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Structure of the Chromosomal Copy of Yeast ARS1†

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Received September 28, 1987; Revised Manuscript Received January 20, 1988

ABSTRACT: We have used deoxyribonuclease I (DNase I) and methidium-propyl-EDTA·Fe(II) digestion to characterize the chromosomal structure of the single-copy autonomously replicating sequence ARS1. The major feature of this chromatin is a region of strong hypersensitivity to both cleavage agents. The hypersensitive region contains most of the DNA sequences which have been suggested by in vitro mutagenesis studies [Celniker, S., Sweder, K., Srienc, F., Bailey, J., & Campbell, J. (1984) *Mol. Cell. Biol. 4*, 2455–2466] to be important in ARS function. It lies at the downstream end of the *TRP1* gene. A chromosomal DNase I footprinting analysis was carried out on the hypersensitive region. These data give direct evidence for several localized DNA/protein contacts within the hypersensitive region. The most prominent of these chromatin-dependent contacts is located on the functionally most important 11 base pairs of ARS DNA. On the *TRP1* side of the hypersensitive region, there are positioned nucleosomes. On the other side of the hypersensitive region, there is a complex (and possibly heterogeneous) structure.

Functionally important regions like centromeres, telomeres, replication origins, and gene regulatory sequences appear to be marked in the chromosome by anomalous chromatin structures. For example, centromeres and telomeres show regions of strong nuclease protection surrounded by precisely positioned nucleosomes (Bloom & Carbon, 1982; Palen & Cech, 1984). Gene regulatory sequences (Elgin, 1982) and replication origins are regions of nuclease hypersensitivity (Varshavsky et al., 1978; Scott & Wigmore, 1978; Borchsenius et al., 1981; Palen & Cech, 1984). The hypersensitive regions around replication origins have received less attention than the hypersensitive regions associated with gene regulatory sequences.

DNA sequences which are able to confer autonomous replication on a plasmid have been identified in yeast [cf. Stinchcomb et al. (1979)]. For this and other reasons (Williamson, 1985; Brewer & Fangman, 1987), these autonomously replicating sequence (ARS)[1] elements are strong candidates for origins of yeast DNA replication.

Chromatin structures of two ARS elements, 2 μm (Livingston, 1982; Veit & Fangman, 1985) and ARS1 (Thoma et al., 1984; Long et al., 1985), have been analyzed. ARS1 is the more thoroughly studied of the two. Using micrococcal nuclease and DNase I, Thoma et al. (1984) concluded that the ARS1 region contains precisely positioned nucleosomes lying on both sides of a central nuclease-hypersensitive region. Long et al. (1985), using only MNase digestion, confirmed nucleosome positioning in one of the regions described by Thoma et al. (1984) but concluded that the other region is nonnucleosomal.

In both of the previous analyses, ARS1 was present on an extrachromosomal plasmid, the *TRP1*–ARS1 circle. Since ARS1 is normally located on the chromosome, it is of interest to determine the structure of the chromosomal copy and to compare it to the structure of the plasmid-associated ARS. This comparison is of general interest, for studies of other eukaryotic replication origins have also involved nonchromosomal origins, e.g., SV40 or polyoma viral origins (Varshavsky et al., 1978; Scott & Wigmore, 1978), or the extrachromosomal rDNA origins of *Tetrahymena* (Borchsenius et al., 1981; Palen & Cech, 1984). This paper describes such a chromosomal analysis of yeast ARS1.

In addition to the differences in subject (chromosomal copy instead of a plasmid-associated ARS), the methodologies used in this study differ in several ways from those used in previous analyses of ARS1. First, we analyze the ARS when present in single copy. This avoids the possibility of structural heterogeneity arising from the presence of multiple copies. In previous analyses of ARS chromatin structure, the ARS sequences were present in multiple copies. Second, this analysis is carried out with yeast nuclei. Previous analyses of ARS1 were carried out on whole cell lysates. Lastly, we use DNase I and the sequence neutral cleavage agent MPE·Fe(II) (Hertzberg & Dervan, 1984) to lessen the problems associated with sequence-specific digestion.

† Supported by U.S. Public Health Service Research Career Development Award Ca00911 and Research Grant GM37788 to D.L. and by Research Grant GM27925 to T.T.

