*Proc. Natl. Acad. Sci. USA*
Vol. 76, No. 3, pp. 1030–1034, March 1979
Biochemistry

# Semihistone protein A24 replaces H2A as an integral component of the nucleosome histone core

(chromatin structure/nucleohistone reconstitution/zero-length crosslinking/nonhistone/preparative gel electrophoresis)

HAROLD G. MARTINSON, ROD TRUE, JOHN B. E. BURCH, AND GARY KUNKEL

Division of Biochemistry, Department of Chemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90024

*Communicated by P. D. Boyer, November 8, 1978*

ABSTRACT **The semihistone protein, A24, was shown to be a stable minor component of purified salt-washed nucleosome core particles. A24 was also shown to become integrated into nucleohistone during reconstitution in a manner characteristic of the core histones. Purified A24 in solution was shown to exhibit the same specificity of interaction with histone H2B as is exhibited by histone H2A. We conclude that A24 in chromatin replaces H2A as a stable integral component of certain nucleosome histone cores.**

The unusual semihistone protein, A24, is a conjugated chromosomal protein derived from histone H2A. In chromatin, a small proportion of the H2A molecules are connected through the $\epsilon$-$NH_2$ of lysine 119 to the nonhistone, ubiquitin (1, 2). Recent data show that A24 is associated with nucleosomes prepared by micrococcal nuclease digestion and sucrose gradient centrifugation in 5 mM EDTA (3). The nucleosomes prepared in this way also had H1 associated with them. Unlike H1, but like H2A, A24 is not extracted from chromatin with 0.6 M NaCl (4); therefore, Goldknopf *et al.* (3) proposed that A24 exists integrated in nucleosome histone cores. Nucleosome cores normally consist only of the "core histones" (H2A, H2B, H3, and H4) complexed tightly with about 144 base pairs of DNA. However, in addition to A24, many purely nonhistone proteins also are resistant to 0.6 M NaCl extraction (5–7). Therefore, it is equally likely that A24 could occupy a peripheral position on nucleosomes where, by virtue of its ubiquitin moiety, it is prevented from becoming incorporated into the histone core but serves some other function in association with the nucleosome.

Initial interest in protein A24 arose from the finding that the content of this protein in rat liver nucleoli dropped rapidly to zero as a consequence of treatments (partial hepatectomy or administration of thioacetamide) which induced nucleolar hyperfunction (8). This observation suggested that A24 may play a role in the modulation of gene activity. If A24 fulfills some regulatory function, it apparently does so in response to a rather specific control mechanism; the overall content of A24 in the *total chromatin* of livers from partially hepatectimized or thioacetamide-treated rats is not detectably affected despite its drastic decrease in the nucleoli (9).

In order to learn more about the role of A24 in chromatin function, we have begun studies to determine the location of A24 in chromatin and the effect of A24 on nucleosome structure. In this report we show that the A24 in chromatin is an integral component of nucleosome cores and that purified A24 exhibits the binding specificity for H2B that is characteristic of H2A. The ubiquitin moiety of A24 therefore does not interfere with its contribution to nucleosome formation but rather must function by altering some specific nucleosomal property. Knowledge of the integral nature of A24 places informative constraints on the manner in which it can be removed from specific regions of chromatin during functional transitions. It also suggests experimental approaches for studying the function of A24 through characterization of the properties of nucleosomes constructed *in vitro* to contain A24 in place of H2A.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

## MATERIALS AND METHODS

**Preparation of Calf Thymus Histones.** Frozen thymus (50 g) was blended in 300 ml of cold 75 mM NaCl/24 mM EDTA at pH 8 with octanol (0.5 ml) and phenylmethylsulfonyl fluoride (4 ml of a 0.1 M solution in dimethylsulfoxide) as foaming and protease inhibitors. The homogenate was filtered and centrifuged. The nuclei were washed once in 200 ml of NaCl/EDTA, twice in 0.35 M NaCl (pH 7), and then resuspended in 40 ml of ice-cold water for extraction with $H_2SO_4$.

