Zinc Deficiency and Metabolism of Histones and Non-Histone Proteins in *Euglena gracilis*†

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ABSTRACT: Histones and most other basic chromosomal proteins are not extracted from zinc-deficient (−Zn) *Euglena gracilis* chromatin either by 0.25 M HCl or by 0.3–0.6 M NaCl/7 M urea. Instead, a class of 3–5-kilodalton (kDa) polypeptides, which is absent in zinc-sufficient (+Zn) cells, is solubilized. These heterogeneous polypeptides are comprised of Asn, Arg, Cys, and Gln. The partial sequence of one of these, which is composed only of Arg and Asn, is Arg-Asn-Asn-Arg-Arg-Asn-Asn-Asn-Asn-Asn-. This demonstrates they are not proteolytic fragments of the histones, proteins which do not contain contiguous Arg-Asn or Asn-Asn sequences. Once −Zn chromatin is depleted of this 3–5-kDa material, nearly all of the histones and most non-histone proteins are extracted. On the other hand, if chromatin first is depleted of, and subsequently is reconstituted with, the 3–5-kDa material, the chromosomal proteins are not solubilized, as observed with intact chromatin. Histone H4 is an exception. It is extracted neither by acids nor by NaCl/urea even after −Zn chromatin is depleted of approximately 90% of the 3–5-kDa polypeptides. Harsher extraction methods, e.g., treatment with sodium dodecyl sulfate and phenol or with protamine, are required to dissociate this histone. Electrophoretic analysis of the solubilized H4 reveals that the degree to which it is acetylated in −Zn is lower than in +Zn chromatin. Jointly, these data indicate that chromosomal proteins bind much more tightly to DNA of −Zn than +Zn cells. The effects of zinc deficiency are not limited to increased binding of these proteins to DNA. The amounts of all histones and at least one set of non-histone proteins are decreased markedly. The histone/DNA weight ratio in −Zn chromatin is 0.44 compared to 1.04 in +Zn chromatin. However, the 3–5-kDa polypeptide fraction maintains the amount of total basic proteins per unit mass of DNA at approximately 1. Further, four non-histone proteins extractable with 5% $HClO_4$ or 0.35 M NaCl and characterized by high electrophoretic mobility have been purified from +Zn nuclei. Only one of these proteins is found in −Zn chromatin. Thus, zinc deficiency induces changes in the amounts and types of histones and non-histone proteins, as well as in their interaction with DNA. These findings are discussed in relation to recent advances in understanding of the role of zinc in replication and transcription.

In response to zinc deficiency, chromatin of *Euglena gracilis* undergoes a number of functional, compositional, and structural changes. In particular, compared with +Zn organisms, it becomes highly condensed and 30-fold more resistant to micrococcal nuclease digestion (Stankiewicz et al., 1983). Furthermore, while acid fails to solubilize histones and many non-histone proteins from −Zn chromatin, it extracts a low molecular weight polypeptide fraction, which is absent from +Zn chromatin (Stankiewicz et al., 1983; Mazus et al., 1984; Falchuk et al., 1986). The condensation of −Zn chromatin is due to the presence of this low molecular weight, basic polypeptide fraction (Stankiewicz et al., 1983), but the basis for the failure to solubilize the histones and non-histone proteins is unknown, though a number of potential causes have been conjectured upon (Mazus et al., 1984).

Hence, among other parameters, we have examined the occurrence of histone and/or non-histone proteins in −Zn chromatin and the effects of the specific low molecular weight basic polypeptides of −Zn cells and/or protein chemical modifications on the DNA binding properties of these nuclear components.

The data demonstrate a low histone/DNA ratio, significantly different from that known for eukaryotic cells, with the exception of sperm. Remarkably, the *total* amount of basic proteins relative to DNA remain unchanged owing to the −Zn-specific low molecular weight protein fraction. In addition, the chromosomal proteins of −Zn cells are bound more tightly to DNA, a circumstance which is primarily a function of the presence in −Zn chromatin of the low molecular weight polypeptide fraction. These observations of the effects of zinc deficiency gain additional importance in light of the role of zinc in the binding of both replication and transcription factors to DNA (Hanas et al., 1983; Miller et al., 1985; Giedroc et al., 1986), a role which has recently moved to center stage in an understanding of mechanisms essential for gene expression.

Materials and Methods

Cell Culture. Euglena gracilis, Klebs strain Z, was grown in the dark in the presence of either 10 μM Zn^{2+}, +Zn cells, or 0.1 μM Zn^{2+}, −Zn cells (Falchuk et al., 1975a). Stationary phase cells were harvested by centrifugation at 500*g*, washed with cold deionized water, pelleted, and frozen at −20 °C.

