TARGETED AND EXTENDED ACETYLATION OF HISTONES H4 AND H3 AT ACTIVE AND INACTIVE GENES IN CHICKEN EMBRYO ERYTHROCYTES

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SUMMARY

Affinity purified polyclonal antibodies recognising the most highly acetylated forms of histones H3 and H4 were used in immunoprecipitation assays with chromatin fragments derived from 15-day chicken embryo erythrocytes by micrococcal nuclease digestion. The distribution of hyperacetylated H4 and H3 was mapped at the housekeeping gene glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and the tissue specific gene carbonic anhydrase (CA). H3 and H4 acetylation was found targeted to the CpG-island region at the 5 end of both these genes, falling off in the downstream direction. In contrast, at the βA-globin gene both H3 and H4 are highly acetylated throughout the gene and at the downstream enhancer, with a maximum at the promoter. Low level acetylation was observed at the 5' end of the inactive ovalbumin gene. Run-on assays to measure ongoing transcription showed that the GAPDH and CA genes are transcribed at a much lower rate than the adult βA-globin gene. The extensive high level acetylation at the βA-globin gene correlates most simply with its high rate of transcription. The targeted acetylation of histones H3 and H4 at the GAPDH and CA genes is consistent with a role in transcriptional initiation and implies that transcriptional elongation does not necessarily require hyperacetylation.
The association of core histone acetylation, particularly of H3 and H4, with transcriptionally active genes is by now a familiar story (1-9), however apparent inconsistencies have arisen between promoter/enhancer-specific acetylation and more widespread acetylation. Mapping the modification at the chicken β-globin locus in 15-day erythrocytes showed 33 kb of acetylated chromatin, having boundaries coincident with the limits of open chromatin (10). Furthermore, the inactive embryonic ε-globin was hyperacetylated as well as the active adult β-globin gene (11). This implied that core histone acetylation was a precondition for transcription, a view supported by the observation that acetylation at the inducible PDGFβ gene was not enhanced upon induction (12) and the modification might therefore be related to the formation or maintenance of accessible chromatin. In a study analysing the basis of aberrant transcription of c-myc genes linked to an enhancer –LCR from the 3’ end of the human immunoglobulin heavy chain (IgH) locus (modeling a well-known translocation in Burkitt’s lymphoma), widespread hyperacetylation was observed both upstream and downstream of the transcriptional start site, with little concentration in the region of the c-myc promoter (13). More recently, analysis of H3 and H4 acetylation at the human β-globin locus in MEL cells containing a complete human chromosome 11, has also shown locus-wide acetylation, particularly of H4. Hyperacetylation of H3 was also widespread but more concentrated at DNaseI hypersensitive sites and at the active β-gene (14). The possibility of direct linkage between core histone acetylation and the passage of RNA polymerase II, thereby generating widespread modification, was raised by the observation that the elongator complex contains a subunit having HAT activity (15). Tracking-mediated chromatin modification has recently been discussed (16).

Such widespread acetylation contrasts with the ‘directed’ or ‘targeted’ acetylation implied by observations that gene activation is often accompanied by the recruitment to promoters/enhancers of protein complexes that include subunits having histone acetyltransferase (HAT) activity (17-21): gene repression frequently results in the recruitment to promoters/enhancers of protein complexes containing components with histone deacetylase (HDAC) activity (22-29). Several papers provide experimental support for targeted acetylation. Using a serum response factor (SRF)-controlled reporter gene construct in mouse NIH3T3 cells, it was shown that extracellular stimulation of gene activation induced rapid acetylation of H4, but not H3, in the region of the SRE (30). In S. cerevisiae,
 promoter-specific hyperacetylation of histone H3 was observed in GCN5-mediated transcription (31), whilst promoter-specific hypoacetylation of histone H4 was found for Sin3/Rpd3-mediated repression (32). A concentration of H4 and H3 acetylation covering only about 3 nucleosomes in the region of the enhanceosome is induced by viral induction of the human IFN-β gene in HeLa cells (33). Mapping of this induced acetylation showed it to extend only marginally into the (rather short) coding sequences. Hyperacetylation of histones H4 and H3 was also noted at the hormone response elements of several oestrogen receptor (ER) target genes in human MCF-7 cells following induction with hormone (34) and at the LCR of the human growth hormone locus (35). Acetylation of histone H4 at K16 by MOF, a Drosophila dosage compensation protein, has been shown to activate transcription when targeted to a his3 reporter gene promoter in yeast (36). In a recent in vitro assay using purified components, SAGA and NuA4 HAT complexes targeted by Gal4-VP16 produced a more restricted region of H3 acetylation than that of H4 (37).

Two mechanisms have been proposed for the consequences of acetylation in the tail regions of core histones. 1) The modification disrupts inter-nucleosomal interactions mediated by core histone tails, thereby opening up higher order structure and rendering the chromatin accessible to the transcriptional apparatus. This mechanism can be logically fitted to the widespread acetylation (often of H4) found, for example, at globin genes (10, 14). It has recently been proposed that transcriptional elongation is required to form, and core histone acetylation to maintain, the open chromatin structure (38). 2) The modification has also been shown to facilitate the access of transcription factors to their DNA recognition sequences in individual nucleosomes at promoters/enhancers/LCRs (39-41). Whilst this mechanism can provide an explanation for the concentration of acetylation (often of H3) in promoter regions, HAT-containing complexes are generally assumed to be recruited by already bound primary transcription factors. Defining the order of binding events is thus crucial to understanding the role of promoter/enhancer/LCR-specific acetylation (42).

In order to further explore the distinction between localised and locus-wide histone acetylation, we have mapped the acetylation of histones H3 and H4 at a housekeeping gene (GAPDH) and a tissue specific gene (CA) in the same cells as used for acetylation mapping at the β-globin locus, i.e. 15-day chicken embryo erythrocytes. The mapping of acetylated histones at housekeeping genes was in part provoked by the observation of Tazi and Bird (43) that chromatin derived from CpG
islands is highly enriched in hyperacetylated histone H4, indicating a concentration of the modification in such regions. At the β-globin locus, where the adult gene does not have a CpG island but the embryonic ρ-gene does, no correlation of acetylation with the presence or absence of a CpG island was observed (10). However, all housekeeping genes and about one half of tissue specific genes have CpG islands, so the enrichment seen by Tazi and Bird (43) could be predominantly from housekeeping genes. The present results show a concentration of H4 and H3 acetylation in the upstream CpG island regions of both the GAPDH and CA genes, in contrast to the β^+-globin gene for which the modification extends throughout the gene and into the 3-enhancer.