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[1] Abbreviations: bp, base pair(s); DNase I, deoxyribonuclease I (EC 3.1.4.5); MPE·Fe(II), methidium-propyl-EDTA·Fe(II); MNase, micrococcal nuclease (EC 3.1.4.7); ARS1, autonomously replicating sequence 1; EDTA, ethylenediaminetetraacetic acid; Hepes, *N*-(2-hydroxyethyl)-piperazine-*N*′-2-ethanesulfonic acid.

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FIGURE 1: *TRP1*–ARS1 region on chromosome IV in yeast. The *TRP1* gene and its direction of transcription are shown by the wavy forward arrow. Several restriction sites used in the work are identified: A = *Alu*I; D = *Dde*I; R = *Eco*RI; H = *Hin*dIII; T = *Taq*I. The rightmost *Dde*I site lies at 1076 bp. The *Taq*I site shown lies at 578 bp. All features (restriction sites, the *TRP1* gene, etc.) are located to scale on this drawing. Tic marks are shown every 200 bp from 0, the *TRP1* proximal RI site, either above (200, 400, 600, 800, and 1000 bp) or below (1200 and 1400 bp) the main line. The *GAL3* in parentheses refers to the fact that the 5′ end of the *GAL3* gene lies just downstream of this RI site. Other symbols are defined in the text.

Materials and Methods

We use methods described in some detail in Lohr (1987). Briefly, spheroplasts are prepared from log-phase [(1–4) × 10^7 cells/mL] or stationary (>20 × 10^7 cells/mL) cells using mercaptoethanol pretreatment and Zymolyase-100, or oxalyticase (Enzogenetics, Corvallis, OR). Spheroplasts are lysed in 18% Ficoll as described by Wintersberger et al. (1973). After a brief (5 min) slow spin (2000 rpm, SS34 Sorvall) to remove unspheroplasted cells, the nuclei are pelleted from the supernatant by a 20-min centrifugation at 30000*g*. The tube is rinsed out once with digestion buffer (1 M sorbitol, 5 mM Hepes, pH 6.5, and 0.05 mM Ca^{2+}), and then the nuclei are resuspended at ~300 μg/mL DNA. For DNase I digestions, $MgCl_2$ to 0.5 mM and DNase I to 2–20 units/mL are added. Digestion is continued for 15–200 s to obtain a series of samples varying in extent of digestion. MPE·Fe(II) digestions are carried out basically as described by Cartwright et al. (1983) at 4–8 μM MPE·Fe(II), 1–2 mM H_2O_2, and 0.6 mM EDTA. Dithiothreitol or sodium ascorbate is added to 2 μM to initiate the reaction, and samples are collected at various times (1–15 min). DNA from the chromatin digests was extracted as described previously and redigested with restriction enzymes (Lohr & Hopper, 1985). In some of the MPE·Fe(II) digests, we treated the purified DNA with S1 nuclease before restriction (Cartwright et al., 1983). Results (autoradiograms) from these digests looked similar to those without S1 treatment. For naked DNA control digests, DNA was resuspended at 300 μg/mL and digested at 0.04 unit/mL DNase I or 1 μM MPE for 15–90 s and then isolated and treated with restriction enzymes. After restriction digestion, the DNA from all samples was reisolated and electrophoresed on 2.5–3.0% polyacrylamide/0.6% agarose nondenaturing gels (Figures 2 and 3) or 4–5% polyacrylamide/7 M urea/0.6% agarose denaturing gels (cf. Figure 5). These gels are described in Lohr and Hopper (1985). DNA was electrophoretically transferred to DBM paper. This paper was hybridized with various ^{32}P end-labeled repaired probes, all as described in Lohr and Hopper (1985). Autoradiograms are taken on Kodak X-OMAT R preflashed film with Lightning Plus intensifying screens at −80 °C. Several strains were used in this work: D585-11C; 21R,7d (Johnston & Hopper, 1982).

