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Histone–Histone Associations within Chromatin. Cross-Linking Studies Using Tetranitromethane†

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ABSTRACT: Treatment of chromatin with the protein cross-linker tetranitromethane (TNM) results in a product identified as an F2a1-F2b dimer. The same product appears after treatment with TNM of HeLa cells growing in culture. Furthermore acid-extracted histones which have been fractionated into the five separate species can be recombined and mixed with DNA to produce a nucleohistone preparation which is also cross-linked by TNM to give the F2a1-F2b dimer. F1 and F3 can be excluded from the reconstitution mixture without effect on the dimer production. In contrast, the presence of F2a2 is essential to the proper reconstitution of F2a1 and F2b with DNA. The specificity of TNM and the characteristics of the reaction suggest that F2a1 and F2b are cross-linked at their specific binding sites. These results provide evidence that F2a1, F2a2, and F2b interact specifically in chromatin.

Recent developments strongly suggest that the structure of chromatin is based on a linear array of subunits (Olins and Olins, 1974; Hewish and Burgoyne, 1973; Axel et al., 1974; Weintraub and Van Lente, 1974; Sahasrabuddhe and Van Holde, 1974; Noll, 1974). X-Ray data which had previously been thought to reflect a supercoil have now been reinterpreted in the context of this new subunit model (Kornberg, 1974). Although some workers still prefer the supercoil concept (Pooley et al., 1974) recent data of Weintraub and Van Lente (1974) argue strongly in favor of histone clusters as a fundamental structural feature of chromatin.

There are no direct data bearing on the stoichiometry of the histones within the putative subunits. However, a number of considerations suggest that for the most part all of the histones, with the possible exception of F1, are represented in each subunit. First of all, in solution, heterologous histone-histone interactions are much stronger than the homologous ones. Moreover the interactions are very specific and can be fit most easily into a scheme which proposes the clustering of the four smaller histones (i.e., F2a1, F2a2, F2b, and F3) into an octamer containing two of each type (D'Anna and Isenberg, 1974b; Kornberg and Thomas, 1974). These considerations are consistent with electron micrographs of chromatin which reveal subunits of a size compatible with a hypothetical histone octamer and about 200 base pairs of DNA (Olins and Olins, 1974). Finally, Kornberg and Thomas (1974) report that the X-ray diffraction pattern characteristic of native chromatin can be obtained from reconstituted chromatin only when all four of the smaller histones are present. Although Richards and Pardon (1970), using a different reconstitution procedure, have regenerated the X-ray pattern with fewer histones, it seems likely that many or most subunits in chromatin contain all four of the smaller histones and probably two of each.

We have studied the arrangement of the histones in chromatin by means of chemical cross-linking and report here the results obtained with the cross-linking agent tetranitromethane (TNM). The mechanism by which this reagent cross-links proteins is not completely clear. Nevertheless the primary site of TNM action is tyrosine and, in those cases which have been studied, dityrosine is recovered from the cross-linked protein (Williams and Lowe, 1971). Probably TNM produces a tyrosine free radical which inserts itself directly onto an appropriately situated and precisely adjacent neighbor. The TNM does not itself become incorporated as a bridge and thus is effectively a zero length cross-linker. The probability that the hydrophobic portions of the histones, where the tyrosine residues are found, are involved in specific and very tight histone-histone interactions in chromatin (Hayashi and Iwai, 1971; Bradbury and Rattle, 1972; Bradbury et al., 1973; Shih and Fasman, 1971; Weintraub and Van Lente, 1974) suggests that TNM, which can penetrate hydrophobic clusters (Myers and Glazer, 1971), may serve as a cross-linking probe specific for complexed rather than merely adjacent histones.

Using TNM we have found the rapid formation of a single cross-linked histone product with the molecular weight of a dimer. This cross-linked product is formed by treatment with TNM of either growing cells in culture, isolated chromatin, or reconstituted nucleohistone. On the basis of reconstitution of DNA with separated radioactive histones the cross-linked product has been identified as an F2b-F2a1 dimer.

