A Special Class of Non-Histone Protein Tightly Complexed with Template-Inactive DNA in Chromatin†

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ABSTRACT: A special class of non-histone protein ("tight protein") is identified in purified HeLa cell chromatin on the basis of its failure to dissociate from the DNA at very high ionic strength (2.5 *M* NaCl–5.0 *M* urea), where over 92% of the total chromatin protein is released. The tight proteins are insoluble in 0.4 *N* H_2SO_4 and lack histones as determined by polyacrylamide gel electrophoresis. They have molecular weights between 14,000 and 85,000 with over 70% of the polypeptide chains between 14,000 and 30,000 mol wt. This is the same size range as the non-histone proteins which others have found to display species-specific DNA binding in vitro. There is approximately one molecule of tight protein per 275 DNA base pairs. The tight proteins are characterized by much higher rates of labeling with amino acids than the histones and non-histone chromatin proteins that are dissociated from the DNA by high ionic strength, but they have the lowest phosphorylation levels. Chromatin fractionation experiments were performed to investigate the distribution of tight proteins between template-active and template-inactive regions. Under specific conditions, spleen DNase (DNase II) selectively shears those portions of HeLa cell chromatin that contain nascent RNA transcripts. This nascent RNA-enriched chromatin fraction also contains a high level of the proteins known to be complexed with heterogeneous nuclear RNA in ribonucleoprotein particles and contains over 70% of the RNA polymerase activity of total chromatin. When this method was employed to investigate the distribution of tight proteins, they were found to be almost entirely confined to the template-inactive fraction. Although these experiments do not elucidate the precise function of these proteins, they identify, for the first time, a particular subclass of non-histone chromosomal protein which is distributed asymmetrically between transcriptionally active and inactive chromatin regions.

Most of the proteins in chromatin are rather loosely bound to DNA, or to one another. This is indicated by the effects of DNase I (Mirsky, 1971; Pederson, 1972), and is demonstrated more clearly by the responses of chromatin to elevated ionic strength. For example, 2.0 *M* NaCl removes essentially all histone from calf thymus chromatin along with about 70% of the non-histone protein (Ohlenbusch et al., 1967). This paper identifies and characterizes a special class of non-histone chromatin protein which remains bound to DNA at very high ionic strength (2.5 *M* NaCl–5.0 *M* urea). These tightly bound proteins possess physical and metabolic properties distinct from other chromatin proteins, and are preferentially disposed in those regions of chromatin that are inactive in gene transcription.

Materials and Methods

Cells and Chromatin Purification. HeLa cells were propagated in suspension culture as detailed previously (Pederson, 1972). All cultures were free of *Mycoplasma*, as assayed microbiologically. Chromatin was isolated from nuclei and purified by sedimentation through 60% sucrose as described in previous communications from this laboratory (Bhorjee and Pederson, 1972, 1973).

Chromatin Dissociation. All steps were performed at 2–4°. Chromatin was dissolved in 0.01 *M* NaCl–0.01 *M* Tris-HCl (pH 7.2) to give an A_{260} of 2.0–4.0 and mixed with two volumes of 3.75 *M* NaCl–7.5 *M* urea (buffered to pH 7.2 with 0.01 *M* Tris-HCl). After 1–2 min the slightly opalescent chromatin sample turns water clear and is centrifuged at 408,000g_{max} for 24 hr to pellet the DNA and tightly bound protein. To examine the proteins dissociated by salt–urea, the supernatant was dialyzed against 0.4 *M* guanidine hydrochloride–6.0 *M* urea–0.02 *M* sodium phosphate buffer (pH 6.1) and then fractionated by chromatography on the weak cationic exchanger, Bio-Rex 70 (Levy et al., 1972). The DNA pellet obtained by ultracentrifugation was rinsed and dissolved in 1% sodium dodecyl sulfate and processed for polyacrylamide gel electrophoresis as described previously (Bhorjee and Pederson, 1973). In some experiments, the pellet was dissolved in buffer without detergent and subjected to a second cycle of 2.5 *M* NaCl–5.0 *M* urea and ultracentrifugation (see Table I).