Results and Discussion

The 1453 bp between *Eco*RI sites define the *TRP1*–ARS1 region ("R", Figure 1). Throughout this paper, locations (of chromatin cleavage sites, restriction enzyme sites, etc.) are given in base pairs from the leftmost RI site, upstream of *TRP1*. The gene *TRP1* is contained from 103 to 777 bp. DNA from 615 to 1453 bp was originally shown to confer on a yeast plasmid the property of autonomous replication

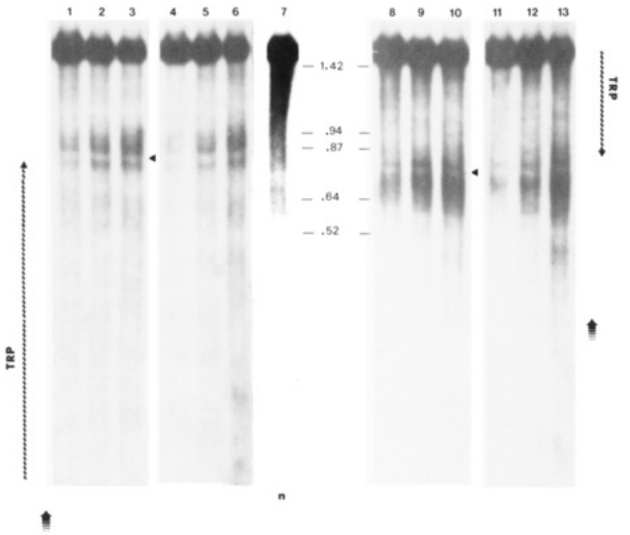

FIGURE 2: DNase I hypersensitive sites in the *TRP1*–ARS1 region. Cleavage sites were mapped toward the *GAL3* side of the *TRP1*–ARS1 region (to the right in Figure 1) from the RI site upstream of *TRP1* using the 95 bp RI–*Alu*I probe (lanes 1–7) or toward the *TRP1* gene (to the left in Figure 1) from the *GAL3* proximal RI site using the 377 bp RI–*Dde*I probe (lanes 8–13) on nondenaturing polyacrylamide/agarose composite gels. Lanes 1–3 and 8–10 are time courses of digestion of nuclei from early log-phase D585-11c cells grown in glucose; lanes 4–6 and 11–13 are time courses of digestion of nuclei from stationary-phase D585-11c cells grown in glucose. Lane 7 shows a naked DNA digest ("n"). Sizes (in kilobases) of some of the phage PM2/*Hae*III restriction fragments used for size standards on the gel are shown. The location of the end of the probe used for mapping is shown by the upward arrows. Electrophoresis is from top to bottom. The *TRP1* transcription unit is marked by wavy arrows. Other symbols are identified in the text.

(Stinchcomb et al., 1979). Subsequent work (Celniker et al., 1984) has suggested that DNA from 750 to 1100 bp contains the sequences needed for ARS function.

DNase I Hypersensitivity. Hypersensitivity was mapped between the two *Eco*RI sites bounding the ARS1 region (Figure 1), by using a 95 bp RI–*Alu*I probe or a 345 bp RI–*Dde*I ("→", Figure 1) to map from the RI stie upstream of *TRP1* or a 377 bp RI–*Dde*I probe ("←", Figure 1) to map from the *GAL3* proximal RI site. In digests from log-phase cells (D855-11C), all probes detect a strong area of hypersensitivity (Figure 2, tracks 1–3 and 8–10). The hypersensitive region lies from ~700 to ~900 bp from the *TRP1* proximal RI site. It thus lies immediately downstream of and includes part of the *TRP1* coding sequence. The region is not hypersensitive in naked DNA digests (Figure 2, track 7). The hypersensitivity is thus a chromatin feature. The hypersensitive region is constitutive: present in chromatin from log-phase or stationary cells (Figure 2, tracks 4–6 and 11–13); from cells grown in galactose, glycerol/ethanol, or dextrose (not shown); in all the several yeast strains analyzed.

Thoma et al. (1984) found this same hypersensitive region in the plasmid-associated ARS1. The plasmid digests also showed another strong hypersensitive region, between 1300 and 1450 bp and between 0 and 100 bp, using the numbering system in Figure 1. The plasmid is circularized at the RI site, so these two regions are contiguous.

