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Composition and Structure of Zinc-Deficient *Euglena gracilis* Chromatin†

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ABSTRACT: The histone content of zinc-deficient (−Zn) *Euglena gracilis* decreases while, concomitantly, DNA content increases and the transcription rate is reduced markedly [Mazus, B., Falchuk, K. H., & Vallee, B. L. (1983) *Biochemistry* (in press); Falchuk, K. H., Fawcett, D. W., & Vallee, B. L. (1975) *J. Cell Sci. 17*, 57–78]. The effects on major constituents of the genome have been examined by studying the rate and extent of hydrolysis of +Zn and −Zn chromatin by micrococcal nuclease, DNase I, or DNase II. The size of hydrolyzed DNA fragments suggests similarity of the +Zn *E. gracilis* chromatin organization to that of other eukaryotes. The major protein constituent of −Zn chromatin is a polypeptide of less than 3000 daltons whose electrophoretic mobility differs from that of any known histone components of chromatin, the latter described elsewhere (K. H. Falchuk et al., unpublished results). This protein profoundly affects the structure of −Zn chromatin, which is about 10–30-fold more resistant to micrococcal nuclease hydrolysis than +Zn chromatin. Moreover, the resultant DNA fragments [2000 base pairs (bp)], are much larger than those of +Zn cells. Under conditions which hydrolyze +Zn chromatin into DNA fragments smaller than 50 bp, only 50% of −Zn chromatin is digested into fragments <2000 bp, i.e., in the range of those expected for oligonucleosomes. Removal of the low molecular weight protein from −Zn chromatin reverses its enhanced resistance to nucleolysis and results in extensive hydrolysis. Conversely, addition of the low molecular weight protein to +Zn chromatin increases the resistance of this complex to digestion. The results demonstrate the critical importance of zinc to the formation, composition, and structure of normal *E. gracilis* chromatin. The implications to genomic function are discussed.

Zinc is critical to the normal composition and function of the *Euglena gracilis* genome, as is apparent from studies of its deprivation. Zinc deficiency greatly increases the amount of DNA (Wacker, 1962; Falchuk et al., 1975a) while causing the virtual disappearance of the histones (Mazus et al., 1983). Ultimately transcription is repressed (Falchuk et al., 1975a). The characteristics of the protein(s) which replaces (replace) the histones, the effects of their substitution on the structure of chromatin in −Zn cells, and the relationship of such changes to the genomic repression observed in these organisms are all unknown.

The nuclei of +Zn and −Zn *E. gracilis* cells have now been isolated to compare pertinent physical and chemical properties of the respective chromatins. Micrococcal nuclease, DNase I, and DNase II were employed as enzymatic probes. The size of the basic nucleosomal unit of +Zn *E. gracilis* and the histone components of its chromatin are all similar to those of other eukaryotes. The chromatin of −Zn is unlike that found

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in +Zn cells or expected for protein-free DNA. It is highly resistant to nuclease digestion, and the resultant DNA fragments are larger than those from +Zn cells. The −Zn chromatin contains a protein whose electrophoretic properties and molecular weight differ from those of normal histones. Its removal renders the nuclease-resistant −Zn chromatin more sensitive, and its addition to +Zn chromatin increases its resistance to nuclease digestion.

The present findings suggest that a new protein replaces the histones of −Zn chromatin resulting in a more compact conformation of DNA which may relate to the extensive repression of the *E. gracilis* genome characteristic of −Zn organisms (Falchuk et al., 1975a,b; Vallee & Falchuk, 1981, 1983).

Materials and Methods

Preparation of Chromatin. *Euglena gracilis* strain Z was grown to stationary phase in the dark in +Zn and −Zn media containing 10 and 0.1 μM Zn^{2+}, respectively, as described (Falchuk et al., 1975a). In addition, aliquots of log phase +Zn cells were centrifuged, the pellets washed and incubated in −Zn media, and the cells harvested 3 days later. These cells are identified as +Zn → −Zn cells. Chromatin from +Zn, −Zn, and +Zn → −Zn *E. gracilis* were prepared by suspending 6 g of cells in 10 mL of buffer A (15 mM Tris-HCl,[1] pH 7.5, 60 mM KCl, 15 mM NaCl, 15 mM β-mercaptoethanol, 0.15 mM spermine, 0.5 mM spermidine, 2 mM EDTA-Na, 1 mM PMSF, and 0.34 M sucrose). The suspension was frozen and thawed 3 times in a dry ice–methanol bath and sonicated subsequently for 15 s up to 8 times. The extent of cell rupture was monitored by light microscopy after each sonication.

