Digestion of the chicken β -globin gene chromatin with micrococcal nuclease reveals the presence of an altered nucleosomal array characterized by an atypical ladder of DNA fragments

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The structure of the chicken adult β -globin gene chromatin in immature and mature erythrocyte nuclei has been analysed using micrococcal nuclease digestion. The resulting DNA fragments were blotted onto DBM-papers and probed with labelled DNA fragments spanning the adult β -globin gene and its 5'- and 3'-flanking regions. The structure of the nucleosomes within and in the regions flanking the adult β -globin gene appears to be altered in at least two ways in erythrocyte chromatin, when compared with either bulk or inactive ovalbumin gene chromatin. First, oligomeric DNA fragments containing the β -globin gene are released faster than those of either bulk or ovalbumin gene chromatin. Second, although the difference in size of the liberated oligomeric DNA fragments is similar to the nucleosomal repeat length of bulk and ovalbumin gene chromatin, the individual oligomers are ~100 bp shorter than their bulk or ovalbumin gene counterparts, most noticeably when the nuclease digestion is performed at 37°C. This results in an atypical ladder of ~300, 500, 700, 900 bp instead of the canonical chicken erythrocyte ladder which is an integral multiple of 207 bp. The same ladder was obtained from immature erythrocytes, in which the β -globin gene is actively transcribed, and from mature erythrocytes, in which it is considered to be inactive with RNA polymerase molecules clustered in the 5' moiety of the gene. This indicates that the alteration of the nucleosomal structure is not due to transcription *per se*. In addition, several sites hypersensitive to digestion with micrococcal nuclease are located in both the 5'- and 3'-flanking regions of the β -globin gene in both immature and mature erythrocytes. Our present results are most easily interpreted by assuming that most of the adult β -globin gene sequences are organized in an altered nucleosomal array characterized not only by the absence of the linker-bound H1 and H5 histones, but also by a modification of the nucleosomal cores resulting in a preferential accessibility of ~40 bp of their DNA to micrococcal nuclease digestion. This preferential accessibility may be due to the absence of (H2A–H2B) dimers which are known to be located in the terminal regions of the core DNA.

Key words: active gene/chromatin structure/DNA repeat length/nucleosome/hypersensitive sites

Introduction

Genes, actively or potentially transcribed by RNA polymerase

B (II) appear to be organized into a chromatin structure that is different from inactive genes (for references see Mathis *et al.*, 1980; Weisbrod, 1982; Igo-Kemenes *et al.*, 1982; Cartwright *et al.*, 1982; Reeves, 1984; Weintraub, 1985). In particular they exhibit a preferential susceptibility to cleavage by DNase I and micrococcal nuclease. In most of the cases which have been studied, some nucleosomal arrangement appears to be preserved, although the structure of 'active' nucleosomes may be different from that of bulk chromatin nucleosomes (for references, see Mathis *et al.*, 1980; Weisbrod, 1982; Baer and Rhodes, 1983; Sargan and Butterworth, 1985).

The existence of a 'regular' nucleosomal array in bulk chromatin of a given cell is revealed by digestion with micrococcal nuclease and analysis of the resulting DNA fragments. The appearance of a ladder of fragments allows one to determine the nucleosomal repeat length which is characteristic of the bulk chromatin of that cell (Kornberg, 1977). Since nucleosome cores invariably contain 146 bp of DNA, differences in repeat lengths are related to variations in linker DNA lengths which vary among organisms and cell types. Improvements of DNA blotting techniques, notably the use of DBM-paper (for references, see Bellard *et al.*, 1982; Kaye *et al.*, 1984), have made it possible to investigate whether unique genes of higher eukaryotes are indeed organized in nucleosome arrays identical to those of bulk chromatin.

We report here the analysis of the DNA fragments resulting from micrococcal nuclease digestion of adult β -globin gene chromatins of immature (reticulocytes) and mature chicken erythrocytes. We demonstrate that in both cases the β -globin gene and its flanking region are apparently contained in an altered nucleosomal pattern characterized by a preferential digestion of the linker DNA and of a fraction of the nucleosomal core DNA. Our results are best interpreted by assuming that the β -globin gene chromatin is depleted in both H1 and H5 linker-bound histones and in (H2A–H2B) core histone dimers.