Isolation of Nuclei. Aliquots of frozen cell pellets were thawed by suspension in nuclear isolation buffer A [15 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 7.6)/10 mM $MgCl_2$/0.6 mg % poly(vinyl sulfate)/1 mM phenylmethanesulfonyl fluoride (PMSF)/5 mM $Na_2S_2O_5$] with 10% (w/v) sucrose and 5% (w/v) Triton X-100. The cell

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suspension was frozen and thawed again and then incubated, with constant stirring, for 1 h at 4 °C. The cells were collected by centrifugation at 500*g* and washed 3 times with buffer A containing 10% sucrose (w/v) and 0.5% (w/v) Triton X-100 by resuspension and centrifugation as above. The final cell suspension was sonicated for 30–120 s and then carefully layered on buffer A with 40% (w/v) sucrose and 0.5% (w/v) TritonX-100. The sonicate was centrifuged at 2000*g* for 20 min. The nuclear pellet was washed 5–8 times with 15 mM Tris-HCl (pH 7.6)/5 mM $MgCl_2$/50 mM KCl/0.6 mg % poly(vinyl sulfate)/1 mM PMSF/10% (w/v) sucrose by gentle homogenization followed by centrifugation at 2000*g* for 10 min to remove any remaining paramylon granules and other cellular debris.

Nuclear Protein Extractions. Total nuclear proteins were extracted by the sodium dodecyl sulfate (SDS)–phenol method as described by LeStourgeon (1977), except that the final dialysis step was replaced by overnight precipitation of proteins with 4 volumes of 95% ethanol at −20 °C. Proteins were recovered by centrifugation, washed 3–5 times in absolute ethanol to remove traces of phenol, and then air-dried.

Nuclear proteins also were solubilized by the protamine release method of Richards and Shaw (1982). Nuclei were suspended in 8 M urea/5% (v/v) acetic acid/5% (v/v) 2-mercaptoethanol. Protamine sulfate (Sigma, histone free) was added to the suspension to a final concentration of 1% (w/v). After 30-min incubation at room temperature, samples were centrifuged for 10 min in an Eppendorf minicentrifuge and aliquots of the supernatant applied directly to an acetic acid/urea gel for electrophoresis, as described below.

Acid-soluble proteins were isolated either directly from the nuclei or indirectly from the phenol-extracted protein fraction by extraction with 0.25 M HCl. Extraction was repeated twice, each time for 1 h at 4 °C. Solubilized proteins were precipitated with trichloroacetic acid at a final concentration of 20% (w/v), washed once with acetone/0.1% (v/v) HCl and twice with acetone, and air-dried. The amounts of histones were quantitated by analysis of the proteins extracted by acid from the phenol-soluble protein fraction.

Another group of proteins were isolated from nuclei by extraction with 5% (w/v) $HClO_4$ as described by Goodwin et al. (1977), followed by selective fractionation with trichloroacetic acid. Proteins soluble in 2% (w/v) trichloroacetic acid and precipitable in 20% (w/v) trichloroacetic acid (Johns, 1982) were taken for further purification. Alternatively, these same proteins were extracted from nuclei with 0.35 M NaCl (Goodwin & Johns, 1973) and precipitated with trichloroacetic acid as described above. These proteins were separated on preparative SDS/polyacrylamide gels. Individual species were purified by electroelution from these gels. Following electrophoresis, side strips of the SDS gel were stained briefly to locate the proteins, followed by excision of the corresponding portions of the unstained gel. Proteins were eluted by electrophoresis into 0.1% SDS/0.5 M ammonium bicarbonate in sealed dialysis tubing (3500 molecular weight cutoff), precipitated for 24 h at −20 °C with 6 volumes of acetone/0.1% (v/v) HCl, centrifuged, dissolved in deionized water, and precipitated for 1 h at 4 °C with 20% (w/v) trichloroacetic acid. After centrifugation, protein pellets were washed with acidified acetone and twice with acetone and dried. The purity of eluted proteins has been judged by analytical SDS/polyacrylamide gel electrophoresis with silver staining.

Dissociation of Chromatin by Salt. +Zn nuclei, 250–300 μg of DNA per sample, were suspended in 10 mM Tris-HCl (pH 7.8)/7 M urea/0.1 M 2-mercaptoethanol and either 0, 0.1, 0.3, 0.6, or 2.0 M NaCl. The suspensions were incubated at 4 °C for 1 h and then centrifuged at 17000*g* for 30 min. Supernatants were collected, dialyzed overnight against 0.9 M acetic acid, clarified by centrifugation, and analyzed by polyacrylamide gel electrophoresis.

−Zn nuclei were divided into three equal aliquots. One (control) was extracted with salt/urea as described above for +Zn nuclei. Two others were extracted with 0.25 M HCl (2 times for 1 h at 4 °C) prior to salt dissociation, to remove the low molecular weight polypeptides. One of the acid-extracted aliquots was treated directly with salt/urea exactly as described above for the +Zn chromatin. The second one was carefully washed in 50 mM Tris-HCl (pH 8)/50 mM NaCl to neutralize acid and then mixed with the low molecular weight peptides dissolved in the same buffer. The suspension was incubated for 30 min at 4 °C and centrifuged, and the resulting pellet (reconstituted −Zn chromatin) was used for salt/urea extraction. All −Zn extracts were analyzed by polyacrylamide gel electrophoresis.