The suspensions were layered on buffer B (15 mM Tris-HCl, pH 7.5, 60 mM KCl, 0.5 mM spermidine, 0.15 mM spermine, 15 mM β-mercaptoethanol, 15 mM NaCl, 0.2 mM EDTA, 1 mM PMSF, and 40% sucrose) and centrifuged for 15 min at 2000*g*. The resultant pellet, composed of nuclei and paramylon, was solubilized in buffer C (buffer A containing 0.4 mM EDTA). This procedure was repeated 3–5 times to separate the chromatin from nuclear membranes.

Chromatin Proteins. To compare the chromatin proteins from +Zn and −Zn cells, chromatin suspensions were mixed with 20 volumes of 0.4 N H_2SO_4 at 4 °C for 2–70 h, followed by centrifugation for 20 min at 8000*g*. The resultant supernatant was precipitated with 5 volumes of 95% ethanol at −20 °C for 48 h each time. The pellet was collected by centrifugation and the supernatant precipitated again with ethanol. The two pellets were combined and used for gel electrophoretic analyses. Samples were dissolved in 0.5 mL of sample buffer I (0.05 M Tris-HCl, pH 6.8, 2.5 mM β-mercaptoethanol, and 1% SDS) and dialyzed for 3 × 24 h against 60 volumes of 0.05 M Tris-HCl pH 6.8, 1.2% β-mercaptoethanol, 0.1% SDS, and 10% glycerol.

Polyacrylamide (12%) with a 5% stacking gel was used for vertical electrophoresis. Tris-HCl, 0.375 M, pH 8.8, and 0.1% SDS were used as gel buffer, while 25 mM Tris-HCl, 0.2 M glycine, pH 8.52, and 0.1% SDS were used as the electrode buffer. Electrophoresis was performed for 3 h at 20 mA. Gels were fixed for 1 h in H_2O and 37% formaldehyde (6:1) stained with Coomassie blue and destained in H_2O, methyl alcohol, and 37% formaldehyde (75:25:1).

Aliquots of protein solutions from −Zn chromatin, extracted with 0.4 N H_2SO_4 as above, were purified further by addition of Cl_3CCOOH to a final concentration of 20%. The solution was kept at 4 °C for 30 min and then centrifuged for 10 min at 8000*g*. Five volumes of ethanol was then added to the supernatant which was kept at −20 °C for 48 h and then centrifuged for 0.5 h at 15000*g*. The protein pellet was dissolved in water and lyophilized. Similar proteins were obtained also from acid homogenates of −Zn cells by the same procedure (K. H. Falchuk et al., unpublished results). Aliquots of these proteins (ranging from 1 to 20 mg) were added to either +Zn chromatin or calf thymus DNA. The resistance to micrococcal nuclease digestion of these protein/DNA or protein/chromatin mixtures was determined as described below. Protein concentration was measured by Bio-Rad Protein Assay Method and/or monitoring at A_{230} (1 mg/mL gives OD of 3.5).

Deoxynucleoprotein (DNP) Preparation from Chromatin. The chromatin pellet was washed 3 times with buffer D (buffer A, without EDTA). $MgCl_2$ and $CaCl_2$ were added to the suspensions to final concentrations of 1 mM. The DNP preparations were obtained from chromatin which was digested with different amounts of micrococcal nuclease (50–500 units) for varying periods of time (30 s to 90 min) at room temperature. Nuclease digestion of chromatin was terminated by adding EDTA to samples for a final concentration of 20 mM. Samples were cooled in ice and then centrifuged for 10 min at 18000*g*. Aliquots of solubilized DNP were chromatographed on Ultragel AcA22. Other aliquots were precipitated twice with 2 volumes of absolute ethanol at −20 °C, then dissolved in 10 mM Hepes and 1 mM EDTA, pH 7.5, and electrophoresed employing a 5% polyacrylamide gel for 2–3 h at 80 V. The electrode and gel buffers were the same as sample buffer. Gels were stained with ethidium bromide and photographed as described below.

The amount of DNA in each aliquot of chromatin used was measured after digestion for 0.5 h with 100 units of nuclease and centrifugation for 20 min at 15000*g*, by measuring OD_{260} in the resultant soluble fraction.