**Preparation of Calf Thymus Nucleosomes and Core Particles.** Nuclei were prepared as described above, except as noted below. Calf thymus (30 g) was homogenized in 700 ml of 25 mM KCl/5 mM $MgCl_2$/50 mM Tris·HCl, pH 6.5. The nuclei were washed in 200 ml of the same buffer which was also 0.1% in Triton X-100 and then in 200 ml of 25 mM KCl/4 mM $MgCl_2$/1 mM $CaCl_2$/50 mM Tris·HCl, pH 7.5. The nuclei were resuspended for digestion in the $CaCl_2$ buffer at an $A_{260}$ of 140 as determined by dilution into 2 M NaCl/5 M urea. Phenylmethylsulfonyl fluoride was used in this and all preceding steps. Sixty milliliters of the nuclear suspension was then warmed to 37°C and digested for about 3 hr with 80 units of micrococcal nuclease (Sigma) giving 25% acid solubility of the DNA (determined in 1 M $HClO_4$/1 M NaCl; uncorrected for hyperchromicity). The chromatin was pelleted at 0°C, 16,000 × *g* for 5 min and then resuspended in 8 ml of 0.55 M NaCl/10 mM EDTA/1 mM Tris·HCl/3 mM $NaN_3$ at pH 7.5. Debris was pelleted at 16,000 × *g* for 30 min, and the supernatant was fractionated on three 2.3-cm × 95-cm Sepharose 4B columns attached in series (10). Elution was at 4°C with 0.55 M NaCl buffer at a flow rate of approximately 8 ml/hr. Fractions were monitored by absorbance and characterized with respect to DNA size and protein content by use of the sodium dodecyl sulfate/polyacrylamide gel procedures described respectively by Todd and Garrard (11) (4% acrylamide) and Bonner and Pollard (12). Aliquots estimated to contain approximately 50 μg of DNA (25–400 μl) were precipitated with 2 ml of 95% ethanol at −20°C overnight. The precipitate was washed with cold 70% ethanol and resuspended in 25 μl of 9 mM Tris·HCl/7.5 mM sodium phosphate/2.5 mM EDTA/10% sucrose/1% sodium dodecyl sulfate at pH 7.8 for analysis directly on either the DNA or protein gels.

For preparation of trimmed core particles, column fractions containing monosomes were pooled, concentrated by use of a Millipore immersible separator, refractionated on Sepharose

4B, pooled, concentrated again, and then dialyzed against 0.1 mM EDTA/0.1 mM Tris·HCl, pH 7.5. This monosome pool (0.12 ml) was added to 1.08 ml of 1 mM Tris·HCl/0.1 mM $CaCl_2$/50 mM NaCl at pH 7.9 (to give $A_{260} = 20$) and digested with 2.4 units of micrococcal nuclease (Sigma) at 37°C to 15% acid solubility of the DNA. The reaction was quenched on ice with the addition of 0.12 ml of 100 mM EDTA, pH 7.0. The sample was centrifuged directly on a 5–20% (wt/vol) sucrose gradient containing 1 mM Tris·HCl/10 mM EDTA/0.55 M NaCl at pH 7.5 (Beckman SW 27.1 rotor, 27,000 rpm, 25.5 hr, 4°C). Fractions (0.6 ml) were collected, monitored for absorbance, and prepared for analysis of proteins or DNA essentially as described above. Our trimmed cores typically sediment at $s_{20,w} = 10.9$ as determined in 25 mM NaCl by analytical ultracentrifugation.

**Rapid Mixing Procedure for Reconstitution of Acid-Extracted Histones with DNA.** Acid-extracted histone (13 mg/ml) was prepared for reconstitution first by effecting complete denaturation by addition of 4 vol of 9 M urea/20 mM HCl and incubation at room temperature for about 0.5 hr. The solution was adjusted to pH 7.5 with 0.25 M $Na_3PO_4$, 2-mercaptoethanol was added to 2%, and phenylmethylsulfonyl fluoride (100 mM in dimethylsulfoxide) was added to 1 mM final concentration. After incubation for 1 hr at 37°C under nitrogen, the urea concentration was reduced to about 2 M by the addition of distilled water, giving a histone concentration of about 0.7 mg/ml. The histones were then renatured in the presence of salt and DNA by mixing directly with an equal volume of DNA dissolved at an $A_{260}$ of 10 in 2 M NaCl/10 mM sodium phosphate/1 mM EDTA, pH 7.5. Thus, at this point, the salt and urea concentrations were both about 1 M. The histones were then deposited on the DNA by reducing the salt concentration of the histone–DNA mixture to 0.6 M by the slow addition with mixing of distilled water at room temperature. The low concentration of urea present at this stage apparently does not interfere with proper nucleosome formation. Reconstitution procedures of this type yield native nucleosomal structures according to various criteria, which will be described in greater detail elsewhere.