In our analysis, neither of these regions is strongly hypersensitive. Since the analysis includes very brief extents of digestion, the lack of strong hypersensitivity in these regions is not due to overdigestion. Hypersensitivity in these regions is apparently not masked by their proximity in the autoradiograms to the very dark band of unit-length DNA; in fainter exposures, in which the unit-length band is much less intense,

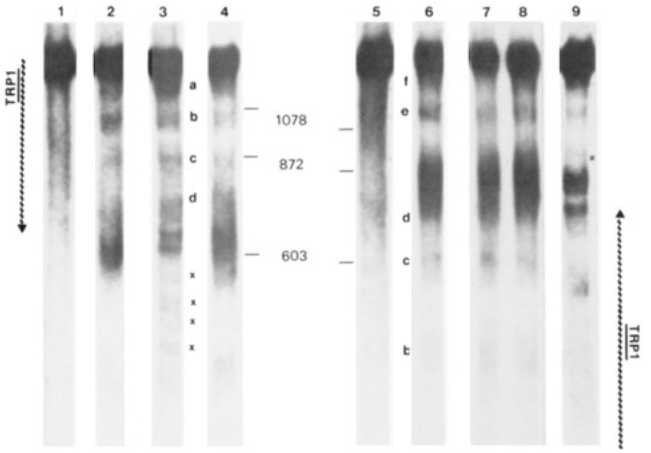

FIGURE 3: MPE·Fe(II) cleavage sites within the *TRP1*–ARS1 region. Cleavage sites were mapped from the *GAL3* proximal RI site using the 377 bp RI–*Dde*I probe described in Figure 2 (lanes 1–4) or from the *TRP1* proximal RI site (lanes 5–9) using the 345 bp RI–*Dde*I fragment. Lanes 1 and 5 show MPE·Fe(II) naked DNA digests; lanes 2–4 and 6–8 show MPE·Fe(II) chromatin digests; lane 9 shows a DNase I chromatin digest. Electrophoresis is from top to bottom. In these gels, ϕX–*Hae*III restriction fragments were used for size markers. The sizes of several of these and their gel mobilities (–) are shown. Lanes 1–4 and 5–9 are from different gels, so the marker fragments are at slightly different locations in the two. The *TRP1* gene is shown by wavy arrows. Other symbols are described in the text. Several MPE·Fe(II) digests are shown, from several different yeast strains and different extents of digestion, to demonstrate the variability in these digests.

these regions show no intensity while the 700–900 bp hypersensitive region can be seen (not shown). Therefore, the strong hypersensitivity in the 0–100 bp and 1300–1450 bp regions reported by Thoma et al. (1984) appears to be plasmid related. Note that the precise level of intensity in this region of the chromosome is strain dependent (cf. Figure 2, track 6, versus Figure 3, track 9). This may also contribute to the difference between our results and those of Thoma et al. (1984).

The very pronounced "coldspot" ~20–25 bp wide within the chromatin hypersensitive region (arrowheads, Figure 2, tracks 3 and 10) suggests protein binding to this region. A direct analysis using chromosomal DNase I footprinting does clearly show protein binding to the hypersensitive region (see below).

MPE·Fe(II): *Hypersensitivity*. In MPE·Fe(II) digests, the strongest cleavage occurs in the same general region of chromatin which is hypersensitive to DNase I, ~700–900 bp from the *Eco*RI site upstream of *TRP1* (cf. Figure 3, tracks 2–4 and 6–8). There is some digest to digest variability in the precise size and appearance of the MPE·Fe(II) hypersensitive region (Figure 3). However, it is generally more extensive than the DNase I hypersensitive region, particularly on the *GAL3* proximal side (cf. Figure 3, tracks 8 versus 9). The MPE·Fe(II) hypersensitive region is a chromatin feature, for it is absent in naked DNA digests (Figure 3, tracks 1 and 5).

MPE·Fe(II): *Nucleosome Positioning*. Analyses of nucleosome positions around ARS1 in plasmids (Thoma et al., 1984; Long et al., 1985) are based on micrococcal nuclease digests. Interpretation of such digests is complicated by the very high preference of MNase to cleave specific sequences of DNA (Dingwall et al., 1981; Hörz & Altenburger, 1981). In the chromosomal analysis, we have used MPE·Fe(II) (Hertzberg & Dervan, 1984) to examine the question of nucleosome positions around the ARS. MPE·Fe(II) has little or no sequence specificity and thus has been very useful in nucleosome positioning studies in other systems [cf. Cartwright et al. (1983)].

