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# A direct link between core histone acetylation and transcriptionally active chromatin

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**An antiserum raised against chemically acetylated histone H4 was found to recognize the epitope ε-*N*-acetyl lysine. Affinity-purified antibodies were used to fractionate oligo- and mononucleosomal chromatin fragments from the nuclei of 15-day chicken embryo erythrocytes. Antibody-bound chromatin was found to contain elevated levels of acetylated core histones. On probing with sequences of α D globin, an actively transcribed gene, the antibody-bound chromatin was 15- to 30-fold enriched relative to the input chromatin. Using ovalbumin sequences as a probe, no enrichment was observed. The results demonstrate directly that transcriptionally active genes carry acetylated core histones.**

*Key words:* histone/acetylation/chromatin

## Introduction

A range of indirect circumstantial evidence exists to support the original proposal of Allfrey *et al.* (1964) that acetylation of the ε-amino group of lysine residues in core histones serves to facilitate transcription of the chromatin containing them. For example, in *Tetrahymena* only the transcriptionally active macronucleus contains acetylated core histones, not the inactive micronucleus (Gorovsky *et al.*, 1973). When lymphocytes are stimulated with the mitogen phytohaemaglutinin, renewed transcription is preceded by a sharp rise in core histone acetylation (Pogo *et al.*, 1966). Biochemical fractionation procedures associate acetylation with active gene sequences: the chromatin rapidly released from nuclei by DNase I [a nuclease known to preferentially attack active genes (Weintraub and Groudine, 1976)] is enriched in acetylated core histones (Sealy and Chalkley, 1978; Vidali *et al.*, 1978). More recently it has been shown that nucleosomal fractionation on the basis of exposed histone H3-sulphydryl groups (Allegra *et al.*, 1987) or solubility in 0.15 M NaCl (Ridsdale and Davie, 1987) also leads to enrichment of both active gene sequences and the acetylated forms of the core histones.

These and other biochemical approaches (for a review see Allfrey, 1980) all observe an enrichment of acetylated core histones in chromatin fractions produced by methods based on properties of the chromatin other than the histone acetylation itself. It is therefore possible that the chromatin selected by such fractionation procedures represents more than a single subset, i.e. one component of the fraction could be enriched in active sequences and another in acetylated histones, rather than both features being present in the same subset. A direct link between active genes and core histone acetylation is therefore lacking. The present work uses an antibody that recognizes acetylated histone to fractionate chromatin. Enrichment of transcribed sequences in the chromatin thus fractionated is shown, thereby establishing this link.

## Results and discussion

### *Antibody characterization*

The antibody was raised in rabbits using a co-precipitate of tRNA and chemically acetylated histone H4 by the method of Stollar and Ward (1970). Serum was tested by ELISA techniques against a variety of 'antigens' to test its specificity. Figure 1 shows the results. Panel A shows that the other core histones are recognized by the antibody when chemically acetylated, whereas the same histones without modification show little response. This could mean either that the antibody recognizes acetyl lysine alone or that there is a related modified sequence in the histones. Panel B shows a comparison of chemically acetylated and unmodified bovine serum albumin (BSA). The strong response with acetylated BSA indicates that the epitope recognized is ε-acetyl lysine. Antibody was therefore fractionated by ammonium sulphate precipitation, DEAE chromatography and then affinity purified using chemically acetylated histone H4 linked to agarose (see Materials and methods). Affinity-purified antibody was tested against acetylated H4 and against completely non-acetylated H4, both extracted and fractionated from calf thymus. Panel C of Figure 1 demonstrates the efficacy of the purification procedure and confirms the conclusion that only modified histone is recognized. It is also clear that α-amino acetyl groups on the N terminus of histones are not recognized by the antibody. To establish whether the antibody will recognize an acetylated core histone when the latter is incorporated in a nucleosome, a comparison was made of core particles from HeLa cells grown in butyrate-containing medium (which therefore contain highly acetylated core histones—data not shown) and mature chicken erythrocyte core particles that contain low levels of histone acetylation. Increasing quantities of core particles were pre-incubated in free solution with a fixed amount of antibody and the excess unbound antibody quantified by ELISA using acetylated H4 as 'antigen'. Panel D of Figure 1 shows that the chicken core particles bind very little antibody but the HeLa core particles bind >50% of the antibody under the conditions used. This result demonstrates that the antibody can recognize an acetylated histone in a core particle in free solution. This is an important prerequisite for chromatin fractionation with the antibody.

Pfeffer *et al.* (1986) have previously reported the generation of an antiserum to a chemically acetylated peptide from