Experimental Section

Materials. Tetranitromethane (TNM) was purchased from Sigma. For reconstitution of calf thymus nucleohis-

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tone commercial calf thymus DNA (Type V) from Sigma and calf thymus histone from either Sigma (Type II) or Worthington (lyophilized) were used.

Chromatin Preparation. PROCEDURE 1 (modification of Bonner et al., 1968). HeLa cells growing in Joklik-modified minimum essential medium (Grand Island Biological) were harvested and washed twice with about 0.1 volume of saline-EDTA (75 m*M* NaCl-25 m*M* EDTA (pH 8)). The cells at 0° were then lysed in saline-EDTA-0.5% NP40 using a Dounce homogenizer. The nuclei were pelleted at about 500 g and washed once with saline-EDTA. The nuclei were then lysed by homogenizing vigorously in 50 m*M* Tris (pH 8). The chromatin was centrifuged down at 25,000*g* for 20 min and then washed once in 50 m*M* Tris. Finally the pellet was resuspended by homogenizing in 1 m*M* EDTA (pH 8) at a concentration of 1–2 mg/ml of DNA. The chromatin was stored at 0° until use and could be stored for many days without detectable change in cross-linking properties.

PROCEDURE 2. Washed HeLa cells suspended in saline-EDTA were disrupted by shear in a French pressure cell (ca. 4000 psi). Nuclei and nuclear fragments were pelleted at 25,000*g* and then washed with saline-EDTA-NP40 followed by saline-EDTA alone. The pellet was then homogenized vigorously in 50 m*M* Tris (pH 8) and the chromatin collected by centrifugation.

Histone Preparation. Histones were extracted from nuclei or chromatin with 0.4 *N* H_2SO_4 at 0° for 15 min or longer. Insoluble materiai was removed by centrifugation. The histones were precipitated overnight with 5 volumes of acetone, collected by centrifugation, and vacuum dried.

Individual histone fractions were prepared by gel exclusion chromatography on columns of Bio-Gel P-30 (Candido and Dixon, 1972). Peak fractions were pooled, concentrated by evaporation under vacuum, precipitated with acetone, and then redissolved at the desired concentration. Up to 35 mg of bulk histone dissolved in about 2 ml of 5 *M* urea–0.5 *M* β-mercaptoethanol were loaded on a three-section column 3.5 m in length and 1.4 cm in diameter; 10 m*M* HCl–10 m*M* β-mercaptoethanol was used for elution. The histones were detected by absorbance at 225 nm and eluted in the order F1, F2a2, F2b, F3, F2a1.

Histone concentrations were determined by absorbance at 230 nm. The A_{230} values of the histones at 1 mg/ml in water were taken to be 3.7 for F2a1, 3.0 for F2a2, 3.9 for F2b (D'Anna and Isenberg, 1974a), 2.0 for F1 (R. D. Cole, personal communication), and 3.5 for whole histone (Bonner et al., 1968). The value for F3 was assumed to be the same as for F2a2 (Johns, 1971).

Polyacrylamide Gel Electrophoresis. The acid-urea system of Panyim and Chalkley (1969) was used with a urea concentration of 2.8 *M* and 15% acrylamide. The 15 × 11 cm slabs were run 4.5 hr at 200 V for the 1.5 mm thick ones and for 12 hr at 100 V for the 5.5 mm thick ones. After electrophoresis the gels were equilibrated with 10% trichloroacetic acid–25% 2-propanol for either 15 (thin slabs) or 60 (thick slabs) min. They were then stained for 3 (thin) or 30 (thick) hr in the same solvent containing 0.1% Coomassie Blue; 7.5% acetic acid was used for diffusion destaining. The gels were scanned on a Joyce Loebl densitometer kindly made available to us by Dr. Stoeckenius. Determination of radioactivity was carried out by excising the gel region of interest, digesting it with Protosol (New England Nuclear), and counting in a toluene-based scintillation fluid.