DNA Extraction from DNP Samples: Analysis of Size of Nuclease Digests. Aliquots of chromatin, washed 3 times with buffer E (same as buffer A but without EDTA or PMSF), were digested with micrococcal nuclease as described above. Conditions resulting in approximately 5–25% solubilization of DNA were selected. The solubilized chromatin was first incubated with 50 μg of ribonuclease A and 20 μg of ribonuclease T/mL for 1 h at 37 °C. Aliquots were adjusted to 1% SDS and incubated for 0.5 h, after which 200 μg of proteinase K was added and the mixture incubated for 3–4 h at 37 °C. The samples then were extracted twice by shaking for 0.5 h with 2 volumes of isoamyl alcohol–chloroform (1:24). Chloroform and water phases were separated by centrifugation for 5 min at 2000*g*. The DNA in the aqueous phase was precipitated with 2 volumes of absolute ethanol at −20 °C.

The precipitated DNA was solubilized in 200 μL of sample buffer (40 mM Tris-HCl, pH 8.0, 5 mM sodium acetate, 1% SDS, 1 mM EDTA, and 5% sucrose) and applied to horizontal 2% agarose gels containing 0.1% SDS. Electrode and gel buffers were 40 mM Tris-HCl, pH 8.0, 5 mM sodium acetate, 0.1% SDS, and 1 mM EDTA. Electrophoresis was performed for 4 h at 100 V. The gels were then washed 3 times for 30 min with 200 mL of water to remove SDS and then stained for 0.5–2 h with 200 mL of 0.5 μg/mL ethidium bromide in water. The stained gels were photographed with 52 or 55 (positive/negative) type Polaroid films. The size of DNA fragments obtained as a result of micrococcal nuclease treatment was calculated by measuring distances of migration on agarose gels with DNA fragments of known molecular

[1] Abbreviations: Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid; PMSF, phenylmethanesulfonyl fluoride; SDS, sodium dodecyl sulfate; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid.

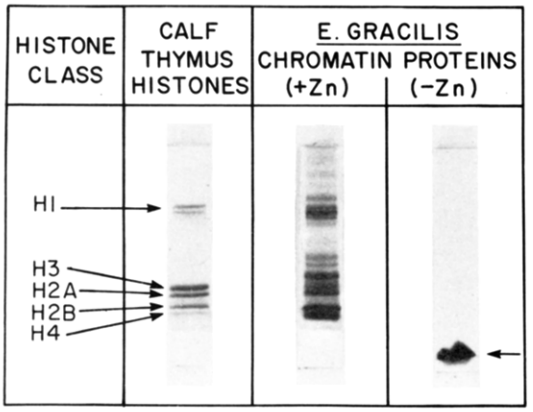

FIGURE 1: Electropherograms of ±Zn *Euglena gracilis* chromatin proteins. Calf thymus histones are electrophoresed under identical conditions to serve as standards for the identification of *E. gracilis* histones. The arrow identifies the sole protein found in −Zn chromatin.

weight (DNA Längenstandards III; Boehringer Mannheim) as standards.

Analysis of Acid-Soluble Nuclease Digests. Chromatin was digested with micrococcal nuclease, DNase I, or DNase II. Aliquots that were digested with DNase I were suspended in 10 mM Tris-HCl, pH 7.6, containing 50 mM NaCl, 0.2 M EDTA, 1 mM $MgCl_2$, and 1 mM $CaCl_2$ while those to be digested with DNase II were suspended in the same buffer except that the divalent cations were omitted. The samples were incubated for varying periods, 1–40 min, at room temperature.

The reaction was stopped by addition of perchloric acid to a final concentration of 5% and cooled for 10 min in ice. The cooled samples were centrifuged for 10 min at 1800*g*, and the amount of material digested by the nucleases was determined by measurement of the OD_{260} of the supernatant, acid-soluble fraction.

Results

While sonication disrupts the pellicle from the −Zn organisms in stationary phase in <45 s, the corresponding +Zn cells require >90 s. However, the −Zn cell nuclei are more resistant to fracture and could, therefore, be isolated more easily than the +Zn ones. The protein composition of each chromatin is distinctive (Figure 1). The major proteins of +Zn chromatin are the histones with electrophoretic properties identical with those reported elsewhere (Mazus et al., 1983). However, in −Zn chromatin, no histones could be detected; instead it primarily contains polypeptides with properties to be described and characteristic molecular weights of 2000–3000 or less (K. H. Falchuk et al., unpublished results). They are not present in +Zn chromatin.

The sensitivity of +Zn and −Zn chromatins to nuclease (50 units) differs markedly. Micrococcal nuclease solubilizes +Zn at a rate 10–30-fold greater than −Zn chromatin (Figure 2). While a 10-fold higher amount of nuclease, i.e., 500 units, digests the +Zn chromatin completely in 10 min, only 20% of the −Zn chromatin is solubilized in this time interval. The rate of digestion of −Zn chromatin by 500 units of nuclease is 7–10 times slower than that observed on incubation of +Zn chromatin with 50 units of nuclease (Figure 2). The addition of −Zn to +Zn chromatin does not affect the digestion of the latter.

The susceptibility of −Zn and +Zn chromatin to two other nucleolytic enzymes, DNases I and II, is different also. The rate and amounts of digestion for the −Zn relative to the +Zn chromatin are reduced 2–4-fold (Figure 3).

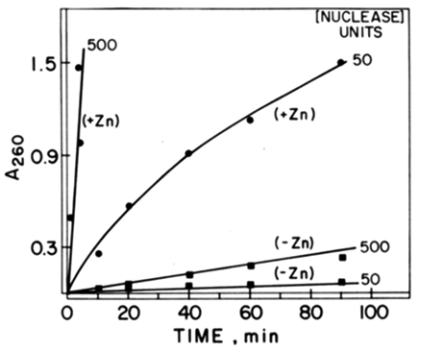

FIGURE 2: Comparison of acid-soluble material resulting from micrococcal nuclease (50 or 500 units) digestion of ±Zn chromatins (400 μg of DNA). The digestion of +Zn chromatin with 500 units of nuclease was nearly complete within 10 min, and hence, the material is solubilized totally. A_{260} was measured in 5% perchloric acid soluble supernatants of these digests.

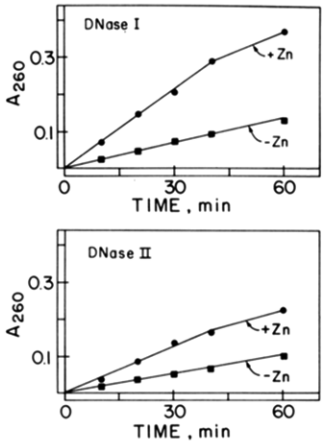

FIGURE 3: DNase I or DNase II (200 μg) digestion of ±Zn chromatins (400 μg of DNA).

Digestion of chromatin from both +Zn and −Zn cells results in deoxyribonucleoproteins, (DNP), of different sizes as demonstrated by their separation either on 5% polyacrylamide gels (Figure 4) or on Ultragel AcA 22 (Figure 5). After 3 min, micrococcal nuclease digests +Zn chromatin into variously sized DNP fragments that distribute along the entire gel (Figure 4), but in 10 min, virtually all of the resultant DNP material has been fragmented to such an extent that it can be found only in the most cathodic region (Figure 4). In accord with this, on chromatography with Ultragel AcA22 the DNP fragments separate into two peaks. The major peak, fractions 20–40 (Figure 5), contains 90% of the material absorbing at 260 nm. In contrast, exposure for 10 min of −Zn chromatin to the same amount of nuclease fails to digest it (Figure 4).

On chromatography on Ultragel AcA22 (Figure 5), when digested with 100 units of nuclease, the DNP fragments from −Zn chromatin appear in the void volume (not shown). Only after −Zn chromatin is incubated for twice this period and with 500 units, i.e., a 5-fold higher enzyme concentration, is it digested into fragments which could be analyzed on the Ultragel column. The −Zn DNP fragments separate into two peaks: the first elutes in fractions 7–12, and hence, they are larger than any of the +Zn material; the second appears in fractions 15–25, suggesting a size equal to that of the +Zn sample (Figure 5).

After removal of the proteins associated with these digested DNP fragments, electrophoretic analysis demonstrates that 50 units of nuclease cleaves the DNA from +Zn chromatin into fragments ranging from 59 to over 1000 base pairs (bp)

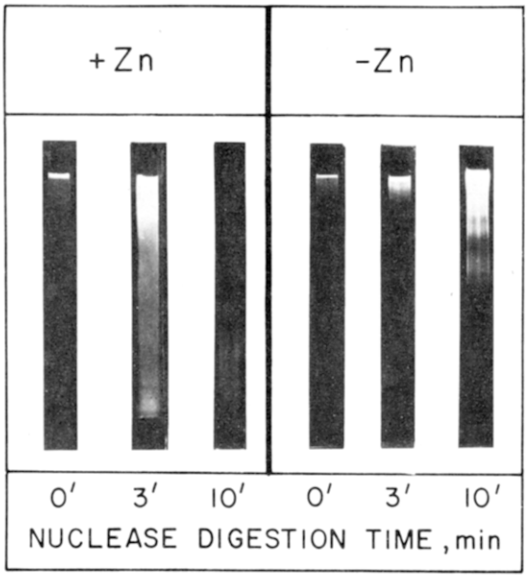

FIGURE 4: Electrophoresis of ±Zn DNP fragments on 5% polyacrylamide gel. Chromatins from ±Zn cells were digested with 100 units/mL micrococcal nuclease and centrifuged at 12000*g*. DNP fragments in the supernatants were precipitated twice with 2 volumes of absolute ethanol for 18 h at −20 °C and electrophoresed as described under Materials and Methods.

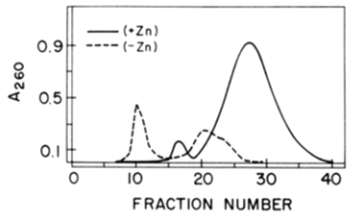

FIGURE 5: Gel chromatography of the soluble products of ±Zn and −Zn chromatins (600 μg of DNA) digested with micrococcal nuclease (100 and 500 units). The +Zn and −Zn samples were incubated for 30 and 60 min, respectively. The soluble DNP was precipitated twice with 2 volumes of ethanol, then dissolved in 0.5 mL of 10 mM Tris-HCl, pH 7.6, plus 0.2 mM EDTA, and chromatographed on Ultragel AcA22 (2 × 30 cm) equilibrated with the same buffer. Fractions of 1 mL were collected, with A_{260} measuring the amount of soluble chromatin present in each.

(Figure 6 and Table I). Under identical conditions the sizes of the DNA fragments resulting from digestion of −Zn chromatin are greater than 2000 bp, and therefore, the fragments do not enter the gel. However, 10-fold higher amounts of nuclease, i.e., 500 units, digest a fraction of the −Zn chromatin into fragments which enter the gel and are of a size either equal to, or slightly larger than, those obtained from +Zn chromatin incubated with 50 units of nuclease (Figure 6, Figure 7, and Table I).

To account for this different nuclease resistance to digestion, the role of the 3000-dalton protein associated with the DNA of −Zn chromatin was examined with chromatin from which either some of the protein had been stripped or which had been reconstituted by combining the protein with chromatins. After 40 min of incubation with nuclease, approximately 25% of intact −Zn chromatin is solubilized (Figure 8). After removal of some, but not all, of the associated proteins, the chromatin is solubilized further, while addition of increasing amounts of the −Zn protein renders the chromatin more resistant to digestion. Moreover, addition of the low molecular weight protein to +Zn chromatin increases its resistance to digestion; the resultant DNP fragments are larger than those produced from control +Zn chromatin (Figure 9). Addition of the low molecular weight protein from −Zn chromatin also induces

Table I: Size of DNA Fragments of +Zn and −Zn Chromatin Digested with Micrococcal Nuclease[a]

nuclease (units)	+Zn (bp)	−Zn (bp)
50	59 ± 14	
	115 ± 9	
	213 ± 12	
	287 ± 10	
	468 ± 24	
	536 ± 33	>2000
	656 ± 23	
	831 ± 2	
	956 ± 50	
500		53 ± 8
		182 ± 17
		260 ± 13
		454 ± 16
	<50	559 ± 27
		690 ± 19
		814 ± 1
		1001 ± 46

[a] The sizes were calculated as described under Materials and Methods by comparison of the migration distance with fragments of DNA of known length as standards. The fragments were identified 5–8 times in the course of 10 experiments, and the mean values ±1 SD are given.

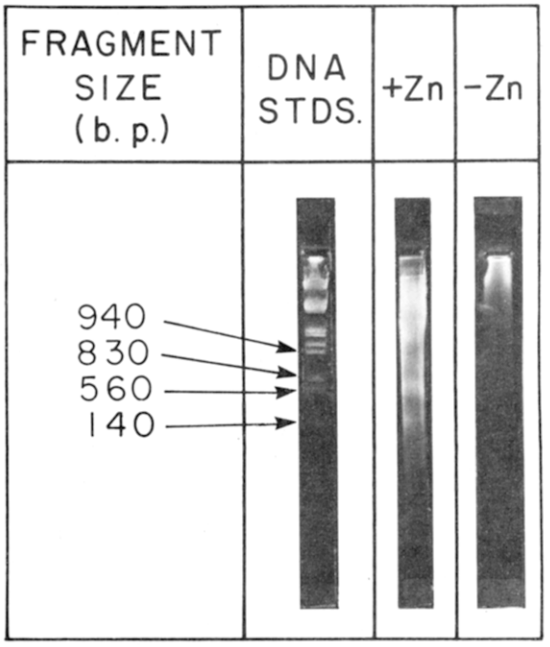

FIGURE 6: Size distribution of nuclease digests of +Zn chromatin. Chromatin (300 μg of DNA) was digested with micrococcal nuclease (50 units) for 5 min. The extracted DNA was electrophoresed on 2% agarose/SDS gels.

increased nuclease resistance in DNA from other sources. It can protect calf thymus DNA from nuclease attack resulting in large DNA fragments, as shown by gel electrophoresis (Figure 9).

The differences in resistance to nuclease digestion between +Zn and −Zn *E. gracilis* chromatin manifest early in the evolution of the zinc deficiency state. Thus, the +Zn → −Zn cells continue to divide after being transferred from +Zn to −Zn media. However, their chromatin is 3 times more resistant to digestion than that of control (+Zn) cells kept in their original medium and still undergoing division (Figure 10).

Discussion

The effects of zinc on the composition, structure, and function of *E. gracilis* chromatin have been examined repeatedly (Wacker, 1962; Falchuk et al., 1975a,b, 1976, 1977;

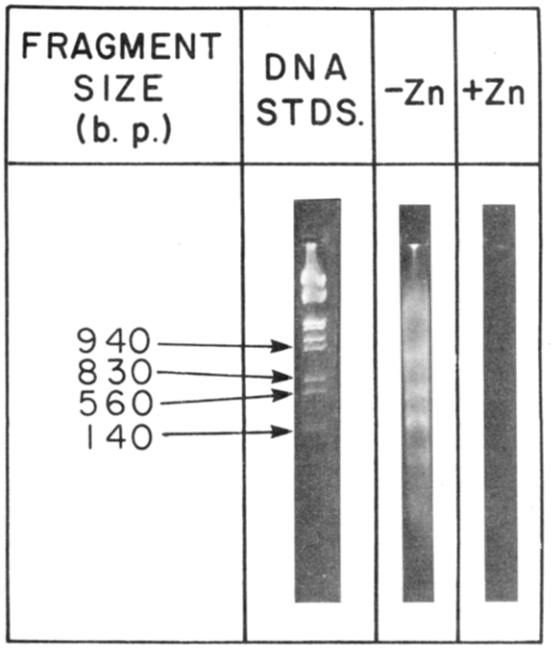

FIGURE 7: Size distribution of nuclease digests of −Zn chromatin. Chromatin (300 μg of DNA) was digested with micrococcal nuclease (500 units) for 15 min. DNA was extracted and electrophoresed on 2% agarose/SDS gel as described under Material and Methods.

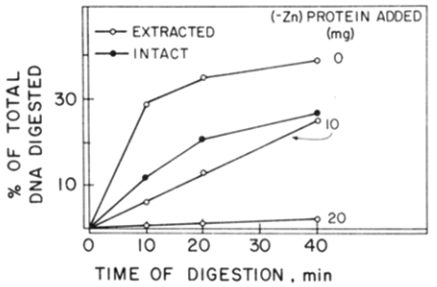

FIGURE 8: Effect of removal of and reconstitution with the low molecular weight, −Zn protein on digestion of Zn chromatin with micrococcal nuclease (100 units). Three different chromatin preparations were used: (a) intact, (b) extracted with ammonium sulfate, as described below, and (c) extracted and mixed with the low molecular weight peptides from −Zn cells. Intact chromatin was extracted for 0.5–2 h at 4 °C with 0.15 M $(NH)_4SO_4$ and then centrifuged for 10 min at 10000*g*. Pellets were resuspended in nuclease digestion buffer, and the extent of solubilization was measured after hydrolysis.

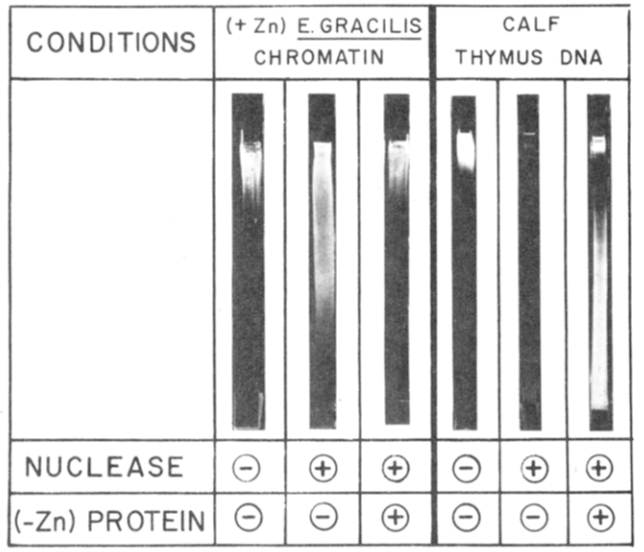

FIGURE 9: Effect of −Zn low molecular weight protein (10 mg) on digestion by micrococcal nuclease of +Zn chromatin (300 μg) and calf thymus DNA (400 μg). Chromatin or calf thymus DNA was digested for 5 min with 50 units of nuclease with or without the protein from −Zn cells. The solubilized material was electrophoresed on 5% polyacrylamide gels.