Results

Oligomeric DNA fragments corresponding to an atypical nucleosomal ladder are released by micrococcal digestion of the adult β -globin gene in mature and immature erythrocytes, whereas the ovalbumin gene DNA fragments are superimposed on the bulk nucleosomal repeat

As previously reported (Compton *et al.*, 1976), the bulk of the DNA in mature erythrocytes of adult chickens is contained in nucleosomes with a DNA repeat length of ~210 bp, irrespective of the temperature conditions during micrococcal digestion (Figure 2A, C and E; digestion at 0, 20 and 37°C, respectively). To investigate whether the adult β -globin gene is contained in a similar nucleosomal pattern, mature erythrocyte nuclei were digested for increasing lengths of time and, after electrophoresis and blotting to DBM-paper, the DNA was hybridized with the intronic probe d (Figure 1b) which hybridizes specifically to the chicken adult β -globin gene (see Robinson and Ingram, 1982).

Fig. 1. Restriction enzyme map of the chicken adult β -globin gene and surrounding sequences (only the relevant restriction sites are indicated; Eco = *EcoRI*). (a) The chicken β -globin gene cluster (Dolan *et al.*, 1981), (b) the chicken adult β -globin gene region; a, b, c, d and e indicate the positions of the DNA fragments which were used as probes (probe b corresponds to a mixture of *SacI*–*XmaI* and *XmaI*–*MspI* fragments), (c) location of the two cDNA fragments A and B which were used as probes (Materials and methods) (both of them cross-hybridize with β H globin sequences). Exons in lanes (a) and (b) are represented by heavy lines.

After autoradiography, the DNA blots were de-hybridized and re-hybridized with a probe for the ovalbumin gene which is transcriptionally inactive (the *XbaI*–*HindIII* segment which contains exons 1–4 and introns B–D, O'Hare *et al.*, 1979). In keeping with the results previously obtained with probes specific for the ovalbumin gene 5' end flanking region (Bellard *et al.*, 1982), the region probed with the *XbaI*–*HindIII* segment is contained in a nucleosomal pattern very similar to that of bulk chromatin, irrespective of the digestion temperature (lanes 0 in Figure 2, panels B, D and F): the repeat length is ~ 210 bp and, at early times of digestion, the sizes of the dimer to pentamer DNA fragments are ~ 410 , 620, 830 and 1040 bp (all sizes are ± 10 bp throughout this paper).

In contrast, the pattern of DNA fragments obtained using the β -globin-specific probe d is atypical in several respects. Firstly, this globin gene chromatin region is more rapidly digested by micrococcal nuclease than the bulk or the ovalbumin gene chromatin. This is especially visible when the digestion is performed at 0 or 20°C, where β -globin DNA was rapidly degraded to fragments < 2000 bp, whereas most of the ovalbumin gene sequences were still contained in larger fragments (compare O and G lanes 1 and 2, in panels B and D). Secondly, although a DNA fragment ladder is observed, it is different from those of the bulk and ovalbumin gene chromatin. For the longest time of digestion at 0°C the sizes of the 'dimer' to 'pentamer' DNA fragment are ~ 350 , 550, 750 and 955 bp, clearly different from those of their ovalbumin gene counterparts (415, 625, 835 and 1045 bp, respectively; Figure 2 panel B, lane 6) by ~ 70 –80 bp. However, it is noteworthy that at very early time of digestion the size of the globin multimeric bands seems to be much closer to that of their ovalbumin gene counterparts (compare the globin and ovalbumin dimer and trimer bands in lanes 1–3 of panel B). A very similar pattern is observed when the micrococcal nuclease digestion is carried out at 20°C (panel D). For the longest time of digestion, the sizes of the β -globin 'dimer' to 'pentamer' fragments are ~ 315 , 520, 725 and 935 bp, respectively. Again, the difference appears to be less pronounced at early times of digestion (lanes 1–3 of panel D). The atypical

β -globin gene ladder was best resolved from the bulk or ovalbumin gene chromatin ladders when the digestion was performed at 37°C (panels E and F). At late times of digestion, the β -globin 'dimer' to 'pentamer' ladder is 300, 500, 710 and 915 bp, whereas that of the ovalbumin gene is 405, 610, 820, 1035, thus different by ~ 110 bp. This atypical nucleosomal ladder obtained for the β -globin gene in erythrocyte nuclei is not related to an intrinsic property of the DNA of this gene, as a typical nucleosomal ladder, similar to that of bulk DNA (~ 196 bp) was obtained when blots of micrococcal nuclease digests of oviduct nuclei were hybridized with a β -globin gene probe (Bellard *et al.*, 1982; see also below).

In contrast to the differences in size between the β -globin and ovalbumin gene multimers, there is no obvious difference in the size of the monomeric band which in both cases extends at 0 and 20°C from ~ 140 to 170 bp at late times of digestion. It is important to stress that due to the stringent conditions of hybridization and washing necessary to prevent non-specific hybridization of the probes with the bulk DNA, the efficiency of hybridization is decreased for DNA fragments in the monomer size range and drastically reduced for smaller fragments (M. Bellard and Sun Yu Lin, unpublished results). It is remarkable that no β -globin material accumulates at 37°C in the monomer size range, whereas the ovalbumin gene monomer band is clearly visible (panels F).