Fractionation of Chromatin into Template-Active and Template-Inactive Regions. Nascent RNA was preferentially labeled by incubating cells (5×10^6/ml) with [^{3}H]-5-uridine (40 μCi/ml) for 45 sec (37°). Chromatin was isolated as above, dialyzed against 0.025 *M* sodium acetate buffer (pH 6.5), and subjected to a limited digestion with DNase II (10 units/1.0 A_{260} unit chromatin DNA; 5 min at 37°). The chromatin was separated into template-enriched and template-deficient fractions by sedimentation for 20 min at 27,000*g* as described previously (Gottesfeld et al., 1974).

Analytical Methods. The procedures used for sodium dodecyl sulfate polyacrylamide gel electrophoresis, protein determination, and liquid scintillation counting have all been described (Bhorjee and Pederson, 1973; Pederson and Kumar, 1971; Pederson, 1974a,b). Protein-bound phosphorus (as incorporated ^{32}P) was measured by making samples 10% in trichloroacetic acid and incubating them at 90° for 30 min (Auerbach and Pederson, 1975). Appropriate controls established that both DNA and RNA are quantitatively hydrolyzed under these conditions and that the material remaining acid-insoluble is phosphorylated protein. HeLa

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Table I: Dissociation of Chromatin by NaCl–Urea.[a]

Fraction	% of Total Protein
Supernatant	90.4
DNA pellet	8.1
2nd DNA pellet	7.5
DNase treated	0.6

[a] Chromatin was dissociated with 2.5 *M* NaCl–5.0 *M* urea as detailed in Materials and Methods and centrifuged in the Beckman Spinco SW56 rotor at 56,000 rpm for 24 hr. We have previously determined that this centrifugation pellets more than 98% of the DNA (Bhorjee and Pederson, manuscript submitted). The pellet was rinsed with 2.5 *M* NaCl–5.0 *M* urea–0.01 *M* Tris-HCl (pH 7.2) and was dissolved in 1% sodium dodecyl sulfate–0.1% 2-mercaptoethanol–0.01 *M* sodium phosphate buffer (pH 7.0) and analyzed for total protein. In some cases, the pellet was dissolved in 0.01 *M* NaCl–0.01 *M* Tris-HCl (pH 7.0) and subjected to a second cycle of 2.5 *M* NaCl–5.0 *M* urea and ultracentrifugation ("2nd DNA pellet" in table). To examine the effects of DNase, chromatin suspended in 0.01 *M* NaCl–1.5 m*M* $MgCl_2$–0.01 *M* Tris-HCl (pH 7.0) was incubated with Sepharose-immobilized pancreatic DNase (500 μg of DNase/ml of final solution) at 37° for 1 hr. These are conditions previously shown to digest over 98% of the chromatin DNA (Pederson, 1972). The DNase was then removed by centrifuging out the Sepharose beads at 600*g* for 7 min. Controls in which Sepharose-DNase was incubated at 37° without chromatin established that less than 1% of the DNase was released from the Sepharose. Incubation of chromatin with blank CNB-Sepharose under similar conditions as above resulted in less than 2% DNA hydrolysis. Aliquots of the DNase-digested chromatin were taken for determination of 0.35 *M* perchloric acid soluble A_{260} and this was compared to values obtained for undigested chromatin, to obtain % DNA digested. The DNase-digested chromatin was then made 2.5 *M* in NaCl and 5.0 *M* in urea and centrifuged in the SW56 rotor as above.

RNA polymerase activity was measured essentially as described by Zylber and Penman (1971), as specified in Table V.

Chemicals and Isotopes. [^{3}H]-5-Uridine, [^{3}H]uridine triphosphate, and ^{32}P (as phosphoric acid) were from New England Nuclear Corp. [^{3}H]-L-Leucine, ^{3}H-reconstituted protein hydrolysate, [*methyl*-^{14}C]thymidine, guanidine hydrochloride, urea, and sucrose were from Schwarz/Mann. Bio-Rex 70 (200–400 mesh) was obtained from Bio-Rad Laboratories (Richmond, Calif.). Porcine spleen DNase (HDAC, RNase-free) and Sepharose-immobilized pancreatic DNase were obtained from Worthington Biochemical Corp. Unlabeled adenosine 5′-triphosphate, cytidine 5′-triphosphate, and guanosine-5′-triphosphate were from Sigma Chemical Co.

