Structural transition in inactive Balbiani ring chromatin of *Chironomus* during micrococcus nuclease digestion

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We have analysed by micrococcus nuclease digestion the chromatin structure of genes in the Balbiani ring (BR) regions of a *Chironomus* cell line. Gel electrophoresis of the DNA fragments reveals a repeating structure which consists of two repeat sizes, a long repeat seen in the large fragments and a small repeat seen in the small fragments. The two repeats hardly overlap, except in a narrow transition zone which is at a different fragment size in the BR 2.2 and the BR 2.1 gene. The sizes of the large repeats fit the repeat of the underlying DNA sequence. The short repeats are between 170 and 180 bp, and after H1 depletion the short repeat in the BR 2.2 gene is 160 bp. Our most favoured interpretation of these data is that in intact chromatin the nucleosomes in the BR genes are phased with respect to the repeating DNA sequence, whereas micrococcus nuclease digestion leads to loss of a nucleosome-positioning constraint and hence to rearrangement of the nucleosomes. Our results imply a possible artefact of nuclease digestion of chromatin, which has to be taken into account in mapping nucleosome positions.

Key words: chromatin structure/micrococcus nuclease digestion/nucleosome phasing/nucleosome rearrangement

Introduction

During the last few years data have been accumulated supporting the notion that at least in certain cases nucleosomes are positioned with respect to the DNA sequence (for reviews see Igo-Kemenes *et al.*, 1982; Cartwright *et al.*, 1982; Reeves, 1984; Eissenberg *et al.*, 1985; Simpson, 1986). A main approach to investigate nucleosome positioning is to digest chromatin with nucleases and to analyse the DNA fragments produced by indirect end labelling (e.g. Bloom and Carbon, 1982; Worcel *et al.*, 1983; Thoma and Simpson, 1985; Thoma, 1986) or, as in the case of the highly repetitive satellite DNA, to examine the DNA arrangement in isolated nucleosome cores (Zhang *et al.*, 1983; Zhang and Hörz, 1984; Böck *et al.*, 1984). A well known complication of the use of micrococcus nuclease is its rather high sequence specificity (Dingwall *et al.*, 1981; Hörz and Altenburger, 1981; Pauli *et al.*, 1981; Hörz *et al.*, 1983; Flick *et al.*, 1986). Little attention has been paid to the possibility that chromatin folding in the nucleus may influence nucleosome positions. Here we describe observations which indicate that an intact chromatin structure may be required for the *in situ* nucleosome positions. Chromatin of the Balbiani ring (BR) genes of *Chironomus tentans* cells, which contain blocks of multiple tandemly repeated sequences, was examined by digestion of isolated nuclei with micrococcus nuclease. In the BR 2.2 gene we found that chromatin fragments longer than the 5-mers have the same repeat size as the repeat of the underlying DNA sequence. However, fragments below the 5-mers, produced after

extensive digestion, reveal a repeat size which is shorter than that of the longer fragments. This short repeat is interpreted to originate from co-operative sliding of phased nucleosomes after extensive cleavage of chromatin fibers. Compatible observations were made in the BR 2.1, BR 1 and BR 6 genes, except that the transition zone between the two repeats is in the region where an 11-mer nucleosome is to be expected. Our results point to an effect of nuclease digestion not realized so far, which may introduce ambiguities in data interpretation, and which in the future has to be taken into account when nucleosome positions are mapped in distinct regions of the genome.