Tritiation of Histones in Vitro. Calf thymus histones F2a1, F2a2, and F2b prepared by Bio-Gel P-30 fractionation were dissolved in water at 1 mg/ml and the solution was adjusted to pH 9.5. To 0.5 ml of each mixture at 0° was added 1 μl of a 1% solution of tritiated formaldehyde (3.3 Ci/g, New England Nuclear) (Rice and Means, 1971). After 30 sec freshly prepared sodium borohydride at 5 mg/ml was added in successive aliquots of 4, 4, 4, 4, and 20 μl. The solutions were acidified by addition of 0.1 ml of 2 *N* H_2SO_4 and the histones precipitated with acetone. The histones were then taken up in water and reprecipitated. The resulting specific activities of the histones were 3000 cpm/μg for F2a1, 3100 cpm/μg for F2a2, and 6500 cpm/μg for F2b.

Results

The reaction of tetranitromethane (TNM) with HeLa chromatin results in rapid formation of a cross-linked histone product which appears as a new species in polyacrylamide gel electrophoretograms of the acid-extracted chromosomal proteins. As the reaction proceeds this new species, together with the histone monomers, disappears as it becomes incorporated into very high molecular weight cross-linked aggregates. These results are illustrated by the gel scans presented in Figure 1. For experiments of this type chromatin was suspended in very low salt (1 m*M* EDTA) in order to minimize protein mobility and chromatin aggregation. The pH was then adjusted to the desired value and a small amount of TNM (0.1–0.01% by volume) was mixed in to start the reaction.

Some important characteristics of the TNM cross-linking reaction should be discussed at the outset. First of all, as illustrated in Figure 1, the reaction is favored by high pH. It can be seen that very little TNM product is formed after 15 min at pH 7.0 whereas at pH 8.9 a prominent new species has appeared among the acid-extracted proteins. Nevertheless the yield of the new cross-linked species is low and, as illustrated in the lower panels of Figure 1, attempts to increase its yield by lengthening the reaction period result only in a general loss of all proteins small enough to enter the gel.

Another important feature of the reaction is that all available tyrosines react with TNM becoming either cross-linked (Williams and Lowe, 1971) or nitrated (Sokolovsky et al., 1966), and these reactions proceed with a release of protons so that in the relatively unbuffered milieu of 1 m*M* EDTA the reaction becomes self-limiting as the pH falls. Thus, for the long reaction times illustrated in the lower panels of Figure 1 periodic adjustments of the pH were necessary. For routine cross-linking, however, pH adjustments were not made since only limited reaction was sought. An initial pH of 8–8.5 and a reaction time of 30–60 min were found to be convenient conditions for chromatin at about 100–200 μg (of DNA)/ml.

In order to determine the in vivo significance of the cross-linked product produced in isolated chromatin, living HeLa cells growing in culture were treated with TNM. The gel patterns shown in Figure 2 demonstrate that an apparently identical cross-linked histone product readily forms in whole cells upon treatment with the reagent. The amount of TNM added to the culture medium was minimized to preclude chemical perturbations of the system although the cells were doubtless rapidly killed. At 50 ppm by volume (0.4 m*M*) the TNM was limiting and the increase in TNM product between 25 min and 2 hr was not very great. On the

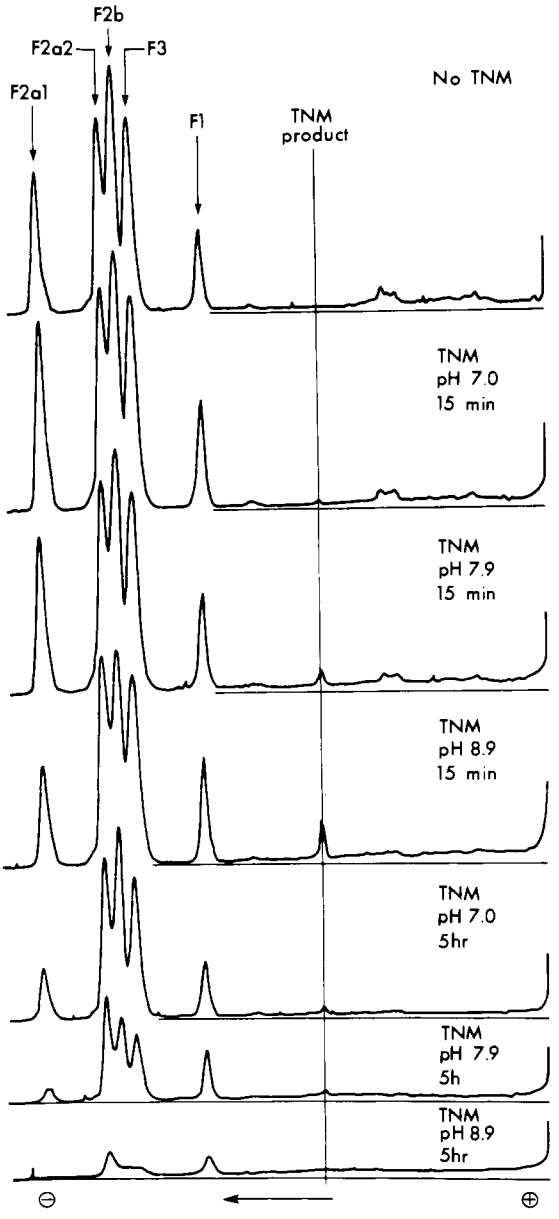

FIGURE 1: Cross-linking of histones in isolated chromatin by TNM. Chromatin prepared by method 1 was suspended in 1 m*M* EDTA (pH 8) at an absorbance (260 nm) of 3 and adjusted to the desired pH with 0.1 *N* NaOH. TNM was then added to a concentration of 200 ppm, mixed in, and allowed to react at room temperature. Protons are released during the reaction necessitating periodic adjustments of pH. The reaction was terminated by extraction of the histones with sulfuric acid. The dried histones were taken up in 8 *M* urea–1 *M* β-mercaptoethanol at a concentration of 5 g/l. and 10 μl were loaded in each 22 mm^2 well of a thick polyacrylamide slab.

other hand, as shown in the bottom panel of Figure 2, when TNM at 100 ppm was used more TNM product, estimated at about 3% of total histone, was produced.[1] Cross-linking for 24 hr in the presence of excess TNM resulted in the disappearance of most of the monomeric histones together with the specific cross-linked product as was the case for chromatin.

[1] Quantitation of the stained protein bands in a gel is not straightforward. The protein species, protein load, destaining duration and so on all affect the relative intensities of the bands. These factors were taken into account in arriving at the estimate of 3% for the yield of TNM product. Thus while variations in peak size within each figure are meaningful, no importance should be attached to relative peak intensities from one figure to the next.

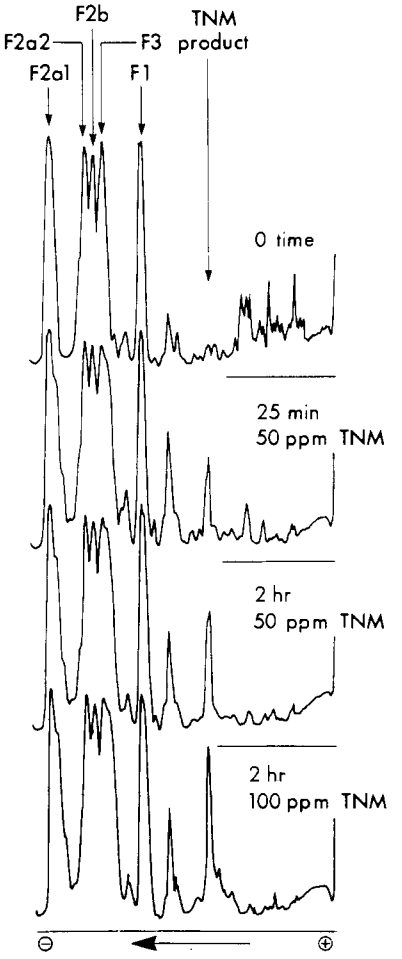

FIGURE 2: Cross-linking of histones in whole cells by TNM. HeLa cells growing in culture at about 8×10^5 cells/ml were treated with TNM at either 50 or 100 ppm by volume (0.4–0.8 m*M*). Aliquots of cells were removed at intervals, chromatin was prepared by method 2, and the histones were isolated and electrophoresed as for Figure 1.