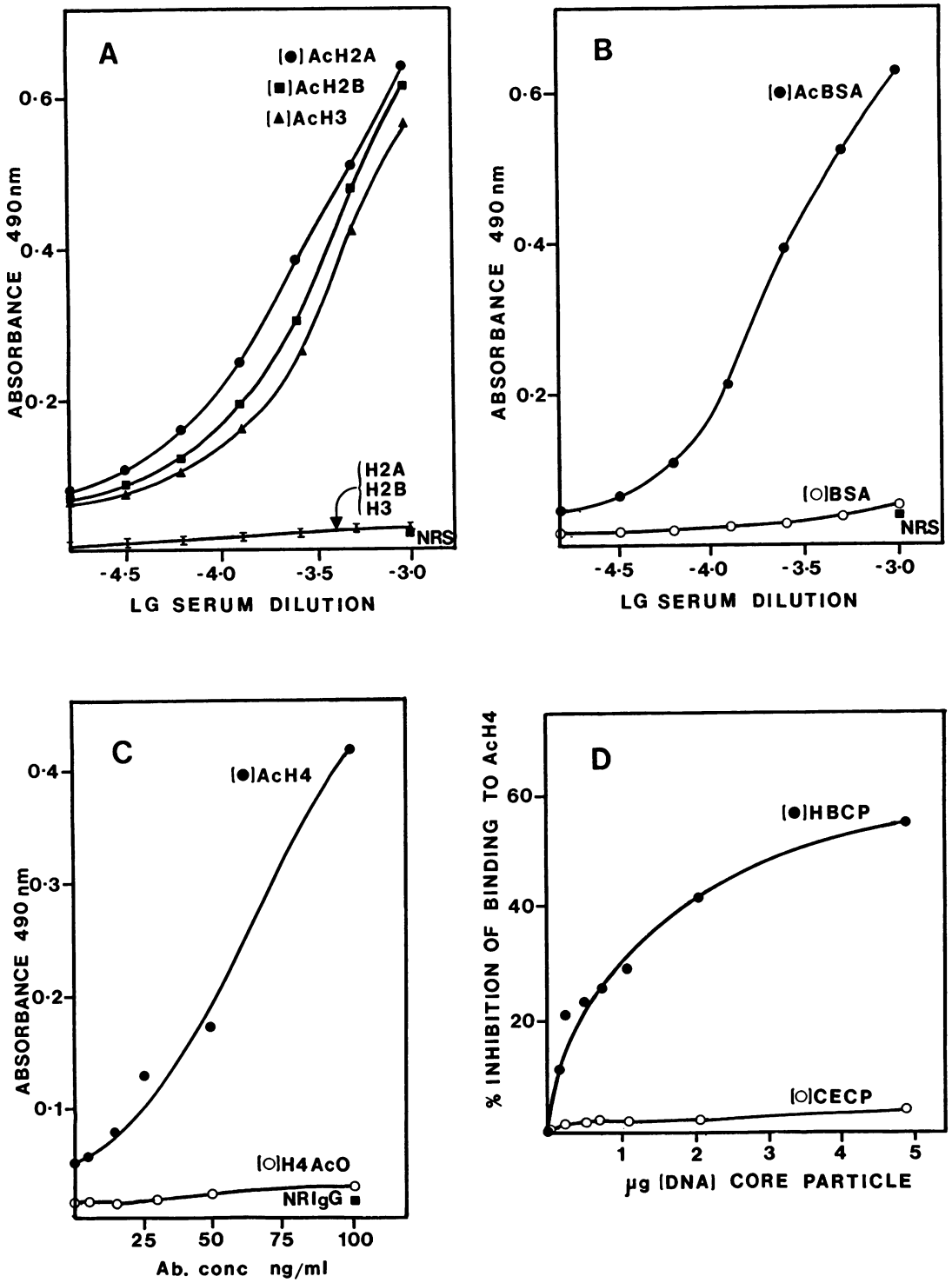


**Fig. 1.** Characterization of the serum and affinity-purified antibody using an ELISA assay. **Panel A:** response of the serum to chemically acetylated histones extracted from calf thymus and averaged values for the three unmodified histones. Equimolar amounts of each 'antigen' histone per well. NRS, average response to all six histone samples by normal rabbit serum. **Panel B:** response of the serum to chemically acetylated bovine serum albumin (AcBSA) compared with unmodified BSA. NRS, averaged response to AcBSA and BSA by normal rabbit serum. **Panel C:** Response of affinity-purified antibody to chemically acetylated histone H4 and to calf thymus derived histone H4 carrying no modified lysine residues, purified by ion-exchange chromatography. NRIgG, average response to AcH4 and H4Ac0 by normal rabbit IgG. **Panel D:** binding of antiserum to core particles containing a high level of natural acetylation (HBCP, derived from HeLa cells grown in 7 mM sodium butyrate), and to core particles containing a low level of natural acetylation (CECP, from mature chicken erythrocytes). Serum at a dilution of 1:2000 was mixed with core particles in free solution and excess unbound antibody back-titrated by ELISA using chemically acetylated histone H4 (AcH4) as 'antigen'.

histone H4 that is likewise able to recognize acetyl-H4 in a highly modified nucleosome. Their serum showed much higher specificity for the tri- and tetra-acetylated H4 species than the other forms of the histone. Muller *et al.* (1987) prepared a panel of monoclonal antibodies to tri-acetylated H4 from cuttle-fish. Of these antibodies, one was specific for acetylated histone H4 associated as an octamer with the other core histones.