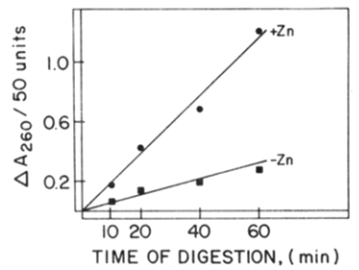

FIGURE 10: Nuclease digestion of chromatin from log phase +Zn *E. gracilis* shifted from +Zn to −Zn medium. The sensitivity to nuclease of chromatin from control log phase +Zn and these shifted +Zn → −Zn cells was compared.

Vallee & Falchuk, 1981; Mazus et al., 1983). With regard to the chromatin of +Zn cells, a number of conclusions can now be reached. While the DNA content of this chromatin changes as the organisms progress through the cell cycle (Falchuk et al., 1975b), on a weight basis the histone/DNA ratio remains approximately equal to one (Mazus et al., 1983). Electron microscopic examination of +Zn *E. gracilis* demonstrates that the chromatin of the nucleus is clustered compactly (Falchuk et al., 1975a). The present studies demonstrate that the rate and extent of its digestion by three different nucleases are similar to those characteristic of chromatins from a variety of eukaryotic cell lines, including those from plants and mammals (Kornberg, 1977; Igo-Klemens et al., 1982) (Figures 2 and 3).

The heterogeneity of the size and distribution during gel electrophoresis of the DNA fragments resulting from nuclease digestion is also in accord with that observed for other chromatins. The results have been ascribed to the specific arrangement of histones along the DNA chain, to variants of these proteins, and their state of chemical modification (Kornberg, 1977; Igo-Klemens et al., 1982; Levy-Wilson & Dixon, 1979; Levy-Wilson et al., 1979; Sperlin & Wachtel, 1981; Albright et al., 1980). The heterogeneous size of the DNA fragments is believed to represent multiples of the nucleosome, the basic unit. The latter is composed of a linker region to which H1 is bound and a core structure with which an octamer of H2A, H2B, H3, and H4 is associated. In different species the size of the linker, commonly 50–60 base pairs, together with the core, varies, ranging from 154 base pairs in *A. niger* to 241 base pairs in sea urchin sperm (Kornberg, 1977). The basic unit of the +Zn *E. gracilis* chromatin has been reported to be in this range (Magnaval et al., 1980), and the size distribution of its DNA fragments obtained after nuclease digestion is consistent with this conclusion (Figure 4) (Table I). Hence, its similarity to that of other eukaryotes can be accepted as the basis for the interpretation of the radical changes in the chromatin of −Zn *E. gracilis* here reported. The DNA content of stationary phase −Zn is double that of +Zn cells, but histones are virtually absent (Wacker, 1962; Falchuk et al., 1975a,b; Mazus et al., 1983). Since the histones play a major role in inducing the DNA to be arranged into compact clusters, the DNA of these histone-depleted −Zn cells might be expected to be dispersed throughout the nucleoplasm. Remarkably, instead, electron microscopic examination of the nucleus of −Zn cells reveals that its chromatin is arranged into clusters at least as compact as those of +Zn chromatin (Falchuk et al., 1975a) and perhaps even more so. This implies that the DNA of −Zn cells interacts

with other proteins which substitute for the missing histones of this −Zn chromatin. The present data confirm this hypothesis and demonstrate, in fact, that a low molecular weight protein is bound to the DNA (Figure 1). This protein appears critical to the 10–30-fold decreased rate and extent of −Zn chromatin digestion by micrococcal nuclease (Figures 5–7 and Table I), which is reversed by its removal from −Zn chromatin. Further, the addition of the protein to +Zn *E. gracilis* chromatin or to a foreign i.e., calf thymus, DNA enhances the resistance of both to digestion by this enzyme (Figures 8 and 9). The results demonstrate that zinc specifically affects the composition and structure of chromatin.