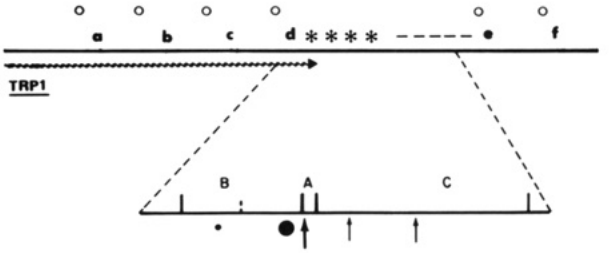

FIGURE 4: Nucleosome positions and other DNA/protein contacts on the *TRP1*–ARS1 region of chromosome IV. MPE·Fe(II)-accessible DNA regions occurring at nucleosome intervals are labeled a–f. These data come from results of the type shown in Figure 3 and correspond to the MPE·Fe(II)-accessible regions so labeled in that figure. The MPE·Fe(II) hypersensitive region is shown by the four asterisks. The anomalous region on the *GAL3* side of the hypersensitive region is shown by the dashed line. For comparison purposes, some of the nucleosome spacer locations from the work of Thoma et al. (1984) and Long et al. (1985) are shown (o). We show their data only for those regions for which our data suggest nucleosome presence. The data to the right of the hypersensitive region reflect data from Thoma et al. (1984) only. The *TRP1* gene is shown by the wavy arrow. Below is an expanded map of the central part of the *TRP1*–ARS1 region. The three separable domains of ARS function from the work of Celniker et al. (1984) are located (A–C). DNase I protections (↑) or enhancements of cleavage in chromatin (●) from chromosomal footprinting experiments of the type shown in Figure 5 are located. The stronger the protection, the heavier the arrow or dot.

Outside the hypersensitive region, major MPE cleavage occurs in three regions on the *TRP1* side ("a–c" track 3, Figure 3) and in two regions on the *GAL3* proximal side ("e–f" track 6, Figure 3). These cleavages are most clearly visible in the higher molecular weight regions of the autoradiograms where hybridization efficiency is higher (Lohr, 1981), but bands in the lower molecular weight ranges can also be seen (cf. Figure 3, track 6). There is again some digest to digest variation, mainly in the intensity of the cleavages (Figure 3). Note that the location of the uppermost cleavage site ("f", Figure 3, track 6) is somewhat uncertain due to its proximity to the unit-length band. This cleavage is not well resolved in track 7, probably due to overdigestion.

DNase I profiles also show weak bands outside the hypersensitive region (cf. Figure 3, track 9). These are usually located at the same positions as the cleavages in MPE·Fe(II) digests. The most consistent exception occurs in the *TRP1* gene, in the region immediately adjacent to the hypersensitive region (cf. Figure 3, track 8 versus 9).

The results of a number of size determinations from MPE·Fe(II) digests are summarized in Figure 4. Distances between MPE·Fe(II)-susceptible regions a and b (=150 bp) and b and c (=150 bp) on the *TRP1* gene are consistent with nucleosome presence. The distance between sites c and d is consistently a bit shorter than a nucleosomal core particle (=130–140 bp). However, this region probably contains a nucleosome. The smaller size-protected region could reflect a nucleosome which is more open to cleavage than a typical core particle. Alternatively, the smaller size could be due to ambiguity in locating site "d". This is measured as the edge of the hypersensitive region and is more difficult to locate unambiguously than the internucleosomal regions. Thus, on the chromosomal *TRP1* gene, MPE·Fe(II) digestion gives evidence for positioned nucleosomes, in agreement with the MNase results of Thoma et al. (1984) and Long et al. (1985) for *TRP1* in plasmids. The ARS1 proximal nucleosome in *TRP1* may have an anomalous structure.

The *TRP1* nucleosome locations we find differ somewhat from the *TRP1* nucleosome locations determined by previous workers (Figure 4). Whether this reflects differences in the

two templates used (chromosomal versus plasmid) or in the analysis techniques [MPE·Fe(II) versus NMase susceptibility] is not clear. Thus, we cannot judge the significance of these location differences. It seems more important that both in the plasmid and in the chromosome there are positioned nucleosomes on *TRP1*.

The *GAL3* proximal side of the hypersensitive region produced a discrepancy between the two analyses of plasmid-associated ARS1. Thoma et al. (1984) suggested this region has three nucleosomes; Long et al. (1985) suggested it is nonnucleosomal. We find an intermediate result. Sites e and f lie 150 bp apart, clearly suggesting a nucleosome. Note the reservation discussed above involving the location of site f. However, site e to the boundary of the hypersensitive region spans ~200–250 bp. This is inconsistent with the yeast nucleosome size of ~170 bp (Lohr et al., 1977). Furthermore, this region shows complex digestion patterns. There is sometimes weak DNase I or MPE·Fe(II) cleavage within the region (cf. "×" in Figure 3, track 3). In some digests, DNase I sensitivity extends into this region from the normal hypersensitive region ("×", Figure 3, track 9). The large size of the "protected" region and its complex digestion make it unlikely that this region is simply nucleosomal in structure.