Results

In the BR 2 region, which in the salivary glands of *Chironomus* larvae forms a large puff in the giant chromosomes, there are two genes (Galler *et al.*, 1985) which code for larval salivary proteins. These genes contain tandemly repeated elements. The known part of the BR 2.1 gene consists of a 220-bp repeat, called the alpha-repeat (cloned in plasmid pCt 63; Sümegi *et al.*, 1982) and the known part of the BR 2.2 gene has a repeat of 195 bp, called the beta-repeat (cloned in plasmid pCp 31; Jäckle *et al.*, 1982). The BR 2 genes are transcriptionally inactive in an established cell line produced from *C. tentans* embryos (L1 cells, Wyss, 1982). We analysed their chromatin structure by digesting purified nuclei from these cells with micrococcus nuclease. The DNA fragments produced were separated by gel electrophoresis and they were visualized by hybridization on Southern blots (Southern, 1975). Figure 1a shows a typical example of the bulk DNA fragments produced by micrococcus nuclease at 37°C (stained with ethidium bromide). A nucleosomal repeat of 180 bp (± 6 bp) is seen (see also Widmer *et al.*, 1984). In order to eliminate the effects of linker trimming, the size of this repeat and all the other repeats analysed in this paper, were determined by subtracting the sizes of the various multiples from one another (Noll and Kornberg, 1977).

The digestion products of the beta-repeat

Hybridizing the DNA of the gel shown in Figure 1a (nuclei digested at 37°C) with a radioactive probe containing the beta-repeat (plasmid pCp 31) reveals a DNA band ladder of striking sharpness and length (Figure 1b). Whereas in the ethidium bromide-stained gel (Figure 1a) about eight nucleosomal bands are seen, in the BR 2.2 gene ~30 multimeric bands, corresponding to fragments up to ~6 kb, are produced. In order to resolve the large number of bands, the sample in track 2 (15 min digestion) was run on additional gels longer and at higher voltages than in Figure 1b (Figure 1c and d, tracks 2, small fragments have run out of the gel). In Figure 1c and d (tracks r) a DNA sample from L1 cells is shown which has been partially cleaved with the restriction enzyme *CfoI*, which cuts once in the beta-repeat unit. It is obvious that the large bands produced by micrococcus nuclease digestion of nuclei (above the 5-mer) comigrate exactly with the bands of repeat size 195 bp produced with deproteinized DNA by the restriction enzyme. In contrast,

Fig. 1. Gel electrophoretic analysis of the DNA fragments produced by micrococcus nuclease digestion at 37°C of the BR 2.2 (beta-type) chromatin. (a) Ethidium bromide staining. (b–d) Autoradiograms of blots hybridized with beta-type probe. (b) Autoradiogram of the blot of the gel shown in (a). Electrophoresis was for 5 h at 80 V (a,b), for 16 h at 45 V (c) and for 16 h at 80 V (d). Numbers indicate number of repeating units. Square brackets with arrow indicate the transition zone between the two repeats. (1–3) Nuclei of L1 cells were digested at 37°C for 4, 15 and 45 min, respectively, as described in Materials and methods. Lanes **r** show a partial digest of L1 DNA with *Cfo*I. Lanes (M₁–M₃) are marker fragments; (M₁) *Eco*RI–*Hind*III digests of lambda DNA with the following sizes: 21266, 5148, 4973, 4277, 3530, 2027, 1904, 1584, 1330, 983, 831 and 564 bp; (M₂) *Hin*fl digest of pBR322 DNA with the following sizes: 1631, 517/506, 396, 344, 298, 211/220 and 154 bp; (M₃) a mixture of lambda DNA digested with *Eco*RI and of lambda DNA digested with *Hind*III with the following sizes, 23130, 21266, 9416, 7421, 6557, 5804, 5643, 4878, 4361, 3530, 2322, 2028 and 564 bp.

the small nucleosomal fragments, the 4-mer and the 3-mer in Figure 1c, track 2, but also the dimer and monomer (see below, Figure 3) migrate clearly faster than the corresponding bands of the DNA repeat produced with the restriction enzyme. The repeat size of the mono- to tetramers is found to be 179 ($\pm$ 5) bp. There is a transition zone between these two clearly different repeats between the 4-mer and the 6-mer (Figure 1b and c). Instead of the 5-mer a broad, smeared region is revealed within which, depending on the extent of digestion, bands of varying intensity can be recognized. This transition zone is best seen in Figure 1b (arrow, tracks 2 and 3). Scanning of the gels allowed us to determine exactly the lengths of the two repeats, as well as to see details in the transition zone (Figure 2). Peak 5' of the 15-min digest in Figure 2A (from track 2 in Figure 1b) is equal to 905 bp and therefore could correspond to the pentamer of the short repeat, whereas peak 5'' of the 45-min digest in Figure 2B (from track 3 in Figure 1b) is equal to 970 bp and therefore could fit the pentamer of the long repeat.

