Histone–Histone Interactions within Chromatin. Cross-Linking Studies using Ultraviolet Light†

Harold G. Martinson,* Martin D. Shetlar,‡ and Brian J. McCarthy

ABSTRACT: Irradiation of either whole cells or chromatin at 280 nm results in the covalent linkage of histones 2A and 2B, presumably at their mutual binding sites. The reaction is specific and proceeds with high yield (about 80%). Irradiation of reconstituted nucleohistone containing only H2A, H2B and DNA also yields the H2A–H2B dimer. The cross-linking event is sensitive to the conformation of the H2A–H2B pair since the histones must be bound to DNA for maximum cross-linking specificity at low ionic strength. However, the histones must first interact with each other before being deposited on the DNA, since separate addition of the histones to the DNA yields no dimer upon irradiation. If irradiation is conducted at 254 nm rather than 280 nm, DNA–histone cross-linking appears to dominate.

The concept of chromatin structure has recently undergone a rapid change (see Elgin and Weintraub, 1975, and Van Holde and Isenberg, 1975, for reviews). The traditional emphasis has been on the physical periodicity of the DNA component in the context of a histone-covered DNA supercoil. Recently, however, many investigators have stressed the periodicity of the histone component in the context of a linear arrangement of chromatin subunits (Olins and Olins, 1974; Baldwin et al., 1975; Van Holde et al., 1974; Weintraub and Van Lente, 1974; Kornberg, 1974; Griffith, 1975; Sollner-Webb and Felsenfeld, 1975; Noll, 1974). The general picture which has emerged is that two each of histones 2A, 2B, 3, and 4 associate strongly with one another, forming a globular structure over or around which are arranged approximately 180 base pairs of DNA. In chromatin, these subunits are linked together by the continuity of the DNA and at physiological ionic strength, the resulting unit fiber resembles a 100 Å wide segmented worm.

The arrangement of histones in chromatin has been studied widely using cross-linking reagents capable of spanning the short distances between neighboring lysine residues in the chromatin (Olins and Wright, 1973; Ilyin et al., 1974; Hyde and Walker, 1975; Chalkley, 1975; Chalkley and Hunter, 1975; Van Lente et al., 1975; Thomas and Kornberg, 1975). Such data yield information on histone proximity presumably over the surface of the subunit, and all of the histones have been shown to become involved in such cross-links. For example, use of formaldehyde, the shortest of these "spanner" cross-linking agents, has shown that H2B is very close to both H2A and H4 in chromatin (Van Lente et al., 1975).

Also required in the study of chromatin structure is information about the internal structure of the subunit and the actual histone–histone interactions involved. A thorough study of the interactions of histones in solution has revealed that the three most significant interactions are those between the pairs H2A–H2B, H2B–H4, and H4–H3 (D'Anna and Isenberg, 1974).

To date the existence of two of these interacting pairs (H2B–H4 and H4–H3) within nuclei has been demonstrated by the use of zero-length crosslinking reagents (tetranitromethane and a carbodiimide, respectively) which convert select noncovalent interactions among the histones into covalent bonds without the interposition of bridges (Martinson and McCarthy, 1975; Bonner and Pollard, 1975). We now report the existence within nuclei of the remaining interaction predicted by D'Anna and Isenberg (1974), namely, H2A–H2B.

The zero-length cross-linking agent we have used in this case is ultraviolet light at about 280 nm. Since the histone chromophore involved is presumably a tyrosine, and since tyrosine is found predominantly in the hydrophobic regions of the histones, this cross-linking event, like that induced by tetranitromethane ($C(NO_2)_4$) for H2B–H4 (Martinson and McCarthy, 1975), probably occurs within a tight hydrophobic binding site. Sequence and structural analysis of such cross-linked histones should eventually lead to a better understanding of the internal conformation of the chromatin subunit.

Materials and Methods

Mouse LA-9 cells were cultured in Joklik-modified minimum essential medium with 5% calf serum. Chromatin was prepared as previously described (Martinson and McCarthy, 1975, procedure 1) by lysing detergent-prepared nuclei in 50 mM Tris, pH 8. The chromatin was pelleted and then resuspended in 1 mM EDTA, pH 8, for storage at 0 °C until use. Histones were prepared by acid extraction and fractionated on columns of Bio-Gel P-30 as previously described (Martinson and McCarthy, 1975). The acid–urea system of polyacrylamide gel electrophoresis was used for identification and quantitation of histone monomers and dimers (Panyim and Chalkley, 1969). Fifteen percent acrylamide containing urea at 2.8 M was cast as 150 × 110 × 0.8 mm slabs. The gels were generally run at 300 V for 2.5–3 h. Staining with Coomassie Blue, destaining, densitometry, and scintillation counting of excised bands were as previously described (Martinson and McCarthy, 1975).

Photolysis. For irradiation at 254 nm, a Rayonet RPR-100 photochemical reactor in a 4 °C room was used. The sample temperature was stable at about 25 °C under irradiation. Irradiation at longer wavelengths was accomplished with a 450-W Hanovia medium-pressure mercury lamp housed

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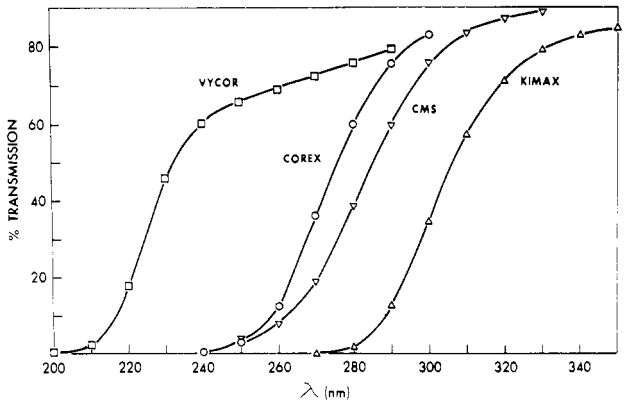

FIGURE 1: Transmission spectra of glass filters used in photolysis experiments. The spectra are from the actual tubes, jackets, or filters used in subsequent experiments and are for a single thickness of the glass.

within a Vycor filter sleeve and surrounded by a quartz water jacket (Ace Glass Co.) for cooling. In early experiments, the samples were placed in 15-mm diameter quartz reaction tubes for irradiation. For the exclusion of short wavelength light and for temperature control in work with the Hanovia lamp, the sample was surrounded by a water jacket made of old Corex glass (see Figure 1). (Corex currently available is of a new formulation and has different spectral characteristics; see Morrison and Maleski, 1972). More recently we have discovered that small samples can be irradiated conveniently in 10 × 75 mm CMS (Curtin-Matheson Scientific) tubes (see Figure 1). When either Corex or CMS glass was used, for convenience we designate the irradiation as being at 280 nm to distinguish it from the 254-nm emission (Vycor filtered) of the Rayonet. Irradiation of cells was in phosphate buffered saline (PBS) (8 g of NaCl, 0.2 g of KCl, 1.15 g of Na_2HPO_4, 0.2 g of KH_2PO_4 all dissolved in 1 l. of water). Irradiation of chromatin was in 1 mM EDTA, pH 8.2.

Results

Irradiation of Cells and Chromatin. Irradiation of whole L cells with ultraviolet light at wavelengths greater than 260 nm results in rapid formation of a cross-linked histone product. This new species appears in the dimer region of acid-urea polyacrylamide gel electrophoretograms of the acid-extracted nuclear proteins (Figure 2). A dimer molecular weight is also indicated by its rate of migration in sodium dodecyl sulfate gels and its elution position after chromatography on Sephadex G-200 (not shown). With increased irradiation of the cells the dimer photoproduct is produced in high yields while histones 2A and 2B preferentially and quantitatively disappear. This suggests that the photoproduct is an H2A-H2B heterodimer, an assignment to be substantiated below. Additional uncharacterized photoproducts, presumably trimers, appear after long periods of irradiation. With the exception of "trimer" production, the irradiation of chromatin at low ionic strength yields nearly identical results.

As shown in Figure 3, the H2A-H2B dimer can be produced by irradiation of chromatin at wavelengths less than 260 nm as well (i.e., at 254 nm). In this case, however, the yield is much lower and all the histones, including the dimer, soon begin to disappear. Presumably the major chromophore excited at wavelengths >260 nm is tyrosine, whereas at 254 nm, it is the bases of DNA. In the former case, proteins become cross-linked to each other, whereas in the latter case, DNA-protein adduct formation may dominate (see below).

All irradiations were performed anaerobically. Control

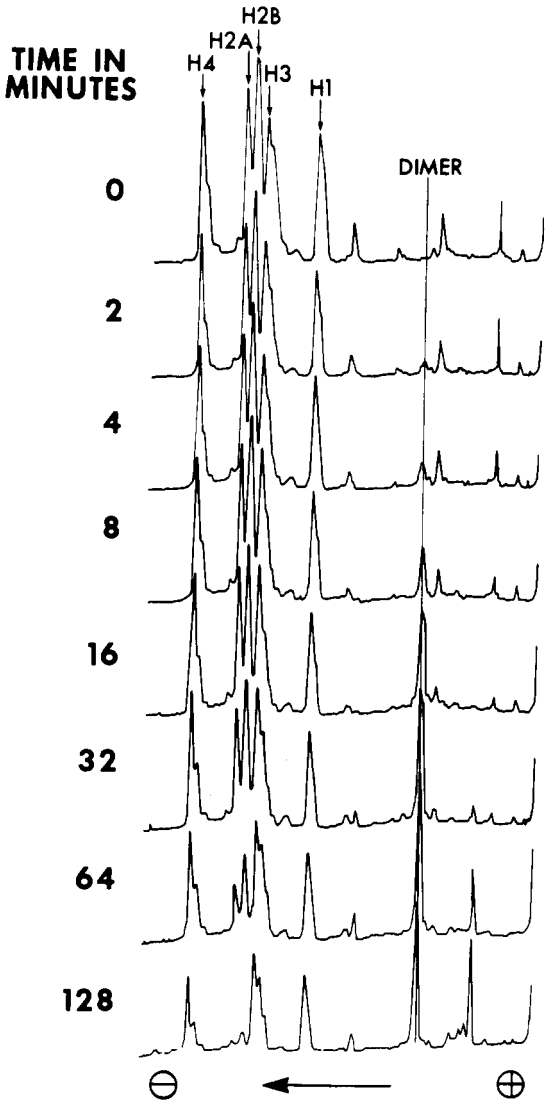

FIGURE 2: Electrophoresis of histones isolated from cells irradiated at 280 nm. About 2-3 × 10^7 cells were spun down, washed in 10 ml of PBS, pelleted again, resuspended with 0.5 ml of PBS, and transferred to a quartz tube. A stream of humid nitrogen was passed over the cell slurry for 5 min. The introduction of air was prevented for the remainder of the experiment. 15 ml of PBS which had been bubbled with humid nitrogen for 0.5 h was then added with mixing and a zero time sample was withdrawn. (Total elapsed time to this point was 30 min. However, identical results were obtained with cells harvested the previous day.) At zero time, the remaining cells were exposed to the 450-W Hanovia uv source through a Corex water jacket maintained at room temperature. Uniform irradiation was ensured by continuous stirring of the sample. At the indicated times, 1.8-ml aliquots were withdrawn and left at room temperature until the last time point. The cells from each time point were then collected for extraction of histones.

experiments showed that recoveries of all histone species were higher when the cells or chromatin were photolysed under an atmosphere of nitrogen rather than air. The effect was particularly striking for the dimer whose relative yield was increased by 50% and whose absolute yield was more than doubled by the exclusion of oxygen. Presumably oxidative side reactions occur which lead to general polymerization and loss of all histones and which, in addition, compete specifically with the H2A-H2B dimer formation.

In Figure 4 we present a quantitative summary of dimer production as a function of elapsed irradiation time. The data for the time points were obtained from polyacrylamide gels by excising the bands, eluting the stain, and taking the absorbance

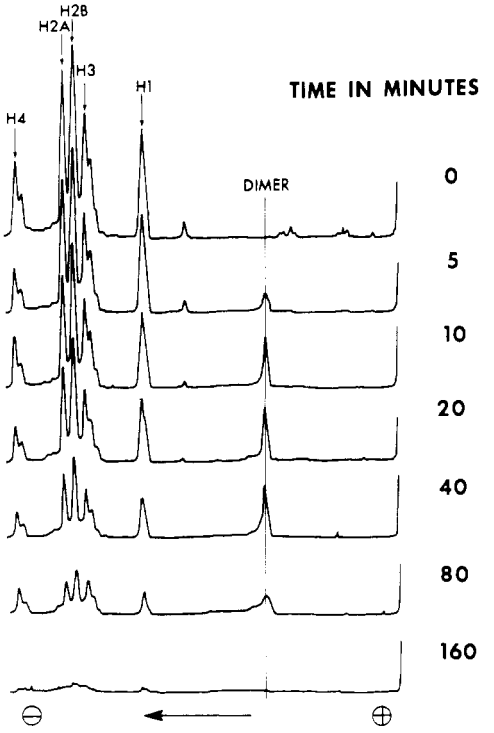

FIGURE 3: Electrophoresis of histones isolated from chromatin irradiated at 254 nm. 0.5 ml of chromatin at an A_{260} of 23 was purged in the quartz reaction tube with a stream of humid nitrogen for 15 min and then mixed with 10 ml of bubbled 1 mM EDTA, pH 8.2, in the absence of air as for Figure 2. This procedure was followed because direct bubbling of a chromatin solution causes surface denaturation and aggregation. A zero time sample was withdrawn, and then the remaining chromatin was irradiated through a Vycor shield at 254 nm. At the indicated times, 1-ml aliquots were withdrawn and the histones extracted.

at 590 nm. The amount of stain eluted from each histone band is expressed as a per cent of the total. No attempt has been made to correct for the small differences in stain:protein ratio which arise from variations in band intensity or protein stain affinity. Such adjustments would not alter the general conclusions to be drawn from Figure 4.

Panels A and B of Figure 4 show that, with the exception of trimer production, the major features of photolysis are the same whether intact cells at physiological ionic strength or isolated chromatin at low ionic strength are irradiated. In whole cells, the decline in rate of dimer production accompanied by the appearance of increasing quantities of the product in the trimer region of the acrylamide gel (see Figure 2) suggests a precursor-product relationship. Whether the production of this presumptive trimer is a reflection of the condensed state of chromatin in vivo has not yet been ascertained.

The solid portion of the bottom line in panel B of Figure 4 demonstrates the consequence of excluding light of 280 nm or less by use of a Kimax filter. The dashed portion of the line shows the result during the first 10 min after removal of the filter. Clearly, filtering out the light between 260 and 280 nm severely depresses dimer production. Since the absorption maximum for histones lies between 270 and 280 nm and a significant emission band of the Hanovia lamp occurs at 280 nm, we infer that absorption of the 280-nm emission into the tyrosine chromophore (histones have no tryptophan) is principally responsible for the histone–histone cross-links. Furthermore, since irradiation at 254 nm is near the DNA absorption maximum and is known to induce DNA–protein cross-links (Strniste and Smith, 1974; Anderson et al., 1975), we attribute the general loss of histones at this lower wavelength to DNA–histone adduct formation resulting from uv excitation of the DNA bases. That irradiation at 254 nm results in loss of each of the histones with very similar kinetics (Figure 3) would be expected for a wavelength absorbed primarily into the DNA to which each of the histones is bound.

The yield of H2A–H2B dimer can be estimated from panels A and B of Figure 4 to be about 80% based on recoverable histone (H2A + H2B decreases from 45 to 8% of the total, while dimer increases to 30%). If the presumptive trimer of panel A is a further photoproduct of the dimer, then 100% of the lost H2A and H2B can be accounted for by these two products. However, it should be recognized that the recovery of histones after photolysis even at 280 nm is incomplete, as would be expected if the action spectrum for histone loss were equivalent to the absorption spectrum of DNA. Thus the yield estimate rests on the assumption that loss is equivalent for all of the histones, monomers and oligomers alike. The data of Figure 3 suggest that this is the case for the drastic losses which occur at 254 nm and the data of Figure 4 when reformulated as shown in Figure 5 lead to the same conclusion for 280 nm. In Figure 5 the effect of irradiation on recoverability is shown for two groups of histones, namely, H2A, H2B, and their photoproducts on the one hand and H1, H3, and H4, which do not yield identifiable photoproducts, on the other. It can be seen that, within experimental error, these two groups of histones are lost with similar kinetics during irradiation of either cells at 280 nm (panel A) or chromatin at 280 as well as 254 nm (panel B). Our estimate of at least 80% efficiency of dimer formation is, therefore, probably valid.

Reconstituted Nucleohistone. In order to identify the dimer unambiguously as H2A–H2B, some simple DNA–histone reconstitution experiments of the type previously reported (Martinson and McCarthy, 1975) were performed. Purified L-cell histones were mixed with DNA at low ionic strength and then irradiated in the usual way. Reisolation and electrophoresis of the histones on polyacrylamide gels showed that even in this crude system good yields of a product with the same mobility as "native" dimer were produced. Furthermore, as shown in Figure 6, only H2A and H2B were required for dimer formation, whereas the absence of either resulted in no dimer. While this further substantiated the H2A–H2B assignment, the possibility still remained that the presence of one of the pair (H2A or H2B) was required to facilitate the production of homodimer from the other. This possibility was examined by the use of radiolabeled H2A or H2B in the reconstitution mixtures. As shown in Table I, equal quantities of both H2A and H2B are incorporated into the dimer. The cross-linked product is thus almost certainly an H2A–H2B heterodimer. The only alternative is that two homodimers, $(H2A)_2$ and $(H2B)_2$, are produced, each of whose cross-linkage is absolutely dependent on the presence of the heterologous histone and whose mobilities on polyacrylamide gels are indistinguishable.

The presence of DNA is not an absolute requirement for dimer production (Figure 6). Nevertheless, if DNA is not present during irradiation, the yield of dimer is low and the background of heterogeneous products in the gel is high. Thus, H2A and H2B associate with each other in the absence of DNA but, under our conditions, apparently only a small proportion of the associated pairs have a conformation resembling that in the native chromosome. Thus, the DNA binding event rectifies the conformations of the various associated pairs of H2A and H2B, improving the yield of dimer and eliminating the nonspecific background. Indeed, as we found previously

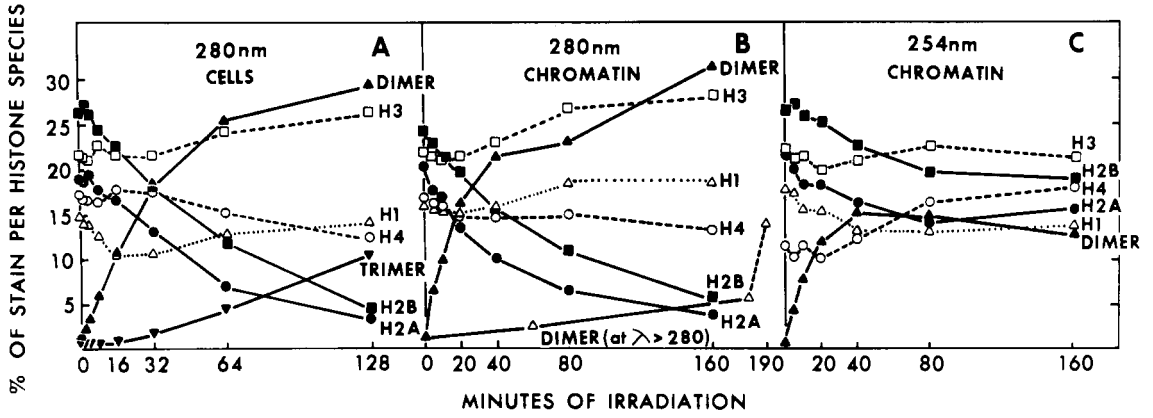

FIGURE 4: Yield of dimer with time of irradiation. Panels A and C are a quantitative summary of the data in Figures 2 and 3. Panel B summarizes an experiment in which chromatin, prepared and sampled as for Figure 3, was irradiated as described for whole cells in the legend to Figure 2. The amount of stain in each band of the polyacrylamide gel was quantitated by incubating the excised band in 0.5 ml of 1% sodium dodecyl sulfate and determining the absorbance of the eluted stain at 590 nm. The amount of stain in a band is expressed as a percentage of the total for all bands at each time point. In panel B, additional data are shown to demonstrate the rate of dimer production in the presence (lower dimer curve, solid line) and subsequent absence (dashed portion of lower dimer curve) of a Kimax filter which is essentially opaque below 280 nm (see Figure 1).

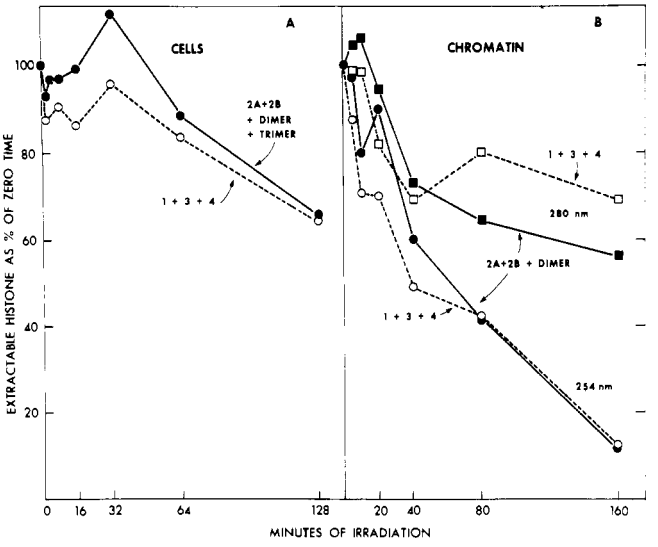

FIGURE 5: Histone recovery as a function of irradiation time at 280 or 254 nm. The data of Figure 4 are transformed to show the percent of histone recovered in the various bands at each time point relative to zero time.

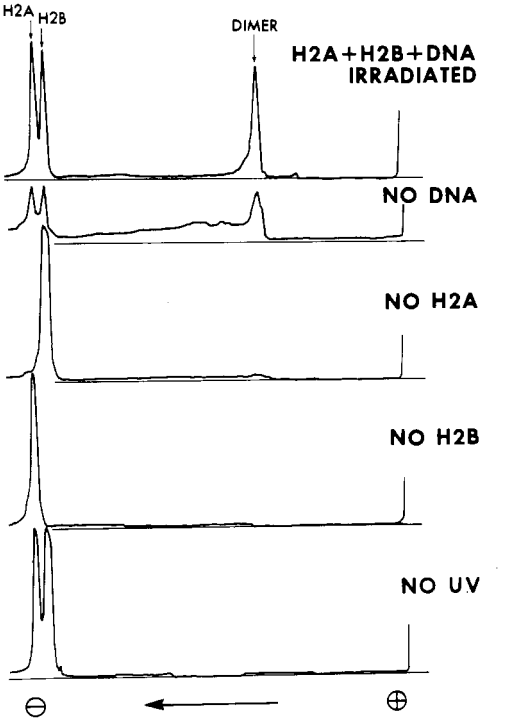

FIGURE 6: Electrophoresis of histones isolated from reconstituted nucleohistone irradiated at 280 nm. For the first panel, purified histone fractions 2A and 2B were mixed first with each other and then directly with DNA in 1 mM EDTA, pH 8, as previously described (Martinson and McCarthy, 1975, Figure 3). 0.5 ml of such a mixture containing 100 μg of each of the two histones was purged with N_2 for 90 min and then irradiated at 280 nm for 20 min in a CMS tube. Subsequent panels in the figure represent parallel samples in which DNA, one of the histones, or the irradiation itself was omitted. The volume as well as the ratio of DNA to total histone in the various samples was kept constant.

(Martinson and McCarthy, 1975), preliminary association of the histones with themselves in solution must precede their deposition on DNA in order for their appropriate native conformations to be adopted. This is illustrated in Figure 7, which documents the effect of adding the histones sequentially (dotted line) rather than simultaneously (solid line) to the DNA. Unless the histones are allowed to interact with each other before being constrained by the DNA, no specific dimer is formed.

Discussion

Irradiation of whole L-cells with uv light induces the formation of high yields of an H2A–H2B dimer. The identity of this dimer was initially inferred from the concomitant disappearance of the H2A and H2B monomers. The assignment was then confirmed through reconstitution experiments which showed that only H2A and H2B together with DNA were required for this dimer to be produced upon irradiation and that both radiolabeled H2A and H2B became incorporated into the dimer.

In the previous cross-linking experiments which we reported (Martinson and McCarthy, 1975), it was shown that the zero-length cross-linker tetranitromethane was a highly sensitive probe of the conformations of interacting histones. Thus the $C(NO_2)_4$-induced H2B–H4 dimer arose in reconstituted nucleohistone only if H2A were also present. Since H2A and H2B are known to interact specifically in solution (D'Anna and Isenberg, 1974), it was proposed that this interaction altered the structure of H2B, and hence of the H2B–H4 part of the

Table I: Dimer Production from Nucleohistone in Which either H2A or H2B Was Radiolabeled.[a]