**Sucrose Gradient-Centrifugation of Reconstituted Nucleohistone.** In order to obtain a uniformly sedimenting complex, we carried out reconstitution for this work with DNA about 2000 base-pairs-long obtained from a preparation of mildly sonicated DNA that had been fractionated on a sucrose gradient. About 1 ml of the reconstitute, prepared as described above, was centrifuged on a 5–20% sucrose gradient containing 0.6 M NaCl and 1 mM EDTA at pH 7 (Beckman SW 27.1 rotor, 27,000 rpm, 14 hr, 5°C). After centrifugation the $A_{260}$ of each fraction was determined, and the histones were collected for electrophoretic analysis by 25% trichloroacetic acid precipitation on ice. The precipitates were dissolved in 20 μl of 2% sodium dodecyl sulfate/0.125 M Tris·HCl, pH 6.8/10% glycerol/5% 2-mercaptoethanol. Five microliters of each fraction was analyzed on 0.8-mm-thick slab gels (12). Staining with Coomassie blue and destaining were as described (13).

**Purification of H2A, H2B, and A24.** Acid-extracted histones were fractionated by Bio-Gel P-30 column chromatography on three 2.3-cm × 95-cm columns attached in series as described (14). The elution positions of the various components of the histone mixture (which also contains A24) were determined by acid–urea gel electrophoresis (13, 15) of lyophilized aliquots taken from the column fractions. The A24 elution largely overlaps H2B in our eluant system (10 mM HCl/10 mM 2-mercaptoethanol). Fractions enriched in H2A, H2B, or A24 were pooled, lyophilized, and (sometimes for H2A and H2B, always for A24) rechromatographed on Bio-Gel P-30. In the case of A24, preparative acid–urea gel electrophoresis on 6-mm-thick gel slabs was also used. The bands to be excised were located by staining the two faces of the thick gel by a brief exposure (15 min) to 0.005% Coomassie blue in 2.8 M urea/0.9 M acetic acid. The protein was recovered by electrophoretic elution in an apparatus based on a design kindly supplied by Irvin Isenberg. It consists of an elution chamber and a collection chamber connected by a short neck into which is cast a plug of 7% acrylamide. Electrical contact with a negative electrode reservoir is maintained across a Spectrapor 1 dialysis membrane, which seals the end of a glass tube extending from the reservoir into the collection chamber. All compartments are filled with 0.9 M acetic acid. The elution chamber is also 2.8 M in urea. Protein is recovered from the collection chamber by lyophilization. We consider the "A24" prepared by this procedure to be identical to the A24 described by Goldknopf *et al.* (3, 4) based on its acid extractability, its presence in nucleosomes, its resistance to 0.6 M salt extraction from chromatin, and its mobility relative to the histones in both sodium dodecyl sulfate and acid–urea gels.

**UV Irradiation of Histone Mixtures.** Photolysis was carried out in a cold room with a 450-watt Hanovia medium-pressure mercury lamp housed within a Corex filter sleeve and surrounded by a quartz water jacket for cooling. The Corex used was of the old formulation (13). The histone samples in 2 M NaCl/2 M urea were placed directly alongside the lamp in 7-mm-diameter stoppered quartz tubes. Hypodermic needles provided inlet and outlet ports for nitrogen purging and the samples were completely deoxygenated prior to photolysis by being maintained under a steady stream of nitrogen for 15–30 min. The lamp was allowed to reach full intensity before photolysis was begun. During the warm-up period the samples were shielded from radiation by aluminum foil.