We repeated the above experiment by digesting nuclei at 0°C (Figure 3). Compatible with the experiment shown in Figure 2, two repeats are seen after long digestion times (Figure 3A). The repeat of the long fragments (above pentamer) fits that of the DNA repeat of 195 bp shown in Figure 3C, whereas that of the mono- to tetramers reveals a repeat of 180–185 bp. However,

after very mild digestion (Figure 3B) an intermediate situation is seen: as expected the sizes of the long fragments correspond to the DNA repeat, but the sizes of the di- to tetramers are equal to 370, 560 and 750 bp, respectively, which is reduced only slightly as compared to the corresponding bands of the DNA repeat (390, 585 and 780 bp, respectively). Thus the repeat of 190 bp appears to be an intermediate between the repeat of the large fragments (195 bp) and the repeat of the small fragments after extensive digestion (180–185 bp) seen in Figure 3A (for summary see Table I). The transition zone in the region of the pentamer is less well visible in Figure 3B than after the longer digestion times in Figure 3A.

Histone H1 stabilizes the nucleosome structure (Thoma and Koller, 1981) and H1 depletion favours nucleosome sliding and rearrangement (Spadafora *et al.*, 1979). We exposed nuclei to 0.5 M NaCl at 0°C to extract histone H1. This procedure leads to disruption and lysis of the nuclei. After centrifugation H1 was found in the supernatant, but not in the pellet (not shown). The H1-depleted nuclei were resuspended in cold digestion buffer and digested with micrococcus nuclease at 0°C. In this case gel electrophoretic analysis (Figure 4) reveals no transition zone. A band pattern with continuously decreasing repeat size, from 195 bp corresponding to the DNA repeat (long fragments) down to 160 bp (short fragments) is seen (see numbers in Figure 4b). Also

Fig. 2. Densitometer scans of autoradiograms. (A) and (B) Scans of tracks 2 and 3 in Figure 1b, respectively. Hybridization with BR 2.2 (beta-type) probe. (C) Scan of track 3 after rehybridization of the blot used in Figure 1b with the BR 2.1 (alpha-type) probe. Numbers indicate number of repeating units. 5' and 5'' designate sub-bands in the pentamer region.

after extensive digestion the repeat size of the short fragments remained unchanged (Figure 4a, track 2).

The digestion products of the alpha-repeat

Blots hybridized with a probe from the alpha-repeat (pCt 63) are shown in Figure 5. (No difference was found, whether nuclei were digested at 37 or at 0°C, not shown). The micrococcus nuclease repeat is again characterized by the sharpness of the bands and the large number of multimeric bands. When the large fragments were resolved after long electrophoresis times (Figure 5b and c) up to 24 bands were seen, corresponding to fragments up to ~5.2 kb. Again the large fragments, in this case above the 11-mer, which now have a repeat of 220 bp, co-migrate exactly with bands produced by partial cleavage of L1 DNA with *Mbo*II which cuts once in the alpha-repeat (tracks r in Figure 5). The small oligomers, in this case up to the 10-mer, migrate faster than the DNA repeat. The oligomer repeat size is 169 (± 9) bp (Figure 5a and b). The scan in Figure 2C, as compared to Figure 2A and B, shows the obvious difference in repeat sizes between the chromatin with the alpha- and the beta-repeat. The transition zone between the two repeats in the alpha-type

Fig. 3. Digestion of BR 2.2 (beta-type) chromatin with micrococcus nuclease on ice. Densitometer scans from the autoradiogram of a blot hybridized with beta-type probe. (A) and (B) Nuclei digested on ice for 6 and 2.5 min, respectively. (C) Partial digestion of L1 DNA with *Hinf*I, which cuts once in the beta-repeat. Electrophoresis was for 5 h and 80 V. Numbers at the bottom give the number of the repeating DNA units.