The present data should be considered in the light of the postulated relationships between composition, structure, and function of chromatin (Barrett, 1976; Nelson et al., 1978; Levy-Wilson & Dixon, 1979; Levy-Wilson et al., 1979; Cusick et al., 1981; Weisbrod, 1982; Wood & Felsenfeld, 1982; Nishio & Uyeshi, 1982; Gazit et al., 1982). The pertinence of the results to this general postulate, on the one hand, and to the reduced metabolic activity and growth arrest of *E. gracilis* induced by zinc deficiency, on the other (Vallee & Falchuk, 1981), is of considerable interest.

The structure of chromatin changes during different functional and physiological states. Thus, chromatin is less compact and resistant to nuclease digestion when DNA is being replicated or transcribed (Seale, 1978; Levy-wilson et al., 1979; Cusick et al., 1981; Wood & Felsenfeld, 1982; Gazit et al., 1982). Conversely, the structure of chromatin is more compact, and its resistance to nuclease digestion is increased in aging cultured mammalian cells, in the course of embryonic development of the sea urchin and during mitosis (Dell'Oreo & Whittle, 1982; Simpson, 1981; O'Meara & Herrmann, 1972; Seale, 1978; Geider & Hoffmann-Berling, 1981; Bojanovic et al., 1981). Chemical modification of histones, e.g., acetylation, methylation, ADP-ribosylation, phosphorylation, and/or replacement of and binding of high mobility group (HMG) proteins, to H1 also induce such structural changes (Vidali et al., 1978; Nelson et al., 1978; Isenberg, 1979; Perry & Chalkley, 1981; Geider & Hoffmann-Berling, 1981; Igo-Klemens et al., 1982; Sperling & Wachtel, 1981; Manzoli et al., 1982; McGhee & Felsenfeld, 1980; Nishio & Uyeshi, 1982; Ryan & Cristofalo, 1972; Bradbury & Matthews, 1982; Weisbrod & Weintraub, 1979; Annuntziato et al., 1982; Weisbrod, 1981; Wood & Felsenfeld, 1982; Noll & Kornberg, 1977; Lawson & Cole, 1982). These alterations in chromatin structure may occur in specific segments of chromatin only and are believed to serve as regulators of their transcription (Nagl, 1982).

Such relationships between structure and function of chromatin are even more apparent in sperm whose chromatin is condensed even more extensively than that of somatic cells. The increased condensation causes general and extensive repression of the genome. Thus, in ram sperm, condensation results from the rearrangement of the somatic type histones (Uscheva et al., 1982), but in rat sperm, specific variants are added to or replace them (Kaye & MacMater-Kaye, 1975; Casas et al., 1981; Kennedy & Davies, 1982; Rao et al., 1982). This replacement may be restricted only to H1 but can include the core histones and involve larger more basic variants (Pospelov et al., 1981; Kennedy & Davies, 1982; Gabrielli & Hancock, 1982; Sperling & Wachtel, 1981). More commonly, the increased compactness of sperm chromatin is accompanied by variable replacement of somatic histones by protamines, the amounts of the latter varying from 10 to 100% of the chromatin proteins (Subirana, 1975; Dixon & Smith, 1968; Kistler et al., 1973; Coellingh, 1975; Calvin, 1975, 1976).

The present findings of increased compactness of −Zn chromatin, together with the decreased overall rate of their RNA synthesis observed previously (Falchuk et al., 1975a), confirm the view that the structural state of chromatin relates critically to its function.

The resemblance of the changes in chromatin structure and function in the course of zinc deficiency to those of chromatin of other cells under specific but different physiological conditions is apparent. In all other instances known, they occur in the course of a variety of physiological processes such as, e.g., spermiogenesis; in the present instance they are a response to zinc deficiency and its metabolic consequences. The pertinence of one to the other may be of major significance. The mechanism by which zinc induces the formation of the low molecular weight non-histone protein[2] and brings about these extraordinary changes in chromatin composition and structure should illuminate the role of zinc in the regulation of chromatin function and its pertinence to physiological processes in which analogous structural changes of chromatin have been observed.

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[2] We have obtained evidence that the composition and size of this protein differs from both histones and protamines. Like the latter, it is rich in arginine which comprises over 50% of its amino acids (K. H. Falchuk, B. Mazus, and B. L. Vallee, unpublished results). This high arginine content could be instrumental in making DNA more compact, since this amino acid is thought to play a specific role in this process (Watson & Maudrianakis, 1982; Balhorn, 1982; Gledhill, 1975; Ischimura et al., 1982).

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