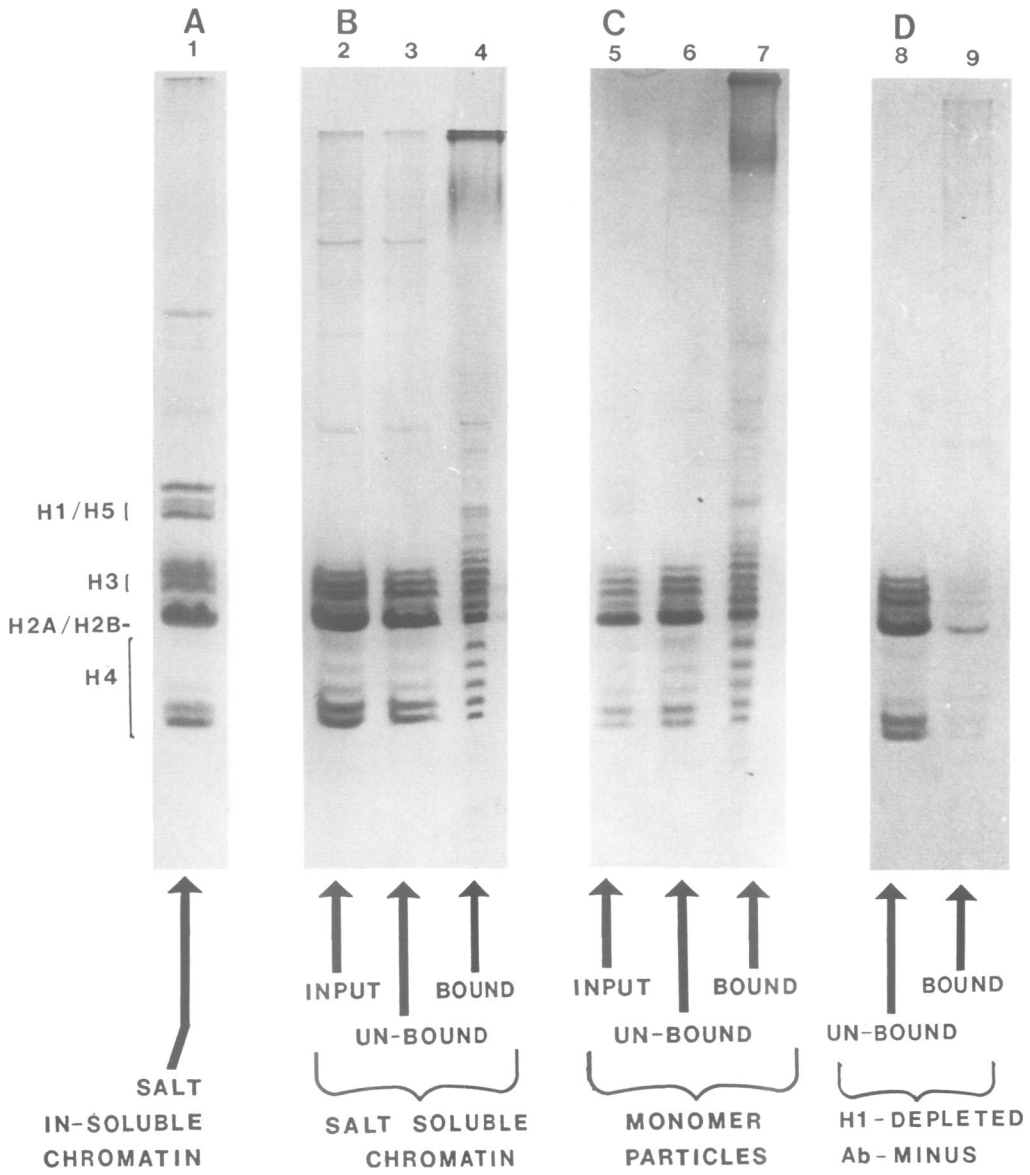


**Fig. 2.** Acetic acid/urea 15% polyacrylamide gels of proteins from several chromatin fractions. **Panel A:** proteins extracted from salt-insoluble (precipitated) chromatin. **Panel B:** proteins from 150 mM salt-solubilized input chromatin (**lane 2**) compared with the antibody-bound (**lane 4**) and unbound (**lane 3**). **Panel C:** experiment using mononucleosomal input chromatin (**lane 5**) fractionated as in panel B into an antibody bound (**lane 7**) and unbound (**lane 6**). **Panel D:** experiment using H1-depleted chromatin in an antibody-minus control. **Lane 8** is the unbound chromatin and **lane 9** is the residual bound chromatin.

### *Chromatin*

Chromatin from the erythrocytes of 15-day-old chicken embryos was taken as the source material and the probe used for transcribed sequences was a genomic DNA fragment from the α D globin gene, known to be active in this tissue (Brown and Ingram, 1974). Erythrocyte nuclei were prepared in buffers containing 10 mM butyrate so as to retain histone acetylation (Candido *et al.*, 1978) and treated with micrococcal nuclease to produce oligonucleosomal chromatin fragments. About 70% of the total nuclear chromatin was obtained in this way. Oligonucleosomes were then treated in one of two ways: (i) they were partially fractionated by addition of NaCl to 150 mM and allowing the larger, H1-containing oligonucleosomes to precipitate. The 'salt-soluble' chromatin in the supernatant and the 'salt-insoluble' precipitate were both recovered, or (ii) they were exposed to low ionic strength (50 mM NaCl) in the presence of a cation-exchange resin, so as to remove histone H1 without inducing any sliding of core particles. The two chromatin fractions—the 'salt soluble' and the 'H1-depleted' (designated 'input' chromatins)—were then separately fractionated using the antibody.