Table I. Summary of the various repeat lengths observed in the BR 2.2 (beta-type) chromatin

	Long fragments (above pentamer)	Short fragments (below pentamer)	Visibility of transition zone
Digestion at 37°C	195 bp	179 (± 5) bp	+
Digestion at 0°C			
Mild	195 bp	190 bp	+
Strong	195 bp	180–185 bp	(+)
Digestion of H1- depleted nuclei at 0°C	195 bp	decreasing from 195 to 160 bp	–

chromatin is most visible in Figure 5b, track 2 (and see also Figure 2C) between the 10-mer of the micrococcus nuclease repeat (indicated as 10n in Figure 5) and the 10-mer of the DNA repeat (indicated as r_{10} in Figure 5). It consists again of a broad smeared region (bracket with arrowhead in Figure 5), in which faint bands are occasionally detected. In Figure 6 we show scans of Figure 5b. The repeat size of the small oligomers (see B) is seen to be smaller than that of the DNA repeat (C), whereas the larger fragments (see A) fit the DNA repeat exactly. The transition zone between the two repeats is visible as a flat region with no obvious peak.

After H1 depletion the alpha-type chromatin revealed the same repeat below the 11-mer as without H1 depletion (not shown). Because of the rather extended digestion (blot of gel of Figure 4), the region above the 10-mer could not be resolved in these experiments.

Sequence-specific cleavage by micrococcus nuclease

It is well established (Dingwall *et al.*, 1981; Hörz and Altenburger, 1981) that micrococcus nuclease has a high DNA sequence preference. We digested L1 DNA both at 37 and 0°C in the same buffer as used for the digestion of nuclei. After gel electrophoresis and blot hybridization with the probe of the beta-

Fig. 4. Micrococcus nuclease digestion of BR 2.2 (beta-type) chromatin after extraction of nuclei with 0.5 M salt. (a) Autoradiogram of a blot hybridized with beta-type probe. (r) Partial digestion of L1 DNA with *Hinfl*. (1 and 2) Salt-extracted nuclei digested on ice for 2.5 and 6 min, respectively. Electrophoresis was for 5 h and 80 V. (b) Densitometer scans from the autoradiogram shown in (a). (A) Scan of track r. (B) Scan of track 1. Numbers at the top give the differences in base pairs of adjacent peaks. Numbers at the bottom indicate numbers of repeating DNA units.

repeat, no bands but only a continuous smear was found (Figure 7a) regardless of the digestion temperature. In contrast, after hybridization of the same filter with the probe of the alpha-repeat, the same periodicity of 220 bp was seen as after cleaving the DNA with an appropriate restriction enzyme (Figure 7b). This suggests that micrococcus nuclease has one preferred cleavage site within the alpha-repeat.

Discussion

Digestion of nuclei of L1 cells with micrococcus nuclease produces distinct DNA fragments in the BR 2 genes. Upon gel electrophoresis these fragments form a long ladder of multimeric bands (up to ~ 30). The repeat size corresponds exactly to the DNA repeat. Beneath a sharp and prominent transition zone a smaller repeat pattern is seen. In the BR 2.2 gene (beta-repeat) the transition zone appears in the region of the penta-nucleosome, while in the alpha-repeat (as well as in the BR 1 and BR 6, not shown) the transition zone is found in the region where an 11-mer nucleosome is to be expected. The small repeat of the BR 2.2 gene is ~ 180 bp and that of the BR 2.1 gene is ~ 170 bp. Thus in all cases two different repeat sizes are produced.