## RESULTS

**A24 Is Present in Salt-Washed, "Trimmed" Nucleosome Core Particles.** Calf thymus nuclei were digested with micrococcal nuclease and the products of digestion were fractionated in the presence of 0.55 M NaCl by Sepharose 4B column chromatography. Aliquots from successive column fractions were ethanol-precipitated and analyzed for DNA and protein on sodium dodecyl sulfate/polyacrylamide gels. Fig. 1*A* shows the protein content of column fractions extending from the oligonucleosome region on the left to the subnucleosome region on the right. It can be seen that A24 was present in all fractions that contained equimolar quantities of the core histones. In contrast, H1, which is not bound to chromatin at this salt concentration, eluted independently in the low-$M_r$ region of the column profile. In this region, neither the core histones nor A24 were evident in appreciable quantities. Thus, A24 is not liberated by digestion of internucleosomal linker DNA by micrococcal nuclease.

Fig. 1*A* also shows that the content of A24 relative to the core histones was independent of particle size. The ratio of A24 to the core histones was not noticeably different in the oligosome region at the left from the two fractions containing predominantly subcore-length DNA just to the right of the monosome peak. Thus, provided there is sufficient DNA to maintain organization of the histone core, A24 also is integrated into similar structures.

In order to rule out unambiguously that A24 could be bound to residual "linker" DNA on nucleosomes, the monosome fractions of Fig. 1*A* were pooled, concentrated, and refractionated on the Sepharose column. The peak fractions, now entirely free of H1 and disome, but still containing the same proportion of A24, were pooled, concentrated, and subjected

