between these complexes (Delange & Smith 1979). The binding of histones to DNA reduces the DNA's capacity to function as a template for both DNA and RNA polymerases. Such an ordered structure has therefore been postulated to represent the inactive state of chromatin, which is converted into one that replicates and transcribes actively through extensive modification of the histones by phosphorylation, acetylation, methylation and/or interaction with other nuclear proteins. The histones are thus believed by many to participate in the regulation of gene expression. Such conclusions are consistent with the appearance of new histone bands or modification of known bands during embryonic development, tissue differentiation, changes in growth and during cell division, and whenever cells require synthesis of new proteins (Panyim & Chalkley 1969; Delange & Smith 1979).

The differences in the electrophoretic patterns of the histones and the accumulation of basic peptides, such as the arginine-rich peptides in (−Zn) cells, is most significant. It is entirely consistent with the postulate of a crucial role for zinc in gene expression and further raises interesting questions about possible sites of action for monitoring the interaction of DNA with proteins known to regulate its function and structure.

A number of possible mechanisms merit exploration. When zinc becomes growth limiting, the resulting set of biochemical responses shift the cell from a state of active division to one of

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

growth arrest. This requires metabolic changes that result in the reduction of protein synthesis, in the utilization of existent cellular energy reserves such as carbohydrates (e.g. β-1,3-glucan), and stabilization and maintenance of existing DNA, which is protected from nucleases while in an inactive state, etc. Such metabolic transformations require selective activation and/or repression of genes, a process that might, perhaps, take place as observed in the *lac* and *tryp* operons (Dickson *et al.* 1975; Bertrand *et al.* 1975). Such processes depend on molecules that bind to DNA, facilitate or hinder RNA polymerase binding and hence which modulate transcription (Ptashne *et al.* 1980).

Zinc deficiency could selectively affect the transcription of genes through a number of such mechanisms. For example, the histones of *E. gracilis* could be modified by metal-dependent methylation or phosphorylation or through interaction with arginine-rich peptides. These could also interact directly with DNA with arginyl residues binding to phosphates of the nucleic acid. In this manner, specific genes could be activated or repressed, and selected proteins and enzymes synthesized or inhibited, resulting in the arrest of cell division (figure 8).

These studies of the function of zinc in *E. gracilis* lay a foundation for the interpretation of relevant data in other organisms, including vertebrates. They show that the pervasive role of this metal in overall growth, development and differentiation can best be understood through gene activation and/or repression, thus providing a rationale for the teratological and genetic abnormalities often observed in zinc deficiency and predicts yet other similar defects that have not as yet been recognized as being attributable to this metal and its metabolism.

This work was supported by N.I.H. grants no. GM–15003 and no. GM–24989.

References (Vallee & Falchuk)

Bertrand, K., Korn, L., Lee, F., Platt, T., Squires, C. L., Squires, C. & Yanofsky, C. 1975 New features of the regulation of the tryptophan operon. *Science, N.Y.* **189**, 22–26.

Chang, L. M. S. & Bollum, F. J. 1970 Deoxynucleotide-polymerizing enzymes of calf thymus glands. IV. Inhibition of terminal deoxynucleotidyl transferase by metal ligands. *Proc. natn. Acad. Sci. U.S.A.* **65**, 1041–1048.

DeLange, R. J. & Smith, E. L. 1979 Chromosomal proteins. In *The proteins*. vol. 4 (ed. H. Neurath & R. Hill), pp. 119–245. New York: Academic Press.

Dickson, R. C., Abelson, J., Barnes, W. M. & Reznikoff, W. S. 1975 Genetic regulation: the lac control region. *Science, N.Y.* **187**, 27–35.

Falchuk, K. H., Fawcett, D. & Vallee, B. L. 1975*a* Role of zinc in cell division of *E. gracilis*. *J. Cell Sci.* **17**, 57–58.

Falchuk, K. H., Krishan, A. & Vallee, B. L. 1975*b* Cytofluorimetry of zinc-deficient cells. *Biochemistry, Wash.* **14**, 3439–3444.

Falchuk, K. H., Hardy, C., Ulpino, L. & Vallee, B. L. 1978 RNA metabolism, manganese and RNA polymerases of zinc-supplemented and zinc-deficient *Euglena gracilis*. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4175–4179.

Fridovich, I. 1974 Superoxide dismutases. *Adv. Enzymol.* **41**, 35–97.