The two repeats could arise from two different populations of chromatin for each gene. We consider this possibility unlikely since the two repeats do not overlap except possibly in the narrow transition zone. Trimming of residual linker DNA as the main cause for the appearance of the short oligomer repeat can be excluded for several reasons. The effects of trimming are best seen the shorter the oligomeric fragments are, especially for the monomer and dimer fragments (Noll and Kornberg, 1977). Moreover, trimming leads to a continuous reduction in fragment size as compared to the corresponding repeat. However, in our case the transition from the long to the small repeat is not continuous (except in H1-depleted nuclei) but occurs at a very defined

Fig. 5. Micrococcus nuclease digestion of the BR 2.1 (alpha-type) chromatin. (a-c) Autoradiograms of Southern blots hybridized with alpha-type probe. (M_1 , (1), (2) and (3) are as described in Figure 1. (r) Partial digest of L1 DNA with *MboII*. 5n, 10n,... denote oligomer numbers of chromatin digests; r_5 , r_{10} ,... denote oligomer numbers of DNA repeats. The square bracket with the arrow marks the transition zone between the two repeats. Electrophoresis in (a) was for 5 h at 80 V, in (b) for 16 h at 45 V, and in (c) for 16 h at 60 V.

Fig. 6. Densitometer scans from the autoradiogram shown in Figure 5b. (A) and (B) 4- and 15-min digests, respectively. (C) Partial digest of L1 DNA with *MboII*. 5, 10n, r_5 and r_{10} as in Figure 5.

fragment length which is different for the alpha- and the beta-repeat and which is in a size range where trimming effects are hardly visible. The repeat lengths were determined by calculating

Fig. 7. Microcococcus nuclease digestion of L1 DNA. (a) Hybridization with beta-type probe. (b) Hybridization with alpha-type probe. (1 and 2) Genomic DNA digested at 37°C for 2.5 and 6 min, respectively. (3 and 4) Genomic DNA digested at 0°C for 2.5 and 6 min, respectively. (r) Partial digest of L1 DNA with *HinfI*.

the difference between multimers, which has been shown to eliminate trimming effects (Noll and Kornberg, 1977). Moreover, after long digestions similar repeat sizes and the same transition zones were observed irrespective of whether the digestion was performed at 37 or 0°C.

Microcococcus nuclease is known to have preferential cleavage sites on DNA (Dingwall *et al.*, 1981; Hörz and Altenburger, 1981; Pauli *et al.*, 1982; Hörz *et al.*, 1983; Flick *et al.*, 1986). Our digestions of deproteinized DNA show one preferential cleavage site within the alpha-repeat (Figure 7b), as well as (data not shown) within the repeat of the BR 1 and BR 6 regions. However, within the beta-repeat no preferential cleavage site is detected. The production of defined DNA fragments by digestion of chromatin is either due to protection of DNA by nucleosomes or other proteins, or to sequence-specific cutting or to a combination of both. Considering our observations first in the context of microcococcus nuclease sequence specificity, at early times of digestion only those linkers which expose the preferred sequence would be cut. This would clearly result in long DNA fragments of a length with a multiple of the DNA repeat, if there is only one preferential cleavage site per repeat. This explanation is not excluded for the long fragments of the alpha-repeat and the BR 1 and BR 6 regions. However, this explanation is very unlikely for the beta-repeat. Once all the preferential sites in linkers had been cut, non-preferential sites in the residual linkers would be attacked leading to fragment lengths corresponding to the nucleosomal repeat. If this explanation were correct, we would not expect such a sharp well defin-

ed transition zone between the two repeats but rather a large zone of overlap as has been observed in the non-transcribed spacer of the ribosomal chromatin of *Dictyostelium* (Ness *et al.*, 1983). For this reason and because of the absence of visible sequence-specific cutting in the DNA of the beta-repeat, we interpret that in the long chromatin fragments and probably in the intact chromatin the nucleosomes are phased with respect to the DNA sequence. Observations which are explained by phasing of nucleosomes have been reported for the highly repetitive satellite DNA's of rat liver and African Green monkey cells (for reviews see Igo-Kemenes *et al.*, 1982; Reeves, 1984; Eissenberg *et al.*, 1985).

