Dimeric Histone Interactions and Histone Packing*

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We have repeated cross-linking experiments originally reported by Thomas and Kornberg ([1975] FERS Lett. 58, 353-358) using Lomant's reagent on rat liver nuclei and other chromatin substrates with special efforts to resolve completely the resulting pattern of core histone dimers. First dimension discontinuous sodium dodecyl sulfate gels resolve dimers formed between arginine-rich histones H3 and H4 and slightly lysine-rich histone H2B. Corresponding dimers involving histone H2A are much less prominent. First dimension acid-urea gels resolve slightly lysine-rich dimers (H2A), and (H2B), from the heterodimer (H2A-H2B). The (H2B)2 dimer can be resolved by ion exchange chromatography from the (H2B-H3) dimer with which it co-migrates in the acid-urea first dimension gel. Core histone dimer cross-linking patterns obtained from soluble chromatin and from purified mononucleosomes are qualitatively indistinguishable on both gel systems from those of nuclei. Cross-linked dimers involving histone H1 have also been observed, as have dimers involving protein A24. These observations are discussed with reference to histone packing models (Carter, C. W., Jr. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 3649-3653) in the light of experiments mapping core DNA-histone (Mirzabekov, A. D., Schiek, V. V., Belyavsky, A. V., and Bavykin, S. G. (1978) Proc. Natl. Acad. Sci. U. S. A. 75, 4184-4188) and histone-histone (Martinson, H. G., True, R., Lau, C. K., and Mehrabian, M. (1979) Biochemistry 18, 1075-1082; De Lange, R. J., Williams, L. C., and Martinson, H. G. (1979) Biochemistry 18, 1942-1948) contact sites.

The chromatin subunit is composed of an octameric histone core containing two each of histones H2A, H2B, H3, and H4, surrounded by about 146 base pairs of DNA. These particles are connected by a DNA linker of variable length, with which 1 molecule of histone H1 is associated (for review, see Ref. 1). Little is actually known at this time regarding the structure of the histone octamer. However, specific proposals concerning the histone packing arrangement have been developed on the basis of nuclease digestion studies and general symmetry considerations (2). According to this scheme the histone octamer is considered to be composed of a ring formed by six histones, the arginine-rich tetramer (3) and one slightly lysine-rich pair, fitted top and bottom with the two remaining slightly lysine-rich histones.

Although it may be useful conceptually (2), differentiation of the latter two histones has proven to be problematical from an experimental point of view. Specifically, one might expect close contacts between various pairs within the set of six histones and between the two sets of histones, but only distant contacts between the latter two histones themselves. Numerous histone-histone cross-linking studies (3-14) have revealed most of the expected dimers. Contrary to expectation, however, all 10 possible dimeric interactions have recently been demonstrated by at least one method of cross-linking and resolution of products (14). Moreover, histone-to-DNA cross-linking experiments (15) also imply close contacts between both pairs of slightly lysine-rich histones which should permit all 10 different dimeric interactions.

We therefore have repeated cross-linking experiments originally done by Thomas and Kornberg (6) and subsequently by Bonner (11) with special efforts to resolve the rather complicated pattern of histone dimers formed under these circumstances. Specifically, we have cross-linked rat liver nuclei at pH 7.4 using Lomant's reagent, a reversible cross-linker (16), and displayed the products using two different "diagonal" electrophoresis systems (6, 17, 18). In addition we have fractionated cross-linked products by ion exchange chromatography prior to electrophoresis.

Our conclusions, some of which have previously been noted by others, are as follows.

1. First dimension discontinuous SDS1 gels resolve dimers formed between arginine-rich histones H3 and H4 and slightly lysine-rich histone H2B. Corresponding dimers involving histone H2A are much less prominent.

2. First dimension acid-urea gels resolve slightly lysine-rich dimers (H2A), and (H2B), from the heterodimer (H2A-H2B). The (H2B)2 dimer can be resolved by ion exchange chromatography from the (H2B-H3) dimer with which it co-migrates in the acid-urea first dimension gel.

3. Histone H1 is cross-linked to form dimers which evidently include the heterotypic form (H1A-H1B), as well as the homotypic forms (H1A), and (H1B), in a tissue which contains two H1 subtype differing in primary structure.

4. Histone H1 also cross-links to core histones (10, 11), suggesting that it binds not only to linker DNA, but also to proteins in the nucleosome core. The broad distribution of H1-core histone dimers in the SDS first dimension gel is indicative of cross-links to more than one core histone, including histone H4 (11).

5. Protein A24, a form of H2A containing ubiquitin in isopeptide linkage (19) has been identified by its migration properties on three gel systems. It apparently cross-links to core histones in chromatin, further supporting the suggestion that A24 substitutes for H2A in certain nucleosomes (20, 21).

6. Cross-linking patterns obtained from soluble chromatin and (except for H1-H1 contacts) from purified mononucleosomes are qualitatively indistinguishable on both gel systems from those of nuclei.

1 The abbreviation used is: SDS, sodium dodecyl sulfate.
On the basis of Results 2 and 6, we conclude that homodimer contacts between both lysine-rich histones do indeed occur within the nucleosome itself. This conclusion can be reconciled with our expectation that a slightly lysine-rich pair occupies a structurally unique position (2) if all core histones are considered to be moderately elongated in a manner suggested by recently published experiments which map specific intranucleosomal histone-DNA (15) and histone-histone (12, 13) contact sites.

**MATERIALS AND METHODS**

Rat liver nuclei were prepared by the method of Burgoyne et al. (22) except that 15 mM sodium phosphate, pH 7.4, 3 mM MgCl₂ were substituted for Buffer A. Tris and 2-mercaptoethanol must be avoided when Lomant's reagent (dithiobis-succinimidyl propionate, obtained from Pierce; Ref. 16) is used because the former destroys the active ester and the latter reduces the disulfide bond in Lomant's reagent.

Nuclei were cross-linked in 0.34 M sucrose, 15 mM sodium phosphate (pH 7.4), 1 mM MgCl₂ at a DNA concentration of 50 or (see below) 500 µg/ml with Lomant's reagent at a concentration of 60 µg/ml for 15 min. Cross-linked nuclei were pelleted, extracted either with 0.2 N H₂SO₄ or with 5%CCI₂COOH or H₃O₂O (23, 24) for 20 min at 4°C, and pelleted, and the supernatants were precipitated with 20% CCl₂COOH. Histone pellets were washed with acetone/0.1 M HCl, then with acetone, and dried under vacuum for analysis using discontinuous SDS-polyacrylamide gel electrophoresis (25) modified by Thomas and Kornberg (26), two-dimensional acid-urea/discontinuous SDS gel electrophoresis (17); this combination was first used by Woodland and Adamson (18), and two-dimensional discontinuous SDS-gel electrophoresis with reversal of cross-links between dimensions. In our hands, inclusion of N-ethylmaleimide in the quenching solution (11) did not appreciably change the pattern of H3-containing cross-links. A control (not cross-linked) sample was also run on a first dimension Triton gel (27) performed by K. K. Kumaroo, University of North Carolina, and on a second dimension discontinuous SDS gel.

Second dimension discontinuous SDS gels 3.3 mm thick were fixed using three 5-h changes of methanol/acetic acid/water (25:5:70) to remove all salt from the gel, stained overnight with 0.1% Coomassie blue in the same solvent, and destained with frequent changes of the same solvent. Gels were photographed with a Wratten 15 yellow filter and Kodak contrast process pan film.

Cross-linked histones were fractionated prior to electrophoresis by ion exchange chromatography as described by Simon and Felsenfeld (28), with modifications. Nuclei cross-linked at a DNA concentration of 500 µg/ml were digested briefly with micrococcal nuclease and lysed (29). The lysed nuclear supernatant (18 mg of histones) was dialyzed against 8 mM potassium phosphate (pH 6.7), 40 mM NaCl, 0.1 mM PPh₃CH₃SO₄, 0.25 mM EDTA, and applied to a hydroxyapatite column (5.5 × 20 cm) equilibrated with 8 mM potassium phosphate (pH 7.6), 40 mM NaCl. After washing with 3 column volumes of starting buffer, histones were eluted with a 1,000-ml NaCl gradient (40 mM to 2.0 M). Histones were recovered from fractions containing appreciable A₂₈₀ absorbance by precipitation with 20% CCl₂COOH and prepared for electrophoresis as described above.

Native chromatin (29) and mononucleosomes (30) were prepared as described and dialyzed into cross-linking buffer (see above for nuclei) prior to cross-linking. Mononucleosomes were repurified by sucrose density gradient sedimentation following cross-linking.

**RESULTS**

Our overall experimental design was first described by Thomas and Kornberg (6), and essentially similar results have been reported by Bonner (11). Lomant's reagent (16) is a bifunctional cross-linking reagent specific for α- and ε-amino groups in proteins. It has a molecular length of 12 Å. Our view is that this cross-linking distance is sufficient to permit cross-linking between lysine groups of histones which may be neighbors around the circumference of the nucleosome core particle. Other reagents (9, 14) may be more effective in cross-linking histones which share more extensive interfaces within the core particle but have separated contact sites on DNA.

**Core Histone Contacts**—Histones cross-linked in fresh rat liver nuclei are displayed in Fig. 1 on a two-dimensional gel with discontinuous SDS electrophoresis in both directions and reversal of cross-links between dimensions. Histone dimers resolved by this system are shown more clearly in the inset and are indicated in the schematic (Fig. 1B). They include two major representatives, (H3)₂ and (H2A-H2B), and at least three minor representatives, (H3-H4), (H2B-H3), and (H2B-H4). In general, the SDS-SDS two-dimensional system tends to reveal heterodimers, the (H3)₂ dimer being an exception, identified because it is formed in such excess over the dimer (H3-H2B) with which it co-migrates in the SDS first dimension gel. A conspicuous feature characteristic of all chromatin substrates we have examined (nuclei, native chromatin, mononucleosomes) is the relative absence of heterotypic contacts formed by histone H2A.

This gel system has important questions unanswered.

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**Fig. 1.** Histones cross-linked in nuclei with Lomant's reagent, run on a two-dimensional discontinuous polyacrylamide SDS gel (18%-18%) with reversal of cross-links between dimensions. Migration in this and subsequent gels is from left to right in the first dimension and from top to bottom in the second dimension. A, a 0.2 N H₂SO₄ extract of the histones cross-linked and electrophoresed described. Inset, the dimer region from a more lightly loaded gel. B, schematic of A. Bands on the diagonal (---) are H1A, H1B, H1α, A24, H3, H2B, H2A, and H4. The top horizontal strip is a noncross-linked control. Selected cross-linked products in the bottom first dimension horizontal strip and second dimension slab gel are identified by vertical lines.
because it may not resolve homodimers of slightly lysine-rich histones H2A and H2B from their heterodimer H2A-H2B. As noted above, the existence of such homodimers is a significant theoretical question. Moreover, Jackson (14) has demonstrated close H2A-H2A and H2B-H2B contacts using formaldehyde cross-linking and high resolution discontinuous SDS gels. We therefore have used additional resolution methods to determine whether or not homodimers containing slightly lysine-rich histones can be formed by reaction with Lomant's reagent.

Fig. 2 shows the result obtained when the cross-linked mixture of Fig. 1 is resolved in the first dimension on an acid-urea gel (17, 18). This two-dimensional gel system shows to advantage the fact that cross-links occur between both pairs of identical slightly lysine-rich histones. A band identified as (H2A)2 migrates faster than the cluster of core histone dimers but considerably slower than H1. It is not accompanied by H4 in the first dimension.

Lighter loading of the gel (Fig. 2, inset) reveals that a band containing histone H2B runs just slower than the major histone H3-containing dimer. That this band contains a mixture of (H3-H2B) and (H2B)2 is shown by the gels in Fig. 3, where we have used ion exchange chromatography to separate histones (and cross-linked products) on the basis of their differential affinity for DNA immobilized as chromatin on hydroxyapatite (28). Lysine-rich histones elute first from such a column, followed by arginine-rich histones. Fig. 3 shows two-dimensional gels (acid-urea first dimension, as in Fig. 2) for fractions eluted in sequence. The first fraction shown (Fig. 3A) contains slightly lysine-rich dimers relatively free from dimers containing histone H3. The two most intense bands correspond to (H2B)2 (contaminated with a small amount of (H3-H2B) (see Fig. 3C) and (H2A-H2B)). The third fraction in sequence (Fig. 3C) shows exclusively (H3-H2B) and (H3)2.

The intermediate fraction (Fig. 3B) contains an apparent mixture of the dimers in preceding and following fractions. A significant portion of the slightly lysine-rich histones appearing as a (H2A-H2B) dimer on the SDS-SDS gel system (Fig. 1), therefore, has been resolved from this heterodimer by the acid-urea first dimension gel and shown to include both (H2A)2 and (H2B)2 homodimers.

Resolution of homodimers on the acid-urea/discontinuous SDS gel system arises from systematic reversals of migration behavior, relative to monomeric histone mobilities. Two dimers, (H2A)2 and (H3)2, migrate faster than expected from the relative histone monomer mobilities in the acid-urea first dimension. This observation is not unprecedented: corresponding oxidized cysteine-containing dimers (H3)2 and (TH2B-X)2 from testis also migrate in reverse order from that of their monomers on acid-urea gels (31). Such reversals of electrophoretic mobility are unlikely to arise from compositional effects and, therefore, probably reflect unique and as yet unspecified conformational properties of the cross-linked dimers (H3)2 and (H2A)2.

Histone H1 Contacts—As pointed out by Thomas and Kornberg (5), H1 dimers migrate between tetramers and pentamers of the core histones. Figs. 1 and 4 show two-dimensional gels which firmly establish the position of H1 dimers with relation to core histone oligomers. Fig. 4 shows a 5% CCl4-COOH extract of nuclei, in which primarily H1 and its oligomers are soluble, run on the gel system of Fig. 1. The putative H1 dimers identified on the basis of migration on two-dimensional gels are, in fact, pure H1 in composition by this extractibility criterion as well.

Histone H1 forms at rat liver are resolved on SDS gels into three subtypes, known as H1A, H1B, and H1o in order of increasing migration. Histone H1o is not evident among the (H1)2 dimers, but both H1A and H1B are represented in the dimer bands. Migration of (H1)2 dimers in the first dimension is notable in that three such dimers are resolved. The leading band is the most sharply resolved and is almost certainly (H1B); as indicated by the second dimension gel. The trailing band is poorly resolved but probably contains (H1A)2. The middle dimer band is sharp, but poorly resolved from the trailing band. The second dimension gel demonstrates that it contains both H1A and H1B, although disproportionately less of the latter. We have not succeeded in eliminating this

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overlap of H1A and H1B in the second dimension by running first dimension gels up to 50% further. We consider it likely, then, that the middle band does contain at least some histone H1B, in addition to H1A and, therefore, that heterotypic (H1A-H1B) dimers have been formed.

A class of cross-linked products containing histone H1 subtypes migrates between H1 monomers and (H1)_2 dimers. In our hands they are insoluble in 5% HClO_4 or CCl_4COOH (they are absent from Fig. 4) but do appear in a 0.2 M H_2SO_4 extract (Fig. 1). Their migration and solubility properties strongly suggest that they contain dimers formed between histone H1 and other core histones, as previously suggested (10, 11). These products co-migrate in the first dimension with core histone trimers, so the presence of core histones underneath H1 in the second dimension cannot provide evidence regarding specific H1-core histone contacts. However, the second dimension shows that histone H1A was resolved in this region of the first dimension gel into two parts, a faster running spot and a slower running broad streak. This pattern mimics the migration of the core histones themselves and is, therefore, consistent with the conclusion that histone H1A is cross-linked to the smallest, most rapidly migrating histone, H4, and to one or more of the remaining core histones which are larger and migrate more slowly on SDS gels.

Protein A24 Contacts—Rat liver nuclei possess one major protein other than H1 and the core histones, which migrates about 1/5 of the way from H1 to H3 on discontinuous SDS gels (Fig. 1). It is stained with Coomassie blue about 20% as intensely as each core histone. It is represented with equal intensity relative to the histones in acid extracts, chromatin prepared by the nuclease method and unfractonated nuclei. It is not soluble in 5% HClO_4 or CCl_4COOH. It therefore seems likely that this protein is a modified histone, probably the same as the protein from calf thymus known as A24 (32) identified as covalently linked H2A and ubiquitin (19).

We have strengthened the case for identifying this protein as A24 by noting its migration (not cross-linked) in a first dimension Triton gel (27) followed by a second dimension discontinuous SDS gel (Fig. 5). The migration of histone H2A is affected significantly in the Triton dimension, showing considerably slower migration than H1. The second dimension gel shows several spots with migration on discontinuous SDS gels similar to that of the putative A24 band. The most intense of these spots migrates slower than H2A on both dimensions, as would be expected for A24 (this reasoning was suggested to us by David B. Ring, University of California, Berkeley) and we therefore believe that this spot represents protein A24.

A cross-linked band containing A24 migrates just faster than (H1-H4) on an SDS gel (first dimension, Fig. 1). We cannot be certain whether this band corresponds to (A24-core histones), to (A24-A24), or to (A24-H1). However, its migration in the SDS first dimension gel is entirely consistent with the first assignment and incompatible with the two alternatives. Qualitatively, this judgment follows from the observation that it migrates ahead of (H1-H4) in the SDS dimension, not behind it as it should if all its components were the size of H1 (M, ≈ 21,000) or A24 (M, ≈ 22,600). In the acid-urea first dimension this cross-linked product also behaves as expected if it were to contain core histones. Protein A24 itself migrates more slowly than histone H1 monomers, and (H1)_2 dimers migrate very slowly. Yet the A24 containing cross-linked product migrates only marginally slower than core histone dimers. Moreover, traces of histones H2B and H3 lie directly beneath cross-linked A24 on both gel systems (Figs. 1 and 2). Since in solution protein A24 forms the same contact to H2B as observed for H2A and H2B by cross-linking with UV light (9, 20), we favor the conclusion that this cross-linked product contains A24 and core histones. This cross-linking behavior would be expected if, as suggested (20, 21), protein A24 is indeed a nucleosomal protein which substitutes directly for histone H2A.

Cross-linking Patterns of Different Chromatin Substrates—Results obtained with histones cross-linked in nuclei...
with Lomant’s reagent are qualitatively indistinguishable from those obtained using substrates in which chromatin superstructures are extensively disrupted. Cross-linking soluble chromatin (29) in dilute (0.1 mg/ml), low ionic strength (I = 0.04, 15 mM Na2PO4, pH 7.5) conditions favoring noninteracting unit fibers produces a pattern of H1 and core histone dimers indistinguishable from that shown in Figs. 1 and 2. An important inference is that H1-H1 contacts occur at the first level of chromatin structure. Strictly speaking, it is also possible that “native” contacts are broken and replaced by others. In either case, some importance may be attached to the occurrence of H1A-H1B heterodimers. We have suggested previously (33) that some portion of histone subtypes H1A and H1B may be replaced coordinately during spermatogenesis by two very similar testis-specific subtypes THlX-A and THlX-B. Alternation of histone subtypes H1A and H1B along the unit chromatin fiber could account for their physical proximity revealed by the cross-linked H1A-H1B heterodimer and for their coordinate replacement during differentiation and/or meiosis.