Fujii, T. 1954 Presence of zinc in nucleoli and its possible role in mitosis. *Nature, Lond.* **174**, 1108–1109.

Fuwa, K., Wacker, W. E. C., Druyan, R., Bartholomey, A. F. & Vallee, B. L. 1960 Nucleic acids and metals. II. Transition metals as determinants of the conformation of ribonucleic acids. *Proc. natn. Acad. Sci. U.S.A.* **46**, 1298–1307.

Jardine, N. J. & Leaver, J. L. 1978 The fractionation of histones isolated from *Euglena gracilis*. *Biochem. J.* **169**, 103–111.

Kawaguchi, H. & Vallee, B. L. 1975 Microwave excitation emission spectrometry. Determination of picogram quantities of metals in metalloenzymes. *Analyt. Chem.* **47**, 1029–1034.

Li, T.-K. & Vallee, B. L. 1980 In *Modern nutrition in health diseases*, 6th edn (ed. R. S. Goodhart & M. E. Shils), pp. 408–411. Philadelphia: Lea & Febiger.

Lieberman, I. & Ove, P. 1962 Deoxyribonucleic acid synthesis and its inhibition in mammalian cells cultured from the animal. *J. biol. Chem.* **237**, 1634–1642.

McLennan, A. G. & Keir, H. M. 1975 Deoxyribonucleic acid polymerases of *Euglena gracilis*. *Biochem. J.* **151**, 227–238.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Panyim, S. & Chalkley, R. 1969 A new histone found only in mammalian tissues with little cell division. *Biochem. biophys. Res. Commun.* **37**, 1042–1049.
Panyim, S. & Chalkley, R. 1971 The molecular weight of vertebrate histones exploiting a modified sodium dodecylsulphate method. *J. biol. Chem.* **246**, 7557–7560.
Prask, J. A. & Plocke, D. J. 1971 A role for zinc in the structural integrity of the cytoplasmic ribosomes of *Euglena gracilis*. *Pl. Physiol.* **48**, 150–155.
Price, C. A. & Vallee, B. L. 1962 *Euglena gracilis*, a test organism for studying zinc. *Pl. Physiol.* **37**, 428–433.
Ptashne, M., Jeffrey, A., Johnson, A. D., Mauver, R., Meyer, B. J., Pabo, C. O., Roberts, T. M. & Sauer, R. T. 1980 How the λ repressor and *Cro* work. *Cell* **19**, 1–11.
Riordan, J. F. 1979 Arginyl residues and anion binding sites in proteins. *Molec. cell. Biochem.* **26**, 71–92.
Sandstead, H. H. & Rinaldi, R. A. 1969 Impairment of deoxyribonucleic acid synthesis by dietary zinc deficiency in the rat. *J. Cell Physiol.* **73**, 81–83.
Scrutton, M. C., Wu, C. W. & Goldwait, D. A. 1971 The presence and possible role of zinc in RNA polymerase obtained from *Escherichia coli*. *Proc. natn. Acad. Sci. U.S.A.* **68**, 2497–2501.
Shin, Y. A. & Eichhorn, G. L. 1968 Interactions of metal ions with polynucleotides and related compounds. XI. The reversible unwinding and rewinding of deoxyribonucleic acid by zinc (II) ions through temperature manipulation. *Biochemistry, Wash.* **7**, 1026–1032.
Slater, J. P., Mildvan, A. S. & Loeb, L. A. 1971 Zinc in DNA polymerase. *Biochem. biophys. Res. Commun.* **44**, 37–43.
Swenerton, H., Shrader, R. & Hurley, L. S. 1969 Zinc-deficient embryos: reduced thymidine incorporation. *Science, N.Y.* **166**, 1014–1015.
Vallee, B. L. 1976 Zinc biochemistry in the normal and neoplastic growth processes. In *Cancer enzymology* (ed. J. Schultz & F. Abnad, pp. 159–199. New York: Academic Press.
Vallee, B. L. 1977 Recent advances in zinc biochemistry. In *Biological aspects of inorganic chemistry* (ed. D. Dolphin), pp. 37–70. New York: Wiley-Interscience.
Vallee, B. L. & Wacker, W. C. C. 1970 Metalloproteins. In *The proteins*, vol. 5 (ed. H. Neurath), pp. 1–192. New York: Academic Press.
Wacker, W. E. C. 1962 Nucleic acids and metals. III. Changes in nucleic acid, protein and metal content as a consequence of zinc deficiency in *Euglena gracilis*. *Biochemistry, Wash.* **1**, 859–865.
Wacker, W. E. C. & Vallee, B. L. 1959 Nucleic acids and metals. I. Chromium, manganese, nickel, iron and other metals in ribonucleic acids from diverse biological sources. *J. biol. Chem.* **234**, 3257–3262.