**EXPERIMENTAL PROCEDURES**

*Preparation and affinity purification of antibodies* Anti hyperacetylated histone H4 serum was prepared by immunising rabbits with chemically acetylated H4 and antibodies were affinity purified over a column of immunogen, immobilised on agarose beads (2, 10, 44, 45). The anti acetylated histone H3 peptide serum was obtained by immunising rabbits with the peptide 1-27 of H3, acetylated at residues 9, 14, 18 and 23, chemically synthesised as a MAPs peptide: antibodies were affinity purified over a column of the same H3 peptide immobilised on CPG beads (Alta Bioscience) using elution conditions as detailed in Ref. 44.

*Western blotting* Acid extracted histones from butyrate treated HeLa cells were resolved by 15% AUT-PAGE (50) and after equilibration in transfer buffer (15 mM glycine, 20 mM Tris, 0.1% SDS and 20% methanol) and electrophoretically transferred using a Biorad transblot apparatus (400 mA, 90 min at 4°C) to nitrocellulose. Membranes were blocked in 5% w/v Marvel in 1xPBS for 1 hr, washed in 1xPBS, 0.1% v/v Tween 20 and incubated with 1:2000 diluted serum for 1hr. After further washing with 1xPBS, 0.1% Tween, chemiluminescent detection was performed using an ECL kit (Amersham).

*Preparation of nucleosomes* Salt soluble chromatin from 15-day chicken embryo erythrocytes was prepared essentially as described in Ref. 2. In brief, a 5 mg DNA/ml suspension of nuclei was digested with MNase at 37°C for 10 min in digestion buffer (10 mM Tris-HCl pH 7.4, 10 mM butyrate, 3 mM MgCl2, 1 mM PMSF, 1 mM benzamidine). Digestion was terminated by the addition of EDTA to a final concentration of 10 mM. Released chromatin was recovered from the supernatant.
S1 after centrifugation (13000g, 1 min). The pellet was resuspended in lysis buffer (0.25 mM EDTA, 10 mM Tris-HCl, 10 mM Na butyrate) to release further material which was recovered in the supernatant S2 after centrifugation. S1 and S2 were pooled and H1/H5-containing chromatin was precipitated by the addition of NaCl to 100 mM. Following centrifugation, the supernatant was layered onto 5-30% exponential sucrose gradients in lysis buffer. Di- and tri nucleosomal fractions were pooled and used as the input chromatin for the experiments since probing with βA-globin and housekeeping sequences showed them to be well represented in this chromatin size class.

**Immunoselection of chromatin** ChIP assays were performed as described in Ref. 10. Typically, 400 µg of input chromatin (as DNA) was mixed with 100 µg of affinity purified antibody in immunoprecipitation (IP) buffer (10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 1 mM EDTA, 10 mM butyrate, 0.1 mM PMSF, 0.1 mM benzamidine) and incubated for 2 hr at 4°C with constant agitation. Immuno-complexes were immobilised using 50 mg of Protein A-Sepharose equilibrated in IP buffer and the suspension incubated for a further hour at 4°C. Unbound chromatin was recovered from the filtrate by centrifugation (6000 rpm, 30 sec) through a 0.45 µm Spin-X filter (SIGMA). After repeat washing with IP buffer, the resin pellet was re-suspended in 150 µl of IP buffer containing 1.5% SDS incubated for 15 min at room temperature and centrifuged to release antibody-bound chromatin in the filtrate. The resin pellet was resuspended in 150 µl of IP buffer containing 0.5% SDS, re-centrifuged and the two Bound’ filtrates pooled. The histones and DNA from the Input, Unbound and Bound fractions were recovered as described in Ref. 10.

**Quantitative PCR** Input, Unbound and Bound DNA samples were subjected to PCR amplification in the presence of 5 µCi of 32P-dCTP and the appropriate primers. Template concentrations and numbers of cycles were determined for each primer pair so that the products fell within the exponential phase of amplification. Typically, 26-28 cycles were used with templates serially diluted from 8.0 - 0.5 ng. Amplification conditions were: 2 min denaturation at 94°C followed by N cycles of: denaturation (94°C for 1 min), annealing (temperature and time optimised for each primer pair) and extension (72°C for 1 min). Products were analysed on 6% native acrylamide gels and quantitated using a Phosphorimager. The signal from the correctly sized product derived from the Input (I) and Bound (B) samples were plotted as a function of template concentration to check for linearity and the B/I value determined as a ratio of the slopes of the two plots in the linear region (data...
not shown). Comparing the Bound signal to the Input normalises for variations in the Input signal that arise from differing susceptibilities to MNase at different points in the genome (or within a single gene). B/I ratios >1 represent 'fold enrichments' achieved by the immuno-precipitation. B/I values <1 represent depletions in the Bound DNA and are plotted as I/B, 'fold depletions'.

**Primer Pairs:**

A1: GTATGGCGCACTCTGGTATAGA; A2: GAGCGGCGCTCTGTGTC; 304 bp product.
A3: ACCTTCTCCAATCTTGCC; A4: ATTCCCTTCTGACTATGCT; 258 bp product.
A5: AAGCGCTAGGAATGTGGTTCC; A6; TTAGTGTACTTGCGACGC; 224 bp product.
A7: ACAAAAGTGAAGGCTTTAATC; A8: TTTTACTCCAGAACATT; 254 bp product.
Ga: GCTCTTTGTCCCGCCC; Gb: CGGGCGATGCGGCTG; 100 bp product.
G1: TCTCGCGCAGGACCGGTGG; G2: GTGTTCCTGCGGGAGAGCG; 244 bp product.
G3: ACCTTGTGTTGTGGGTGCC; G4: GCCAGAGGAGCGGCAGCCC; 246 bp product.
Gc: GAGTCCACTTGTTGCTCTACG; Gd: GAGATGATAACAGCCTAGC; 250 bp product.
CA1: TCGAGCTGGACACAGGGAGCATT; CA2: AGTTGAATCACCACTCCACGGCT; 273 bp product.
CA3: TACGCCAGCCACAACCGGTGA; CA4: CTCAGGCCTGGCATCTCAAGGT; 145 bp product.
CA5: ACTGCTTCTCCAGACACTGC; CA6: TTTCCAGCACCATTCTCAAGGT; 100 bp product.
CA7: CTGGATGGAGTCTCAGGAGGAA; CA8: GCAAAGCAGCAAATGGCTG; 249 bp product.
OvA: TTTGTCTCATTATGTCTCCTGCC; OvB: TCGAGTTACAACCAGATAATGGG; 201 bp product.
Ov1: ACAGGCAAGGACAGCTAATA; Ov2: AAGTTGCTGGCAAGGCTG; 175 bp product.
Ov3: AACTCATGGATGAAGGCTTAAGG; Ov4: TTGTCAGCATAGGAATGGTTGG; 220 bp product.

**Nuclear Run-On Analysis** The run on analysis was performed essentially as described in Refs. 51 and 52. 15-day chicken embryo erythrocyte nuclei were prepared as follows: blood was collected in to 1xPBS, 10 mM Na-butyrate, 5 mM EDTA, 0.1 mM PMSF and 0.1 mM benzamidine and filtered through sterile gauze. Erythrocytes were pelleted at 2200g for 5 min at 4°C and washed and pelleted in the same buffer a further two times. The final pellet was resuspended in ice-cold RSB buffer (10mM Tris-HCl pH 7.4, 1.5 mM MgCl2 10 mM KCl, 0.5% NP-40) and incubated for 5 min on ice with vigorous agitation to lyse the cells. Nuclei were pelleted at 2000g for 5 min at 4°C and the supernatant removed by aspiration. Pellets were resuspended in glutamate run-on buffer (125 mM K-glutamate,
10 mM HEPES pH8.0, 5 mM MgCl₂, 2 mM DTT, 1 mM EGTA, 40% glycerol), snap frozen in liquid nitrogen, then stored at −80°C. Nuclei for analysis (100 µg DNA) were thawed quickly, placed on ice, then 1.0 µl of 1 M creatine phosphate in 10 mM HEPES pH 8.0, 2.4 µl 2 mg/ml creatine kinase, 1 µl 100 mM ATP, 1 µl AGC (25 mM of each of GTP, ATP, CTP), 8 µl 32P UTP (160 µCi, at 800 Ci/mmol) and 50 U RNAsin (Promega) were added, followed by incubation at 37°C for 15 min. CaCl₂ was added to a final concentration of 10 mM together with 50 U RNase-free DNase1 followed by incubation at 37°C for 20 min. 5 µl of 10xSET buffer (10% SDS, 100 mM Tris-HCl pH 7.5, 50 mM EDTA) and 150 µl of 1xSET buffer were added along with 10 µl of 10 mg/ml proteinase K and incubated at 37°C for 45 min. RNA was extracted using phenol-chloroform and precipitated with isopropanol. The RNA pellet was resuspended in 90 µl of 1 mM EDTA, 0.5% SDS, and 15 µl of 2M NaOH was added followed by incubation on ice for 10 min to partially fragment the RNA. Samples were neutralised by adding 0.48 M HEPES and heated to 100°C for 5 min. before applying to the filter. 5 µg of both sense and anti-sense single stranded DNA was slot-blotted onto Biodyne B membrane for each of the GAPDH, CA, ovalbumin and βA-globin genes. The hybridisation buffer was 50% deionised formamide, 6xSSPE buffer 0.1% SDS, 100 µg/ml tRNA. Prehybridisation was for 2 hr at 42°C, hybridisation overnight at 42°C. Washing was for 10 min in 2xSSPE, 0.1% SDS at room temperature, 20 min in 0.2xSSPE, 0.1% SDS at 68°C.

RT-PCR Total RNA was extracted from 15-day chicken erythrocytes using an RNAqueous kit (Ambion) and DNase 1 treated (1U/µg, 30 min, 37°C). First strand cDNA was prepared from 2µg aliquots using random hexamer primers (Promega) and Superscript II enzyme (GIBCO-BRL) under the following conditions: 90°C for 3 min, add 300 units Superscript II, 37°C for 60 min, 95°C for 3 min. Products were amplified by PCR (94°C for 2 min; followed by 94°C for 1 min, 55°C for 1 min, 72°C for 2 min, for 28 cycles; finally 72°C for 7 min) with gene specific primers which where designed to reveal the presence of contaminating genomic DNA in the RNA preparations.

RESULTS

Characterisation of antibodies The two immunogens used to raise the antibodies utilised in the ChIP assays were chemically acetylated histone H4, in which essentially all the lysine residues of H4 become modified (2, 10, 44, 45) and the peptide 1-27 of histone H3 acetylated at residues 9, 14, 18
and 23 chemically synthesised as a MAPs peptide (Alta Bioscience). Western blots were conducted to characterise the specificity of the sera, using histones extracted from butyrate-treated HeLa cells and in each case a Coomassie stained marker lane of the these histones is shown for comparison. Figure 1 shows the results for each serum using both SDS gels and acetic acid/urea (AU) or acetic acid/urea/Triton (AUT) gels. The SDS gels in panels A and C show that both sera are highly specific for the histone used as immunogen, although careful inspection shows that the specificities are not absolute. For example, the anti acetylated H4 serum shows a weak recognition of H3 at the higher loading that amounts to about 10% of activity against H4, whilst the anti acetylated H3 peptide serum shows a very weak recognition of H4 and H2B histones. This weak cross-reactivity is almost certainly due to the presence of anti acetyl lysine activity in the sera, as previously documented for sera derived from chemically acetylated H4 (44). When the acetylated sub-species are spread out using AUT or AU gels the specificity is more precisely revealed. For histone H4, about half the activity is directed at the tetra (fully) acetylated species, with the remainder against Ac3 and Ac2, whilst for histone H3 the activity is about equally directed against the Ac4 and Ac3 species (though a low activity against Ac2 can also be detected). These antisera can most simply be described as being against hyperacetylated H4 and H3. Antibodies from both sera were then affinity purified using columns carrying the immobilised immunogens, i.e. chemically acetylated histone H4 and the acetylated H3 peptide. All the ChIP experiments described below were carried out using these affinity-purified antibodies.

**Chromatin immunoprecipitation assays** Nucleosomal fragments from 15-day chicken embryo erythrocyte nuclei were prepared by micrococcal nuclease digestion and fractionated on a sucrose gradient. The di- and tri-nucleosomal components were pooled and used as ‘input’ chromatin for ChIP assays. In a typical assay, 400 µg of input chromatin (as DNA) was mixed with 100 µg of affinity purified antibodies (see Materials and Methods). Immuno-complexes were immobilised on Protein A Sepharose, washed to remove the unbound chromatin and the bound chromatin was released by a SDS-containing buffer. Typically about 5-10 µg of DNA was recovered from the bound fraction, i.e. about 1.25-2.5% of the input. The content of acetylated histones in the two precipitated chromatin fractions was assessed by comparing the ‘input’ (I) and ‘bound’ (B) samples on stained AUT gels (Fig 2). When the anti-acetylated H4 antibodies were used, the ‘bound’ chromatin was indeed much enriched in multiply acetylated H4 species as compared with the ‘input’ chromatin, as expected. Close
inspection also shows some increase in multiply acetylated H3 species. In the experiment using the anti-acetylated H3 antibodies, an enrichment in multiply acetylated H3 species can be seen, as expected, but in addition there is a considerable rise in the acetylation level of the histone H4 that is present. The tight histone specificity of the anti-acetylated H3 peptide antibodies (Fig. 1) means that the co-isolation of other multiply acetylated histone species must be due to their presence in chromatin fragments selected by virtue of their acetylated H3 content, i.e. there is co-habitation of hyperacetylated H4 with hyperacetylated H3 at the di/tri nucleosomal level.

*The adult β^A^-globin gene and its 3 enhancer.* DNA from the ChIP assays using both anti acetylated H3 and H4 antibodies was analysed by quantitative PCR for chicken β^A^-globin gene sequences. The gene has 3 exons, is about 2.0 kb in length and the enhancer is located just 3 of the end of the major transcript. The amplicons are: a) at the promoter (primers A1/A2), b) spanning the boundary between exon II and the large intron (primers A3/A4) and c) at exon III (primers A5/A6). The 4th amplicon is in the enhancer region, approximately 360bp downstream of the transcription termination site, primers A7/A8, (see Fig. 3). In the exponential phase of PCR amplification, the ratio of the amount of product from antibody-`Bound’ to ‘Input’ DNA (the B/I ratio), gives a measure of the enrichment achieved, i.e. the relative acetylation level at the different regions of the gene (see Materials and Methods). The promoter region shows a 9.5 fold enrichment using the anti-acetyl H4 antibodies and an 8.1 fold for H3 acetylation. In the transcribed region, both primer pairs showed enrichments of 4↑6 fold with both antibodies and similar enrichments were observed at the enhancer. There is thus a high level of H4 and H3 acetylation throughout the chicken β^A^-globin gene, including its enhancer but the promoter exhibits higher levels of modification. Strikingly, levels of H4 and H3 acetylation follow a similar distribution, in accord with the generalised observation from PAGE analysis of co-habitation of the two modified histones (Fig. 2).

*The glyceraldehyde 3-phosphate dehydrogenase gene (GAPDH).* The chicken housekeeping gene GAPDH contains 12 exons, is about 4.5 kb long and has a CpG island of about 1.5 kb in length at its 5’ end (46). The primers pairs chosen for analysis (Fig 4) were: a) Ga/Gb, located in the promoter just upstream of the TATA box, b) G1-G2, spanning the translational start, i.e. largely in the 5’-UTR and within the CpG island, c) G3-G4, covering all of exon III and surrounding intron sequences and d) Gc-Gd, covering exons VI and surrounding intron sequences. The two most 3’ amplicons are
outside the CpG island whilst the other two pairs are within it. Quantitative PCR analysis showed a 4-fold enrichment for H4 acetylation at the promoter but only half of this for H3 acetylation (B/I~2). About 600bp into the transcribed region (G1-G2), the H4 acetylation has dropped markedly (B/I~2), whereas a 2-fold depletion in the ‘Bound’ DNA (B/I~0.5) is observed using the anti acetylated H3 antibodies. Further into the transcribed region, (G3-G4 and Gc-Gd), depletions are observed using both antibodies and the effect is more pronounced as regards H3 acetylation, reaching a 4-fold depletion at only about 1.2kb from the transcriptional start (G3-G4). The distribution of acetylation is thus different from that at the βλ- globin gene in that although the promoter is acetylated on both H3 and H4, the transcribed region essentially lacks acetylated H3 and H4, with the exception of modest H4 acetylation at the most 5' end (G1-G2).

The carbonic anhydrase (CA) gene The distribution of acetylated H3 and H4 histones was also monitored across the chicken carbonic anhydrase gene. This is an active tissue-specific gene in 15-day erythrocytes that possesses a CpG island. The gene covers about 18 kb and comprises 7 exons, (Fig. 5, Ref. 47). The amplicons used were firstly in the promoter region just upstream of the TATA box (primers CA1/CA2) and located just upstream of the CpG island. These primers gave a 2.0-fold enrichment using both the anti-acetylated H4 and H3 antibodies. The second amplicon, (primers CA3/CA4), is within the CpG island covering the first intron and a small amount of exon II: this gave a 16.5-fold enrichment using the anti-acetylated H4 antibodies and an ~7-fold enrichment using anti-acetylated H3 antibodies. The third amplicon (primers CA5/CA6) is located ~1.5 kb into the transcribed region and both antibodies gave an ~2.4-fold enrichment. This is in marked contrast to the GAPDH gene for which, at a similar distance from the transcriptional start (primers G3-G4), there is a 1.5-fold depletion using the anti-acetylated H4 antibodies and a 4-fold depletion using anti-acetylated H3 antibodies. The fourth amplicon, (primers CA7/CA8), covers exon III and the most 3’ amplicon, (primers CA9/CA10) is within the final exon (VII). For both these amplicons, a low enrichment was observed using the anti-acetylated H4 antibodies, which although not high is still 1.5^† fold at exon VII, 16 kb downstream of the transcriptional start. For the anti acetylated H3 antibodies, modest depletions (1.2 and 1.3 fold) are observed at exon III and exon VII of the CA gene: this is again very different from the GAPDH gene for which a 2.1-fold depletion is observed only 600bp into the gene (primers G1-G2).

The ovalbumin gene The 9.5 kb ovalbumin gene is inactive in this tissue, does not have a CpG island and was chosen as a negative control for these experiments. The three amplicons used, primers pairs OvA-OvB, Ov1-Ov2 and Ov3-Ov4, are located at the promoter, in the 5’-UTR and at exon VII
of the gene, respectively. For both the anti H4 and H3 antibodies increasing depletions are observed in a downstream direction, considerably more so for H3 than H4 (Fig 6). At the promoter, a B/I value of close to unity is observed using the anti acetylated H4 antibodies: this implies a low but non zero level of hyperacetylation at this point which decreases to a 2.1-fold depletion near the end of the gene (Ov3-Ov4). As regards H3 acetylation, a significant depletion is observed at the promoter but this becomes more pronounced in a 3′ direction, reaching a 5.3-fold depletion at exon VII. Levels of H4 and H3 acetylation on the ovalbumin gene are clearly very low but the considerable changes in depletion measured along the gene suggest that there may be some acetyl groups at the 5′ end of the gene.

The pattern of histone acetylation at the βA-globin, GAPDH, CA and ovalbumin genes is different in each case: throughout for βA-globin gene, very concentrated at the promoter of the GAPDH gene and whilst maximal at the 5-end of the CA gene, not falling off so sharply in a 3′ direction as at the GAPDH gene. A very low level of H3 and H4 acetylation is found at 5′ end of the silent ovalbumin gene. Both the GAPDH and CA genes have CpG islands, whilst the βA-globin and ovalbumin genes do not. The differences between the acetylation patterns prompted us to determine to what extent the acetylation pattern observed reflects the transcriptional status of the genes in 15-day chicken erythrocytes.

The transcriptional status of the studied genes Total RNA was extracted from 15-day erythrocytes and RT-PCR was used with primer pairs specific for the studied genes in order to establish the amount of each mRNA present, i.e. the pool levels of the four messages in 15-day erythrocytes. Fig. 7A shows ethidium bromide stained agarose gels of the RT-PCR products with multiple loadings of the βA-globin and CA products. Qualitatively, the 460bp βA-globin product is very much stronger (>50-fold) than the 420bp product from the GAPDH housekeeping gene. The 368bp product from the tissue specific CA gene is about 20-fold weaker than the βA-globin product. As expected, there is no evidence for the presence of any ovalbumin mRNA. Although the PCR reactions used were not accurately quantified, the amplified sequences were all about 50% GC, the amplicon lengths were similar to each other and a limited number of cycles (33) was used: product intensities should therefore approximately reflect mRNA pool sizes.

Variable rates of mRNA degradation mean that pool sizes cannot be equated with actual rates of transcription. In vitro run-on assays were therefore used as a direct approach to defining the actual levels of transcription at the different genes. Preparation of nuclei results in losses of nucleotide
precursors and stalling of engaged transcription complexes: on incubation of nuclei in the presence of radiolabeled UTP and unlabeled other NTPs, these complexes complete transcription of the gene but do not re-initiate (48). The labeled transcripts were then used to probe immobilised gene sequences, in this case single stranded DNA corresponding to the antisense sequence (complementary to mRNA), with sense sequences also dotted as controls. Fig. 7B shows the results for all the four studied genes. The very strong signal from $\beta$A-globin sequences demonstrates the high level of transcription on this gene. In marked contrast, very weak but above background levels of CA and GAPDH transcripts can be detected, whilst no signal at all is detected for ovalbumin transcription, as expected. Thus transcription from the tissue specific $\beta$A-globin gene is at a very much greater rate than from the tissue specific CA gene and transcription from the housekeeping GAPDH gene is at similar levels to that from the CA gene.

**DISCUSSION**

The nuclei used in these experiments were made from 15-day embryo erythrocytes directly after blood collection and then used to prepare chromatin for ChIP experiments. The observations should therefore relate to the natural state of the chromatin at the four single copy genes in these cells and not be subject to any of the caveats that inevitably apply to tissue culture cells, transgenic cells and transfected cells. Nevertheless, in considering the detail of the acetylation mapping results, it is important to bear in mind the spatial resolution of the experiments. Having in mind that di/tri nucleosomes were antibody selected and the amplicons were typically of about mononucleosomal length, the ‘worst case scenario’ is that the amplification comes from the next but one nucleosome to the acetylated nucleosome that gave rise to the immuno-precipitation. The centers of these two nucleosomes would be about 400 bp apart and this could be considered as the resolution of the experiments. In the case of the primer pairs Ga-Gb and G1-G2 at the GAPDH gene, the centers of the two amplicons are about 500 bp apart and it is noteworthy that with the anti acetylated H3 antibodies the former gave a 2-fold enrichment but the later a 2-fold depletion. So the spatial resolution is empirically seen to be better than 500 bp. If shorter chromatin fragments are used in ChIP assays in order to improve the resolution and, necessarily, reduced DNA lengths are amplified, then the spatial modulation of acetylation levels (the observed enrichments B/I) would probably be enhanced. However, the resolution in the present experiments allows very large differences in B/I ratios to be detected over quite short distances, e.g. comparing the H4 acetylation mapped with the CA1-CA2 and the CA3-CA4 primer pairs at the carbonic anhydrase gene that are centered about 1 kb (5 nucleosomes) apart.
The enrichment (B/I) or depletion (I/B) values obtained from a single immuno-precipitation experiment represent relative levels of acetylation with a good degree of accuracy, both within a gene and between genes. Indeed, for the present experiments all the data on the distribution of H4 acetylation were obtained from a single immuno-precipitation and the same is true for the H3 acetylation data. If a ChIP experiment is repeated to test the overall reproducibility of the modulation of the enrichments, then variations in the batch of antibody, the chromatin preparation and the efficiency of the actual immuno-precipitations can lead to significant variations in the measured B/I values. However, the relative values at different points along a gene or locus remain essentially the same (our unpublished observations). For this reason we present B/I values obtained from a single IP. To assess the repeatability of the enrichments, multiple PCR amplifications were performed using template DNAs from the same immuno-precipitation, repeating the dilutions as well as the amplifications. For the βA-globin promoter amplicon (A1-A2) in the anti acetylated H4 immuno-precipitation this gave a B/I value of 9.5 with an RMS deviation of 1.2. This indicates the repeatability of B/I measurements from a single IP, i.e. +/- 15%.

However, caution must be exercised when using B/I values to compare levels of H4 acetylation with levels of H3 acetylation since the efficiency of immuno-precipitating modified nucleosomes may be very different for the two antibodies and even for a given antibody there are variations between individual immuno-precipitations. Thus although the enrichments found using the anti acetylated H3 antibodies are frequently less than for the anti acetylated H4 antibodies (though not at the βA-globin gene), this does not necessarily mean that there is more H4 acetylation than H3 acetylation, e.g. at the GAPDH promoter (Ga-Gb) or at the first intron of the CA gene (CA3-CA4). It is clear however that the H4 acetylation at the first intron of the CA gene is about 8-fold more than at the promoter, whilst the H3 acetylation is only 3-4-fold more than at the promoter.

It is important to note the practical advantages of using quantitative PCR for sequence content analysis, rather than hybridisation, in particular for detecting depletion of signals in the Bound DNA. Slot blots frequently show (variable) backgrounds even in the absence of any sample DNA, making it difficult to measure depletions with any accuracy. However, when linear relationships between amplicon intensity and template concentration are obtained in quantitative PCR, there is no background problem. So a depletion (I/B) of, say, 5.3-fold is readily distinguishable from a depletion of 2.7-fold and 1.2-fold – to quote the values observed for H3 acetylation at the ovalbumin gene. It is also important to note that an enrichment of 1.04-fold is experimentally fairly close (~20%) to a depletion of 1.2-fold – the results for H4 and H3 at the OvA-OvB amplicon - but very distinct from
the 5.3-fold depletion for H3 at the Ov3-Ov4 amplicon.

Since B/I values give the change in concentration (representation) of a given sequence between the Input and the Bound DNA samples, it is worth noting that with a range of reliably measured B/I ratios from substantially >1 to significantly <1, a value of unity has no special significance. This is because whilst the representation of a sequence in the Input is roughly the same as in total genomic DNA, the Bound DNA is a completely different collection of sequences, selected for their characteristic and variable acetylation status.

With these considerations in mind, several clear observations stand out. There is a high level of both H3 and H4 acetylation throughout the βA-globin gene, including its downstream enhancer, with an approximately two-fold increase at the promoter, but this is not a characteristic feature of all tissue specific genes since it is not observed for the CA gene. The continuous acetylation is certainly not an obligatory feature of CpG island-containing genes, since the βA-globin gene does not have an island.

The continuous and extensive acetylation of the βA-globin gene does appear to correlate with its high level of transcription in 15-day embryos, as seen in the run-on experiments. However, the silent embryonic βρ-globin gene is also highly acetylated, as seen from experiments using a hybridisation probe (P5) that covers most of the βρ transcribed sequences (10), so there is no simple correlation between hyperacetylation and a high level of transcription over transcribed sequences. At the GAPDH and CA genes, the H4 and H3 hyperacetylation is concentrated within the CpG island region at the 5' ends of these genes and it is striking that at the CA gene a peak of very intense acetylation is observed not at the promoter (as for the GAPDH gene) but in the middle of the CpG island part of the transcribed region. At the GAPDH gene the H3 acetylation falls off more rapidly than that of H4, as seen particularly from the B/I values at the G1-G2 amplicon (located in the 5’-UTR) for which a 2-fold enrichment is observed using the anti acetylated H4 antibodies but a 2-fold depletion seen using anti acetylated H3 antibodies: this is in contrast to the promoter for which enrichments are found in both H3 and H4 immuno-precipitations. We have previously reported that H4 acetylation is also concentrated in the region of the CpG island for the housekeeping gene thymidine kinase (TK) in the same cells as used here, falling off rapidly in a 3’ direction, as for the GAPDH gene (49). The contrast between the GAPDH and CA genes is considerable in that although both exhibit a falling off of acetylation in a 3’ direction, the overall acetylation patterns are rather different. The CA gene is very much longer than the GAPDH gene yet there is still a small (x1.5) but significant enrichment for H4 at exon VII of the CA gene (~16kb from the transcriptional start), whereas only 1.2kb from the
transcriptional start of the GAPDH gene there is a 1.5-fold depletion for H4 acetylation. To decide whether this is a distinguishing difference between tissue specific and housekeeping genes will require the study of several more genes in both categories.

The results for the ovalbumin gene demonstrate very low levels of both H4 and H3 acetylation, as expected for an inactive gene and as shown by our earlier results using hybridisation, (2, 10, 11). Importantly however, it is clear that acetylation is not completely absent since not only is there a B/I value of >1 for H4 at the promoter but the changing levels of depletion in a 3’ direction with both antibodies are well outside the experimental error. In fact, the pattern of decreasing H4 and H3 acetylation in a 5’ to 3’ direction on the ovalbumin gene looks similar to that at the GAPDH gene, with the difference that the levels of acetylation are everywhere much lower at the ovalbumin gene.

The results overall suggest that acetylation, particularly of H4, is concentrated at the CpG island regions of active genes, as suggested by the observations of Tazi and Bird (43), rather than just being targeted at promoters. In the case of the CA gene, a peak of hyperacetylation is seen within the CpG island (CA3-CA4) and only modest enrichments (2-fold) are observed in the promoter region (CA1-CA2). For this gene the promoter amplicon is located outside of the CpG island. At the GAPDH gene, where the promoter is within the CpG island, there is acetylation of both H3 and H4 in the region of the promoter. In this particular case therefore, H3 acetylation appears to be restricted to the promoter, whereas H4 acetylation is somewhat more extensive, a situation not dissimilar to that at the human $\beta$-globin locus (14) and that consequent upon Gal4-VP16 recruitment (37). So whilst both the GAPDH and CA genes exhibit hyperacetylation of H3 and H4 only at the 5-end of the genes in contrast to the $\beta^A$-globin gene it is not at present possible to conclude that this is a promoter-specific effect, particularly as regards histone H4. In fact, the data obtained for GAPDH suggest that the H4 hyperacetylation could be a CpG island phenomenon, whereas H3 hyperacetylation is a feature of active promoter/enhancers.

The correlation of the modification with chromatin structure at the chicken $\beta$-globin locus (10) suggested a direct relationship of H4 acetylation to either the formation or maintenance of the open conformation of the chromatin. Assuming that an open chromatin structure over the whole of a gene is required for transcription, then the spatially restricted H4 and H3 acetylation observed for the housekeeping gene GAPDH and the tissue specific gene CA must have another function. The observed localisation of hyperacetylated H4 and H3 at the 5’ end of the GAPDH and CA genes, as well as the TK gene (49), is qualitatively similar to that mapped at the promoter regions of genes for which the recruitment of acetyltransferase-containing complexes on induction is well documented and the
consequent targeted H3 acetylation (31), or H4 and H3 acetylation (33) has been demonstrated. This is in marked contrast to the extensive high level H4 and H3 acetylation found for the βA-globin gene, which has the appearance of a promoter-targeted component lying on top of a high level continuum of acetylation throughout the gene and into the enhancer. Of the genes studied here only the β-globin transcripts produce a strong signal in a run-on assay, whilst the housekeeping gene (GAPDH) and the other tissue specific gene (CA) are detected only weakly. Similarly, previous transcription through these genes, as seen from the transcript pool size (RT-PCR, Fig. 7A), also shows a much stronger signal for the βA-globin gene than for the GAPDH or CA genes. Clearly in the 15-day erythrocytes the predominant active transcription is that from the βA-globin gene and this may be related to the high levels of acetylation.