Results

Characterization of Tight Proteins. Table I shows that 8% of the protein in purified HeLa cell chromatin is not dissociated by 2.5 *M* NaCl–5.0 *M* urea. More than 92% of this protein fraction is retained by the DNA during a second cycle of NaCl–urea treatment (Table I), indicating that it is a specific subfraction of chromosomal protein and not merely trapped adventitiously in the DNA pellet. For clarity, this fraction is hereafter termed "tight protein", although it is understood that this is a purely operational phrase (ionic strength) and not one based on thermodynamic measurements of the DNA–protein interaction.

That the tight proteins are actually a part of the chromatin structure is shown by the effects of pancreatic DNase. If the chromatin is digested until more than 98% of the DNA is hydrolyzed (Pederson, 1972), over 92% of the tight protein is abolished (Table I). Thus, the tight protein does not merely cosediment with the DNA during ultracentrifugation, but is apparently attached to it. A similar conclusion is reached by equilibrium centrifugation in cesium sulfate density gradients, in which a substantial fraction (over 35%) of the tight protein is observed to band with the DNA at 1.39–1.41 g/cm^3 (not shown).

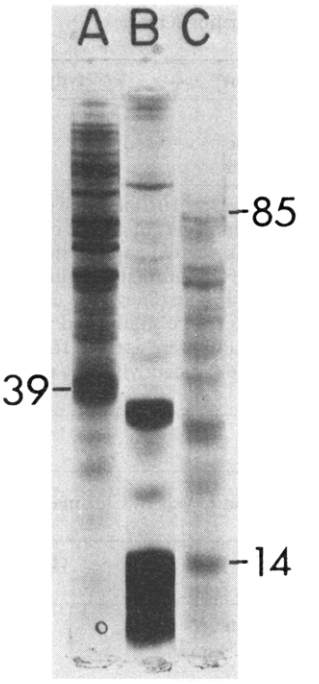

FIGURE 1: Electrophoresis of non-histones, histones, and DNA-bound proteins. The supernatant proteins obtained by ultracentrifugation of NaCl–urea–dissociated chromatin (Table I) were separated into acidic and basic proteins by chromatography on Bio-Rex 70. Proteins remaining bound to DNA after ultracentrifugation (i.e., "pellet", Table I) were solubilized by 1.0% sodium dodecyl sulfate (control experiments established that under these conditions the detergent completely dissociates the proteins from the DNA). All samples were then prepared for electrophoresis as described previously (Bhorjee and Pederson, 1973). Direction of electrophoresis is from top to bottom. (A) 35 μg of non-histones. (B) 30 μg of histones. (C) 15 μg of DNA-bound proteins. In gel C, controls with tight proteins from ^{32}P-labeled chromatin showed that no DNA entered the 10,000–85,000 mol wt region of the gel under these electrophoretic conditions (see also additional controls reported in Bhorjee and Pederson, 1972). The numbers refer to molecular weight multiplied by 10^{-3}, as determined by the migration of protein standards.

Figure 1C shows the molecular weight distribution of the tight proteins as determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. For comparison, gels are also shown of the proteins that are dissociated from chromatin in NaCl–urea, after chromatography on Bio-Rex 70 (see Materials and Methods). The dissociated proteins (Figure 1A and B) resemble the non-histone and histone protein fractions obtained by extraction of intact HeLa chromatin in dilute acid (Bhorjee and Pederson, 1972, 1973). The tight proteins (gel C) are devoid of histones (gel B), as expected from the earlier studies on calf thymus chromatin (Ohlenbusch et al., 1967). Further, they are insoluble in 0.4 *N* H_2SO_4 and display p*I*'s of 5.0–6.0 in isoelectric focusing (not shown). Although the tight proteins do contain a low molecular weight component that coelectrophoreses with histone ARE[1] (ca. 14,000), this is

[1] Histone nomenclature used in this report is that developed at the 1972 Gordon Conference on Nuclear Proteins, Chromatin Structure and Gene Regulation: f1 = I = KAP; f2a2 = IIb1 = LAK; f2b = IIb2 = KAS; f3 = III = ARE; f2a1 = IV = GRK.