### *Immunofractionation of chromatin*

Chromatin fragments were first incubated with affinity-purified antibody in a butyrate-containing medium. Immuno-complexes were isolated by adding these mixtures to formalin-fixed *Staphylococcus aureus* cells that retain active protein A in the outer membrane. After incubation, cells were pelleted by centrifugation, resuspended in buffer and repelleted. The two supernatants were combined and designated the 'unbound' chromatin fraction. Chromatin complexes bound to the *S.aureus* cells were removed by suspension in the same buffer containing 1.5% SDS and

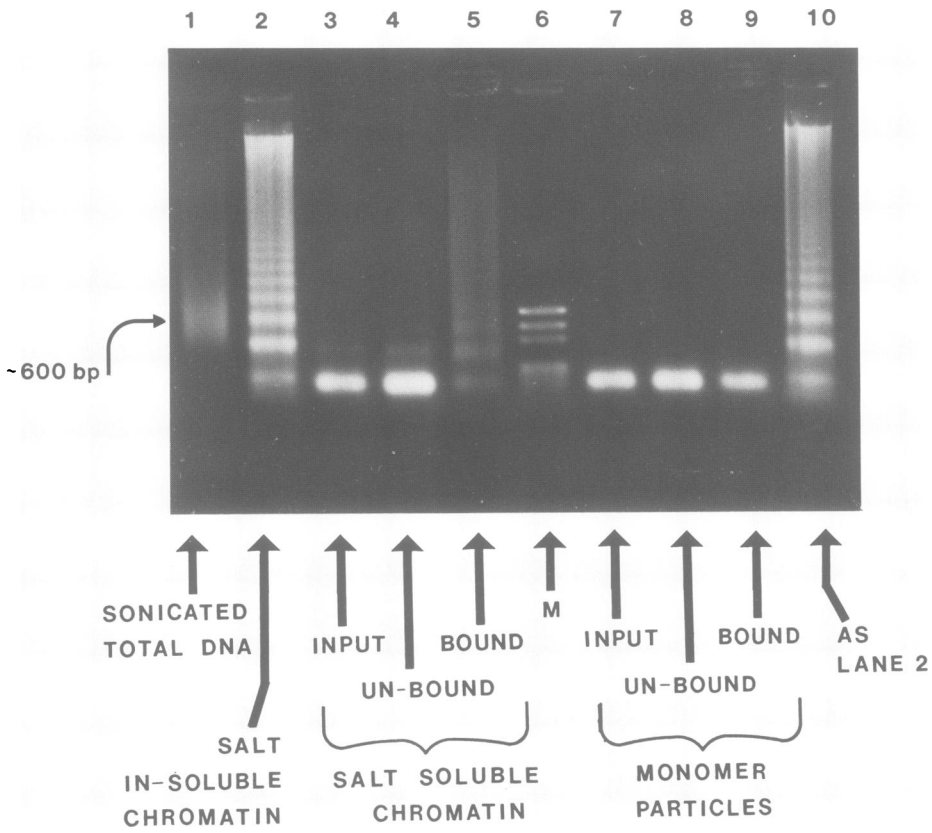


**Fig. 3.** Double-stranded 1.2% agarose gel of DNA from several chromatin fractions. **Lanes 2** and **10** are from the chromatin insoluble in 150 mM NaCl and act as size markers. **Lane 1:** sonicated chicken embryo DNA of ~600 bp; **Lanes 3–5:** experiment using 150 mM NaCl salt-soluble chromatin as the input for antibody fractionation; **lane 6:** pAT153/*Hpa*II marker; **lanes 7–9:** experiment using sucrose gradient fractionated mononucleosomes as input for antibody fractionation.

pelleting the cells. The chromatin in this supernatant was designated the 'bound' fraction. From both bound and unbound fractions, as well as from the input chromatin, DNA was extracted for dot-blot hybridizations and for size class estimation by electrophoresis. Histones were extracted for acetic acid/urea gel electrophoresis in order to assess levels of core histone acetylation.

### *Modification levels of bound and unbound chromatin fractions*

Figure 2 shows an acetic acid/urea gel of histones from the salt-soluble input chromatin used for immunofractionation (lane 2). A modest level of H4 and H3 acetylation is evident. In the case of H4 there is ~40% of the monacetyl species, falling away to 6% tetra-acetyl. Overall this represents an average of about two out of eight possible H4 sites modified per nucleosome. This is much the same level of modification as found in the salt-insoluble nuclear chromatin of the chicken embryo cells (lane 1). H1/H5 is not detected in the salt-soluble input chromatin, as expected. Lanes 3 and 4 show histones from the unbound and bound chromatin and it is apparent that the chromatin not bound to the Ab/*S.aureus* ('unbound') has a similar level of modification as the input chromatin. Since, however, only ~5% of the input chromatin remains in the bound fraction (estimated from its DNA content), this is as expected. The bound fraction, in contrast, shows greatly enhanced levels of acetylation in both H3 and H4. Additional strong bands of modified H2B and H2A are also apparent and were confirmed by a second dimension SDS gel (data not shown). In the case of H4 there is ~20% of each of the five species. This represents an average of about four out of eight possible H4 sites acetylated per nucleosome in the antibody-bound chromatin.

### *Chromatin fragment sizes*

Figure 3 shows a gel of dsDNA from the salt-soluble input chromatin (lane 3) and from the precipitated salt-insoluble chromatin (lane 2). As expected, the salt-insoluble material contains a broad range of multi-nucleosomal sizes, and appears to be depleted in monomers, whereas the salt-soluble chromatin is largely monomer. With the exception of the monomer fraction, the salt-insoluble chromatin in lane 2 represents the material released in the initial micrococcal nuclease digest. Lanes 4 and 5 show the DNA from the chromatin fractionated by the antibody: whilst the unbound fraction, being the bulk of the material, is largely monomer, the antibody-bound chromatin contains a higher size class.