Two other features evident in Figure 2 merit comment. At first sight the presence of the component between the TNM peak and F1 appears to be TNM dependent. However, closer scrutiny shows that, although the size of the TNM peak increases steadily with reaction time and reagent concentration, the magnitude of this other peak exhibits no such correlation. In fact material at this position is a variable component of most of our histone preparations and has also been observed by others (Garrard and Bonner, 1974; Goodwin et al., 1973). The other interesting feature of Figure 2 is the almost complete disappearance of the peaks in the nonhistone region of the gel after treatment with TNM. This phenomenon is also evident in the first few panels of Figure 1. Presumably the non-histones are more readily cross-linked into high molecular weight material by TNM than are histones.

Having obtained the same TNM product in whole cells as in isolated chromatin we next asked whether reconstituted nucleohistone might yield similar results. We found not only that reconstituted nucleohistone yields a TNM product but that sophisticated reconstitution procedures are unnecessary. Rapid mixing of the acid-extracted histones with DNA produced a DNA–histone complex which, when treated with TNM in the standard manner, yielded the cross-linked product. Moreover, commercial histone and DNA preparations were equally effective. The electropho-

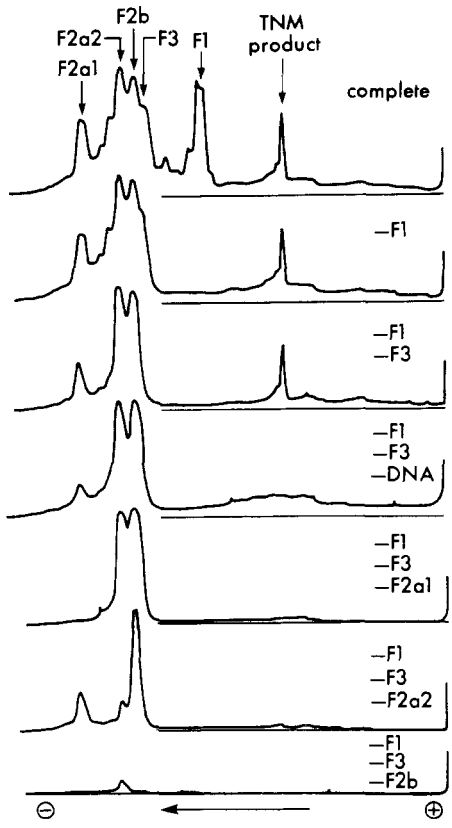

FIGURE 3: Cross-linking of histones in reconstituted nucleohistone by TNM. Calf thymus histones were fractionated on P-30 and the individual species were adjusted to a concentration of 0.5 g/l. and pH 8 in water. The histones were then recombined with mixing according to the stoichiometry of Garrard et al. (1974) to give both a complete mixture containing 45 μg of histone as well as additional mixtures lacking specific histones but supplemented with water to give the same final volume as the complete mixture; 0.2 ml of 1 m*M* EDTA (pH 8) was then added to each mixture. Finally 40 μg of DNA (0.5 g/l. in 1 m*M* EDTA (pH 8)) was added and immediately mixed in. For the control which lacked DNA an equal volume of 1 m*M* EDTA was added. The mixtures were then adjusted to pH 8.1 and 1 μl of TNM was added at room temperature. After 30 min the histones were extracted and loaded on a thin polyacrylamide slab (4 mm^2 well).

retic mobility of the TNM product obtained from mixed commercial calf thymus histones and DNA was indistinguishable from that of the TNM product obtained from HeLa cells in culture. Similarly, mixed HeLa histones and HeLa DNA also yielded the cross-linked product when treated with TNM but the high molecular weight DNA prepared in the laboratory had to be sheared somewhat to prevent precipitation on addition of histones. The simplicity of the reconstitution procedure encouraged us to investigate the properties of the system further using pure preparations of the individual histones obtained by gel exclusion chromatography of total calf thymus histones on columns of Bio-Gel P-30.