The above observations suggest that in spite of its atypical nucleosomal ladder, the β -globin region corresponding to the intronic probe d is organized in an array of nucleosome-like structures, which however are highly sensitive to further micrococcal nuclease digestion at 37°C when released in the monomer state. The existence of a nucleosome-like structure is also supported by results obtained when more extensive micrococcal nuclease digests were analysed using agarose-formamide gels (Sun *et al.*, 1982) to achieve a better resolution in the 120–200 bp DNA fragment range. As shown in panels G and H of Figure 2, DNA fragments of ~ 120 and 140 bp were revealed using the β -globin and ovalbumin probes, suggesting that the β -globin gene sequences are organized into nucleosomal- and subnucleosomal-

Fig. 2. Comparison of micrococcal nuclease digestion pattern of the DNA of the chicken adult β -globin gene (G) and of the ovalbumin gene (O) in chicken erythrocyte chromatin. Erythrocyte nuclei were digested with micrococcal nuclease in the absence of spermine-spermidine (buffer A – Materials and methods) at 0°C or 20°C (300 U/mg DNA) or 37°C (100 U/mg DNA). Digestion times at 0°C were 5, 15, 30, 60, 100 and 150 min for tracks 1–6, respectively; digestion times at 20°C or 37°C were 1, 1.5, 2, 2.5, 3 and 4 min for tracks 1–6, respectively, except for tracks 3 and 6 in G and H where it was 6 and 12 min, respectively. Purification of DNA, agarose gel electrophoresis (70 μ g DNA/track, 2% agarose gel for panels A–F and 4% agarose 50% formamide gel for panels G and H), DNA transfer to DBM-paper, hybridization to 32 P-labelled probes, autoradiography, dehybridization (Bellard *et al.*, 1980), and measurement of DNA fragment lengths in base pair (bp), were as described in Materials and methods. Panels A, C, E and G correspond to the ethidium bromide stainings (shown as negative prints) of bulk DNA from micrococcal nuclease digests before transfer to DBM-paper (only a small fraction of DNA fragments >4 kb remained in the gel after the DNA transfer). The DNA blots were then successively hybridized with the intronic β -globin probe d (Figure 1b), the ovalbumin gene probe *Xba*I–*Hind*III (Materials and methods) and the β -globin cDNA (A) probe (Figure 1c). To achieve a better comparison of the DNA fragments obtained from the two genes after each time of the micrococcal nuclease digestion (tracks 1–6, see above), the two autoradiograms obtained for a given track with the intronic globin (d) probe and the ovalbumin probe are presented side by side (G and O at the top of the tracks correspond to the globin and ovalbumin probes, respectively). The autoradiogram obtained with the cDNA (A) globin probe is shown here only for track 6 [labelled Gc at the top of the track; identical results were obtained with the cDNA (B) probe – not shown].

like structures. Extensive micrococcal nuclease digestion of the globin gene chromatin at 37°C also results in the appearance of a minor DNA band in the dimer region in addition to the major 300 bp band (panel H, lanes 6). It is unknown whether this additional band belongs to the 'tight' or 'compact' dimers previously characterized, which contain from 240 to 280 bp of DNA and may result from nucleosome 'sliding' in H1-depleted regions of the chromatin during preparation of nuclei or micrococcal digestion (see Spadafora *et al.*, 1979 for references). Similar results to those shown in Figure 2 for mature erythrocytes were obtained with immature polychromatic erythrocytes (also called here reticulocytes for the sake of simplicity) (results not shown).

The autoradiogram shown in Figure 3 shows that the atypical nucleosomal ladder obtained for the micrococcal nuclease digest of the β -globin gene in adult erythrocyte chromatin is not particular to the region corresponding to probe d which was used throughout the experiments shown in Figure 2. Although no strict quantitative comparison can be made because the specific ac-

tivities of the various probes are not identical, it is clear that similar ladders are obtained using any one of the five probes (a, b, c, d and e, see Figures 3 and 1b after micrococcal nuclease digestion at 20°C. This shows that the altered chromatin structure extends beyond the gene itself, since probe a corresponds to sequences located between 600 and 1000 bp upstream from the cap site. In addition to a common altered ladder, the autoradiograms obtained with probes a–e reveal some interesting differences. First, the ladder obtained with probe b is blurred by a high background when compared with those revealed by the other probes. This may be related to the presence of several sites hypersensitive to micrococcal nuclease in this region (see below). Second, the distribution of the dimer, trimer and tetramer bands is different with the various probes, suggesting that the kinetics of digestion with micrococcal nuclease is not the same throughout this region. It is likely that these differences are related to local variations in the structure of this chicken chromatin region, since we have not noticed any strong preferential