The fact that (except in H1-depleted nuclei) the change in repeat size is abrupt and occurs at a defined chromatin length suggests that a co-operative structural change might be responsible for the change in repeat size. It is well known that upon digestion of nuclei oligonucleosomal particles can be enriched because they are more resistant to nucleases than bulk chromatin (Hozier *et al.*, 1977; Zentgraf and Franke, 1984). We have to consider that fragments produced by digestion might adopt its optimal conformation, which then will influence the subsequent cutting within the new structure. In the BR chromatin such a structural transition appears to occur rapidly as soon as a fragment below a critical size (5-mer or 10-mer) is produced. Except for the critical size, which appears to have a metastable structure leading to the transition zone, the fragments are predominantly built up either of presumably phased or rearranged nucleosomes. One might be tempted to speculate that the fact that the sharp transition occurs around the 5- or the 10-mer reflects the stability of one, respectively two turns of a solenoidal fiber structure (Thoma *et al.*, 1979). However, we emphasize that at this point we do not want to relate our observations to a particular model of chromatin higher order structures. Our results imply that in intact nuclei and presumably in long fragments chromatin exists in a conformation which exhibits a nucleosome-positioning constraint. Upon cleavage below a critical fragment size this constraint would be lost due to possible change in conformation and the nucleosomes therefore would be allowed to occupy different positions. Several possible nucleosome positions have been identified in isolated and in reconstituted nucleosome cores from satellite chromatin implying that a nucleosomal repeat outside the frame of the underlying DNA repeat is not excluded once nucleosomes rearrange (Zhang *et al.*, 1983; Zhang and Hörz, 1984; Böck *et al.*, 1984; Linxweiler and Hörz, 1985; Neubauer *et al.*, 1986). The fact that the transition zone between the two repeats is seen at different fragment lengths for different BR genes suggests structures and nucleosome positions of different local stabilities. An increased stability of the BR 2.2 chromatin as compared to the other BR gene regions is suggested by the fact that the transition in BR 2.2 is at a smaller oligomer size than in the other BR genes.

A rearrangement causing a decrease in repeat size must lead to stretches of free DNA. One could argue that degradation of the free DNA stretches results in a smearing of the bands. However, stretches of free DNA must be rapidly degraded. The extent of smearing depends upon the difference in speed of degradation between the free and the nucleosome-associated DNA. This difference must be considerable, otherwise no nucleosome band pattern could ever be visualized. Moreover, the gels do not allow the amount of background in the region of the fragments with the long repeat to be compared with that in the region of the fragments with the short repeat.

One could expect that the speed of rearrangement is reduced

at 0°C compared to 37°C. In agreement with this expectation (Figure 3) we found that when nuclei were digested on ice, under mild conditions (curve 3B), the repeat size of the small oligomers was only slightly shorter than the DNA repeat and it decreased further upon further digestion (curve 3A). In addition, we have digested nuclei at 0°C which had been washed with 0.5 M NaCl. The observation that under these conditions the short repeat in the BR 2.2 gene decreased even further down to 160 bp is again in favour of nucleosome sliding. Unstable nucleosome positions have been described by Thoma *et al.* (1984) who showed that nucleosomes in the TRP1 gene on a yeast plasmid rearranged upon increasing digestion with micrococcus nuclease. Our results imply a possible effect of micrococcus nuclease (and probably other nucleases) which may occur even under very mild conditions of chromatin digestion (data of BR 2.1, BR 1 and BR 6) and which therefore has to be taken into account when nucleases are used to determine nucleosome positions.

Our results can be interpreted in the sense that they could indicate a mechanism for how nucleosomes could change a given position upon activation during transcription and replication. In both processes topoisomerases appear to be involved (Sundin and Varshavsky, 1980; Fleischmann *et al.*, 1984; Bonven *et al.*, 1985; Kmiec and Worcel, 1985; Muller *et al.*, 1985; Weaver *et al.*, 1985; Gilmour *et al.*, 1986; Ness *et al.*, 1986) which are known to transiently nick the DNA and, therefore, could lead to a change in a nucleosome positioning constraint.