The observations of targeted acetylation suggest that when gene transcription first starts, hyperacetylation of H4 and H3 occurs in the promoter or CpG island region and persists whilst the gene is active. A possible explanation for this persistence would be that hyperacetylation of histones H4 and H3 is required for every re-initiation of a transcribed gene. Such a model is not the same as assuming H4/H3 hyperacetylation plays the role of rendering the chromatin permissive for the binding of gene-specific primary transcription factors when transcription is first initiated (as implied by in vitro nucleosome binding assays, Refs. 39-41) since such factors presumably remain promoter-bound on active genes, or at least are not required to re-bind at every re-initiation. It is quite possible that hyperacetylation of H4/H3 is important in both contexts, i.e. it is required for the initial binding of transcription factors and must also be maintained for subsequent re-initiations. Further experiments to determine the timing of acetylation during the assembly of transcriptionally competent complexes at promoters/enhancers/LCRs will be necessary to define the role of hyperacetylation at different stages of gene induction and continuing transcription.

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REFERENCES
1. Allfrey, V. G., Faulkner, R. and Mirsky, A. E. (1964) *Proc. Natl. Acad. Sci. USA* **51**, 786-794
2. Hebbes T. R., Thorne A. W. and Crane-Robinson C. (1988) *EMBO J.* **7**, 1395-1402
3. Jeppeson, P. and Turner, B. M. (1993) *Cell* **74**, 281-289
4. Grunstein, M. (1997) *Nature* **389**, 349-352
5. Struhl, K. (1998) *Genes and Dev.* **12**, 599-606
6. Workman, J. L. and Kingston, R. E. (1980) Annual Rev. Biochem. 67, 545-579

7. Imhof, A. and Wolffe, A. P. (1998) Current Biol. 8, R422-4

8. Kuo, M.-H. and Allis, C. D. (1998) Bioessays 8, 615-26

9. Strahl, B. D. and Allis, C. D. (2000) Nature 403, 41-45

10. Hebbes T. R., Clayton, A. L., Thorne A. W. and Crane-Robinson C. (1994) EMBO J. 13, 1823-1830

11. Hebbes T. R., Thorne A. W., Clayton, A. L. and Crane-Robinson C. (1992) Nucleic Acids Res. 20, 1017-1022

12. Clayton, A. L., Hebbes, T. R., Thorne, A. W. and Crane-Robinson, C. (1993) FEBS Lett. 1, 23-26

13. Madisen, L., Krumm, A., Hebbes, T. R., and Groudine, M. (1998) Mol. Cell. Biol. 18, 6281-6292

14. Schubeler, D., Francastel, C., Cimbro, D. M., Reik, A., Martin, D. I. K. and Groudine, M. (2000) Genes Dev. 14, 940-950

15. Wittschieben, B. O., Otero, G., de Bizemont, T., Fellows, J., Erdjument-Bromage, H., Ohba, R., Li, Y., Allis, C. D., Tempst, P. and Svejstrup, J. Q. (1999) Mol. Cell 4, 123-128

16. Travers, A. (1999) Proc. Natl. Acad. Sci. USA 96, 13634-13637

17. Brownell, J. E., Zhou, J., Ranalli, T., Kobayashi, R., Edmondson, D. G., Roth, S. Y. and Allis, C. D. (1996) Cell 84, 843-851

18. Yang, X. J., Orgyko, V. V., Nashikama, J. L., Howard, B. and Nakatani, Y. (1996) Nature 382, 319-324

19. Orgyko, V. V., Schiltz, R. L., Russanova, V., Howard, B. and Nakatani, Y. (1996) Cell 87, 953-959

20. Mizzen, C. A., Yang, X. J., Kobuko, T., Brownell, J. E., Bannister, A. J., Owen-Hughes, T., Workman, J., Berger, S. L., Kouzarides, T., Nakatani, Y. and Allis, C. D. (1996) Cell 87, 1261-1270

21. Bannister, A. J. and Kouzarides, T. (1996) Nature 384, 641-643

22. Taunton, J., Hassig, C. A. and Schreiber, S. L. (1996) Science 272, 408-411

23. Heinzel, T., Lavinsky, R. M., Mullen, T.-M., Söderström, M., Laherty, C. D., Torchia, J., Yang, W.-M., Brard, G., Ngo, S. D., Davie, J. R., Seto, E., Eisenman, R. N., Rose, D. W., Glass, C. K. and Rosenfeld, M. G. (1997) Nature 387, 43-48

24. Alland, L., Muhle, R., Hou Jr, H., Potes, J., Chin, L., Schreiber-Agus, N. and DePinho, R. A.
25. Hassig, C. A., Fleischer, T. C., Billin, A. N., Schreiber, S. L. and Ayer, D. E. (1997) *Cell* **89**, 341-347
26. Laherty, C. D., Yang, W. M., Sun, J. M., Davie, J. R., Seto, E. and Eisenman, R. N. (1997) *Cell* **89**, 349-356
27. Zhang, Y., Iratnl, R., Erdjument-Bromage, H., Tempst, P and Reinberg, D. (1997) *Cell* **89**, 357-364
28. Kadosh, D. and Struhl, K. (1997) *Cell* **89**, 365-371
29. Nagy, L., Kao, H. Y., Chakravati, D., Lin, R. J., Hassig, C. A., Ayer, D. E., Schreiber, S. L. and Evans, R. M. (1997) *Cell* **89**, 373-380
30. Alberts, A. S., Geneste, O. and Treisman, R. (1998) *Cell* **92**, 475-487
31. Kuo, M.-H., Zhou, J., Jambeck, P., Churchill, M. E. A. and Allis, C. D. (1998) *Genes and Dev.* **12**, 627-639
32. Kadosh, D. and Struhl, K. (1998) *Mol. Cell. Biol.* **18**, 5121-5127
33. Parekh, B. S. and Maniatis, T. (1999) *Mol. Cell* **3**, 125-29
34. Chen, H., Lin, R. J., Xie, W., Wilpitz, D. and Evans, R. M. (1999) *Cell* **98**, 675-686
35. Elefant, F., Cooke, N. E. and Liebhaber, S. A. (2000) *J. Biol. Chem.* **275**, 13827-13834
36. Akhtar, A. and Becker, P. B. (2000) *Mol. Cell* **5**, 367-375
37. Vignali, M., Steger, D. J., Neely, K. E. and Workman, J. L. (2000) *EMBO J.* **19**, 2629-2640
38. Walia, H., Chen, H. Y., Sun, J.-M., Hoth, L. T. and Davie, J. R. (1998) *J. Biol. Chem.* **273**, 14516-14522
39. Lee, D. Y., Hayes, J. J., Pruss, D. and Wolffe, A. P. (1993) *Cell* **72**, 73-84
40. Vettese-Dadey, M., Grant, P. A., Hebbes, T. R., Crane-Robinson, C., Allis, C. D. and Workman, J. L. (1996) *EMBO J.* **15**, 2508-2518
41. Vitolo, J. M., Thiriet, C. and Hayes, J. J. (2000) *Mol. Cell. Biol.* **20**, 2167-2175
42. Ito, T., Ikehara, T., Nakagawa, T., Kraus, W. L. and Muramatsu, M. (2000) *Genes and Dev.* **14**, 1899-1907
43. Tazi, J. and Bird, A. (1990) *Cell* **60**, 909-920
44. Hebbes, T. R., Turner, C. H., Thorne, A. W. and Crane-Robinson, C. (1989) *Mol. Immunol.* **26**, 1085-1093
Figure 1. Characterisation of the antibodies by Western blotting using SDS, AUT and AU PAGE. The marker lanes (M) represent Coomassie stained tracks of the HeLa butyrate histone mixture used for all panels.