Fig. 3. Micrococcal nuclease digestion pattern of the DNA of the chicken adult β -globin gene in adult erythrocyte chromatin using different probes. Erythrocyte nuclei were digested at 20°C for 17 min with micrococcal nuclease (100 U/mg DNA) in the absence of spermine-spermidine (buffer A) as described in Materials and methods. All subsequent steps were carried out as described in the legend to Figure 2. 70 μ g of DNA was loaded in each track of a 2% agarose gel and transferred to a single DBM-paper after electrophoresis. The bands of paper corresponding to the various tracks were cut out and hybridized individually with either one of probes a–e (Figure 1b). The corresponding autograms are shown in lanes a–e.

micrococcal nuclease cutting sites in the DNA regions corresponding to probes c, d and e (see below).

Cloned adult β -globin cDNA probes were also used to probe the micrococcal nuclease digest of mature erythrocyte chromatin. Although the cDNA probe cross-hybridizes to some extent with the hatching globin gene β H (see Bellard *et al.*, 1986; Roninson and Ingram, 1982), which may complicate the interpretation of the results, it is clear that the same oligomeric ladder was obtained whether a genomic or a cDNA probe was used (only one time of each kinetic is shown here — lanes labelled Gc in panels B, D, F and H in Figure 2 — but results in keeping with those obtained with the genomic probe were also obtained at earlier times of digestion). The only marked difference between the results obtained with the cDNA and genomic probes concerns the relative intensity of the various bands, since the monomeric band is markedly underrepresented using the cDNA probe. This is most likely related to very inefficient hybridization, the lengths of the hybrids formed between monomeric DNA and a cDNA probe (which corresponds only to the mRNA sequences) being too short to be stable under the stringent hybridization conditions used in this study.

Since spermine and spermidine are known to slow down markedly the kinetics of chromatin digestion by micrococcal nuclease, presumably by binding to DNA regions readily accessible to the nuclease in their absence (for references, see Billett and Hall, 1979), we have also analysed the micrococcal nuclease digestion pattern of the adult β -globin gene in mature and immature (reticulocyte) erythrocytes at 20°C in the presence of these polyamines. For both mature erythrocytes and reticulocytes, the ovalbumin and the bulk DNAs are contained in the same oligomeric DNA bands, multiples of ~ 210 bp. In keeping with the results obtained in the absence of polyamines, the adult β -globin

gene region corresponding to probe d is digested faster than the bulk or ovalbumin chromatin to fragments < 2000 bp (compare panels A, B and C, and panels D, E and F in Figure 4). However, a series of discrete bands with sizes ranging from ~ 900 bp to 2000 bp are clearly resolved, while they were much less distinct in the absence of polyamines (compare Figure 4, lanes 1 and 2 of panels C and F with lanes 1G and 2G of panel B and D in Figure 2). An enlargement of lanes 1 and 2 of panel C is shown in Figure 5 (these fragments were not seen when incubating in the absence of micrococcal nuclease — results not shown). As in the absence of polyamines, the DNA bands which result from digestion of the β -globin gene in mature erythrocyte or reticulocyte nuclei are present in a ladder of dimer to hexamer fragments which, as those of the ovalbumin gene, differ from one another by ~ 200 bp, but which are smaller than their ovalbumin counterparts by ~ 70 – 100 bp (compare panels B and C, and panels E and F, and the ovalbumin and globin lanes in panels I and J in Figure 4). However, these bands are superimposed on a background which is much stronger than that observed in the absence of polyamines. It is noteworthy that this increase in background is not observed for the ovalbumin gene digest in reticulocyte or mature erythrocyte chromatin (panels B and E), nor for the β -globin gene digest in laying hen oviduct chromatin where its oligomeric ladder is perfectly superimposed on that of bulk chromatin with a DNA repeat length typical of oviduct (196 bp, panels G and H, see also Bellard *et al.*, 1982). Note that, in contrast to the situation of erythrocyte or reticulocyte chromatin, no discrete bands can be seen at early times of digestion of the adult β -globin gene in oviduct chromatin.