Materials and methods

L1 cells were grown as described by Wyss (1982). Preparation of nuclei was as described by Widmer *et al.* (1984). The pelleted nuclei were washed with buffer A4 (60 mM KCl, 15 mM NaCl, 0.15 mM spermidine, 0.5 mM spermine, 0.1 mM EDTA, 15 mM Tris-HCl, pH 7.4). For the extraction of histone H1 nuclei were resuspended in 0.5 M NaCl, 0.1 mM EDTA, 10 mM Tris-HCl (pH 7.5) at a concentration corresponding to 2 A_{260} /ml. After 10-min on ice the lysed nuclei were pelleted and resuspended in buffer A4 (10 A_{260} /ml).

Digestion of nuclei with micrococcus nuclease

To nuclei in buffer A4 (10 A_{260} /ml) $CaCl_2$ was added to 1 mM. For digestion at 37°C the samples were pre-incubated at 37°C for 2 min, then micrococcus nuclease (Worthington) was added to 40 U/ml. For digestion at 0°C the samples were kept on ice and micrococcus nuclease was added to 3000 U/ml. Digestion of salt-extracted nuclei was carried out on ice with 2000 U/ml. At the times indicated in the figure legends aliquots were removed and the reaction stopped with EDTA and SDS, at final concentrations of 5 mM and 0.5%, respectively.

Digestion of DNA with micrococcus nuclease

Genomic DNA was dissolved in buffer A4 (250 μ g/ml) containing 1 mM $CaCl_2$. For digestion at 37°C, the samples were pre-incubated for 2 min, then micrococcus nuclease was added to 4 U/ml. For digestions on ice an enzyme concentration of 250 U/ml was used.

DNA extraction, electrophoresis in 1.5% agarose gels, Southern blotting onto DBM paper, nick translation of excised insert DNA and hybridizations were performed as described (Widmer *et al.*, 1984). Scanning of X-ray films was carried out on a Shimadzu chromatogram densitometer CD 50.

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References

- Bloom, K.S. and Carbon, J. (1982) *Cell*, **29**, 305–317.
 Böck, H., Abler, S., Zhang, X.-Y., Fritton, H. and Igo-Kemenes, T. (1984) *J. Mol. Biol.*, **176**, 131–154.
 Bonven, B.J., Gocke, E. and Westergaard, O. (1985) *Cell*, **41**, 541–551.