Polyacrylamide Gel Electrophoresis. SDS/polyacrylamide gel electrophoresis was carried out as described by Laemmli (1970) in 0.1% SDS/15% acrylamide/0.4% bis(acrylamide) gels. Proteins were visualized by staining with Coomassie brilliant blue. Gels were scanned in a densitometer. The peaks corresponding to the histones were excised and weighed, and the results were used for analysis of the relative amounts of these proteins in various nuclear preparations.

Apparent molecular weights of $HClO_4$-extracted proteins were determined from a logarithmic plot of electrophoretic mobility versus the molecular weights of the calf thymus histones. Acetic acid/urea/polyacrylamide gel electrophoresis was performed according to the method of Panyim and Chalkey (1969), with the modifications described by Hurley (1977), in 2.5 or 6.25 M urea/15% acrylamide/0.5% bis(acrylamide) gels.

Amino Acid Analysis. Amino acid analyses were performed on a Durrum D-500 amino acid analyzer equipped with a Hewlett Packard 3390A integrator after hydrolysis of the samples in vacuo with 6 N HCl and 0.1% phenol at 100 °C for 20 h (Sanger & Thompson, 1963). No corrections were made for hydrolytic loss.

Determination of Protein and DNA. Histones were determined in acid extracts of phenol-soluble nuclear proteins by the method of Bradford (1976). Calf thymus total histone fraction (Worthington) was used as a standard. Corrections were made for the presence of acid-soluble non-histone proteins (see Figure 6), based on densitometry measurements of proteins resolved on the SDS/polyacrylamide gel.

The proteins in acid extracts of nuclei were quantified by using the biuret and phenol reagents as described by Ohnishi and Barr (1978) with bovine serum albumin as a standard.

DNA was determined according to Burton (1956), using calf thymus DNA as a standard.

Results

Dilute mineral acids extract both histones and a number of non-histone proteins from +Zn *E. gracilis* nuclei. Instead, the predominant material which is released from the nuclei of −Zn organisms is composed of a heterogeneous population of polypeptides migrating as 3000–5000-dalton species that is not detectable in +Zn chromatin (Figure 1), as previously described (Stankiewicz et al., 1983; Mazus et al., 1984). This fraction is resolved into at least 10 bands on acetic acid/urea gels (not shown). The amino acid composition of the total peptide fraction is Asn (55 mol %), Arg (35 mol %), Cys (5 mol %), and Gln (5 mol %). The partial N-terminal sequence

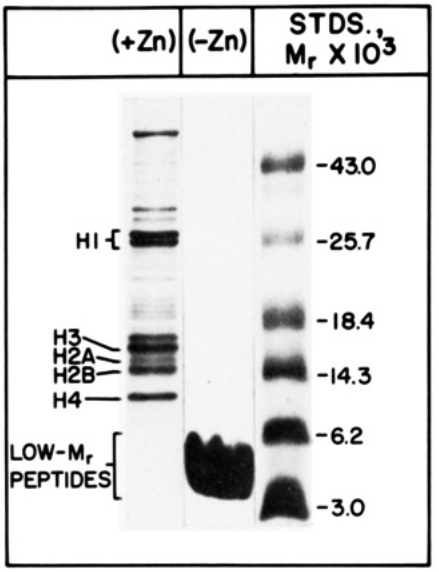

FIGURE 1: Acid-soluble chromosomal proteins of +Zn and −Zn *E. gracilis*. The proteins were extracted from isolated nuclei with 0.25 M HCl and electrophoresed on an SDS/polyacrylamide gel. Nomenclature of *Euglena* histones follows that of Jardine and Laever (1978) and Mazus et al. 1984). Protein standards used for molecular weight calibration are ovalbumin (43 000), α-chymotrypsinogen (25 700), β-lactoglobulin (18 400), lysozyme (14 300), bovine trypsin inhibitor (6200), and insulin (3000) (Bethesda Research Laboratories).

of 1 of the 10 bands is Arg-Asn-Asn-Arg-Arg-Asn-Asn-Asn-Asn-.

This low molecular weight polypeptide fraction affects the extractability of histones and other chromatin proteins. In +Zn chromatin, which does not contain these polypeptides, treatment with increasing concentrations of NaCl in the presence of 7 M urea dissociates chromosomal proteins. NaCl (0.3 M) initiates the dissociation of the core histones followed by the concurrent extraction, with 0.6 M NaCl, of histone H1 and a number of non-histone proteins (Figures 2 and 3). In contrast, treatment with increasing NaCl concentration up to 0.6 M dissociates neither histones nor non-histone proteins from DNA of intact −Zn chromatin, which contains the 3–5-kDa polypeptides. Much higher salt concentrations, e.g., 2 M NaCl, release some non-histone proteins, 3–5-kDa peptides, and small amounts of histones (Figure 2, control). Following the removal of 80–90% of the 3–5-kDa polypeptides from −Zn nuclei by acid extraction, salt/urea treatment dissociates both histones and non-histone proteins at concentrations of salt between 0.3 and 0.6 M, analogous to +Zn chromatin (Figures 2 and 3, acid extracted). Importantly, reconstitution of the chromatin initially depleted of low molecular weight peptides with this latter material restores the behavior of intact −Zn chromatin; histones and other proteins are no longer extractable with 0.3–0.6 M NaCl (Figure 2, reconstituted). On removal of the 3–5-kDa material from −Zn chromatin, significantly lower proportions of histones H2A and H2B are released, compared to +Zn chromatin. Histone H4 is not solubilized even with 2 M NaCl (Figures 2 and 3).