Discussion

J. K. Chesters (*Rowett Research Institute, Bucksburn, Aberdeen U.K.*) I believe that mRNAs are derived from much larger nuclear RNA species by a process of excision of RNA sequences that commonly contain unusual bases. Does Dr Vallee think that the unusual bases in the mRNA fraction from zinc-deficient *Euglena* indicate a failure of the excision process and thus of mRNA maturation?

B. L. Vallee. There is no way in which one could answer this hypothetical question – either affirmatively or negatively – without suitable experimental evidence.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

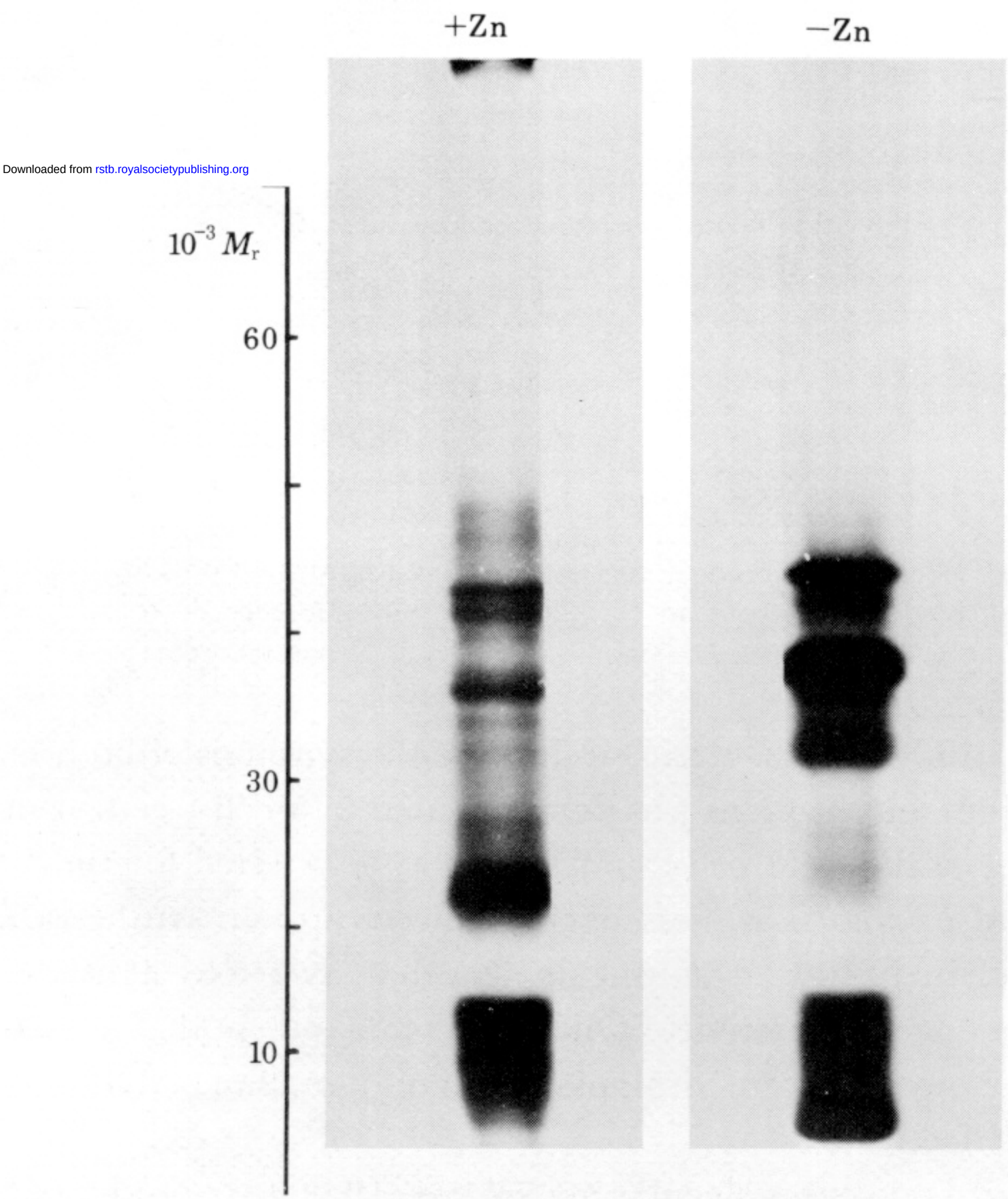
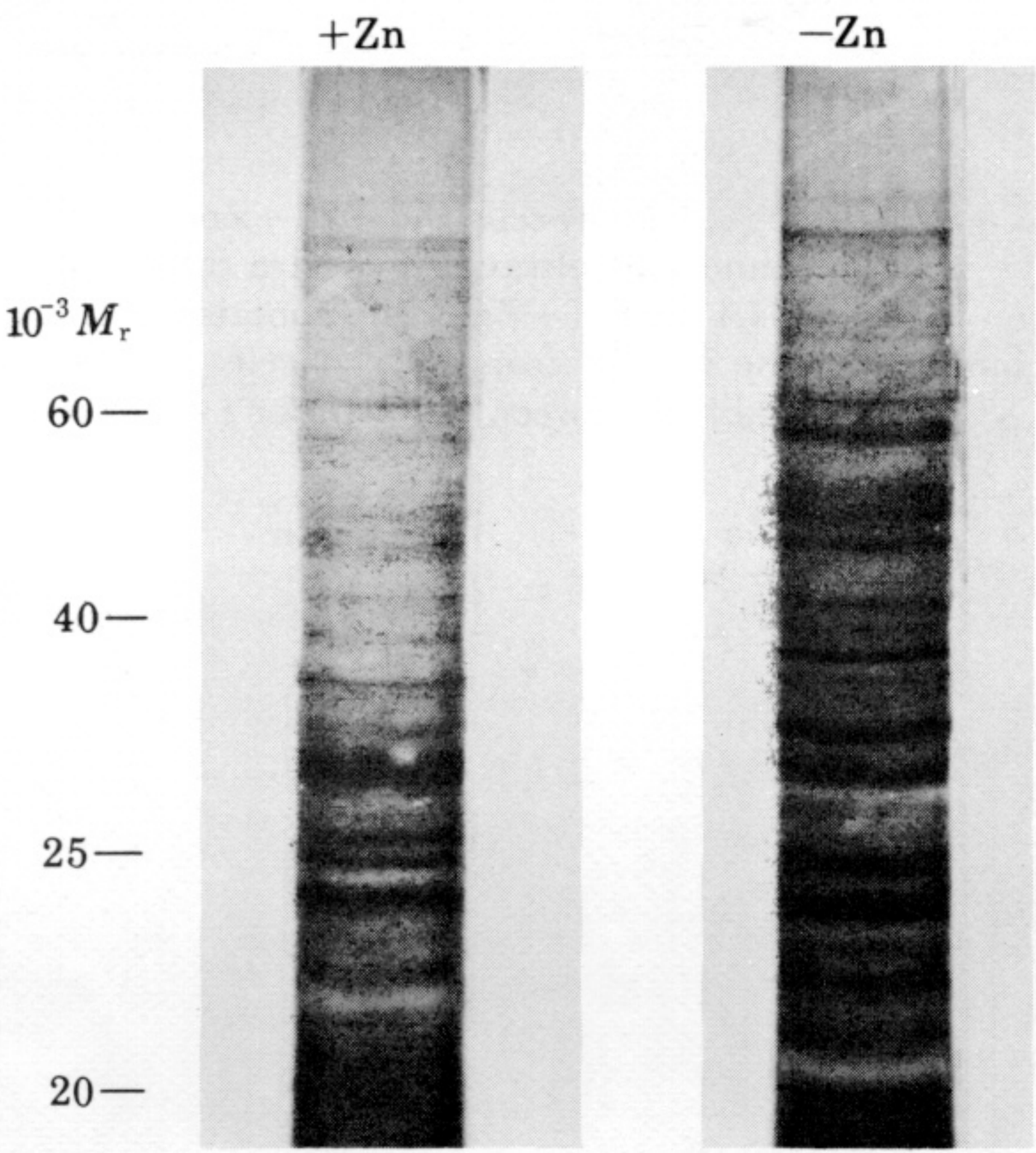
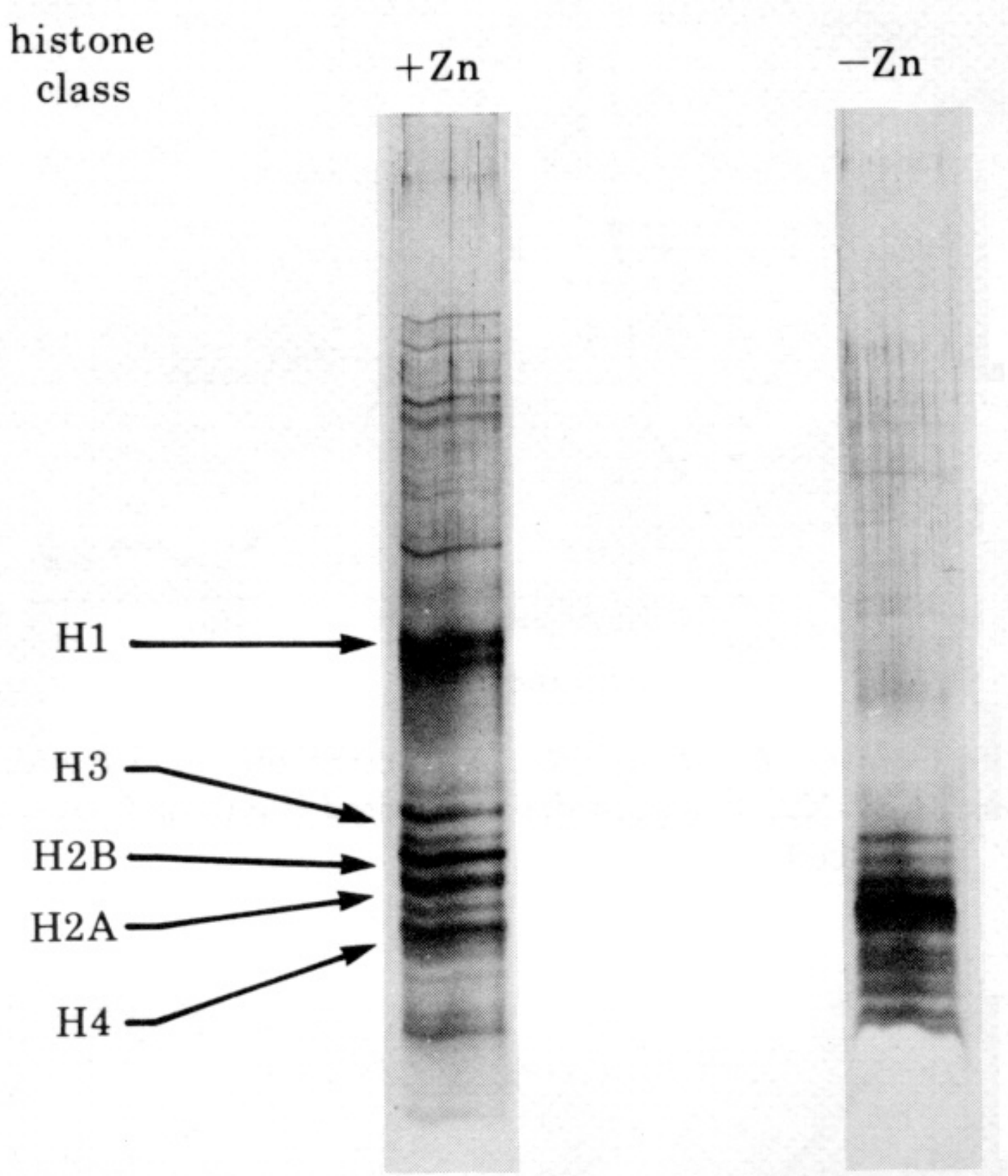


FIGURE 3. Cell-free translation products of mRNAs from (+Zn) and (−Zn) *E. gracilis* in a reticulocyte cell S_{30} lysate. Autoradiographs of the [^{35}S]methionine-labelled polypeptides were made after their separation by electrophoresis in 12.5% polyacrylamide gels.



GURE 4. (+Zn) and (−Zn) *E. gracilis* cell homogenates were chromatographed on CM-Sephadex A-50. The polypeptides were separated by electrophoresis on 12.5% polyacrylamide gels. Sections of the resultant gels are compared.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES



GURE 7. Histone fractions from (+Zn) and (−Zn) *E. gracilis* were electrophoresed on 15% polyacrylamide gels (0.1% sodium dodexyl sulphate, pH 8.8). Identification of histones was by the criteria of Panyim & Chalkley (1971) and Jardine & Leaver (1978).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES