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Histone 2B can be modified by the attachment of ubiquitin

Michael H.P.West and William M.Bonner

Laboratory of Molecular Pharmacology, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, MD 20205, USA

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SUMMARY

Histone 2B in mouse and man can be modified by the post-translational addition of ubiquitin. In mouse Ll210 cells, both H2B variants are modified, however only to the extent of 1-1.5% compared with about 11% of H2A. Analysis of cyanogen bromide peptides shows that ubiquitin is attached to the C-terminal part of the histone.

INTRODUCTION

It is now well established that histone 2A can be modified by the post-translational addition of ubiquitin through an isopeptide linkage to lysine at position 119 to form the protein known as A_{24} (for a review see Goldknopf and Busch (1). We have recently shown that mouse Ll210 cells, in addition to the known H2A variants, contain two heteromorphous H2A variants (2), all four of which can undergo modification by ubiquitin. We have suggested the terminology uH2A for these forms. The function of this modification is still unclear, however it has been suggested that there is a correlation between the presence of uH2A and the transcriptional competency (although not the transcriptional activity) of the nucleosome (3).

We show here that histone 2B can also be modified by the addition of ubiquitin to its C-terminal region. In the identification of uH2B, we have used a technique whereby the tryptic peptides of proteins can be electrophoresed in polyacrylamide gels. This enables two proteins to be compared, and their relationship determined.

MATERIALS AND METHODS

The sources of materials, and the preparation of nuclei from mouse Ll210 cells, have been described elsewhere (2). Most non-histone proteins were extracted with 0.35 M NaCl. This caused lysis, but not dispersal of the

chromatin, which was then pelleted by centrifugation. The chromatin was acid-extracted and the soluble material prepared for electrophoresis by the methods previously described in detail in Bonner et al. (4). For obtaining radioactively labeled proteins, 50 ml of the cells were generally grown overnight with 250 μCi of the ^{14}C amino acid, with the content of unlabeled amino acid in the culture medium reduced to 14% of the normal level.

Protein Purification and Identification

Proteins of interest were purified by electrophoresis in acetic acid-urea-Triton X-100, acetic acid-urea-CTAB, and SDS gels. These gel techniques have been described in detail in Bonner et al. (4) and West and Bonner (2). The proteins were extracted and digested overnight at 37°C with trypsin (TPCK treated, Worthington) at 0.1 mg/ml in 50 μl of 0.4% ammonium bicarbonate. The amount of radioactively labeled sample used in each digestion was determined by its content of the particular amino acid, with approximately 800 cpm per residue giving good autoradiographs after a 2 week exposure. The tryptic digests were prepared for electrophoresis by drying and resuspending in 10 μl of 8 M urea, 5% acetic acid, 45 mM NH_4OH. Electrophoresis was carried out in 50% polyacrylamide gels with a discontinuous buffer system as described in West and Bonner (2). The resolving gel was then dried onto paper under vacuum with slight heating, and X-ray film (Kodax XR-5) was exposed to the gel for an appropriate length of time.

Cyanogen Bromide Cleavage

Samples were reduced by overnight incubation in 5% 2-mercaptoethanol, dried, and dissolved in 70% formic acid. Cyanogen bromide (Fisher) was sublimed and prepared as a stock solution (40 mg/ml in 70% formic acid). It was diluted to give a final concentration of 4 mg/ml, and incubation was for 3 hours at 37°C, after which the samples were dried, redissolved in 8 M urea, 1 M acetic acid, 45 mM NH_4OH, and electrophoresed in an acetic acid-urea gel with 25% acrylamide; 0.04% N,N'-methylene-bis-acrylamide. After electrophoresis, the resolving gel was impregnated with scintillant (DMSO-PPO (5) or En^{3}Hance - New England Nuclear) dried, and X-ray film was exposed to it at -70°C for an appropriate length of time. After visualization of the bands, 1 mm strips from the band regions and 2 mm strips from the other regions were digested overnight in 30% hydrogen peroxide/conc. ammonium hydroxide (9.6:0.4). Scintillation fluid was then added and the radioactivity determined.

RESULTS

Figure 1 shows a two dimensional analysis of mouse L1210 histones from nuclei which had been extracted with 0.35 M NaCl to remove most of the non-histone proteins. All the proteins on this gel except two minor proteins (labeled 1 and 2) have been previously identified as histones or ubiquitin adducts of histones. These two minor proteins (1 and 2) have approximately the same mobility in acetic acid-urea gels as do the ubiquitin adducts of H2A and like them are not extracted by 0.35 M NaCl.

While mouse L1210 has two proteins in this position, a human line HL60 has one (Figure 2). Since mouse has two H2B variants and HL60 only one, these results suggest that the unknown proteins may be ubiquitin adducts of H2B.

AUT gels, as usually formulated, selectively retard the H2A's and H3.1. However as the urea in the AUT dimension is lowered from 8 M to 1 M with the Triton remaining constant, all core histones including H2B are retarded (6). Figure 3 shows that the H2B's were retarded, and eventually co-

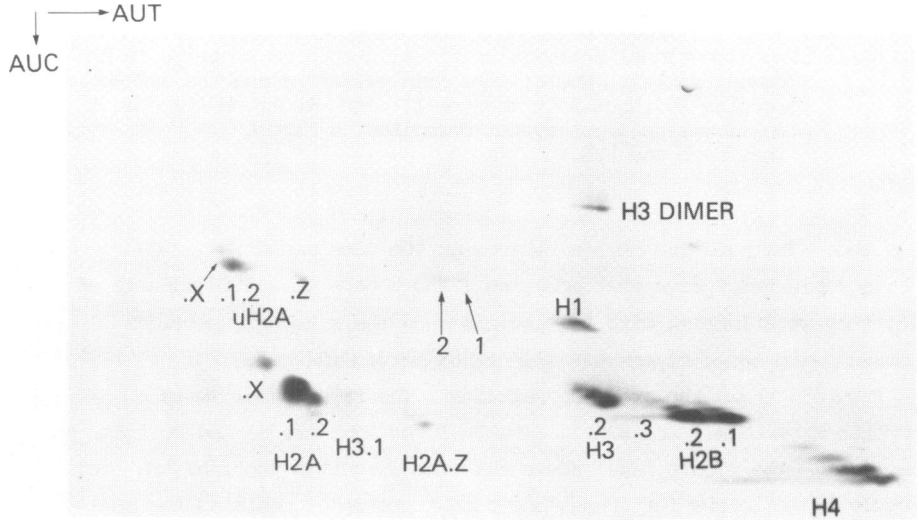

FIGURE 1: Two dimensional analysis of the acid-soluble proteins in L1210 nuclei after salt treatment. Nuclei were extracted with 0.35 M NaCl, and the chromatin was centrifuged and acid-extracted. The acid soluble proteins were electrophoresed in a first dimension acetic acid-urea-Triton X-100 gel (AUT) with 8 M urea and 8 mM Triton, then in a second dimension gel (AUC) (4). The proteins were stained with Coomassie Blue. Proteins 1 and 2, H2B.1 and H2B.2 are indicated; other proteins have been identified elsewhere (2).

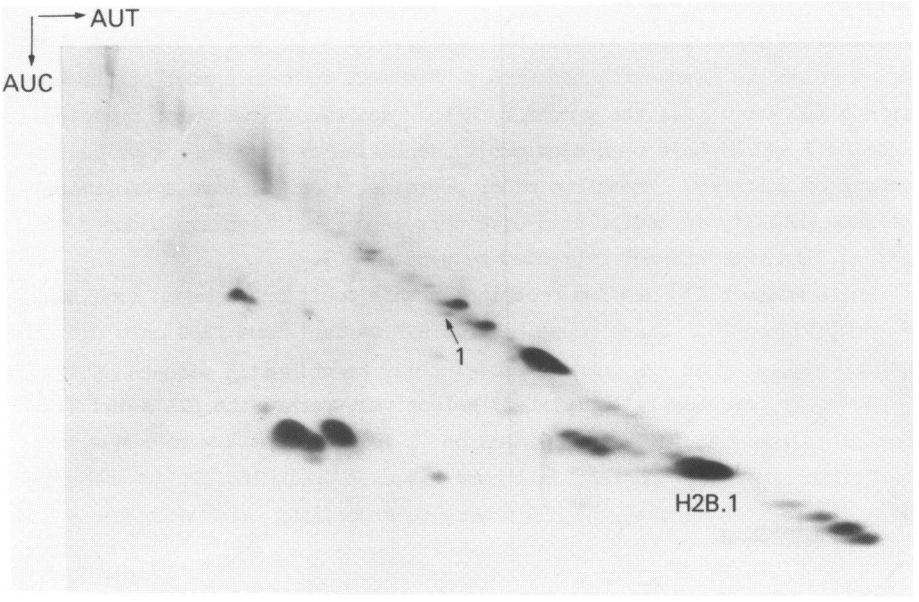

FIGURE 2: Two dimensional analysis of the acid-soluble proteins in HL60 (human) nuclei. Nuclei were acid extracted and the acid-soluble proteins (without salt treatment) were electrophoresed in the two dimensional gel system described in Figure 1. Human cell lines have one H2B.

migrated. The putative uH2B's behaved in the same manner.

To definitely prove that proteins 1 and 2 were ubiquitin adducts of H2B, they were labeled with ^{14}C-arginine and their tryptic peptides compared with those of the H2B's in a 50% acrylamide gel.

Figure 4 shows the arginine peptides. The right panel shows a comparison of uH2A.1 and H2A.1. The 2A.1 peptides are marked by ticks on the right. The middle panel shows that the ubiquitin peptides are found in protein 1, while the left panel shows that protein 1 contains the same arginine peptides as does H2B.1, (these are indicated by ticks on the left). Therefore the arginine peptide pattern of protein 1 is the sum of the protein patterns of H2B.1 and ubiquitin, showing that protein 1 is uH2B.1, a ubiquitin adduct of H2B.1.

Figure 5 shows a comparison between H2B.1 and H2B.2. The arginine peptide pattern reflects a known difference between the two variants. This difference occurs at position 75, with serine in the .2 variant replacing

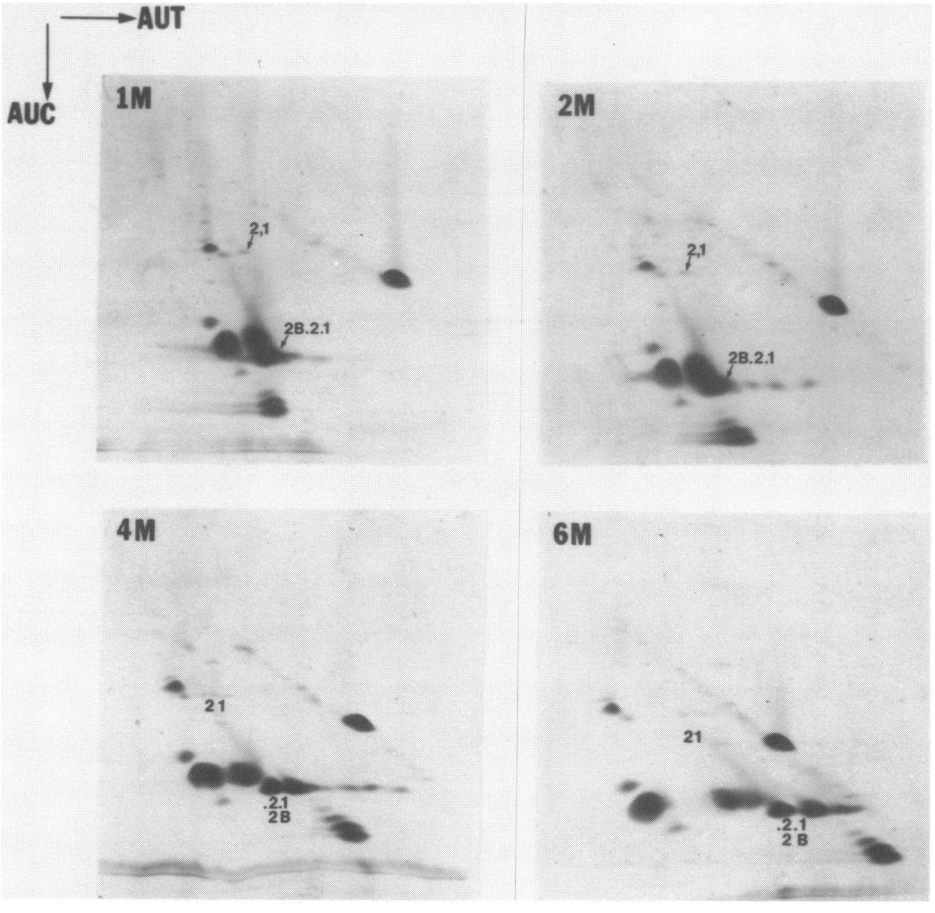

FIGURE 3: Migration of proteins 1 and 2 under differing urea concentrations. Acid-soluble proteins from L1210 nuclei were electrophoresed under the conditions described in Figure 1 except that the urea in the first dimension gel was lowered to either 6, 4, 2 or 1 M as indicated. The proteins of interest are labeled.

glycine of the .1 variant. Protein 2 had the peptides characteristic of H2B.2 and of ubiquitin (data not presented), and was therefore uH2B.2.

H2B from mouse has methionines at positions 59 and 62, a property which allows H2B to be cleaved into two large pieces using cyanogen bromide. Ubiquitin has methionine at position 1, so cyanogen bromide treatment does not significantly alter its size. We carried out a cyanogen bromide cleavage of the uH2B to determine which end of the H2B molecule had

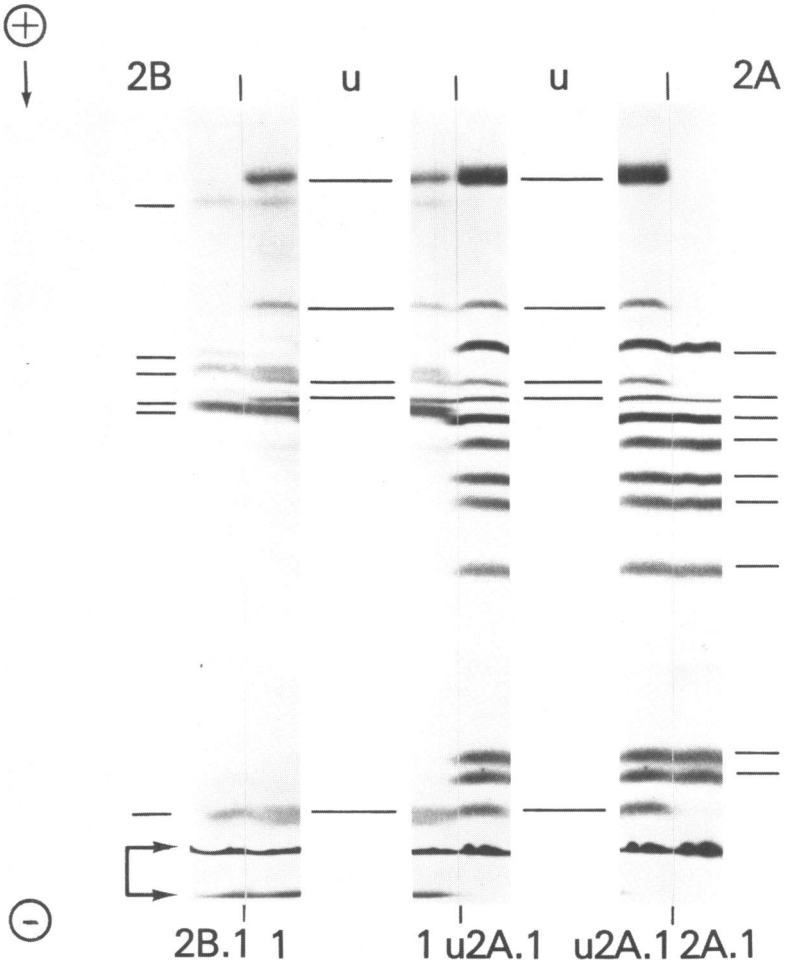

FIGURE 4: Separation of ^{14}C-arginine-labeled peptides in a 50% polyacrylamide gel. Tryptic peptides from protein 1, H2B.1, and uH2A.1 as indicated were electrophoresed in adjacent slots of a gel as described in Materials and Methods. This figure shows the autoradiograph of the arginine peptides. Peptides common to H2A and uH2A are indicated under "H2A", ubiquitin peptides are shown under "u," and H2B peptides under "H2B." The 2 arrowed bands near the bottom of the gel represent unresolved dipeptides and free arginine.

ubiquitin attached (Figure 6). The inset of the Figure shows the migration (left to right) of mass standards of intact H2B and its C and N terminal portions (supplied by Dr. E. W. Johns). They were electrophoresed

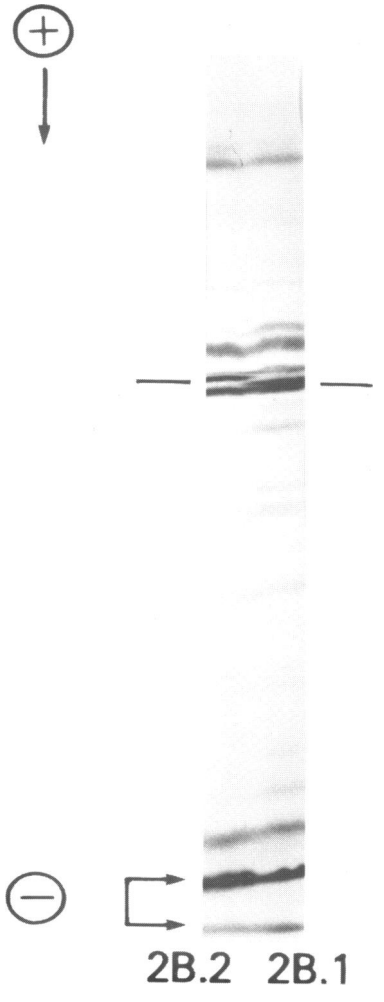

FIGURE 5: Comparison of H2B.1 and H2B.2. The arginine peptides from H2B.1 and H2B.2 were electrophoresed in a 50% acrylamide gel as described under Materials and Methods. The indicated peptides differ slightly in mobility. By labeling with other amino-acids, these bands have been identified as Ile-Ala-Gly-Glu-Ala-Ser-Arg in H2B.1 and Ile-Ala-Ser-Glu-Ala-Ser-Arg in H2B.2. The positions of dipeptides and free arginine are also indicated by the arrows.

in the same gel as digested and control samples of H2B and uH2B which had been labeled with ^{14}C arginine. The distribution of radioactivity in the uH2B digest is depicted here; H2B digestion products migrated in the

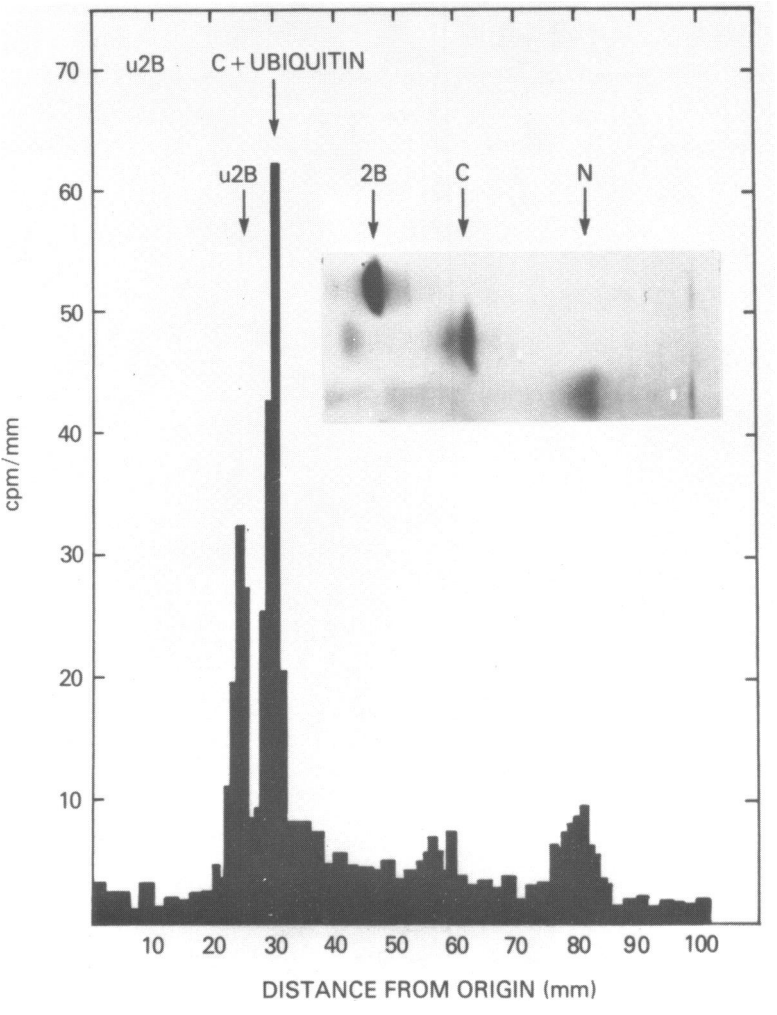

FIGURE 6: Cyanogen bromide cleavage of uH2B. Fragments of uH2B prepared as described under Materials and Methods were electrophoresed from left to right in a 25% acrylamide gel containing acetic acid and urea (8 M). After fluorography, radioactivity in the bands was determined by the procedure described in Materials and Methods, background has been substracted. The stained standards (H2B, C and N-termini) are depicted in the inset. The positions of these and undigested u2B are indicated.

expected positions. Cleavage by cyanogen bromide was not complete, the most slowly migrating peak here represented undigested material (this comigrated with the control sample). After cleavage of uH2B, the N-

terminal portion of H2B was found but little if any of the C-terminal portion was found. The H2B C-terminal fragment has 5 arginines and the N-terminal 3; a comparison of the radioactivity in the H2B digest gave close to the expected 5:3 ratio (data not shown). Addition of ubiquitin to the C-terminal fragment would change the ratio to 9:3 (3:1) and the measured radioactivity in the bands labeled C + ubiquitin and N satisfied that ratio very closely (3.1:1.0).

By using techniques of analysis similar to those described in West and Bonner (2) u2B's were found in nucleosomes including monosomes. Quantitatively, the uH2B forms comprise 1.0-1.5% of each variant and presumably replace H2B in the nucleosome.

DISCUSSION

In the mouse L1210 cell line, H2B can undergo a similar post-translational addition of ubiquitin as can H2A. All variants of H2A become modified to the same extent (about 11%). Both H2B variants are modified, however their ubiquitin forms only amount to between 1 and 1.5%. It has been reported (7) that calf and other mammalian tissues contain an H2B.3 variant which comigrates with the H2B.2 variant; in this study we were not able to identify this component on the basis of its altered sequence, however, by reducing the urea concentration so that the H2B's were retarded, three other protein spots could be distinguished. These co-migrated with H2B in the second dimension (Figure 3). It is possible that these represent other H2B variants and we are presently attempting to identify these proteins.

It is clear, from the difference in the relative proportions of ubiquitin forms (between H2A and H2B) that uH2A can occur in a nucleosome which does not contain uH2B, but the reverse is not necessarily true. Also, if there are domains in chromatin which contain uH2A's exclusively, then the H2B complement cannot be completely modified. It is therefore difficult to reconcile these observations with the hypothesis that modification of the nucleosome by the addition of ubiquitin transfers it from a transcriptionally incompetent to a transcriptionally competent but inactive configuration (3) unless there is only a requirement for one of the four H2A's or H2B's to contain ubiquitin.

ACKNOWLEDGEMENTS

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REFERENCES

1 Goldknopf, I. L. and Busch, H. (1978) In The Cell Nucleus: Chromatin Part C, 149-180
2 West, M. H. P. and Bonner, W. M. (1980) Biochemistry 19(14), 3238-3245
3 Goldknopf, I. L., Sudhakar, S., Rosenbaum, F. and Busch, H. (1979) J. Cell Biol. 83(2) Part 2, p. 159
4 Bonner, W. M., West, M. H. P. and Stedman, J. D. (1980) Eur. J. Biochem. 109, 17-23
5 Bonner, W. M. and Laskey, R. A. (1974) Eur. J. Biochem. 46, 83-88
6 Zweidler, A. (1978) Methods in Cell Biology XVII, 223-233
7 Franklin, S. G. and Zweidler, A. (1977) Nature 266, 273-275