- Cartwright, I.L., Abmayr, S.M., Fleischmann, G., Lowenhaupt, K., Elgin, S.C.R., Keene, M.A. and Howard, G.C. (1982) *Crit. Rev. Biochem.*, **13**, 1–86.
 Dingwall, C., Lomonosoff, G.P. and Laskey, R.A. (1981) *Nucleic Acids Res.*, **9**, 2659–2673.
 Eissenberg, J.C., Cartwright, I.L., Thomas, G.H. and Elgin, S.C.R. (1985) *Annu. Rev. Genet.*, **19**, 485–536.
 Fleischmann, G., Pflugfelder, G., Steiner, E.K., Javaherian, K., Howard, G.C., Wang, J.C. and Elgin, S.C.R. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 6958–6962.
 Flick, J.T., Eissenberg, J.C. and Elgin, S.C.R. (1986) *J. Mol. Biol.*, **190**, 619–633.
 Galler, R., Rydlander, L., Riedel, N., Kluding, H. and Edström, J.-E. (1984) *Proc. Natl. Acad. Sci. USA*, **81**, 1448–1452.
 Galler, R., Saiga, H., Widmer, R.M., Lezzi, M. and Edström, J.-E. (1985) *EMBO J.*, **4**, 2977–2982.
 Gilmour, D.S., Pflugfelder, G., Wang, J.C. and Lis, J.T. (1986) *Cell*, **44**, 401–407.
 Hörz, W. and Altenburger, W. (1981) *Nucleic Acids Res.*, **9**, 2643–2658.
 Hörz, W., Fittler, F. and Zachau, H.G. (1983) *Nucleic Acids Res.*, **11**, 4275–4285.
 Hozier, J., Renz, M. and Nehls, P. (1977) *Chromosoma*, **62**, 301–317.
 Igo-Kemenes, T., Hörz, W. and Zachau, H.G. (1982) *Annu. Rev. Biochem.*, **51**, 89–121.
 Jäckle, H., de Almeida, J.C., Galler, R., Kluding, H., Lehrach, H. and Edström, J.-E. (1982) *EMBO J.*, **1**, 883–888.
 Kmiec, E.B. and Worcel, A. (1985) *Cell*, **41**, 945–953.
 Linxweiler, W. and Hörz, W. (1985) *Cell*, **42**, 281–290.
 Muller, M.T., Pfund, W.P., Miehta, V.B. and Trask, D.K. (1985) *EMBO J.*, **4**, 1237–1243.
 Ness, P.J., Labhart, P., Banz, E., Koller, Th. and Parish, R.W. (1983) *J. Mol. Biol.*, **166**, 361–381.
 Ness, P.J., Parish, R.W. and Koller, Th. (1986) *J. Mol. Biol.*, **188**, 287–300.
 Neubauer, B., Linxweiler, W. and Hörz, W. (1986) *J. Mol. Biol.*, **190**, 639–645.
 Noll, M. and Kornberg, R.D. (1977) *J. Mol. Biol.*, **109**, 393–404.
 Pauli, U.H., Seebeck, T. and Braun, R. (1982) *Nucleic Acids Res.*, **10**, 4121–4133.
 Reeves, R. (1984) *Biochem. Biophys. Acta*, **872**, 343–393.
 Simpson, R.T. (1986) *BioEssays*, **4**, 172–176.
 Southern, E.M. (1975) *J. Mol. Biol.*, **98**, 503–517.
 Spadafora, C., Oudet, P. and Chambon, P. (1979) *Eur. J. Biochem.*, **100**, 225–235.
 Sümegei, J., Wieslander, L. and Daneholt, B. (1982) *Cell*, **30**, 579–587.
 Sundin, O. and Varshavsky, A. (1980) *Cell*, **25**, 659–669.
 Thoma, F. (1986) *J. Mol. Biol.*, **190**, 177–190.
 Thoma, F. and Koller, Th. (1981) *J. Mol. Biol.*, **149**, 709–733.
 Thoma, F. and Simpson, R.T. (1985) *Nature*, **315**, 250–252.
 Thoma, F., Koller, Th. and Klug, A. (1979) *J. Cell Biol.*, **83**, 403–427.
 Thoma, F., Bergmann, L.W. and Simpson, R.T. (1984) *J. Mol. Biol.*, **177**, 715–733.
 Weaver, D.T., Fields-Berry, S.C. and DePamphilis, M.L. (1985) *Cell*, **41**, 565–575.
 Widmer, R.M., Lucchini, R., Lezzi, M., Meyer, B., Sogo, J.M., Edström, J.-E. and Koller, Th. (1984) *EMBO J.*, **3**, 1635–1641.
 Wieslander, L., Sümegei, J. and Daneholt, B. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 6956–6960.
 Worcel, A., Gargiulo, G., Jessee, B., Udvardy, A., Louis, C. and Schedl, P. (1983) *Nucleic Acids Res.*, **11**, 421–439.
 Wyss, C. (1982) *Exp. Cell Res.*, **139**, 309–319.
 Zentgraf, H.W. and Franke, W.W. (1984) *J. Cell Biol.*, **99**, 272–286.
 Zhang, X.Y. and Hörz, W. (1984) *J. Mol. Biol.*, **176**, 105–129.
 Zhang, X.Y., Fittler, F. and Hörz, W. (1983) *Nucleic Acids Res.*, **11**, 4287–4306.

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