When −Zn nuclei are treated with more potent dissociating methods, either SDS/phenol or protamine in the presence of high concentrations of urea, the amount of solubilized protein compared to that extracted with acids increases by a factor of 5 or greater. The protein extraction from −Zn nuclei, however, is still less effective than that from +Zn chromatin. Thus, with the SDS/phenol method, approximately 92.3% ± 4% (SD, $n = 3$) of the total proteins present in +Zn nuclei are solubilized contrasted with 68.7% ± 13% (SD, $n = 6$) obtained from the −Zn ones.

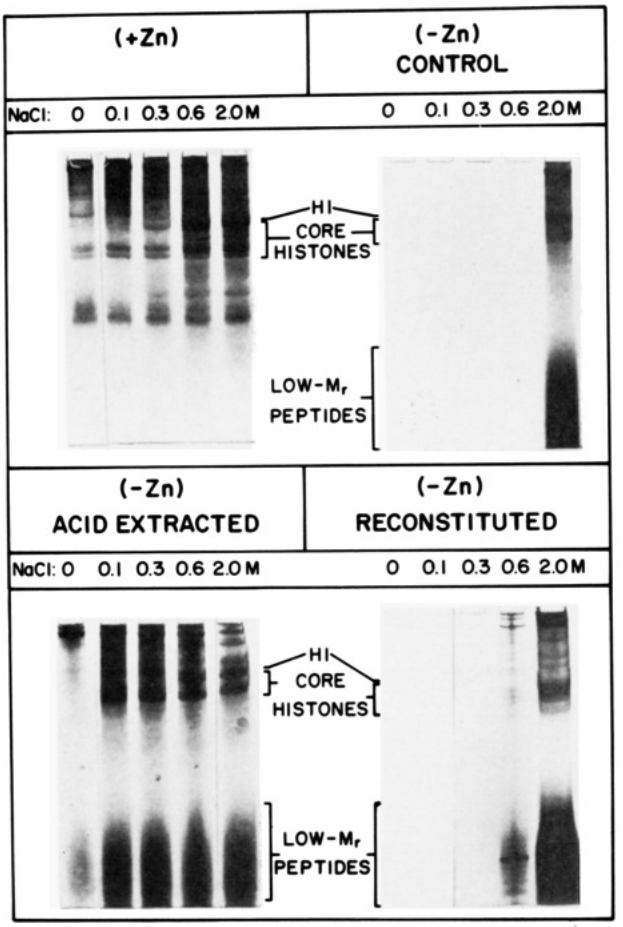

FIGURE 2: Salt dissociation of *E. gracilis* chromatin in the presence of urea. Four types of preparations were used: intact +Zn and −Zn nuclei (control); −Zn nuclei preextracted with 0.25 M HCl (acid extracted); and −Zn nuclei extracted with acid and then reconstituted with 3–5-kDa polypeptides (reconstituted). Nuclei were incubated at various NaCl concentrations in the presence of 7 M urea as described under Materials and Methods. Proteins dissociated from DNA were analyzed by electrophoresis on an acetic acid/6.25 M urea gel under conditions which allow the 3–5-kDa peptides to be retained on the gel.

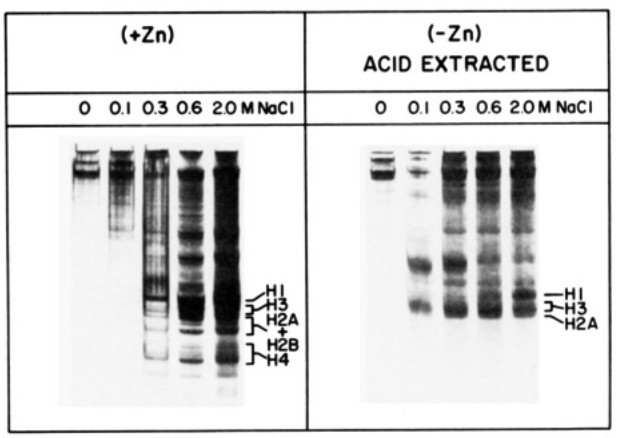

FIGURE 3: Extended electrophoresis of proteins dissociated from +Zn and −Zn acid-preextracted chromatin by various NaCl concentrations in the presence of 7 M urea. Electrophoresis was on an acetic acid/2.5 M urea gel.

The material from both cell types, extracted by using either the SDS/phenol (Figure 4) or the protamine release method (Figure 5), is composed of a heterogeneous set of chromosomal proteins, ranging in molecular weights from 10 000 to greater than 100 000. In addition, the 3–5-kDa acid-soluble polypeptides are detected among the proteins released from −Zn

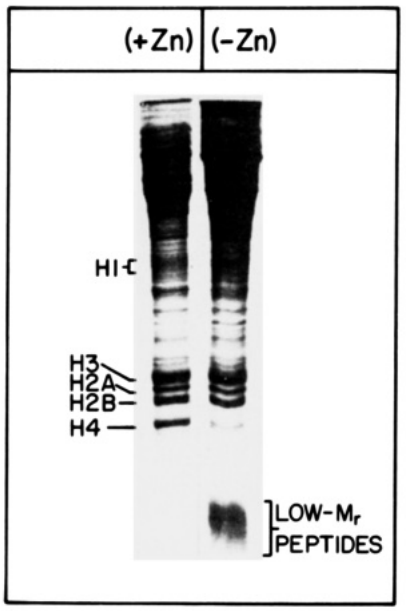

FIGURE 4: Total nuclear proteins of +Zn and −Zn *E. gracilis*. The proteins were extracted from the isolated nuclei by the SDS/phenol method and separated on an SDS/polyacrylamide gel. Before electrophoresis, a portion of 3–5-kDa polypeptides has been removed from the −Zn sample by treatment with 20% (w/v) trichloroacetic acid.

FIGURE 5: Nuclear proteins of +Zn and −Zn *E. gracilis* prepared by the protamine release method. The proteins were electrophoresed on an acetic acid/6.25 M urea gel. The 3–5-kDa polypeptides of −Zn cells have been eluted from the gel. The assignment of histone bands in this gel system was established by analysis of the histone region on a second dimensional SDS gel (result not shown). E1, E2, E3, and E4 denote the positions of four non-histone proteins.

chromatin. Both the −Zn and +Zn nuclear extracts contain all histone species including histone H4, each with its characteristic electrophoretic mobility (Jardine & Laever, 1978). However, the relative proportion of the latter histone is still markedly reduced in extracts from −Zn compared to those from +Zn nuclei (Figures 4 and 5). The decreased content of histone H4 is observed in all preparations independent of the extraction method used. No consistent differences in the relative amounts of the other histones are noted. Once histones are dissociated from DNA, either by SDS/phenol or by protamine, they readily dissolve in acid solutions and exhibit their typical behavior on SDS gel electrophoresis (Figure 6).

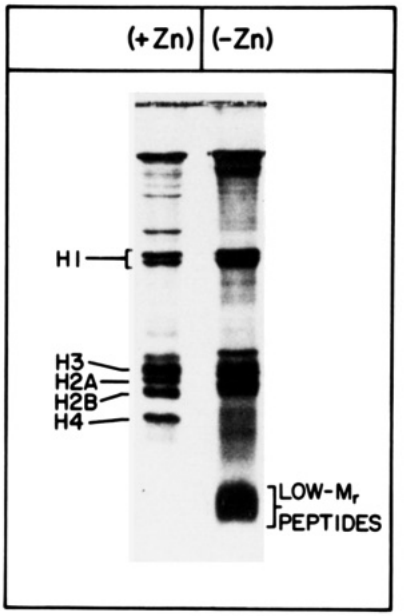

FIGURE 6: Acid-soluble chromosomal proteins of +Zn and −Zn *E. gracilis*. The proteins were extracted from the phenol-soluble protein fraction with 0.25 M HCl and electrophoresed on an SDS/polyacrylamide gels.

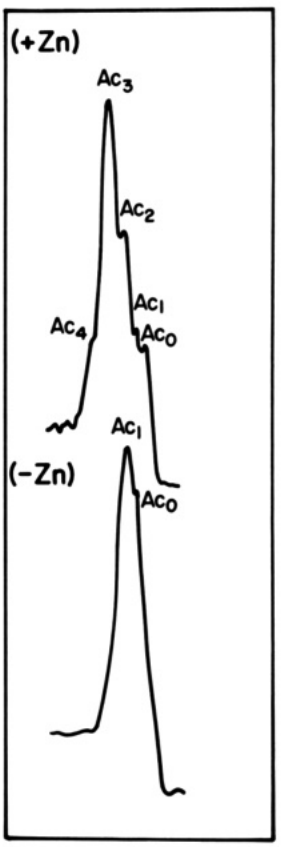

FIGURE 7: Densitometer tracings of the H4 regions of an acetic acid/urea gel. Histones were isolated from +Zn and −Zn nuclei by the protamine release method and electrophoresed on an acetic acid/2.5 M urea gel. Ac_4, Ac_3, Ac_2, Ac_1, and Ac_0 correspond to mobilities expected for tetra-, tri-, di-, mono-, and unacetylated H4, respectively.