It is interesting that both of the chromatin regions lying immediately adjacent to the hypersensitive region show anomalies in their digestion patterns. This may reflect an interaction of these regions with (components of) the hypersensitive region. Some of the complexity in these immediately adjacent regions, particularly on the *GAL3* proximal side, may also be due to heterogeneity in chromatin structure. Since we have analyzed a single-copy ARS1, such heterogeneity would have to be in the cell cycle stage or other cellular process, rather than heterogeneity involving copy number.

Chromosomal DNase I Footprinting. In order to characterize more fully the chromatin structure within the hypersensitive region, we carried out a DNase I chromosomal footprinting analysis of the region lying between 770 and 1000 bp from the *Eco*RI site upstream of *TRP1* (Figure 5). DNA from DNase I digests of nuclei (and of naked DNA) was isolated and restricted to completion with *Taq*I. These DNA samples were electrophoresed on a polyacrylamide/agarose/7 M urea denaturing gel (Lohr & Hopper, 1985) to obtain a single strand pattern. DNA was electrophoretically transferred to DBM and the paper hybridized with a 55 bp *Hin*dIII–*Dde*I probe (Figure 1). The resultant autoradiogram gives a pattern of DNase I protected and exposed DNA bands. The sizes of these bands correspond to their distance in base pairs from the *Taq*I site at 578 bp (Figure 1). Comparison of patterns from the naked DNA and chromatin digests allows a direct assessment of the DNA/protein contacts on this region of the chromosome in isolated nuclei. Such an analysis has not been previously applied to ARS or extrachromosomal replication origins.

We find three regions of strong cleavage in naked DNA digests ("+", Figure 5, track n) which are very weakly cleaved in chromatin (Figure 5, tracks c). These lie at 860 bp, at 900 bp, and at 960–980 bp. These chromatin-dependent protections demonstrate that there is protein binding to the DNA in these regions of the chromosome. The region at 860 bp is the most strongly protected of the three. There is also weak protection extending somewhat beyond (upward in the gel) 980 bp. The 860 and 900 bp contacts lie within the DNase I hypersensitive region.

There are two regions which show enhanced cleavage in chromatin ("+", Figure 5, track c) compared to naked DNA,

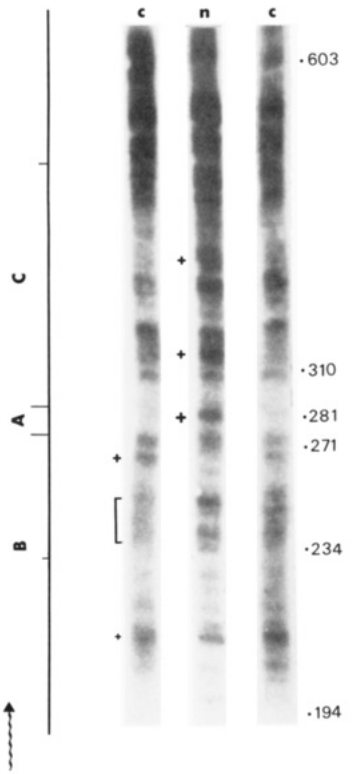

FIGURE 5: Chromosomal footprint of the 3′ end of *TRP1* and the adjacent ARS region. DNase I cleavage sites were mapped from the *Taq*I site at 578 bp (Figure 1) using the 55 bp *Hin*dIII–*Dde*I probe. Note that the probe was made by repair synthesis of the probe ends using [^{32}P]dATP, [^{32}P]dTTP, and reverse transcriptase (Goodman, 1980). This *Dde*I site has three repairable nucleotides while the *Hin*dIII site has one. Thus, the footprint is not strand specific. Lane 2 shows a naked DNA digest ("n"); lanes 1 and 3 are chromatin digests ("c"). Sizes of the restriction marker fragments used (ϕX–*Hae*III) are shown. Electrophoresis is from top to bottom. "+" to the left of track 2 shows sites of strong cleavage in naked DNA which are protected in chromatin digests; "+" to the left of track 1 shows regions of cleavage enhancement in chromatin. The bracketed region is discussed in the text. There is some uncertainty in the extent of the B domain (Celniker et al., 1984); the boundary of the minimal B domain (46 bp wide) is shown by a tic mark. The 3′ end of *TRP1* is shown by the wavy arrow. These profiles are from 21R cells.