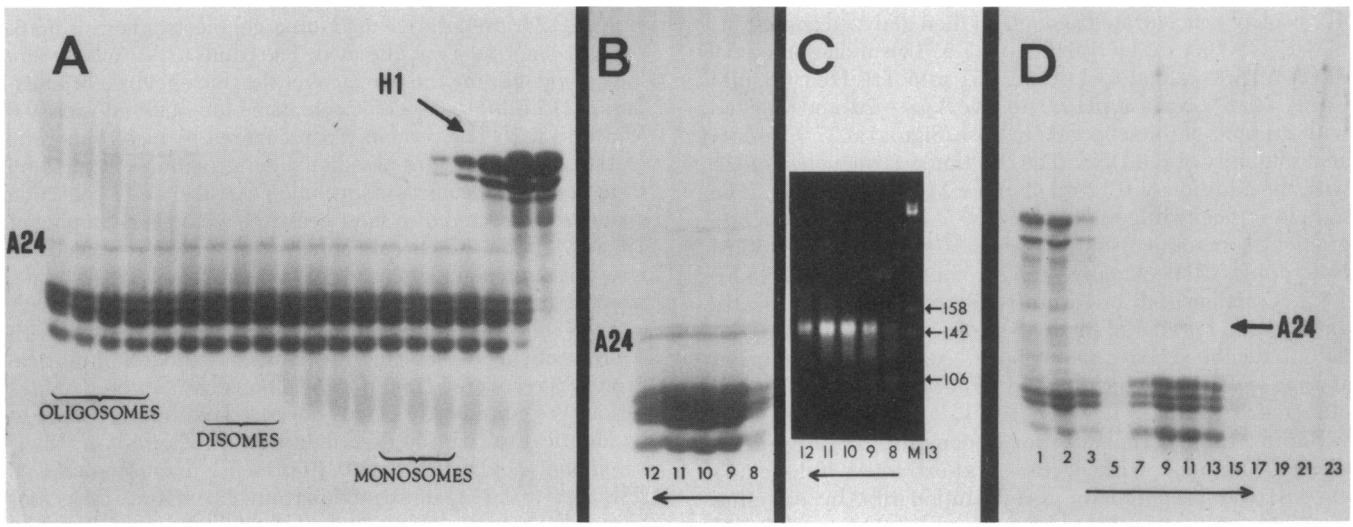


FIG. 1. Presence of A24 together with the core histones in (*A*) salt-washed nucleosomes; (*B, C*) trimmed core particles; and (*D*) reconstituted nucleohistone. (*A*) The nuclease digestion products of calf thymus nuclei were fractionated in 0.55 M NaCl by Sepharose 4B column chromatography. The fractions were characterized with respect to protein and DNA content as described. Only the protein gel is shown. The regions of the elution profile containing predominantly oligosome, disome, or monosome length DNA are indicated. DNA stains weakly with Coomassie blue and can be seen as a faint background extending approximately diagonally from upper left to lower right in the figure. The four core histones are the dark bands below A24. (*B*) The protein content is shown for fractions across a sucrose gradient peak of trimmed core particles. The direction of sedimentation is indicated by the arrow. The peak of $A_{260}$ was at fraction 10 (out of 24). Larger proportions of the fractions flanking the peak were loaded on the gel for clear visualization of the protein composition. (*C*) The DNA sizes are shown for the same fractions as in *B*. The relative proportions of each fraction analyzed are also the same. In the right-hand lane is a display of *Hae* III restriction fragments from phage M13 replicative-form DNA kindly provided by Dan Ray. The size of the 142-base-pair fragment is known from direct sequencing (16) and the 158- and 106-base-pair fragments were sized by comparison of the M13 restriction map with the known sequence of phage fd DNA, which is almost identical to that of M13 (16–18). (*D*) Acid-extracted histones were reconstituted with calf thymus DNA, and the complex was separated from free histones by sedimentation in the presence of 0.6 M NaCl. Equivalent volumes from each fraction were assayed by sodium dodecyl sulfate gel electrophoresis as described in the reconstitution sections of *Materials and Methods*. The lower arrow indicates the direction of sedimentation. Subsequent to fraction 3, only alternate fractions were assayed. The gradient displayed a symmetrical peak of DNA absorbance centered at fraction 10.

to additional nuclease digestion. The resulting trimmed core particles were then sedimented on a sucrose gradient in 0.55 M NaCl. Except for the soluble DNA which remained at the top of the gradient, all of the DNA sedimented as a single peak.

The protein and DNA compositions of the fractions across this peak are shown in Fig. 1*B* and *C*. It is evident from the protein gel of Fig. 1*B* that A24 was present, along with the core histones, in each of the fractions. The gel in Fig. 1*C* shows that the DNA in the core particle peak was not only well trimmed, but much of it was clipped internally as well. By comparison with the M13 restriction fragment markers, it can be seen that the core particle DNA was centered at a size of 144 base pairs. Moreover, for fractions 8 and 9, which show no evidence of diminished relative A24 content (Fig. 1*B*), no DNA of size greater than 149 base pairs is visible on the gel (Fig. 1*C*). The core particles were intentionally "overdigested" with nuclease for this particular preparation in order to make this point. This observation has been confirmed by using both higher DNA loads and longer photographic exposure times. We conclude, therefore, that A24 is bound tightly to the nucleosome core.

**A24 Reconstitutes with the Core Histones onto DNA.** If A24 is normally part of a nucleosomelike core structure in chromatin, it may then be expected to be able to reassemble into nucleosomes together with the core histones. Fig. 1*D* illustrates that when bulk acid-extracted histones were reconstituted with DNA and then sedimented on a sucrose gradient in the presence of 0.6 M salt, A24 sedimented with the reconstituted complex. This complex contains the core histones in the usual equimolar ratios and lacks H1. Because excess core histones in nonequimolar ratios remained at the top of the gradient (to the left), it seems unlikely that the A24 was merely adventitiously bound. Thus, A24 becomes assembled with DNA in a manner similar to that characteristic of the core histones themselves. The experiment of Fig. 1*D* was conducted with DNA about 2000 base pairs long. Identical results have been obtained with DNA isolated from nucleosome monomers.

**A24 Binds Specifically to H2B.** We have shown that A24 is associated tightly with trimmed nucleosome cores. However, it could be bound to the DNA on the outside of the core particle, or it could be an integral part of the histone core itself. Levy W. *et al.* (19) have presented strong evidence that a trout nonhistone protein is bound to the outside of core particles, and we (Chao, M. V., Gralla, J., and Martinson, H. G., unpublished results) have found that the *lac* repressor can bind tightly to cores reconstituted from a 144-base-pair restriction fragment containing the *lac* operator. If A24 is indeed a constituent of the *histone* core of nucleosomes (in contrast to being merely bound to core particles), then it would be predicted that A24 should be able to interact strongly with H2B. Therefore, we addressed the question of whether the ubiquitin moiety of A24 affected the ability of the H2A moiety of A24 to interact with H2B under reconstitution conditions.