### *Hybridization*

The probe used to screen for transcribed gene sequences was plasmid pα2R7 (Dodgson *et al.*, 1981) that contains the complete genomic sequence of the chicken α D globin gene, incorporated into the plasmid pBR322. The probe for non-transcribed sequences was plasmid pOv230 (McReynolds *et al.*, 1978) that incorporates cDNA of the chicken ovalbumin gene in plasmid pMB9. The results are shown in Figure 4A and B. Hybridizations were performed with a fixed amount (500 ng) of DNA on the filters and $^{32}$P-random primer-labelled probes. Differences in the activity of the two probes were assessed by using 500 ng of total chicken DNA,

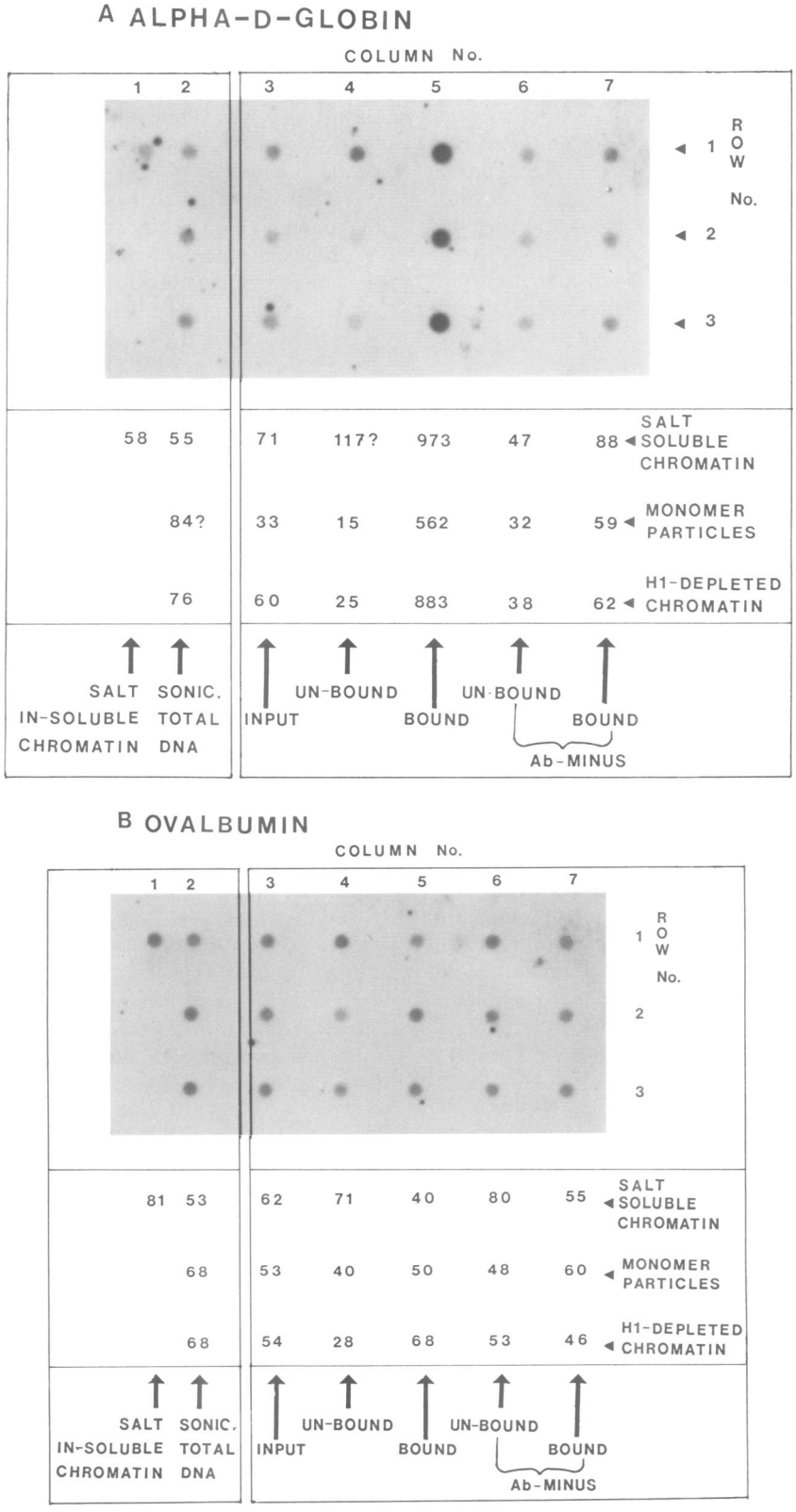


**Fig. 4.** Dot-blot hybridization of chromatin DNA probed with: (**A**) $\alpha$ D globin sequences; (**B**) ovalbumin sequences. The right-hand side of the filters (**columns 3–7**) uses chromatin DNA from antibody fractionations. The left-hand side of the filters (**columns 1** and **2**) uses control DNA from salt-insoluble chromatin and total DNA from nuclei. Each dot contains 500 ng of chromatin DNA. Each row in the right-hand panels gives chromatins from one of three different experiments, namely 150 mM salt-soluble input (**row 1**), mononucleosome input (**row 2**) or H1-depleted total micrococcal nuclease derived chromatin (**row 3**). Each column represents chromatins from different stages of an experiment. **Columns 3:** input DNA of all antibody fractionation experiments; **columns 4** and **5:** unbound and antibody-bound DNA respectively; **columns 6** and **7:** unbound and bound DNA in antibody-minus experiment. *Control hybridizations.* **Columns 1:** DNA from chromatin insoluble in 150 mM NaCl; **Columns 2:** identical dots of ~600 bp DNA from sonicated 15-day embryo nuclei. The scanning densitometry results given below the filters are at a fixed gain so that comparison is possible between dots on a single filter. Comparison between filters A and B is not possible. The two dots with a ? contain contaminating radioactivity, as subsequently shown by reprobing.