Mapping of the micrococcal nuclease cleavage sites in the chicken adult β -globin gene region from mature erythrocyte and laying hen oviduct chromatins

The presence of discrete DNA fragments at early time of micrococcal nuclease digestion of the adult β -globin in mature erythrocyte or reticulocyte nuclei (see above) suggests that some chromatin sites should be more accessible to cleavage by the nuclease. We have mapped these sites using the indirect end-labelling technique of Wu (1980). Since it is known that micrococcal nuclease cuts purified naked DNA at preferential sites (for references see Dingwall *et al.*, 1981; Hörz and Altenburger, 1981), we have also mapped these sites in naked DNA of the adult β -globin gene region and compared their location with that of the preferential sites which may exist in the adult β -globin region of oviduct chromatin. The DNA purified from micrococcal nuclease digests of naked DNA and erythrocyte and oviduct chromatins was cut with endonuclease *Hind*III and after agarose gel electrophoresis the DNA fragments were blotted onto DBM-paper. Two α - 32 P-labelled probes (see Figure 6) were then used to map the location of the preferential micrococcal nuclease cuts in the 5' (*Hind*III–*Bg*III probe) and 3' (*Hind*III–*Sac*I probe) directions (see also legend to Figure 6). The lower part of Figure 6 shows the bands which were generated when naked DNA (A), oviduct nuclei (B) and erythrocyte nuclei (C) were digested by micrococcal nuclease at 20°C in the presence of polyamines (a schematic representation of the location of the cuts and of their relative intensity is shown in the central part of Figure 6 — see legend to the figure).

In the 5' moiety of the adult β -globin region some of the micrococcal nuclease cleavage sites found in naked DNA are also present in both oviduct and erythrocyte chromatin (sites g, e and a). On the other hand, there are some striking differences. Firstly, site e, which is located at ~ 230 bp upstream from the adult β -globin cap site is by far the most prominent site in erythrocyte

Fig. 4. Effect of spermine and spermidine on the micrococcal nuclease digestion pattern of the chicken adult β -globin gene and the ovalbumin gene in reticulocyte and erythrocyte chromatin. Erythrocyte, reticulocyte or oviduct nuclei (Materials and methods) were digested at 20°C with micrococcal nuclease in the presence of spermine and spermidine (buffer B – Materials and methods) as follows. Reticulocyte: 100 U/mg DNA for 12, 20, 30, 60, 80 and 100 min (panels A–C, lanes 1–6, respectively); erythrocyte: 150 U/mg DNA for 3, 5, 8, 14, 20 and 30 min (panels D–F, lanes 1–6, respectively); oviduct: 150 U/mg DNA for 3, 5, 10, 15, 20 and 30 min (panels G and H, lanes 1–6, respectively). All subsequent steps were as described in the legend to Figure 2 with 70 μ g of DNA/track (2% agarose gel). Panels A, D and G correspond to the ethidium bromide stainings (shown as negative prints) before transfer to the DMB-paper. The 'reticulocyte' and 'erythrocyte' blots were successively hybridized with the ovalbumin gene probe *Xba*I–*Hind*III (autoradiograms in panels B and E) and the intronic β -globin probe d (autoradiograms in panels C and F, see Figure 1b and legend to Figure 2). The oviduct blot was hybridized with the intronic β -globin probe d only (autoradiogram in panel H). Panels I and J are comparisons side by side of lanes 6 of panels B and C (arrows), and of lanes 5 of panels E and F (arrows), respectively.

chromatin, whereas it is much less important in naked DNA or in oviduct chromatin (the same e site was also the most prominent site when it was mapped in the other direction using as probe the *Eco*RI–*Sac*I fragment 'a' located at $\sim$ 1000 bp from the cap site – result not shown). Secondly, site f, which is located $\sim$ 100 bp upstream from the cap site, is clearly visible in the

digest of erythrocyte chromatin, whereas it appears to be absent in naked DNA and oviduct chromatin digests. Thirdly, site d which is located at $\sim$ 480 bp upstream from the cap site in digests of naked DNA and oviduct chromatin, is absent from the erythrocyte digest and replaced by a new site d' with no apparent counterpart in naked DNA or oviduct chromatin digests. Four-

Fig. 5. Micrococcal nuclease digestion pattern of the chicken adult β -globin gene in chicken reticulocyte chromatin at early time of digestion in the presence of spermine and spermidine. This figure is an enlargement of the autoradiograms shown in Figure 4, panel C, lanes 1 and 2.

thly, site c is very weak, if present at all, in oviduct chromatin, whereas it is present in both naked DNA and erythrocyte chromatin digests. Finally, site b, which is not seen in naked DNA is present in oviduct and erythrocyte chromatin.

The situation is simpler in the region 3' to the poly(A) site of the adult β -globin gene. With the exception of site k, which is present in naked DNA and erythrocyte chromatin digests but seems to be absent from the oviduct chromatin digest, all of the cleavage sites are found both in naked DNA and oviduct and erythrocyte chromatin digests. There are, however, two major differences: sites i and j, which are minor cutting sites in naked DNA and oviduct chromatin, are by far the most prominent in erythrocyte chromatin.