Analysis of histones in acid/urea/polyacrylamide gels reveals that the extent to which histone H4 is modified differs in +Zn and −Zn cells (Figures 5 and 7). In this electrophoretic system, H4, obtained from +Zn cells, separates into five distinct protein bands with progressively decreasing mo-

Table I: Weight Ratios of Basic Chromosomal Proteins to DNA in +Zn and −Zn Chromatin

type of protein	type of chromatin	
	+Zn	−Zn
histones	1.04 ± 0.07 (3)[a]	0.44 ± 0.05 (6)
3–5-kDa polypeptides		0.63 ± 0.06 (3)

[a] Micrograms of protein per microgram of DNA, mean ± SD. Number of determinations is included in parenthesis.

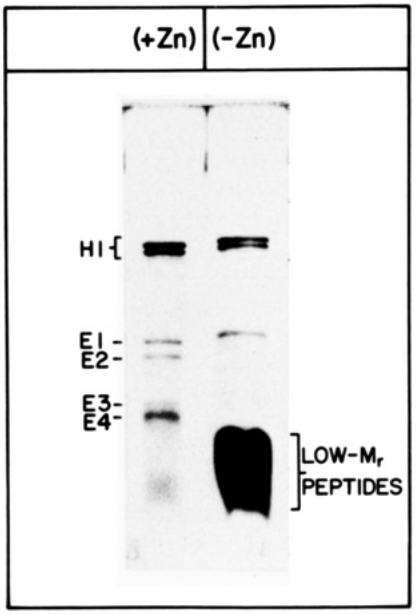

FIGURE 8: 5% $HClO_4$-soluble chromosomal proteins from +Zn and −Zn *E. gracilis*. Isolated proteins were electrophoresed on an SDS/polyacrylamide gel.

bilities (Figure 7). Such electrophoretic behavior has been shown to be due to acetylation of from zero to four lysyl residues in this histone (Panyim & Chalkley, 1969; Allfrey et al., 1984). In contrast, histone H4 from −Zn cells migrates either as an unmodified or as a monoacetylated form. No distinct differences in the extent to which other histones are modified are detected.

The effects of zinc deficiency on chromatin are not limited to alterations in DNA binding properties of chromosomal proteins, as described above, but also extend to quantitative differences in both histones and non-histone proteins. Thus, the weight ratio of histones relative to DNA is decreased in −Zn compared to +Zn chromatin (Table I). The decreased proportion of histones appears to be compensated by the 3–5-kDa polypeptides, since the mass ratio of *total* basic proteins—histones plus the 3–5-kDa polypeptides—to DNA approximates unity.

The majority of non-histone proteins from +Zn and −Zn nuclei behave similarly upon extraction and on gel electrophoresis (Figures 2, 4, and 5). However, a group, characterized by high electrophoretic mobility in acid/urea gels (Figure 5, bands marked E1 to E4), differs. These proteins are isolated from nuclei by extraction with 5% $HClO_4$ followed by selective fractionation with trichloroacetic acid. The 5% $HClO_4$ extract of +Zn nuclei separates into six bands upon electrophoresis in SDS gels (Figure 8). The two bands exhibiting the slowest migration correspond to the very lysine-rich H1 histone, one of the major $HClO_4$-soluble proteins in most organisms (Johns, 1964). Its amino acid composition (see Table II) is typical for this protein (Jardine & Laever, 1978). The other four proteins, labeled E1, E2, E3, and E4 in order of increasing electrophoretic mobility, have apparent molecular

Table II: Amino Acid Composition[a] of 5% $HClO_4$-Soluble *E. gracilis*

	proteins				
	E1	E2	E3	E4	histone H1
Asx	6.6	6.4	7.1	7.1	1.0
Thr	3.1	3.3	3.9	5.2	3.3
Ser	5.8	7.6	7.5	8.9	4.8
Glx	6.8	9.5	8.8	6.5	3.3
Pro	4.5	3.1	6.6	14.5	8.7
Gly	18.3	14.0	13.1	12.8	4.0
Ala	7.1	4.7	9.2	6.9	20.0
Val	3.7	3.7	4.2	7.0	5.3
Cys	N.D.[b]	N.D.	N.D.	N.D.	N.D.
Met	1.4	1.9	2.0	0	1.0
Ile	2.6	3.5	3.7	2.4	1.5
Leu	4.6	6.2	6.2	4.9	4.5
Tyr	0	0	0	6.1	2.0
Phe	1.7	2.5	2.4	2.4	1.3
His	1.5	1.1	1.2	0	0.8
Lys	27.2	28.6	20.3	12.0	36.3
Arg	5.1	3.9	3.8	3.3	3.2
Asx + Glx	13.4	15.9	15.9	13.6	4.3
basic amino acids	33.8	33.6	25.3	15.3	40.3

[a] Mole percent. [b] N.D., not determined.

weights of 17000, 15300, 10800, and 10000, respectively. In this electrophoretic system, E2 and E3 migrate similarly to histones H3 and H4, respectively. However, both their behavior on acetic acid/urea (Figure 5) and two-dimensional acid/urea–SDS gels (not shown) and also their amino acid composition (Table II) differentiate them from these histones.