The separated histones were mixed with each other in various combinations and then DNA was added and cross-linking was carried out. The results, illustrated in Figure 3, confirmed that the cross-linked TNM product was produced when the five histones were combined with DNA in this way. Moreover both F1 and F3 could be excluded from the reconstitution mix without effect on the yield of cross-linked product. However if F2a1, F2a2, or F2b was omitted no TNM product was detectable. Also if DNA was omitted a high background resulted but no specific TNM product

Table I: Production of TNM Complex from Nucleohistone Prepared with Separated Radioactive Histones.[a]

Radioactive Histone in Reconstituted Nucleohistone	cpm in TNM Region	pmol of [^{3}H]Histone in TNM Region
F2a1	326	11.7
F2a2	52	1.4
F2b	824	11.4

[a] Reconstitution of DNA with separated histones was carried out as described for Figure 3 except that each of the three mixtures contained a radioactive histone species as indicated. After treating the mixtures with TNM the histones were extracted and electrophoresed on a thin polyacrylamide slab. The stained bands corresponding to the TNM product were excised and the amount of radioactivity was determined.

was evident. Thus these results show that any or all of histones F2a1, F2a2, and F2b were contained in the TNM product and that furthermore, in our buffer system, the presence of DNA was required in order for the correct histone configurations to be assumed for specific cross-linking by TNM.

It will be noticed that in the last panel of Figure 3 no F2a1 peak is present. This result probably arises from the tendency of F2a1 to undergo extensive self-aggregation or from its specific configuration when bound cooperatively to DNA (Ziccardi and Schumaker, 1973). Controls have shown that TNM can cross-link F2a1–DNA complexes with extraordinary efficiency, rapidly converting the F2a1 quantitatively to polymeric material. However, mixing F2a1 with F3 or F2b prevents F2a1 self-aggregation while F2a2 is much less efficient in this regard (D'Anna and Isenberg, 1974b). Thus when only F2a1 and F2a2 are deposited on DNA apparently TNM can very rapidly polymerize all of the F2a1 (Figure 3).

In order to determine which histones actually constitute the TNM product, radioactive F2a1, F2a2, or F2b were individually incorporated into separate reconstitution mixtures for cross-linking. The results, summarized in Table I, show that when either radioactive F2a1 or radioactive F2b is present the TNM product is radioactive, but that when radioactive F2a2 is used the TNM product does not incorporate significant radioactivity. Nevertheless, if the F2a2 is omitted from the reaction mixture, as shown in Figure 3, no TNM product forms. Thus F2a2 facilitates the formation of the TNM complex but does not itself become incorporated. This shows that the TNM product contains only F2a1 and F2b and would appear to be a histone–histone dimer.

The dimer assignment is borne out in Figure 4 which shows that the electrophoretic mobility of the TNM product in acid–urea polyacrylamide gels is slightly greater than that of authentic F3 dimer. The histone preparation used for this experiment was extracted from HeLa cells which had been cross-linked in culture for 2 hr with 100 ppm of TNM. The histones, at 14 mg/ml, were allowed to oxidize in air at 5° for several days. This procedure promotes the oxidation of F3 to give the disulfide linked F3 dimer, a reaction which, for HeLa histones, only F3 can undergo (Panyim et al., 1971). The top panel of Figure 4 shows the presence of F3 dimer which migrates slightly more slowly

than the TNM product. The lower panel shows the results after treating the oxidized histones with β-mercaptoethanol. It can be seen that this treatment reduces the F3 dimer completely to monomer but that the TNM peak has remained unchanged. Similar results were obtained using SDS gel electrophoresis at higher pH. The TNM product is thus unaffected by mild oxidation or reduction and appears to have a molecular weight which is lower than that of F3 dimer. These are the characteristics to be expected of a cross-linked F2a1-F2b dimer.