Discussion

Our present data show clearly that the structure of the adult β -globin gene chromatin is altered in chicken mature and immature (reticulocytes) erythrocytes, when compared with both those of bulk and inactive ovalbumin gene chromatin. In agreement with previous reports (for references see Komaiko and Felsenfeld, 1985; Wood and Felsenfeld, 1982), we found that chromatin of the β -globin gene and of its flanking regions is more rapidly digested with micrococcal nuclease in erythrocytes than either bulk or ovalbumin gene chromatin. This does not reflect

Fig. 6. Comparison between micrococcal nuclease cleavage sites in naked chicken DNA (A), laying hen oviduct chromatin (B) and mature erythrocyte chromatin (C), in the adult β -globin region. Micrococcal nuclease digestions (1 mg DNA/ml, 20°C, in buffer B) were as follows. Naked DNA (line A) purified from erythrocyte nuclei: 25 U/mg DNA, for 15 s; oviduct (line B) and mature erythrocyte (line C) nuclei, 100 U/mg DNA for 1 min. DNA was then extracted with phenol, ethanol-precipitated and digested with *Hind*III. Electrophoresis (1.7% agarose gel, 70 μ g DNA per track), blotting to DBM-paper and hybridization were as in the legend to Figure 2. The cleavage pattern was analysed in the 3' to 5' direction from the *Hind*III site with the probe *Hind*III–*Bgl*II (autoradiograms at the lower left). Mapping in the 5' to 3' direction from the *Hind*III site was performed with probe *Hind*III–*Sac*I (autoradiograms at the lower right). The size of the bands was determined by parallel electrophoresis of *Hind*III + *Hind*III fragments of SV40 (not shown). The letters (a–q) on the side of the autoradiograms indicate the DNA bands which were resolved after *Hind*III digestion of: line A, naked DNA digested with micrococcal nuclease; line B, DNA from micrococcal nuclease-digested oviduct nuclei; line C, DNA from micrococcal nuclease-digested erythrocyte nuclei. The position of the DNA bands is diagrammatically represented above the autoradiograms (lines A, B and C) below a schematic representation of the gene where the exons are represented by the three open rectangles, the 5' end of the gene by a filled triangle, and the two probes used to map the micrococcal nuclease cleavage points by two divergent arrows. The lengths of the vertical bars in A–C correspond roughly to the relative intensity of the DNA bands seen in the autoradiograms. The hatched area in the n–q regions in lines B and C indicate that the bands were embedded in a smear. The arrowheads in line C point to bands the intensity of which is greatly increased in digests from erythrocyte nuclei. The diagrams shown in lines A–C were established from many autoradiograms similar to those shown at the bottom of the figure. No additional differences were seen when the micrococcal nuclease digestion step was carried out at different enzyme concentrations and/or in the presence of spermine and spermidine. No bands were seen when micrococcal nuclease was omitted during the digestion period. The distance between the 5' end of the gene and sites a, b, c, d, d', e and f are (in bp $\pm$ 10): 1190, 870, 770, 480, 460, 233 and 107, respectively; the distances between the 3' end of the gene and sites h, i, j, k, l, m, n, o, p and q are (in bp $\pm$ 10): 120, 272, 425, 515, 560, 725, 970, 1080, 1210 and 1290, respectively.

peculiarities in the β -globin gene DNA sequence, since it is not attacked faster than bulk chromatin in the oviduct where the gene is inactive. Strikingly, this preferential digestion is accompanied by the appearance of an atypical DNA ladder which is particularly obvious when the digestion is performed at 37°C in the absence of the polyamines spermine and spermidine. Although the difference in size of the liberated oligomeric DNA fragments is similar to the nucleosomal DNA repeat length of the bulk or ovalbumin gene chromatin, the individual oligomers are ~100 bp shorter than their bulk or ovalbumin gene counterparts. This results in a ladder of ~300, 500, 700, 900 bp instead of the canonical chicken erythrocyte chromatin ladder which is a multiple of 207 bp (Compton *et al.*, 1976). It is important to note that this altered ladder is very different from that obtained when nucleosomes slide along the DNA to form tightly packed oligomers of core particles with no intervening linker DNA (Spadafora *et al.*, 1979). That DNA fragments, with sizes corresponding to those of both bulk and ovalbumin gene nucleosomal and subnucleosomal particles, can nevertheless be seen in β -globin gene micrococcal nuclease digests, and the existence of a 200-bp ladder (even though it is atypical), strongly suggest that the β -globin gene DNA sequences are organized in some type of regular nucleosomal array. This suggestion is supported by the observation that, although they are released faster, the β -globin gene oligomeric DNA fragments have sizes much closer to those of the corresponding bulk or ovalbumin gene fragments at early stages of micrococcal nuclease digestion, particularly when the latter is carried out at 0 or 20°C, instead of 37°C. This may reflect the fact that the exonuclease activity of micrococcal nuclease is slowed down relative to the endonuclease activity for digestions carried out at low temperature (Noll and Kornberg, 1977).