reduced by sonication to ~600 bp length (see lane 1 of Figure 3). All the dots in column 2 of Figure 4A and B are thus of identical DNA. Rows 1 of the right-hand side of the Figure 4A and B compare the bound and input DNA from the salt-soluble chromatin (columns 5 and 3). Whereas with the ovalbumin probe (Figure 4B) there is little difference between bound and input signals, with the α D globin probe (Figure 4A) the bound/input ratio is ~14. This emphasizes the high selectivity of the antibody for chromatin containing the transcribed α D globin sequences. The signal from the salt-insoluble chromatin (column 1) is similar to that from the input chromatin, indicating that the micrococcal digestion and addition of 150 mM NaCl have not provided any significant enrichment of transcribed sequences.

We conjectured that the degree of enrichment of active chromatin might be increased if the ratio of chromatin to antibody was raised, due to increased selectivity for the most highly acetylated nucleosomes. Antibody fractionation of salt-soluble chromatin was therefore carried out at three different ratios: 400, 800, 1200 μg of chromatin per 75 μg of antibody. DNA (500 ng) from the three bound, unbound and input chromatins was then tested with both globin and ovalbumin probes. The enrichment of globin sequences in the bound fraction relative to the input was >30-fold in all three cases. Again there was no enrichment of ovalbumin sequences in the bound chromatin (data from this experiment not shown). The amount of antibody used in the fractionation does not therefore seem to be a critical factor over the range used.

### Controls

*Antibody-minus.* The observed enrichments could possibly be due to a specific affinity of active chromatin for the *S.aureus* cells themselves, unrelated to the presence of antibody. An 'antibody-minus' experiment was therefore carried out in which the affinity-purified antibody was omitted from the buffer solution with which the salt-soluble chromatin was incubated prior to addition of the *S.aureus* cells. Columns 6 and 7 of Figure 4A and B show the hybridization results. There is no significant difference between the signals from the bound (or unbound) DNA and the input DNA with respect to transcribed or non-transcribed sequences.

*Monomers.* Figure 3 (lane 5) shows that the antibody-bound fraction represents largely the higher multimers of the salt-soluble chromatin. Differences in the hybridization signals might thus depend on this size difference. A sucrose gradient was therefore used to remove all of the higher multimers from the salt-soluble chromatin, and the purified mononucleosome preparation was then fractionated and probed as above. Lane 7 of Figure 3 confirms the absence of higher multimers in the input for this fractionation (cf. lane 3), and lane 9 shows that the antibody-bound chromatin is also only monomer size (cf. lane 5). The unbound chromatin (lane 8) is also, of course, monomer only. Protein gels were obtained of the input monomers and their histones compared with those of the antibody-bound and unbound monomers (Figure 2, panel C). The levels of acetylation in the bound monomer fraction are similar to those observed when no sucrose gradient fractionation was used; i.e. panels B and C are very similar. It is notable, however, that the input monomers are depleted of all other high mol. wt proteins. This gives reassurance that fractionation is not due to some other minor unknown, but acetylated, protein. The larger proteins in the gel of the bound chromatin (lanes 4 and 7) come from the *S.aureus* cells and antibody itself. The hybridizations of the mononucleosome preparations are shown in row 2 of Figure 4A and B. Again a very high (17-fold) enrichment of α D globin sequences is seen in the bound fraction relative to the input, whilst the unbound chromatin shows a depletion of α D globin sequences relative to the input. With the ovalbumin probe there is essentially no distinction made between the input and bound chromatins. An antibody-minus control was also carried out with the sucrose gradient purified mononucleosomes (columns 6 and 7 in rows 2 of Figure 4A and B). The hybridization results are not therefore a consequence of the different DNA size classes found for the bound and input fractions of the total salt-soluble chromatin.

*H1-depletion.* To compare the monomer results with chromatin of a much higher average size class, the initial micrococcal nuclease-released chromatin (approximately as in lane 2 of Figure 3) was depleted of H1 at low ionic strength and then antibody fractionated as before. Rows 3 of Figure 4A and B give the hybridization results. Again, α D globin sequences show a high (15-fold) enrichment in the bound chromatin with no differences detectable using the ovalbumin probe. In this experiment it was important to check that H1 had been fully removed and to assess whether any specific or non-specific binding of long chromatin to *S.aureus* cells occurs. An antibody-minus control was therefore made and the protein gel is shown in Figure 2, Panel D. The unbound chromatin (essentially the input material also) is seen to be very free of all but the core histones (lane B). Despite it being an antibody-minus control, a very low level of bound core histone was, however, recovered from the *S.aureus* cells.