The equivalent 5% $HClO_4$-soluble fraction from −Zn nuclei contains only histone H1 and E1 (M_r = 17000) but not the other three proteins found in the +Zn material (Figure 8). As with other acid extracts of −Zn chromatin, the 3–5-kDa polypeptides constitute the predominant fraction. Essentially the same results as those described for the 5% $HClO_4$ extraction are obtained when +Zn and −Zn nuclei are treated with 0.35 M NaCl (not shown). The four proteins of +Zn cells (E1, E2, E3, andd E4) contain high levels of both basic (15–34%) and acidic (13–16%) residues (Table II). The composition of the two larger proteins, E1 and E2, is very similar, suggesting that these proteins may be homologous. E1 isolated from −Zn cells is identical with its counterpart from +Zn cells (data not shown), and they are, therefore, considered to be the same protein.

Discussion

Zinc is present in the cell nucleus (Fujii, 1954) and is involved in diverse aspects of the functions of the major constituents of this organelle, and we proposed that zinc deficiency alters their metabolism (Vallee & Falchuk, 1981). The processes underlying gene action might then induce the biological abnormalities characteristic of zinc deficiency (Vallee & Falchuk, 1981; Crossley et al., 1982). We have continued to examine this hypothesis experimentally by studies of the effects of zinc deficiency on the metabolism of chromatin constituents including DNA, RNA polymerases, and nuclear proteins, in general, and the basis for the absence of histones in acid extracts of −Zn *E. gracilis* (Mazus et al., 1984), in particular.

We have demonstrated previously that the inability of extracting histones with acids is characteristic of −Zn *E. gracilis*; it is not observed with +Zn organisms or cells deprived of Fe, Mn, or Mg or exposed to cold shock (Mazus et al., 1984; Falchuk et al., 1986). By using conditions which are more effective than acid in releasing proteins from DNA, e.g., extraction by SDS and phenol (LeStourgeon & Beyer, 1977) or exposure to protamine in the presence of a high concen-

tration of urea (Richards & Shaw, 1982), it has been shown that most, but not all, chromatin proteins present in +Zn, including histones, also are found in −Zn cells (Figures 4 and 5). Once dissociated from DNA, the histones from −Zn chromatin readily dissolve in acids, in marked contrast to their behavior when bound to the nucleic acid. Therefore, one general conclusion of the present work is that the absence of chromatin proteins in acid or salt extracts (Figures 1–3) must be due to the induction of tighter binding of these proteins to DNA in −Zn chromatin. At least two biochemical variables which could account for this tighter binding are identified.

First, the 3–5-kDa basic polypeptides, the major acid-soluble fraction of −Zn nuclei, appear to be the predominant factor(s) involved in generating this phenomenon (Figures 2 and 3). Their removal from chromatin by acid extraction results in the subsequent dissociation from DNA of the remaining chromosomal proteins, including histones, by low salt/urea, as observed with +Zn chromatin from *E. gracilis* (Figures 2 and 3) and from other sources (Bartley & Chalkley, 1972). On the other hand, reconstitution with the peptides of the depleted chromatin once again prevents protein dissociation until the concentration of salt reaches 2 M, as observed with intact −Zn chromatin (Figure 2).

The need to invoke the existence of at least a second mechanism for tighter binding of chromatin proteins to DNA derives from the observation that some proteins, in particular histone H4, resist dissociation from −Zn chromatin even after removal of about 90% and 100% of the 3–5-kDa polypeptides by acid (Figures 2 and 3) and SDS/phenol (Figure 4), respectively. It has been recognized that acetylation of histones weakens interactions between the protein and the associated DNA by neutralization of positive charges of the lysine side chains (Doenecke & Gallwitz, 1982). Therefore, the lesser extent of acetylation of H4 from −Zn chromatin (Figure 7) favors tighter binding of at least this histone to DNA and raises the possibility that this modification could play a role in the behavior observed for histone H4. This and yet other possibilities await experimental confirmation. Differences in the extent to which the other histones derived from +Zn and −Zn chromatin are modified have not been identified as yet. Their existence might provide additional explanations for the altered binding of some of these chromosomal proteins, e.g., histone H2A and H2B, to DNA.

While the data show that −Zn cells contain all of the histones and most non-histone proteins found in +Zn organisms, they also demonstrate that zinc deficiency affects the quantity of these proteins in *E. gracilis* cells. First, in regard to histones, during S phase of the cell cycle there is a concurrent, presumably linked synthesis of these proteins and DNA (Sheinin & Humbert, 1978). At the termination of DNA synthesis, nuclei contain one set of preexisting histones and another of newly formed ones. In this manner, the histone/DNA weight ratio of 1 is preserved. The DNA content of −Zn nuclei is that characteristic of late S/G_2 cells (Falchuk et al., 1975b). The present data, however, demonstrate that, despite a doubling of DNA, the nuclei contain only *one* set of histones (Table I). This supports the view that zinc deficiency may impair histone synthesis itself. Alternatively, if histones are synthesized, then they either do not combine with or are displaced from chromatin and undergo proteolytic degradation, as previously postulated (Mazus et al., 1984). The absence of the full complement of histones in −Zn chromatin, however, is not associated with the existence of naked DNA by virtue of the formation of the 3–5-kDa polypeptides. Their amounts are equivalent to or even slightly higher than those of histones (Table I). This allows the proper ratio of basic proteins to DNA to be maintained in −Zn chromatin. The nature of these polypeptides and their DNA binding will be discussed (M. Czupryn, K. H. Falchuk, and B. L. Vallee, unpublished results). At present, it can be concluded that, on the basis of their amino acid composition and partial sequence of one of these polypeptides, they are not degradation products of any of the known chromatin proteins, including histones. Although the amino acid sequence of *Euglena* histones has not been established yet, none of the histones from the other sources contains contiguous Asn/Arg-rich regions (Isenberg, 1979).