Acid-extracted calf thymus histones were irradiated in the presence or absence of added purified A24. Acid–urea gel electrophoretic analysis of the products shows (Fig. 2) that bulk histones, in the absence of added A24 (Fig. 2, panel A), gave rise to only one major crosslinked product—the H2A–H2B dimer (13). In contrast, in the presence of added A24 (Fig. 2, panel B), two prominent crosslinked components were produced. It will be shown below that the component of lower mobility was an A24–H2B dimer. It can be seen that the A24–H2B dimer was formed at the expense of the H2A–H2B dimer whose yield was reduced considerably.

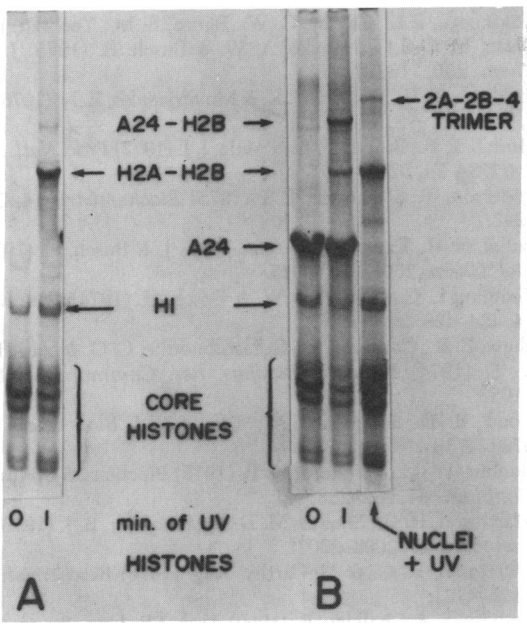


FIG. 2. UV crosslinking of total histones alone or in the presence of added A24. Acid-extracted histones, dissolved in water at 13 mg/ml, were mixed with an equal volume of 8 M urea/20 mM phosphate, pH 7.3. For panel A, 10 μl of this was mixed with 200 μl of 2 M urea/2 M NaCl/10 mM phosphate (pH 7.3) for crosslinking. One-half of this solution was irradiated for 1 min and the other half was maintained as a zero time control. One milliliter of 0.4 N $H_2SO_4$ was then added to each portion, and they were dialyzed in Spectrapor 1 dialysis tubing in the cold overnight against 0.4 N $H_2SO_4$ to remove the salt and urea. The histones were precipitated with several volumes of acetone at −20°C overnight, collected by centrifugation, and then analyzed by acid–urea gel electrophoresis. The experiment for panel B was conducted in exactly the same way except that 20 μl of A24 at about 6 mg/ml in 5 M urea was also added to the histone mixture before crosslinking. For comparison, the electrophoretic pattern of histones isolated from UV-irradiated calf thymus nuclei (13) is also shown.

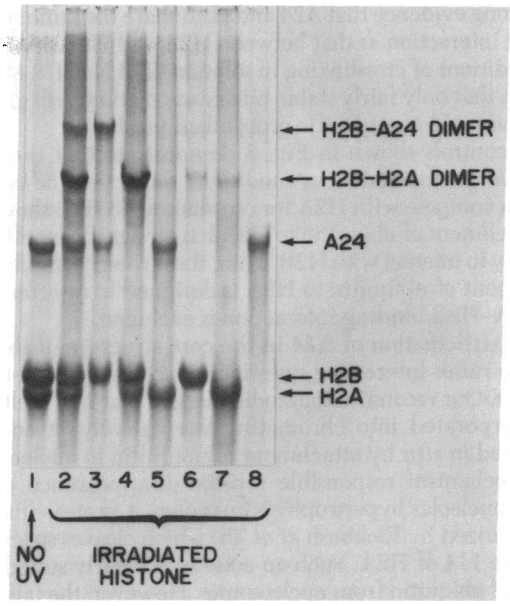


FIG. 3. Specific crosslinking of A24 to H2B. Approximately 15–30 μg each of A24, H2A, and H2B were dissolved in 30 μl of 5 M urea either individually or in various combinations as shown in the figure. Two hundred microliters of 2 M urea/2 M NaCl/10 mM phosphate (pH 7.3) were added to each of the histone solutions, and all were irradiated for 1 min except for the zero time control. The histones were recovered for acid–urea gel electrophoretic analysis by dialysis against distilled water and lyophilization.

The extreme right-hand lane of Fig. 2 shows the electrophoretic pattern of histones isolated from UV-irradiated nuclei. The trimer previously reported to be induced by UV (13) has now been identified (20) and is labeled in the figure. It can be seen that, although the A24–H2B dimer migrated in the "trimer" region of the gel (as would be expected, because A24 is itself a dimer consisting of H2A linked to ubiquitin), it is nevertheless not the same as the H2A–H2B–H4 trimer induced in nuclei by UV irradiation (13). The A24–H2B dimer also differs in mobility (not shown) from the H2A–H2B–H4 trimer induced by successive UV and tetranitromethane treatment (14).

To show that it is H2B with which A24 interacts giving rise to the formation of the new crosslinked dimer, we have subjected A24 to crosslinking in the presence of purified H2A and H2B. A24, H2A, and H2B were purified individually and then irradiated alone (Fig. 3, lanes 6–8) or in various combinations (lanes 2–5), and the products of crosslinking were assayed by acid–urea polyacrylamide gel electrophoresis. As can be seen in Fig. 3, only when H2B was present together with A24 (lanes 2 and 3) was the new dimer of low mobility formed by UV irradiation. No prominent discrete dimer component was formed when any of the three proteins was irradiated separately (lanes 6–8). Nor did irradiation of a mixture of A24 and H2A yield a discrete dimer component (lane 5). Irradiation of an H2A–H2B mixture gave rise to the usual H2A–H2B dimer (lane 4). Irradiation of an A24–H2B mixture yielded only the new lower mobility dimer as a discrete component (lane 3). Significantly, when A24 was mixed with both H2A and H2B, the A24–H2B dimer was formed in competition with the H2A–H2B dimer (lane 2) despite the fact that H2A was present in approximately 4-fold molar excess (2-fold weight excess) over the A24. These results show that A24 interacts in solution with H2B in a manner equivalent to the H2A–H2B interaction.

We conclude that the attachment of ubiquitin to H2A in A24 does not interfere with the ability of the H2A moiety of A24 to interact with H2B. The presence of A24 in salt-washed core particles as well as in reconstituted nucleosomes indicates that, in chromatin, A24 is a constituent of the nucleosome histone core, substituting for H2A in those nucleosomes in which it occurs.

## DISCUSSION

We have shown that A24 is an integral component of the nucleosome histone core. A24 is present in salt-washed nucleosome core particles and it reconstitutes onto DNA with the core histones under conditions that exclude adventitious binding. Most importantly, A24 behaves like H2A with respect to the specificity of binding to H2B in solution.

We note in Fig. 1*B* that trimmed, salt-washed core particles contain, in addition to A24, several other minor components. Some of these can be seen in Fig. 1*A* as well. Whether these other proteins are integral or peripheral components of the trimmed core particles is an interesting question which we hope to address in the near future.

We have studied the A24–H2B binding interaction by means of UV crosslinking. This method is particularly well suited to the study of binding specificity because it has been shown to give specific and nearly quantitative crosslinking of H2A to H2B in the nuclei of intact cells (13). The nature of the reaction is such that UV light can be considered a histone–histone binding-site probe (13, 14, 21). Moreover, we have found (22) that plant chromatin is crosslinked with equivalent specificity by UV light and that calf and plant histones 2A and 2B crossreact specifically by this crosslinking test. We thus consider that the production of a specific A24–H2B dimer with UV light is

very strong evidence that A24 and H2B share the same specific binding interaction as that between H2A and H2B. Moreover, our conditions of crosslinking in solution (2 M NaCl/2 M urea) are such that only fairly stable binary associations will give rise to a good yield of a specific crosslinked product.