Table II: Amino Acid Labeling Kinetics.[a]

Protein Fraction	dpm/mg of Protein
I. Histone	163,744
II. Non-histone	193,338
III. DNA-bound	819,438

[a] 1000 ml of HeLa cells (3×10^5/ml) were grown for 3 hr with a mixture of ^{3}H-labeled amino acids at a total concentration of 1.67 μCi/ml. Chromatin was dissociated and fractionated as detailed in Materials and Methods. After chromatographic separation on Bio-Rex 70, the histones and non-histones were concentrated by lyophilization. Protein samples were solubilized in 1% sodium dodecyl sulfate and duplicate aliquots were assayed for total protein. Trichloroacetic acid insoluble radioactivity was measured in parallel aliquots also in duplicate.

Table III: Phosphorylation Levels of Chromatin Protein Fractions.[a]

Fraction	cpm of Protein-Bound ^{32}P/mg of Protein
Intact chromatin	91,723
Non-histone	166,200
Histone	63,570
DNA-bound proteins	16,142

[a] Two liters of cells at 3×10^5/ml were grown for 24 hr with ^{32}P (2 μCi/ml). Chromatin was dissociated and fractionated as detailed in Materials and Methods. Protein samples were solubilized in 1% sodium dodecyl sulfate and assayed in duplicate for total protein. Parallel aliquots (duplicates) were used to determine protein-bound phosphorus as described in Materials and Methods.

actually an acidic, tryptophan-containing protein (Bhorjee and Pederson, 1972, and unpublished results). There are also several differences between the tight proteins and the non-histones that are dissociated by salt–urea (gel A). The tight proteins do not have molecular weights above 85,000, while the dissociated non-histones have components as high as 180,000. Moreover, several of the salt–urea-dissociated nonhistone components in the 40,000–80,000 mol wt range are absent from the tight proteins. Particularly conspicuous in this regard are the 38,000–41,000 mol wt species, which are nascent hnRNA-associated proteins (Pederson, 1974a). It is of particular interest that the tight proteins are specifically enriched in 14,000–30,000 mol wt components (gel C). These low molecular weight proteins comprise over 70% of the total polypeptide chains in the tight protein population. It is unlikely that their low molecular weight is due to proteolysis since the dissociated non-histone and histone proteins (gels A and B) were subjected to the same preparative procedures (high salt–urea, ultracentrifugation etc.) with no sign of degradation (compare with patterns for acid-fractionated chromatin in Bhorjee and Pederson, 1973). It is also of interest to note that studies of DNA-binding proteins from rat liver chromatin (van den Broek et al., 1973) demonstrate that it is precisely the 15,000–30,000 mol wt non-histone components which display the highest levels of species-specific DNA binding in vitro.

These electrophoretic results show that the tight proteins are a nonrandom subfraction of total chromatin protein, a conclusion that is confirmed and extended by their metabolic properties. Table II shows the specific activities of histones, non-histones (salt-dissociated), and tight proteins after 3 hr of continuous labeling with ^{3}H-labeled amino acids. The tight proteins had a specific activity five times higher than either the histones or non-histones. The data also imply that the salt–urea-dissociated non-histones do not turn over appreciably faster than histones in exponentially growing HeLa cells. This contrasts with the high rates of non-histone labeling that typify nondividing cell populations (Daly et al., 1952; Pederson, 1974b).

Table IV: Composition of Chromatin Fractions.[a]

	% of DNA	% of Nascent hnRNA	Non-Histone/DNA
Intact chromatin	100	100	0.65
"S"	22	86	0.91
"P"	78	14	0.65

[a] Chromatin was fractionated as described in Materials and Methods. Following sedimentation at 27,000g for 20 min, the amount of DNA in the supernatant was determined by measurements of absorbance at 260 nm. Similar values were obtained by measurements of acid-precipitable radioactivity in experiments with [^{14}C]thymidine-labeled chromatin. Nascent RNA was measured as acid-precipitable radioactivity using chromatin from cells labeled for 45 sec with [^{3}H]uridine (see Materials and Methods). Non-histone protein was prepared by extracting the intact, S, and P chromatin fractions in 0.4 N H_2SO_4 as described previously (Bhorjee and Pederson, 1973) and collecting the acid insoluble proteins by sedimentation at 37,000g (15 min).