Non-histone proteins are a second group affected. The identities of the four proteins isolated from +Zn nuclei are unknown. Their extractability and solubility as well as electrophoretic migration are similar to those of high-mobility group (HMG) proteins (Johns, 1982; Mayes, 1982; Nicolas & Goodwin, 1982) (Figures 5 and 8; Table II). In addition, they contain relatively high amounts of both basic and acidic residues, a feature characteristic of all HMG proteins (Mayes & Johns, 1982). However, the amino acid composition of HMG proteins isolated from lower and higher eukaryotes, including mammalian ones, is not highly conserved (Mayes & Johns, 1982). Therefore, this criterion is not helpful in categorizing the proteins from *E. gracilis* as HMG species. In contrast to +Zn chromatin, E1 is the only protein of this type present in −Zn nuclei. The absence of the three proteins of this class suggests that, like histones, they either are not formed or are degraded in −Zn cells.

These findings extend those of previous data on the effects of zinc on chromatin metabolism. Thus, the reversible condensation of chromatin in −Zn *E. gracilis* must occur not only by virtue of the association with 3–5-kDa polypeptides (Stankiewicz et al., 1983). On the basis of information presently available, potentially, effects of other chromatin proteins must be considered, for example, the binding properties of histones to DNA as well as differences in the extent of chemical modification of these proteins, particularly histone H4.

This would be analoguous to the way by which condensation of chromatin occurs in other cells where this event depends also on alterations in the types of chromosomal proteins bound to DNA and/or their chemical modifications. For example, in highly condensed sperm chromatin of different species, DNA is associated either with protamines which partially or completely displace histones (Honda et al., 1975) or with unmodified (unacetylated) histones (Ruiz-Carrillo & Palau, 1973). Acetylation occurs in histone N-terminal regions (Allfrey et al., 1984) which are involved in generating the higher order structure of the bulk of chromatin, the 30-nm coil (Igo-Kemenes et al., 1982). The presence of unmodified (unacetylated) species is presumed to promote chromatin condensation. In sperm, however, the molecular mechanisms which induce the changes in the protein components and structure of chromatin are unknown. In *E. gracilis*, they are triggered by zinc deprivation and are entirely reversed by zinc supplementation.

In this regard, the emerging understanding of the known roles of zinc in the metabolism of the cell nucleus is pertinent here (Vallee & Falchuk, 1981). Zinc stabilizes the native structure of RNA (Wacker & Vallee, 1959; Fuwa et al., 1960) and DNA (Shin & Eichhorn, 1968); it is essential for the catalytic activity of the RNA polymerases (Scrutton et al., 1971; Auld et al., 1976; Falchuk et al., 1976, 1977, 1985) and for the function of at least two chromatin proteins: TFIIIA, which is essential to transcription (Hanas et al., 1983; Miller

et al., 1985), and g32P, involved in replication (Giedroc et al., 1986). The zinc binding domains of both TFIIIA and g32P proteins depend upon -Cys-X(n)-Cys- sequences present either as single units or as repeats, the latter referred to as "zinc fingers" (Miller et al., 1985; Berg, 1986). Zinc is believed to be involved in maintaining the protein conformation of the DNA binding domains required for function (Hanas et al., 1983; Miller et al., 1985; Giedroc et al., 1986).

The demonstration that zinc and its deficiency exert such profound effects on the DNA binding properties of other nuclear proteins, in addition to TFIIIA and g32P, points to the fact that zinc plays yet other, more general role(s) in interaction among the chromatin components, including the major ones, i.e., histones. The findings moreover confirm our previous suggestions (Vallee & Falchuk, 1981; Crossley et al., 1982) that zinc modulates the binding properties of regulatory proteins to their particular genes either directly when the metal is a component of the protein or indirectly by affecting the function of zinc-activated kinases capable of chemically modifying histones and non-histone proteins (Kang et al., 1974) among other possibilities. The functional consequences of such effects on DNA binding properties, particularly in the control of transcription itself, have also been detailed previously (Falchuk et al., 1975; Vallee & Falchuk, 1981; Crossley et al., 1982). It seems quite likely to us that the multiple effects of zinc deficiency on chromatin structure and function relate to, or can be explained by, the involvement of zinc in the function of nuclear proteins which regulate the activity of genetic material. The extensive information available on zinc and nucleic acid metabolism of *E. gracilis* makes it exceptionally suitable for the experimental study of this fundamental biological question.

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