Radioactive Histone(s) in Reconstituted Nucleohistone	Specific Act. (cpm/pmol)	cpm in Dimer Region	A_{640} of Dimer Extract × 10^2	cpm/A_{640} × 10^{-1}
2A	45	60	5.7	106
2B	90	140	8.3	168
				Sum = 274
2A + 2B	45 and 90	226	8.7	260

[a] Reconstitution was performed as described for Figure 6. Tritium labeled histones were those previously described (Martinson and McCarthy, 1975). After irradiation and polyacrylamide gel electrophoresis, the stained dimer bands were excised. The stain was extracted into 0.5 ml of 20% trichloroacetic acid–25% 2-propanol and quantitated by taking the absorbance at 640 nm. (The use of 640 rather than 590 nm was inadvertent but satisfactory.) The gel slices were subsequently removed and counted in Aquasol containing 10% Protosol.

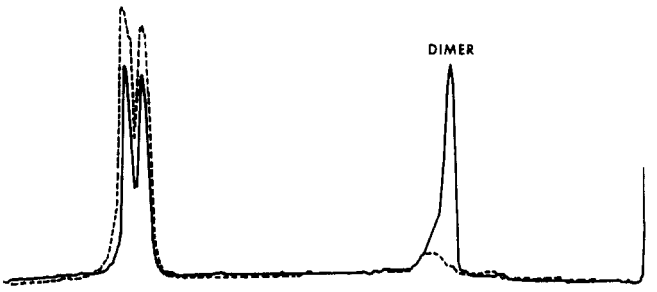

FIGURE 7: Effect of order of addition in direct mix reconstitution. The solid line represents a reconstitution–irradiation experiment of the type described for the first panel of Figure 6. For this reconstitution, the histones were mixed first with each other and then with the DNA. The dashed line represents a different reconstitution–irradiation experiment in which the DNA was mixed first with one of the histones (2A) and then with the other.

complex, in such a way as to render 2B and 4 susceptible to cross-linking by the $C(NO_2)_4$ when deposited on DNA. The results reported in this communication are consistent with this interpretation and suggest an assembly heirarchy. Thus the association of H2A with H2B can proceed with fidelity in the absence of other histones (present results) and is, moreover, apparently a prerequisite for the correct association of the H2B with H4 (previous results). H3 would presumably be the fourth member of the series.

Weintraub et al. (1975) have recently reported the association of H2A, H2B, H3, and H4 to yield the expected tetramer when they are mixed under conditions of high salt and pH (1–3 M NaCl, pH 7–9). The histones in this tetramer exhibited properties indistinguishable from the histones of chromatin by several tests but underwent drastic conformational changes when the salt concentration was lowered by dialysis. These changes were prevented when DNA was present during dialysis. These results confirm not only the role of DNA in maintaining histone nativity but also the existence of the 2A–2B–3–4 tetramer predicted by the above assembly hierarchy and originally postulated on the basis of histone pair associations at low ionic strength (D'Anna and Isenberg, 1974).

We do not believe that the less stringent requirement for proper H2A–H2B reconstitution compared to that for H2B–H4 reflects a correspondingly lower degree of specificity for the uv reaction relative to that of $C(NO_2)_4$. The primary site of action for both cross-linking agents is presumably a tyrosine residue, yet each agent gives rise to a different cross-linked histone pair. Furthermore, CNBr peptide mapping (unpublished) has shown that the cross-linkages in H2A–H2B and H2B–H4 involve different sites on the H2B. Finally, both cross-linking reactions, even when conducted on whole cells, yield only a single dimer as their major oligomeric product. This is in contrast to reagents of low specificity such as imidoesters or aldehydes, which yield mixtures of dimers and oligomers (Olins and Wright, 1973; Ilyin et al., 1974; Hyde and Walker, 1975; Chalkley, 1975; Chalkley and Hunter, 1975; Van Lente et al., 1975; Thomas and Kornberg, 1975). It should be emphasized that the cross-linking agents we have used are sensitive specifically to conformation at the histone–histone binding sites. The data, therefore, do not imply that our direct-mix reconstitution procedure yields nucleohistone which is native in all aspects of conformation.

The high yield with which the uv cross-linking reaction proceeds suggests that most, and perhaps all, of the H2A and H2B molecules in chromatin are associated with one another. Furthermore, beneath whatever conformational variations H2A and H2B may exhibit in chromatin, it appears that the presumptive binding site between the two is relatively invariant in structure. Experiments are currently under way to characterize the uv and $C(NO_2)_4$ sites of cross-linkage and to determine more precisely the stoichiometry of histone–histone interactions in nuclei.