All of our results are most easily explained by assuming not only that the β -globin gene chromatin lacks the linker-bound H1 and H5 histones, but also that its nucleosome core particles are altered in such a way that ~40 bp of their DNA are very easily digested by micrococcal nuclease at 37°C. The absence of linker-bound H1 and H5 histones in the β -globin gene chromatin of chicken erythrocytes has been previously proposed in order to account for its kinetics of digestion and release as soluble chromatin when exposed to nucleases (Kimura *et al.*, 1983; Komaiko and Felsenfeld, 1985). It has also been suggested that the DNA of transcribed genes may be associated with an altered histone core, deficient in H2A and H2B histone dimers (Baer and Rhodes, 1983). In addition, several recent reports have pointed out that histones H2A and H2B can readily exchange in and out of nucleosomal structures (Louters and Chakley, 1985; Schwager *et al.*, 1985). Since the (H2A–H2B) dimers are located in the terminal regions of the core DNA (for references see Richmond *et al.*, 1984; Read *et al.*, 1985; Shick *et al.*, 1985), it is easy to envisage how the possible absence of at least one (H2A–H2B) dimer, together with the absence of the linker-bound H1 and H5 histones, may result in a DNA ladder which would be ~100 bp shorter than that of inactive chromatin. That the bulk canonical nucleosomal chromatin structure is disrupted over transcribed or potentially transcribed genes has been repeatedly reported during the past few years. Bellard *et al.* (1982, 1986 and unpublished results) have found that digestion of the transcribed ovalbumin gene chromatin and of its 5'- and 3'-flanking regions with micrococcal nuclease results in the release of DNA fragments with sizes very close to those reported here. Fragments of ~300 bp and 500 bp were also observed by Levy and Noll (1981) in micrococcal nuclease digests of the active *Drosophila* heat shock

genes *hsp70*. Similarly, Rose and Garrard (1984) have reported the presence of di- and trinucleosomal fragments of 328 and 470 bp, respectively, following digestion of active immunoglobulin light chain genes in lymphoid B cells. In other cases (Bloom and Andersson, 1982; Cohen and Sheffery, 1985; see Rose and Garrard, 1984 for additional references), it has been reported that digestion of active gene chromatin with micrococcal nuclease generates a much more smeared hybridization pattern. It is, however, possible that such smears are related to the conditions of digestion used in these experiments, since our present results indicate that the temperature of digestion and especially the absence of polyamines are important to obtain a clear altered repeating pattern (see also below).

It is unlikely that the altered nucleosomal pattern results from transcription *per se* as it is found throughout the β -globin gene region extending into both 5'- and 3'-flanking sequences. We have previously shown that while polymerase molecules are distributed throughout the β -globin transcription unit in reticulocytes, they are clustered at its 5' end in erythrocytes (Gariglio *et al.*, 1981). In addition Bellard *et al.* (1986 and unpublished results) have observed a similar disruption of chromatin structure throughout the ovalbumin gene transcription unit and its 5'- and 3'-flanking non-transcribed regions in oviducts, as well as in the regions containing the X and Y ovalbumin-related genes which are transcribed at a much lower rate (LeMeur *et al.*, 1982). These observations exclude a simple mechanism in which transcribing RNA polymerases would displace (H2A–H2B) dimers in regions containing active genes. Multiple histone modifications and the possible intervention of the non-histone proteins, most notably high mobility group proteins (HMGs), have been postulated to play a role in the generation of altered nucleosomal structures over actively or potentially transcribed genes (for reviews, see Mathis *et al.*, 1980; Cartwright *et al.*, 1982; Weisbrod, 1982; Reeves, 1984). Whether and how they could participate in the putative (H2A–H2B) dimer displacement remains to be established.

The presence of the micrococcal nuclease hypersensitive site located at ~200 bp upstream from the cap site (site e in Figure 6) of the β -globin gene in chicken erythrocytes has been reported previously (McGhee *et al.*, 1981). It is situated at the 5' border of a segment which appears to be devoid of histones (McGhee *et al.*, 1981). The newly found site f (Figure 6) may correspond to the 3' border of this segment. It is likely that it is the existence of these sites and the absence of a nucleosome between them (McGhee *et al.*, 1981; Emerson and Felsenfeld, 1984), which prevents the detection of the altered nucleosomal ladder when using probe b which covers this region. This suggests that at least some of the smears observed when hybridizing the DNA of micrococcal nuclease digests of active gene chromatin with specific probes could be due to the presence of hypersensitive sites within the probed regions.