Table V: HeLa RNA Polymerase Activity.[a]

Fraction	RNA Polymerase Activity/unit of DNA
Intact chromatin	100
"S"	525

[a] Chromatin was fractionated as described in Materials and Methods. $MgCl_2$ was added to the S fraction (27,000g supernatant) to a final concentration of 0.01 M and it was dialyzed for 14 hr (4°) against 200 volumes of 0.01 M KCl–0.01 M $MgCl_2$–0.05 M Tris-HCl (pH 7.9). The "P" fraction (27,000g pellet) could not be reliably assayed for polymerase activity due to its aggregated state. Intact (unsheared) chromatin was therefore dialyzed as above and used as the reference sample. Each reaction mixture contained, in 1.0 ml, 1.0 A_{260} unit of either intact or sheared chromatin, 10 nmol of KCl, 10 nmol of $MgCl_2$, 50 nmol of Tris-HCl (pH 7.9), 40 nmol each of unlabeled ATP, CTP, and GTP, and 1.5 μCi of [^{3}H]UTP. Reactions were carried out in a water bath equilibrated to 26° (±0.5°). After 30 min, 10 ml of 5% (w/v) trichloroacetic acid containing 0.01 M sodium pyrophosphate was added to each sample. After 15 min at 4°, the acid-insoluble material was collected on glass fiber filter discs, which were dried and counted in a toluene-based scintillation fluid. All data were corrected for the low level conversion of [^{3}H]UTP to acid-insoluble form that takes place at 4°. The values shown are the averages of triplicates. Similar results were obtained in two separate experiments.

A second distinctive metabolic property of the tight proteins is their very low level of phosphorylation in vivo, as compared to the other chromatin proteins (Table III). After 24 hr of cell growth in the presence of ^{32}P, the tight proteins displayed only one-tenth the amount of protein-bound phosphorus as the dissociated non-histones, and only one-fourth as much as the histones.

Distribution of Tight Protein in Template Active vs. Inactive Chromatin. Fractionation techniques previously reported (Gottesfeld et al., 1974) were employed to examine the distribution of tight protein in transcriptionally active and inactive chromatin regions. These experiments reveal that tight proteins are distributed nonrandomly in chromatin, with over 96% being confined to the genetically inactive regions.

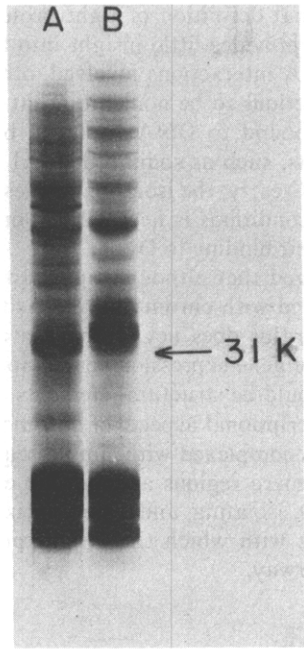

FIGURE 2: Electrophoresis of proteins from template-enriched and template-deficient chromatin fractions. The proteins of each fraction were solubilized in 1% sodium dodecyl sulfate and prepared for electrophoresis. (A) 50 μg of protein from template-inactive fraction. (B) 50 μg of protein from template-enriched fraction. Arrow indicates the position of histone KAP, which has an apparent molecular weight of 31,000 in the presence of sodium dodecyl sulfate.

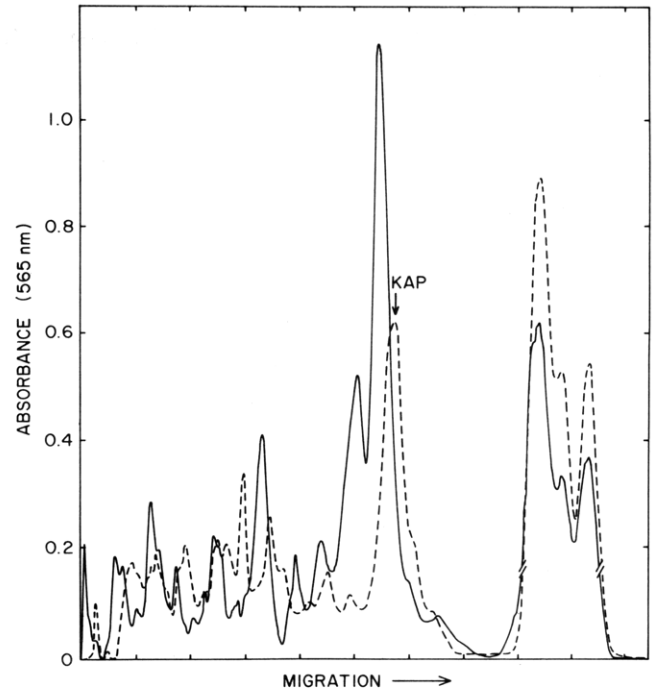

FIGURE 3: Electrophoretic distribution of proteins from template-enriched (—) and template-deficient (- - -) chromatin fractions.