Discussion

We have shown that growing HeLa cells, isolated chromatin, and reconstituted nucleohistone can all be treated with tetranitromethane (TNM) for short times to yield a unique cross-linked product with the molecular weight of a histone–histone dimer. On the basis of reconstitution experiments in which individual radioactive histone fractions were used the cross-linked dimer was identified as a complex of F2a1 and F2b.

The cross-linking reaction we have described is extraordinarily specific. This is to be expected of a cross-linker which is of essentially zero length and which acts at the hydrophobic regions of the histones. Thus not only is the F2a1-F2b dimer the only low molecular weight cross-linked product to appear, but it can be formed during reconstitution only in the presence of F2a2 and DNA. D'Anna and Isenberg (1974b) have shown that specific conformational changes occur when histones become complexed to each other in solution and that the increase in α-helical content is greatest for the F2b-F2a2 and F2b-F2a1 pairs. The α-helical content of the histones also increases upon interaction with DNA (Wagner, 1970; Shih and Fasman, 1971; Ramm et al., 1972). Clearly all of these effects come into play in order to arrive at the final chromatin structure, and cross-linking with TNM is apparently acutely sensitive to some aspect of the final configuration.

Kornberg and Thomas (1974) as well as Roark et al. (1974) contend that acid extraction causes "irreversible" denaturation of histones. D'Anna and Isenberg (1974c) have argued cogently against this point of view. The present reconstitution results now show that acid-extracted histones can indeed reassemble into their native configuration with high specificity. Furthermore Richards and Pardon (1970) have obtained the X-ray diffraction pattern characteristic of chromatin using similar methods of reconstitution.

Recent results of D'Anna and Isenberg (1974b) suggest that the histones may form their specific complexes first in solution and that it is these complexes which then become specifically associated with the DNA. This interpretation was verified by mixing the individual histones sequentially with the DNA and then cross-linking with TNM. Reconstituted in this way the nucleohistone yielded no F2a1-F2b dimer. Thus premixing the histones before the addition of DNA is essential. These results suggest that, in addition to the specific pairwise associations of the various histones (D'Anna and Isenberg, 1974b), at least three different histones (F2a1, F2a2, and F2b) can also associate with each other in solution to give a specific complex. Moreover the apparent fidelity with which these histones can become attached to DNA by simple mixing suggests that no special mechanism, such as the modifications invoked by Louie et al. (1973), may be necessary for correct deposition of the histones on DNA *in vivo*.

In view of the stringent requirement for the presence of F2a2 in the formation of the F2a1-F2b dimer it is notable that the presence of F3 is unnecessary since all four of these histones appear to be associated in chromatin (see introduction). The lack of involvement of F1, on the other hand, is not surprising since F1 differs fundamentally from the other histones (Bradbury et al., 1973; Hnilica, 1972). However, the four smaller histones themselves fall clearly into two classes with evolutionary stability, amino acid composition, ease of removal from chromatin, relative affinities for each other, and relative accessibilities to proteolytic enzymes all distinguishing F3 and F2a1 on the one hand from F2b and F2a2. Thus, unlike F2b and F2a2, F3 and F2a1 have undergone very few amino acid sequence changes throughout evolution (Brandt et al., 1974; Strickland et al., 1974; Panyim et al., 1971; also see Kornberg and Thomas, 1974), and they contain more arginine than lysine (Hnilica, 1972; Panyim et al., 1971). Moreover the two pairs are removed from DNA as separate classes by certain chromatin dissociation procedures (Kleiman and Huang, 1972; Bartley and Chalkley, 1972; Ilyin et al., 1971). More recently it has been shown that in solution, the affinities of F2a1 and F3 for each other exceed their affinities for F2b or F2a2 whereas F2b and F2a2 have equal or greater affinities for each other than for F3 or F2a1 (D'Anna and Isenberg, 1974b). Finally, when chromatin is digested with proteolytic enzymes, F3 and F2a1 are attacked coordinately followed by F2b and F2a2 which are also attacked coordinately