Inspection of lane 9, however, shows that this bound chromatin is not enriched in the acetylated histones. Thus although the *S.aureus* cells themselves do bind a low level of the longer chromatin, there is no indication that acetylated components are preferentially retained.

## Conclusions

The experiments described, in particular that with the salt-soluble chromatin, were repeated several times and the enrichment of α D globin sequences in the antibody-bound chromatin relative to the input was always in the range of 15- to 30-fold. Furthermore, no enrichment of ovalbumin sequences was ever found in the bound chromatin. These results demonstrate directly that in the 15-day embryonic chicken erythrocyte the transcribing α D globin chromatin, but not the inactive ovalbumin chromatin, contains a significant level of core histone acetylation. The 2-fold variation in the enrichment factor observed for the globin chromatin is not unexpected, bearing in mind the uncertainties of quantification of the hybridization experiments. Enrichment values of the antibody-bound chromatin relative to the sonicated total cellular DNA (column 2 of Figure 4A and B) were observed to be in the same range.

On a simple model of the nuclear chromatin, a 20-fold enrichment would represent 5% of the genome being in a transcriptionally active or potentially active conformation and having its histones uniformly acetylated to a high enough

level to be recognized by the antibody. Such a figure for the proportion of the chicken embryo genome in a transcriptionally active state is not unreasonable (Weintraub and Groudine, 1976). As a model to explain the observed enrichments it also assumes that core histone acetylation is not a major factor in other chromosomal processes. An assumption of uniformly high acetylation throughout the transcriptionally active and potentially active chromatin is, however, not substantiated, and antibodies of the type described here will be required for the detailed mapping of acetylation in active and potentially active gene regions.

## Materials and methods

### *Antigen preparation*

Pig thymus histone H4, 0.5 mg/ml, in 50 mM sodium bicarbonate pH 8.0, was chemically acetylated with 2 mM acetic anhydride for 30 min on ice. The reaction was terminated by the addition of Tris−HCl, pH 8.0, to a final concentration of 10 mM. The absence of any signal from unmodified lysine resonance at 3.06 p.p.m. in the proton NMR spectrum of the acetylated H4 confirmed complete acetylation of all 11 lysine residues in the protein.

### *Antibody generation and purification*

Six New Zealand white rabbits were immunized with 200 μg of the chemically acetylated H4 complexed with tRNA, 3:1 w/w (Stollar and Ward, 1970). The antigen was emulsified with an equal volume of Freund's complete adjuvant and injected into five subcutaneous sites on the back of the animals. Stimulations, in incomplete adjuvant, were repeated fortnightly, and the response screened by ELISA.

The total IgG fraction from the rabbit serum was obtained by ammonium sulphate precipitation followed by DE 52 anion-exchange chromatography (Reif, 1969). Affinity chromatography was used to select the anti-acetyl-H4 antibodies. Histone H4, incompetely acetylated with acetic anhydride such that only 75% of the lysine residues had been modified, was coupled to an amino Sepharose CL 4B (derivatized with the *N*-hydroxy succinimidyl ester of bromo-acetic acid), using the remaining unmodified lysine residues. Bound antibodies were eluted from the acetyl-H4-resin using 3.5 M potassium thiocyanate in 30 mM phosphate buffer pH 7.2, and desalted using a Sephadex G25 column prior to lyophilization.

### *Enzyme-linked immunoadsorbant assay (ELISA)*

Wells of polystyrene microtitre plates were coated with antigen in 50 mM sodium carbonate, pH 9.6, at 4°C for 15 h and then blocked with 0.5% gelatin solution in phosphate-buffered saline (PBS), pH 7.4, for 30 min at 37°C. After washing five times with PBS/0.1% Tween 20, PBS-diluted serum was added and incubated for 30 min at 37°C. After five further washings in PBS, a second antibody was added (horseradish peroxidase conjugated goat anti-rabbit IgG) at a dilution of 1 in 10 000, and incubated for a further 30 min at 37°C. After further washing, bound second antibody was visualized by addition of 0.2 mg/ml *o*-phenylenediamine, 0.001% $H_2O_2$ in 50 mM $Na_2HPO_4$, 20 mM citric acid, pH 5.2. Absorbance at 490 nm was then recorded after termination with 0.5 M $H_2SO_4$.

### *Pre-incubation of serum with core particles*

A 1:2000 dilution of the immune serum was pre-incubated for 15 h at 4°C with 0−5 μg of core particles. Samples were then centrifuged to remove any immunoprecipitate. Excess antibody was back-titrated by ELISA, using the chemically acetylated H4 as 'antigen'.

### *Chromatin preparation for immunofractionation*

Blood from 15-day-old chicken embryos was collected into PBS containing 5 mM $Na_3EDTA$, 10 mM sodium butyrate and 0.1 mM PMSF. Cells were lysed in 0.25 mM sucrose, 80 mM NaCl, 20 mM Tris−HCl pH 7.6, 10 mM sodium butyrate, 6 mM $MgCl_2$, 0.1 mM PMSF, 0.1% Triton X-100, and nuclei washed in the same buffer without Triton.

*Nuclease digestion.* Nuclei were suspended in digestion buffer, (10 mM NaCl, 20 mM Tris−HCl, pH 7.6, 10 mM sodium butyrate, 1 mM $CaCl_2$, 0.1 mM PMSF) at a concentration of 5 mg/ml DNA, and digested with micrococcal nuclease at 200 U/ml for 10 min at 37°C. Digestion was terminated by the addition of $Na_3EDTA$ to a final concentration of 5 mM and nuclei lysed by sonication. Insoluble nuclear material was removed by centrifugation. The chromatin-containing supernatant was recovered and treated as described below.