The pH optimum of spleen DNase (DNase II) is 4.8. Under conditions of limited enzyme activity (viz., pH 6.5), DNase II selectively attacks and shears those portions of HeLa cell chromatin that contain nascent RNA transcripts. These sheared chromatin fragments, which contain 15–22% of the total DNA, can be readily separated from the remaining, unsheared chromatin by sedimentation at 27,000*g*. The use of the term shear derives from the observation that less than 2% of the total DNA is hydrolyzed during the reaction, as measured by the conversion of DNA to perchloric acid soluble material. As shown in Table IV, the unsheared chromatin fraction ("P") typically contained 75–82% of the DNA and had the non-histone/DNA stoichiometry of intact, unfractionated chromatin. In contrast, the nonsedimentable, selectively sheared chromatin contained only 22% of the DNA but most of the nascent RNA. This template-enriched chromatin fraction also contained approximately 50% more non-histone protein (0.4 *N* H_2SO_4 insoluble) than unfractionated chromatin, and was also enriched more than fivefold in RNA polymerase activity (Table V). Figure 2 shows the polyacrylamide gel electrophoresis patterns of proteins from the P and S fractions. The P proteins (gel A) correspond closely to those of unfractionated chromatin (Bhorjee and Pederson, 1972, 1973), both quantitatively and qualitatively. In contrast, the proteins of the nascent RNA-enriched fraction are similar to those previously shown to be complexed with heterogeneous nuclear RNA in HeLa cells (Pederson, 1974a), the only difference being that histones (and DNA) are absent in the latter case since the purified hnRNP particles are chromatin free. The lack of homology between the non-histone proteins (i.e., those having molecular weights greater than 31,000) of the template-enriched and template-deficient chromatin fractions can be seen more clearly by comparison of the respective densitometric gel scans (Figure 3).

The template-rich chromatin fraction (Figure 3, solid line) has a very high content of 38,000–41,000 mol wt polypeptides (those running just behind histone KAP), which are also the major proteins of purified hnRNP (Pederson, 1974a). The scans also show that the template-enriched fraction is practically devoid of histone KAP. Each of the other four histones was present at the same level, per unit of DNA, in the two chromatin fractions. A reduced content of histone KAP was also noted by Simpson and Reeck (1973) in an "extended chromatin" fraction, which was later shown to be partially enriched for RNA polymerase binding sites (Simpson, 1974). In contrast to the report of Gottesfeld et al. (1974) on rat liver chromatin, we found no evidence for a decreased content of histone GRK in the template-active fraction of HeLa cell chromatin.

It is of interest to know whether the presence of nascent RNA in the template-active chromatin fraction is in fact due to the action of the added DNase, or instead reflects the action of the very small amount of ribonuclease activity which is present in porcine spleen DNase as supplied commercially. This was investigated by exploiting the known inhibitory effect of magnesium on DNase II. In 10 m*M* Mg^{2+}, DNase II activity is inhibited by more than 99%, as assayed on [^{14}C]thymidine-labeled chromatin. However, this magnesium concentration does not substantially affect the small amount of contaminating ribonuclease activity (assayed on [^{3}H]adenosine-labeled HeLa cell hnRNA). When chromatin was incubated with DNase in 10 m*M* Mg^{2+}, no sheared chromatin or nascent RNA was found in the nonsedimentable fraction. The same result was obtained with heat-inactivated DNase (90°, 15 min), in which the DNase activity is abolished with practically no effect on the ribonuclease. Finally, when chromatin was incubated at 37° in sodium acetate buffer without added enzyme, no DNA or nascent RNA was released to the nonsedimentable fraction, showing that the usual result cannot be due to the action of endogenous nucleases. These controls establish that the presence of nascent RNA in the nonsedimentable frac-

Table VI: Distribution of "Tight Protein" between Template Active and Inactive Chromatin.