Core histone dimer cross-linking patterns from purified mononucleosomes on both two-dimensional gel systems are shown in Fig. 6. These patterns do not show (H1)2 dimers but are in all other respects virtually indistinguishable from those obtained using nuclei (Figs. 1 and 2). We conclude that cross-linked core histone dimers formed in nuclei, in particular the (H2A)2 dimer, reflect intranucleosomal histone-histone contacts.

**DISCUSSION**

Constraints on histone packing models arising from the necessity to complement core particle DNA geometry have been discussed previously (2). Histone octamer models consistent with these symmetry requirements have been limited further by elegant histone-to-DNA cross-linking experiments (15) and peptide mapping of specific histone-histone contact sites (12, 13). A three-dimensional realization of these histone-DNA and histone-histone contacts, based on the DNA geometry proposed by Finch et al. (34) (Fig. 7), illustrates schematically how contacts between identical histones could occur, and how individual histones may be oriented along the core particle DNA.

**Histone-DNA Cross-linking Sites Suggest How Homodimer Contacts Arise**—Mirzabekov et al. (15) found that H2A molecules contact core particle DNA at two sites, roughly 70 and 120 bases from each 5’ end. Assuming that the length of each turn of DNA is approximately 80 base pairs in the core particle (34) and that points one turn apart are helically displaced from each other, it seems likely that the H2A molecules are highly elongated. Contacts with DNA 70 bases from the 5’ end are very close to each other; those at 120 could approach only in neighboring nucleosomes. Identification of an intranucleosomal (H2A-H2A) cross-link strongly implies a contact site close to the H2A-DNA contact near base 70. Cross-links between histone H3 and DNA reveal contacts ranging from base 70 to base 100, comprising a total length of roughly 75 Å at a radius of 32 Å. It is, therefore, likely that this histone is also elongated. Binding domains on the two DNA strands extend in opposite directions along the circumference of the DNA superhelix. Consequently histone H3, like histone H2A and unlike histone H2B, can approach itself only end-to-end. Finally, histone H2B-DNA cross-links are spaced one full turn apart along the DNA superhelix, permitting H2B-H2B contacts at one or more points within this histone.

**Unique Orientations Can Be Specified for All Four Histones in the Nucleosome Core Particle**—Several histone-histone contacts have been mapped using cross-linking and protein chemistry to specific sites within histone primary sequences (12, 13). The packing arrangement shown in Fig. 7 affords a mutually consistent rationale for these contacts.

A zero-length cross-link formed by UV light between histones H2A and H2B is located in the NH-terminal third of both proteins (13). From histone-DNA cross-linking the closest approach of these two histones is located at a DNA site roughly 120 bases from the 5’ end (15). This orientation would place the COOH terminus of elongated H2A molecules close to the DNA site near base 70 and permit H2A-H2A cross-links between sites close to this end (Fig. 7, upper left).

Multiple cross-linking sites in histone (H2B-H4) dimers...
have been mapped to clusters near the COOH termini of both proteins (12). These histones approach neighboring DNA sites near base 40 (15). This juxtaposition of histone-histone and histone-DNA sites is consistent only with moderately elongated H2B molecules oriented in an antiparallel fashion about the nucleosomal dyad (Fig. 7, upper left).

Histone H3 from avian erythrocytes contains a single cysteine residue, Cys110, near its COOH terminus. Camerini-Otero and Felsenfeld (35) have shown that oxidized H3-H3 dimers can be incorporated into reconstituted nucleosomes indistinguishable by various criteria from native nucleosomes. They argue from this result that Cys1 should lie close to the nucleosome dyad. As indicated in Fig. 7 (upper left), this interpretation implies that histone H3 is probably elongated along core DNA in a direction antiparallel to that of histone H4.