*Salt fractionation.* 4 M NaCl was added to a rapidly stirred solution of chromatin fragments to a final concentration of 150 mM and incubated once for 10 min to precipitate H1-containing material. This was then removed by centrifugation, leaving the 'salt-soluble' chromatin. H1-free monomers were isolated from this salt-soluble chromatin by centrifugation (24 h, 40 000 r.p.m., 4°C, Beckman SW40) in 5−30% exponential sucrose gradients using the digestion buffer, but replacing the calcium with 0.5 mM $Na_3EDTA$.

*Low ionic strength H1 depletion.* NaCl was added to chromatin fragments at 5 mg/ml DNA to give a final concentration of 50 mM. Dry Sephadex C-25 CM resin was added to a concentration of 30 mg/ml chromatin solution (Libertini and Small, 1980). Depletion was achieved by rolling for 2 h at 4°C. The resin was removed by centrifugation of the mixture over a 50% w/w sucrose cushion containing 40 mM NaCl, 10 mM Tris−HCl, pH 7.6, 10 mM sodium butyrate and 5 mM $Na_3EDTA$. The top layer containing the depleted chromatin was removed.

### *Immunofractionation of chromatin*

Chromatin was immunofractionated using a modification of the method of Dorbic and Wittig (1986). Glycerol or sucrose was removed from stored chromatin using a Centricon C30 (Amicon). Chromatin fragments containing 400 μg DNA were incubated with 75 μg of affinity-purified anti-acetyl-H4 antibody in 750 μl of incubation buffer, 50 mM NaCl, 20 mM Tris−HCl, pH 7.4, 20 mM sodium butyrate, 5 mM $Na_3EDTA$, 0.1 mM PMSF for 3 h at 37°C under constant agitation. The chromatin/antibody mixture was added to the cell pellet obtained from 200 μl of a 10% w/v suspension of formalin-fixed *S.aureus* cells (Sigma) and the whole suspension incubated at room temperature for 1 h with constant agitation. Complexes were collected by centrifugation and the unbound fraction in the supernatant retained. The pellets were resuspended in 250 μl of incubation buffer, centrifuged and the resulting supernatant combined with the first unbound fraction. Pellets were washed five times in 1 ml of incubation buffer by repeated resuspension and centrifugation.

Bound material was eluted by resuspending the final pellets in 250 μl of incubation buffer containing 1.5% SDS and incubating at room temperature for 15 min. *S.aureus* cells were removed by centrifugation and the supernatants containing the released chromatin and antibody (the 'bound' fraction) collected. The cell pellets were re-extracted with a further 250 μl of the same buffer, pelleted and the two supernatants pooled. DNA from all chromatin fractions was obtained by two phenol/chloroform and one chloroform extraction. The DNA was ethanol precipitated, redissolved and treated with 50 μg/ml RNase A and subsequently with 50 μg/ml proteinase K before final precipitation with ethanol.

Proteins from all chromatin fractions were isolated from the phenol/chloroform phase of the first phenol/chloroform extraction (Mathew *et al.*, 1979) by the addition of 1/100th the volume of 10 M HCl followed by precipitation with 12 vols of acetone. Precipitates were washed once with acidified acetone (1:6, 100 mM HCl/acetone) and finally three times with dry acetone before drying under vacuum.

### *Dot-blot hybridization*

Heat-denatured DNA samples were applied to Hybond-N filters (Amersham) using a manifold (BioRad) and 500 ng DNA/dot. The filters were air dried, covered in Saran wrap and the DNA cross-linked to the filters by exposure to UV irradation for 5 min. Filters were probed with chicken α D globin sequences, as an active gene, and ovalbumin as an inactive gene. Plasmid DNA containing these sequences was restricted (with *Eco*RI for the globin insert and with *Pst*I and *Xba*I for the ovalbumin-containing plasmid) to release the gene inserts and these were purified by electrophoresis in a 1% agarose gel. The gene fragments cut from the gel were labelled by the random primer method (Pharmacia oligo-labelling kit) to produce probes with a specific activity of $1 \times 10^8$ c.p.m./μg, which were used at $0.5 \times 10^6$ c.p.m./ml. After hybridization, filters were washed twice in 2 × SSC, 0.1% SDS at room temperature for 15 min, once with 2 × SSC, 0.1% SDS at 65°C for 1 h, once with 0.2 × SSC, 0.1% SDS at 65°C for 30 min, and finally twice with 2 × SSC, 0.1% SDS at room temperature for 10 min. The filters were blotted dry and autoradiographed. Enrichment factors were obtained by flying-spot densitometry of the exposed film.

### *Electrophoresis*

DNA was analysed in 1.2% agarose gels employing 0.08 M Tris-phosphate, 2 mM EDTA buffer containing 0.5 μg/ml ethidium bromide. Gels were visualized and photographed under UV light. Proteins were analysed on 15% polyacrylamide gels containing 0.9 M acetic acid, 4 M urea in the running gel and 0.9 M acetic acid, 8 M urea, 7.5% polyacrylamide in the upper gel. The protein gels were scanned directly using a microdensitometer.

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