	Inactive	Active
% of DNA	78.0	22.0
% of Tight Protein	96.2	3.8
Tight/DNA ratio	*1.23*	*0.17*

[a] 1800 ml of cells (3×10^5/ml) were labeled for 24 hr with [^{14}C]-thymidine (0.005 μCi/ml) and [^{3}H]leucine (1.67 μCi/ml) in medium containing one-half the usual amount of L-leucine. Cell growth was normal under these conditions (3×10^5/ml to 6×10^5/ml in 24 hr). Chromatin was isolated and fractionated as detailed in Materials and Methods. The P and S fractions were made 2.5 *M* in NaCl and 5.0 *M* in urea (0.01 *M* Tris-HCl (pH 7.2) and allowed to stand 30 min at 4°. Aliquots were removed for determination of ^{14}C and ^{3}H radioactivity and the remainder of each sample was centrifuged at $408{,}000g_{max}$ for 24 hr. Measurements of ^{14}C (DNA) radioactivity in the 408,000*g* supernatants verified that all of the DNA was pelleted under these conditions. The pellets were rinsed gently with 2.5 *M* NaCl–5.0 *M* urea–0.01 *M* Tris-HCl (pH 7.2) and then with 0.01 *M* NaCl–0.01 *M* Tris-HCl (pH 7.0) and finally dissolved in 1% sodium dodecyl sulfate–0.01 *M* sodium phosphate buffer (pH 7.0). Triplicate aliquots were taken for measurement of trichloroacetic acid insoluble radioactivity. The data shown are the averages of three separate experiments. Similar results were obtained in a fourth experiment in which the amounts of DNA were determined by absorbance at 260 nm and protein by chemical analysis.

tion is due to the selective shearing of template-active chromatin regions by the action of DNase II, not by some other exogenous or endogenous enzyme activity.

To investigate the distribution of tight protein in the template-active and inactive chromatin fractions, cells were labeled for 24 hr with [^{3}H]leucine and [^{14}C]thymidine. The chromatin was fractionated as above and assayed for tight protein by measuring the relative amounts of protein and DNA in the pellet obtained after ultracentrifugation of salt–urea-dissociated samples. As shown in Table VI, over 96% of the tight protein present in the initial chromatin was localized in the template-inactive fraction. Relative to the template-active fraction, the inactive fraction was enriched more than sevenfold in tight protein per unit of DNA.

Discussion

The present experiments identify and characterize a subfraction of HeLa cell non-histone chromatin protein that remains complexed with the DNA in 2.5 *M* NaCl–5.0 *M* urea. Data from a number of additional experimental systems show that this class of chromatin protein is a general property of eukaryotic chromosomes (T.P., unpublished results). In HeLa cells, the "tight proteins" comprise 8% (mass) of the total chromatin protein and 20% (mass) of the total non-histone protein. Assuming the spatial distribution of tight protein in chromatin to be uniform (except for its exclusion from template-active regions), there would be 5.6 molecules of tight protein/10^6 daltons of DNA.[2] This corresponds to 1 molecule for every 275 nucleotide pairs.

[2] As shown previously (Pederson, 1974b) there are 700,000 daltons of non-histone protein per 10^6 daltons of HeLa cell chromatin DNA; 20% of this is tight protein, or 140,000 daltons. The mole average molecular weight of tight proteins is 25,000, corresponding to 140,000/25,000, or 5.6 molecules.

The operational definition of tight proteins used in this communication provides little insight into the chemistry of the protein–DNA interactions involved, other than indicating these interactions to be nonionic. That the proteins are not covalently bound to DNA is shown by the effects of strong detergents, such as sodium dodecyl sulfate (see legend, Figure 1). Clearly, the isolation of these proteins under nondenaturing conditions is necessary in order to elucidate the details of their binding to DNA.

It is emphasized that although tight proteins are preferentially associated with chromatin regions that are inactive in transcription, this does not establish a role for them in the physiology of gene expression per se. Some, or all, of the tight proteins could be structural elements, or factors relating to nontranscriptional aspects of chromosome dynamics. Special proteins complexed with simple-sequence (satellite) DNA at centromere regions are but one example. Experiments aimed at isolating and characterizing the specific DNA sequences with which these tight proteins are complexed are underway.

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