at 790 and 850 bp. Such features also suggest chromosomal protein/DNA interactions, on or around these DNA sequences. The footprint differences at ~790 bp may be related to the proximity of the *TRP1* termination region.

Between each of the DNA/protein contacts (i.e., a protected or an enhanced cleavage in chromatin), there is one (or more) band(s) where the chromatin and naked DNA tracks show similar intensity. Thus, the protein contacts must be localized to small regions of DNA.

The distance over which the contacts are spread suggests the involvement of multiple protein factors. However, the two most striking features in the profile, the protection at ~860 bp and the enhancement at ~850 bp, lie close together. It is possible that a single factor could be responsible for both of these effects. However, even in this case, there is DNA between the two features which is unaffected by the factor(s).

In some regions (cf. the bracketed area, Figure 5) of the chromatin digest, bands are less well resolved than the bands in the same region of the naked DNA digest. This feature may also reflect DNA/protein interactions; an enhanced cleavage in a region of chromatin could diminish the relative clarity of the strongly cut naked DNA bands in the parts of the chromatin profiles corresponding to this region.

Note that the sizes of the DNA bands, and thus the locations of DNA/protein contacts, were determined by comparison to

the mobilities of restriction fragments and are not known to single-nucleotide precision. However, the locations of contacts up to about 900 bp should be quite accurate because there are several restriction fragments corresponding to bands in this size range. Above that, the locations are less certain.

Figure 5 shows two chromatin profiles digested to extents which bracket the extent of digestion in the naked DNA digest. This illustrates the point that the footprint features described here are general features, present at any extent of digestion suitable for these mapping studies. These features are also present in a variety of yeast strains (not shown). There are other differences between the chromatin and naked DNA profiles (cf. Figure 5), but these are minor compared to the differences above and/or are strongly digestion extent dependent.

Celniker et al. (1984) have defined the regions essential for ARS1 function by in vitro mutagenesis. These regions are located on the map shown in Figure 4 and on the footprint in Figure 5. The most highly conserved and the most essential region, A, lies from 857 to 868 bp. This region thus contains the naked DNA band (~860 bp) protected most strongly in chromatin digests. We would suggest that this footprint feature could reflect chromosomal binding of a protein involved in the function of ARS1. In agreement with this suggestion, Brewer and Fangman (1987) have recently mapped the origin of replication in ARS1-containing plasmids to lie near the *Bgl*II site in ARS1, which is at 853 bp.

Regions surrounding the ARS also play an important role in ARS function (Celniker et al., 1984): a 46–109 bp region, "B", on the *TRP1* side of region A; an ~200 bp region, "C", on the opposite side of region A. Domain B is located from 740 or 803 to 857 bp and domain C from 868 to 1068 bp with respect to the *Eco*RI site upstream of *TRP1*. Domain B includes sites of enhanced cleavage in chromatin (790 and 850 bp) and a region where band resolution is strongly affected in chromatin digests (~815–845 bp). Two of the chromatin-protected regions (900 and 960–80 bp) lie in domain C. The DNase I/MPE·Fe(II) hypersensitive region includes all of domains A and B and some of domain C.

In summary, the ARS1 region when present in the chromosome is characterized by several structural features. It is strongly DNase I and MPE·Fe(II) hypersensitive; it is the only hypersensitive region within a stretch of at least 1450 bp. The hypersensitive region of ARS1 is not nucleosome covered but does contain (localized) DNA/protein interactions. There are clearly positioned nucleosomes on the *TRP1* side of ARS1. The other side has an anomalous structure. The general organizational features of ARS1 (hypersensitive region downstream of *TRP1*, positioned nucleosomes on *TRP1*) appear to be similar in the chromosome and the plasmid.

ACKNOWLEDGMENTS

We thank Dr. Peter Dervan for a generous gift of MPE·Fe(II) and D. Wolf and A. Leon for typing the manuscript.

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