The controls shown in Fig. 3 demonstrate that the crosslinking is specific under our conditions. Moreover, the fact that A24 can compete with H2A for crosslinking to H2B shows that the attachment of ubiquitin to H2A has no significant effect on its ability to interact with H2B. Thus, the obvious possibility that attachment of ubiquitin to H2A is designed to interfere with the H2A–H2B binding interaction is excluded.

The participation of A24 in the core structure of the nucleosome raises interesting questions concerning its metabolic turnover. Our reconstitution studies suggest that A24 itself could be incorporated into chromatin. Alternatively, it could be generated *in situ* by attachment of ubiquitin to nucleosomes. The mechanism responsible for the disappearance of A24 during nucleolar hypertrophy is unknown. A protease has been characterized by Eickbush *et al.* (5) which cleaves specifically at valine 114 of H2A. Such an activity is clearly suited to removal of ubiquitin from nucleosomes. However, the fate of the resulting nucleosomes, which would lack the 15 COOH-terminal residues of H2A, cannot be predicted.

A24 in chromatin may serve as a recognition signal for various proteins. Alternatively, because A24 is an integral component of nucleosome cores, its function may be related to modification of the normal structural role of nucleosomes in chromatin.

We thank Shirley Phillips for preparation of the manuscript. This work was supported by Grant PCM 76-00712 from the National Science Foundation and by U.S. Public Health Service Training Grant GM 07185 (J.B.E.B. and G.K.).

1. Hunt, L. T. & Dayhoff, M. O. (1977) *Biochem. Biophys. Res. Commun.* **74,** 650–655.
2. Goldknopf, I. L. & Busch, H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 864–868.
3. Goldknopf, I. L., French, M. F., Musso, R. & Busch, H. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5492–5495.
4. Goldknopf, I. L., Taylor, C. W., Baum, R. M., Yeoman, L. C., Olson, M. O. J., Prestayko, A. W. & Busch, H. (1975) *J. Biol. Chem.* **250,** 7182–7187.
5. Eickbush, T. H., Watson, D. K. & Moudrianakis, E. N. (1976) *Cell* **9,** 785–792.
6. Musich, P. R., Brown, F. L. & Maio, J. J. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 3297–3301.
7. Pederson, T. & Bhorjee, J. S. (1975) *Biochemistry* **14,** 3238–3242.
8. Ballal, N. R., Kang, Y.-J., Olson, M. O. J. & Busch, H. (1975) *J. Biol. Chem.* **250,** 5921–5925.
9. Yeoman, L. C., Taylor, C. W. & Busch, H. (1974) *Cancer Res.* **34,** 424–428.
10. Shaw, B. R., Corden, J. L., Sahasrabuddhe, C. G. & Van Holde, K. E. (1974) *Biochem. Biophys. Res. Commun.* **61,** 1193–1198.
11. Todd, R. D. & Garrard, W. T. (1977) *J. Biol. Chem.* **252,** 4729–4738.
12. Bonner, W. M. & Pollard, H. B. (1975) *Biochem. Biophys. Res. Commun.* **64,** 282–288.
13. Martinson, H. G., Shetlar, M. D. & McCarthy, B. J. (1976) *Biochemistry* **15,** 2002–2007.
14. Martinson, H. G. & McCarthy, B. J. (1976) *Biochemistry* **15,** 4126–4131.
15. Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130,** 337–346.
16. Suggs, S. V. & Ray, D. S. (1978) *Cold Spring Harbor Symp. Quant. Biol.* **43,** in press.
17. Horiuchi, K., Vovis, G. F. & Model, P. (1978) in *Single Stranded DNA Phages,* eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 113–137.
18. Schaller, H. & Takanami, M. (1978) in *Single Stranded DNA Phages,* eds. Denhardt, D. T., Dressler, D. & Ray, D. S. (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 139–163.
19. Levy W., B., Connor, W. & Dixon, G. H. (1979) *J. Biol. Chem.,* in press.
20. Martinson, H. G., True, R., Lau, C. K. & Mehrabian, M. (1979) *Biochemistry,* in press.
21. DeLange, R. J., Williams, L. C. & Martinson, H. G. (1979) *Biochemistry,* in press.
22. Martinson, H. G. & True, R. J. (1979) *Biochemistry,* in press.