Panel A: SDS gel and Western blot (loadings x1 and x4) using serum from a rabbit immunised with chemically acetylated histone H4 and detected using a goat anti-rabbit horseradish peroxidase conjugated secondary antibody with a chemiluminescent substrate (ECL). The bulk of the activity is against histone H4 and ~10% against histone H3.

Panel B: AUT gel and Western blot using serum from a rabbit immunised with chemically acetylated histone H4 and detected using a goat anti-rabbit horseradish peroxidase conjugated secondary antibody with a chemiluminescent substrate (ECL). The activity is against tetra, tri and di-acetylated H4 with very weak activity against the histone H3 sub-fractions 2 and 3 being detected on very long exposures (data not shown).

Panel C: SDS gel and Western blot (loadings x1 and x2) using serum from a rabbit immunised with
the acetylated histone H3 N-terminal peptide and detected using a goat anti-rabbit horseradish peroxidase conjugated secondary antibody with a chemiluminescent substrate (ECL). The bulk of the activity is against histone H3, together with very weak recognition of histones H2B and H4.

**Panel D:** AU gel and Western blot using serum from a rabbit immunised with the acetylated histone H3 N-terminal peptide and detected using a goat-anti-rabbit horseradish peroxidase conjugated secondary antibody with a chemiluminescent substrate (ECL). The activity is against tetra, tri and, at much lower level, di-acetylated H3 with little evidence of activity against other histones.

**Figure 2.** AUT analysis of histones in the ‘Input’ di/tri nucleosomal chromatin used for the immuno-precipitations and the histones isolated from the antibody-Bound chromatin. Panel A shows the proteins from the anti hyperacetylated H4 immuno-precipitation stained with Coomassie and Panel B shows the proteins from the anti hyperacetylated H3 immuno-precipitation stained with silver.

**Figure 3.** Distribution of H4 and H3 acetylation at the chicken beta-adult βA-globin gene and its 3’ enhancer using four primer pairs A1-A2, A3-A4, A5-A6 and A7-A8 whose positions in the gene are indicated by the red lines. Quantitative PCR analysis was used of DNA taken from Input and antibody-Bound fractions obtained from ChIP assays that used affinity purified anti-acetyl H4 (AcH4) and H3 (AcH3) antibodies. PCR products were quantitated using a Phosphorimager and the signals from the correctly sized product from Input and Bound template samples were plotted as a function of template concentration. Values of (B/I) were determined as the ratio of the slopes of the two plots and are given in the bottom panel as enrichments for each set of primer pairs.

**Figure 4.** Distribution of H4 and H3 acetylation at the chicken GAPDH gene. Four primers pairs Ga-Gb, G1-G2, G3-G4 and Gc-Gd were used in quantitative PCR analysis, as for the βA-globin gene in Fig. 3. Measured B/I values greater than 1 are given as Fold Enrichments (as in Fig. 3) and values less than 1 are given as Fold Depletions, i.e. I/B.

**Figure 5.** Distribution of H4 and H3 acetylation at the chicken carbonic anhydrase (CA) gene. Four primer pairs CA1-CA2, CA3-CA4, CA5-CA6 and CA7-CA8 were used in quantitative PCR
analysis, as for the $\beta^A$-globin gene in Fig. 3. Measured B/I values greater than 1 are given as ‘Fold Enrichments’ (as in Fig. 3) and values less than 1 are given as ‘Fold Depletions’, i.e. I/B.

**Figure 6.** Distribution of H4 and H3 acetylation at the chicken ovalbumin gene. Three primer pairs, OvA-OvB, Ov1-Ov2 and Ov3-Ov4 were used in quantitative PCR analysis, as for the $\beta^A$-globin gene in Fig. 3. Measured B/I values greater than 1 are given as Fold Enrichments (as in Fig. 3) and values less than 1 are given as Fold Depletions, i.e. I/B.

**Figure 7. Panel A:** RT-PCR detection of mRNA pool sizes in 15-day chicken erythrocyte nuclei. Relative loadings are given below each lane: $x1 = 5\mu l$ from a $50\mu l$ PCR amplification. Product sizes correspond to those expected: $\beta^A$-globin, 460bp; GAPDH, 420bp; ovalbumin, 488bp; carbonic anhydrase, 368bp. The intensity of the ethidium staining indicates a much greater pool size for $\beta^A$-globin than for the other genes.

**Panel B:** Nuclear run-on experiments to assess relative rates of transcription at the studied genes. $^{32}$P-labeled run-on transcripts were used to probe $5\mu g$ of antisense and sense single stranded DNA fragments from the studied genes, immobilised on Biodyne B membrane. There is no evidence of transcription from the ovalbumin gene, whilst the housekeeping gene (GAPDH) and the tissue specific gene (CA) give a weak signal. The signal from $\beta^A$-globin is more than 2 orders of magnitude greater.
Figure 1.
Figure 2.
Figure 3

Chicken βA Gene

Amplicon

| Amplicon | A1-A2 | A3-A4 | A5-A6 | A7-A8 |
|----------|-------|-------|-------|-------|
| Bound AcH4 | | | | |
| Input AcH4 | | | | |
| Bound AcH3 | | | | |
| Input AcH3 | | | | |

Fold enrichment

- A1-A2: 9.5
- A3-A4: 8.1
- A5-A6: 5.9
- A7-A8: 5.0

- AcH4
- AcH3
Figure 4
Figure 5.
Figure 6.
Figure 7.
Targeted and extended acetylation of histones H4 and H3 at active and inactive genes in chicken embryo erythrocytes

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