The possibility of studying both histone–histone and histone–DNA interactions by using uv light of different wavelengths appears promising. Analysis of histone–DNA complexes isolated from 254-nm irradiated cells should yield useful information on the nonelectrostatic component of the histone–DNA interaction in chromatin.

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Purification of Proteins from the 50S Ribosomal Subunit of *Escherichia coli* by Ion-Exchange Chromatography†

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ABSTRACT: Thirty-three proteins have been isolated from the 50S ribosomal subunit of *Escherichia coli* by a technique based solely upon ion-exchange chromatography. The procedure can be adapted to a wide range of sample sizes, requires no prefractionation of the subunit proteins, and employs readily regenerated chromatographic media. The molecular weights, purity, and immunological properties of the individual proteins have been characterized. More than 20 of the proteins were judged to be at least 95% pure by electrophoretic analysis; the remaining proteins were generally over 90% pure. Methods for the immunological identification of small amounts of ribosomal proteins are described.

From 27 to 34 chemically distinct proteins have been identified in the 50S ribosomal subunit of *Escherichia coli* (Traut et al., 1969; Hindennach et al., 1971; Mora et al., 1971; see review by Wittmann, 1974). Development of techniques for the purification and characterization of these components has contributed significantly to investigations of their role in protein synthesis. A variety of specific 50S proteins have now been implicated in critical subunit functions including tRNA binding, peptidyl transferase and GTPase activities, interaction with elongation and termination factors, and structural integration of the 5S RNA (see reviews by Pongs et al., 1974; Cantor et al., 1974; Stöffler, 1974; Möller, 1974; Monier, 1974). It has also been possible to identify the proteins which lie in or near the binding sites for several antibiotics (Nierhaus and Nierhaus, 1973; Pongs et al., 1973a,b; Sonenberg et al., 1973; Wittmann et al., 1973). Owing to the discovery of conditions for the total reconstitution of the *E. coli* 50S subunit in vitro (Nierhaus and Dohme, 1974), the rapid extension of such studies can be expected.

Since purified 50S subunit proteins are required for many kinds of investigations, efficient means of resolving them are of considerable importance. Several techniques for the isolation of individual 50S subunit proteins have been described, all of which entail prefractionation of the proteins, either by treatment of the subunits with high concentration of LiCl or urea (Traut et al., 1969; Hindennach et al., 1971; Möller et al., 1972; Pearson et al., 1972), or by fractional precipitation of the total 50S protein mixture with ammonium sulfate (Hindennach et al., 1971; Mora et al., 1971) before purification by chromatography or electrophoresis. Prefractionation techniques, however, do not always separate the proteins into mutually exclusive groups and a single protein is frequently found in more than one of the fractions.

We presently describe a method for obtaining more than 30 proteins of the 50S subunit in a state of high purity by ion-exchange chromatography alone. Since no prior fractionation of the proteins is required, losses are reduced to a minimum and any given protein is recovered quantitatively at the appropriate stage of the procedure. The method is applicable to both small and large quantities of material and, because ion-exchange columns are used throughout, sample concentration is not critical. Most of the proteins isolated by this procedure have been correlated with those purified according to Hindennach et al. (1971) by measurements of electrophoretic mobility, molecular weight, and immunological specificity.

Materials and Methods

Bacterium and Culture Cultures. Escherichia coli strain MRE600 (Cammack and Wade, 1965), growing exponentially at 37 °C in 500 ml of medium containing 7 g/l. $NaH_2PO_4 \cdot 2H_2O$, 3 g/l. K_2HPO_4, 0.5 g/l. NaCl, 1.0 g/l. NH_4Cl, 1.32 g/l. $(NH_4)_2SO_4$, 0.25 g/l. $MgSO_4 \cdot 2H_2O$, 0.18 g/l. $CaCl_2 \cdot 4H_2O$, 4 g/l. glucose, and 0.2 g/l. casamino acids (Difco, vitamin-free), was incubated for five generations with 3–6 mCi of [^{3}H]amino acid mixture (New England Nuclear, NET-250). Ribosomal proteins were assumed to be labeled to equal spe-

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