The presence of several hypersensitive sites downstream from the poly(A) addition site of the β -globin gene in erythrocyte chromatin is also noteworthy. Together with the hypersensitive sites located in the 5'-flanking region, their presence accounts for the appearance of DNA fragments of ~2 kb at early times of micrococcal digestion. That these fragments are better seen when the digestion is carried out in the presence of polyamines is probably related to a decreased rate of digestion of the DNA present in the nucleosome-like structures, presumably because these are more stable in the presence of spermine and spermidine. Whether the 3'-flanking hypersensitive sites reflect the continuation of transcription beyond the polyadenylation site and

transcription termination several hundreds of base pairs downstream from it, as in the case of the ovalbumin gene (Bellard *et al.*, 1986), remains to be established. Finally, we note that almost all of the sites which are preferentially cut by micrococcal nuclease in the naked β -globin gene DNA, are also cut in the β -globin gene chromatin of either erythrocytes or oviduct cells, which suggests that nucleosomes are not phased in this region of the chicken genome.

In summary, our results indicate that the structure of nucleosomes within and in the regions flanking the adult β -globin gene is altered in at least two ways in both mature erythrocytes where the gene is transcribed at a very low rate, if at all, and in the immature erythrocytes where it is actively transcribed. Our data are most easily interpreted by assuming that both the linker histones H1 and H5, and at least one H2A–H2B dimer are missing. A number of observations made by others suggest that such a structure may be typical of genes transcribed or potentially transcribed by RNA polymerase B. Since the presence of 'normal' nucleosomes prevent both efficient initiation and elongation of RNA chains (Wasylyk and Chambon, 1979, 1980), it remains to be seen whether such structural alterations of the nucleosome structure would be sufficient to allow RNA polymerase to efficiently transcribe through them.

Materials and methods

Isolation of nuclei, nuclease and restriction endonuclease digestion

Reticulocyte, erythrocyte and oviduct nuclei were prepared from laying hens as described previously (Bellard *et al.*, 1980, 1982; Gariglio *et al.*, 1981). Nuclei were resuspended (1 mg DNA/ml), in digestion buffer A containing 15 mM Tris-HCl (pH 7.5), 60 mM KCl, 15 mM NaCl, 340 mM sucrose, 15 mM 2-mercaptoethanol and 1 mM CaCl₂ or in buffer B (buffer A containing 0.15 mM spermine and 0.5 mM spermidine) and digested with micrococcal nuclease (Worthington) at 0, 20 or 37°C as indicated in the legends to the figures. DNA was purified from nuclei as previously described (Bellard *et al.*, 1980). Restriction endonuclease *Hind*III (BRL) was used according to the supplier's instructions.

DNA electrophoresis, transfer to diazobenzoyloxymethyl (DBM) paper and hybridization of nick-translated probes

'Ordinary' agarose gels (1.7% and 2%) were run as described (Bellard *et al.*, 1980). SV40 DNA *Hind*II and *Hind*III restriction fragments were used as external or internal size markers (Bellard *et al.*, 1982). Agarose–formamide gels (4% agarose–50% formamide) were run as previously described (Sun *et al.*, 1982). Electrophoresis with buffer containing 50% formamide was carried out at room temperature at 80 V until bromophenol blue was ~1 cm from the bottom of the gel. *Hpa*II fragments of pBR322 DNA were used as size markers. Transfer to DBM-paper, nick-translation of the probes and hybridization were carried out as described (Bellard *et al.*, 1982; Sun *et al.*, 1982).

DNA probes

Chicken adult β -globin cDNA probes were prepared from plasmid pCG18- β which contains ~600 bp of the chicken adult β -globin cDNA [Figure 1C (B), Reynaud *et al.*, 1980] or from a pBR322 clone containing a shorter cDNA fragment [Figure 1C (A), Bellard *et al.*, 1980]. The plasmid p β 1BR15 which contains the *Bam*HI–*Eco*RI chicken adult β -globin genomic segment present at the right of λ C β G1 was a gift from J.D. Engel (Dodgson *et al.*, 1979). Probes a–e [Figure 1, line (b)] were prepared from this *Bam*HI–*Eco*RI fragment. The ovalbumin gene probe was the *Xba*I–*Hind*III fragment (O'Hare *et al.*, 1979). All probes were purified after excision with restriction enzymes as described by Dretzen *et al.* (1981). Labelling by nick-translation with [³²P]deoxyribonucleotides, hybridization and autoradiography were as described (Bellard *et al.*, 1980, 1982).

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