Orientation of the histones in this manner provides a convincing rationale for the observation that HeLa core particles digested sequentially with trypsin and DNAse I show decreased protection of core DNA segments 20 to 35 and 60 to 80 bases from the 5' terminus (36, 37). From Fig. 7 it is apparent that the NH2 termini of all four histones lie within just these segments.

Do Protein-Nucleic Acid Interactions in the Nucleosome Core Facilitate Recognition and Regulatory Phenomena—General features expected for histone packing in the nucleosome core particle have been described previously (2). It is appropriate here to recall briefly two of these with reference to Fig. 7.

1. Elongation of histones H3, H4, and H2B along the circumference of the DNA supercoil affords the potential for three extensive vertical dimer contacts along the superhelix axis. Histones H2B can form a homodimer; histones H3 and H4 can pair to form two heterodimers. It is notable, however, that the relative abundance of contacts between H2B and the two arginine-rich histones (Fig. 1, inset) and the well known contacts which stabilize the arginine-rich histone tetramer (3) suggest that there may be rather extensive protein-protein interfaces between all six histones composing this helical ring.

Antiparallel orientation of the arginine-rich histones could afford pseudo-2-fold rotation symmetry normal to the superhelix axis. To the extent that it exists, this feature would require the NH2 termini of both histones H3 and H4 to have similar conformations and, likewise, for their COOH termini.

2. The fourth histone, H2A, occupies a structurally distinct site above and below the ring formed by the other six histones. The relative absence of cross-links between histone H2A and the arginine-rich histones H3 and H4 (Fig. 1, inset) suggests that corresponding protein-protein contacts at the core DNA-histone interface are also structurally dissimilar and perhaps weaker than those involving H2B. Nonpolar contacts between H2A and other histones at other sites within the core can, however, be inferred from the repeated observation that H2A can stabilize interactions between other core histones (7, 9, 38).

Histone-to-DNA cross-linking (15) has revealed the unexpected result that intranucleosomal H2A-H2A contacts might occur, arising from close approach of both H2A molecules to proximal DNA sites near base 70. We have demonstrated formation of a cross-linked (H2A)2 dimer, presumably arising from these contacts. The H2A-DNA contact at base 70 and the apparent localization of the H2A-H2A contact to this region underscores the fundamental importance of this DNA segment and its complementary histone configuration. Considerable structural emphasis seems to be focused here, perhaps because it is the site of screw dislocation (2).

Taken together, these two observations reinforce the earlier suggestion (2) that the histone-related architecture of the core particle might differentiate structural features near base 70 from those elsewhere along core DNA as recognition sites for regulatory interactions. In this context, it is especially noteworthy that the ubiquitin moiety in A24 is located close to the COOH terminus of A24 (position 119 out of 137 amino acid residues (32)). Such a large perturbation of H2A as the attachment of ubiquitin (M, ≈ 8,000) close to the position 70 nucleotides from the 5' end of core particle DNA would almost certainly affect the H2A-DNA contact at this site (Fig. 7, upper right). Histone-to-DNA cross-linking by the procedure of Mirzabekov et al. (15), therefore, might be expected to reveal contacts between A24 and DNA only near base 120. Furthermore, the ubiquitin moiety could interfere with the H2A-H2A contact. Although we cannot identify from our two-dimensional gels any individual A24-core histone contacts formed by Lomant's reagent, it seems likely that the strongest H2A contact, H2A-H2B, would be preserved for A24 as an A24-H2B cross-link as demonstrated by Martinson et al. (13) for nucleohistone complexes reconstituted from DNA and a mixture of core histones enriched in A24. Thus, alteration in nucleosome structure and function arising from the presence of A24 might be localized to the central region of the core, possibly resulting in a different wrapping of DNA with consequently altered accessibility to other DNA-binding proteins.

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Dimeric histone interactions and histone packing.
C W Carter, Jr, L F Levinger and F Birinyi

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