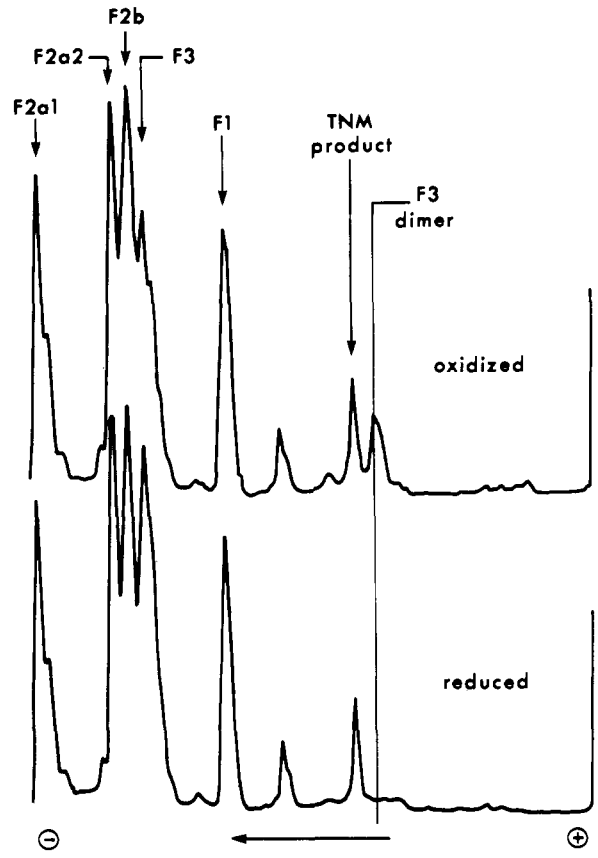

FIGURE 4: Coelectrophoresis of the TNM product with F3 dimer. Histones were isolated from HeLa cells treated with 100 ppm of TNM for 2 hr as described for Figure 2. The histones were dissolved at a concentration of 14.6 g/l in water and allowed to oxidize by standing for several days at 5°. Before loading on the gel an aliquot was treated with 1 *M* β-mercaptoethanol in 4 *M* urea at room temperature for 1 hr; 14 μg of both oxidized and reduced histones were then electrophoresed on a thin polyacrylamide slab.

(Weintraub and Van Lente, 1974). A completely different pattern of digestion is observed with the histones free in solution. All of these observations, taken together, implicate the pairwise associations of F2b with F2a2 and F3 with F2a1 in chromatin.

Thus it was unexpected that F2a2 but not F3 was required in order to adequately assemble a complex which involved F2a1. These results do not, of course, mean that F3 has only peripheral importance to the structure of the putative chromatin subunit. Obviously the data concern only one, albeit very fundamental portion of histone–histone association within chromatin. F2a2 is critically important to the structure of one particular F2a1–F2b binding site but other regions of interaction, where no TNM reaction occurs, may similarly be dependent on association with F3. On the other hand, these results do question the idea put forward by Kornberg (1974) that F3 and F2a1 are more fundamental to the structure of the chromatin subunit than are F2a2 and F2b. Most likely all of the histones interact in a very precise way to yield the total subunit.

Unfortunately in chromatin, as in reconstituted nucleohistone, our data do not allow a quantitative conclusion to be drawn. We can observe a maximum of only about 10% conversion of F2a1 and F2b to dimer after which the specific cross-linking apparently becomes overwhelmed by nonspecific cross-linking and competed by tyrosine nitration. Thus we cannot say whether all F2a1 and F2b in chromatin is capable of specific cross-linking by TNM. However, while it seems likely that most of the subunits contain the same histones, it is probable that the various subunits are structurally heterogeneous owing to interactions with nonhistones as well as to specific histone modifications such as acetylation or phosphorylation.

Qualitatively our results support the implications drawn by D'Anna and Isenberg (1974) from their data on histone–histone interactions in solution. They determined that the most significant interactions were F3-F2a1, F2a1-F2b, and F2b-F2a2 and suggest that all four histones participate as an uninterrupted functional unit in chromatin. The TNM cross-linking data now demonstrate very directly that, although the F3-F2a1 and F2b-F2a2 domains are apparently distinct, they are nevertheless contiguous and specifically interjoined in the chromosome.

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