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Transcription-induced nucleosome 'splitting': an underlying structure for DNase I sensitive chromatin

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Utilizing yeast strains containing promoter mutations, we demonstrate that transcription of the *HSP82* gene causes nucleosomes toward the 3'-end to become DNase I sensitive and 'split' into structures that exhibit a 'half-nucleosomal' cleavage periodicity. Splitting occurs even when only a few RNA polymerase II molecules are engaged in basal level transcription or during the first round of induced transcription. The split nucleosomal structure survives nuclear isolation suggesting that it may be stabilized by post-translational modifications or non-histone proteins, and may require DNA replication for reversal to a whole nucleosomal structure. Split nucleosomes represent a structure for DNase I sensitive chromatin and are probably of common occurrence but difficult to detect experimentally. We suggest that transient positive supercoils downstream of traversing RNA polymerase lead to nucleosome splitting.

Key words: chromatin/DNase I sensitivity/gene regulation/heat shock/nucleosome

Introduction

A wide variety of experimental approaches indicate that in eukaryotic cells, active genes are associated with nucleosomes, both upstream and downstream of traversing RNA polymerase II molecules. For example, the particles released from actively transcribed immunoglobulin genes of plasmacytoma cells upon micrococcal nuclease treatment of nuclei comigrate with nucleosomes during gel electrophoresis (Huang *et al.*, 1986). Histones can be chemically crosslinked to heavily transcribed heat-shock inducible genes in *Drosophila* nuclei (Solomon *et al.*, 1988; Nacheva *et al.*, 1989). Immunoelectron microscopic studies reveal that the nucleosome beads upstream and downstream of transversing RNA polymerase II molecules contain core histones (McKnight *et al.*, 1978). Microinjection of antibodies against core histones inactivates transcription (Einck and Bustin, 1983). Finally, psoralen crosslinking experiments reveal that transcribing SV40 minichromosomes have a nucleosomal organization (De Bernardin *et al.*, 1986). Thus, while it is formally possible that histones become transiently displaced at the point of RNA polymerase II passage (see Lorch *et al.*, 1988; Jackson, 1990), it seems clear that the surrounding environment exhibits a nucleosomal structure.

A priori, one might predict that the topologically constrained DNA associated with histone octamers within nucleosomes would impede RNA polymerase movement. Therefore, the chromatin fiber itself would be expected to undergo conformational alterations to facilitate transcription. In fact, it has been known for many years that transcriptionally active or potentially active genes have an increased DNase I sensitivity in chromatin (Weintraub and Groudine, 1976), suggesting that their corresponding chromatin fibers are conformationally more open than those of inactive genes. Such an increased DNase I sensitivity also appears to be associated with torsional stress within active chromatin domains (Villeponteau and Martinson, 1987). It is, however, still ambiguous whether such changes are a cause or an effect of transcription. Furthermore, although it is clear that the histone–DNA interactions and compositions of histones associated with active genes are altered (see Discussion), the nature of these alterations at the level of nucleosome structure is not known.

Here, we address the question of how transcription affects chromatin structure, utilizing yeast strains containing engineered promoter mutations altered in transcription of the heat-shock inducible gene, termed *HSP82*. We demonstrate that transcription causes the DNA sequence-positioned nucleosomes associated with the *HSP82* gene to become DNase I sensitive and exhibit a 'half-nucleosomal' cleavage periodicity, which we define here as nucleosome 'splitting'. Such splitting occurs during the onset of transcriptional induction, and the structures are stable to nuclear isolation. Split nucleosomes are probably of general occurrence and represent a molecular basis for the preferential DNase I sensitivity of active genes in chromatin. We suggest that positive supercoiling ahead of the transcription apparatus splits nucleosomes to pave the way for RNA polymerase passage.

Results

Transcriptionally variegated alleles of the yeast HSP82 gene

In the present investigation, we address the role of transcription on generating DNase I sensitivity and nucleosome alterations in chromatin. We focus on the yeast heat-shock inducible *HSP82* gene whose chromatin structure and transcriptional regulation have been well characterized previously (Szent-Gyorgyi *et al.*, 1987; McDaniel *et al.*, 1989) and utilize haploid strains containing promoter mutations. We also analyze the chromatin structure of *CIN2*, a flanking gene that affects microtubule function (Stearns *et al.*, 1990; T.Stearns and D.Botstein, personal communication) whose transcription is repressed by heat-shock (see below). Figure 1 summarizes the physical maps and transcription patterns of these genes in the three yeast strains that we have employed. The yeast strain carrying the wild type heat-shock gene, designated HSP(WT), exhibits upon heat-shock an ~16-fold increase in the level of *HSP82* gene

mRNA concomitantly with a corresponding drop in *CIN2* gene transcripts. The strain designated HSP(P2) contains a 2 bp change within the promoter that eliminates basal level but not heat-shock inducible transcription (McDaniel *et al.*, 1989), while the strain termed HSP(URA) contains an *HSP82* gene inactivated by replacement of its 5′-half with the *URA3* gene. In both mutant strains the *CIN2* gene is still repressible by heat-shock (Figure 1). To summarize the properties of these transcriptionally variegated alleles of the *HSP82* gene: HSP(WT) is both basally and inducibly transcribed, HSP(P2) lacks basal transcription but is inducible, and HSP(URA) is completely inactive.

Promoter mutations that eliminate transcription abolish DNase I sensitivity within the HSP82 gene

To examine the relationship between transcription and DNase I sensitivity, we prepared nuclei from control and heat-shocked cells and mapped the nuclease cleavage sites at low resolution by indirect end-labeling (Wu, 1980). As shown in Figure 2 (filled bars), the 3′-region of the *HSP82* gene of HSP(WT) cells is markedly sensitive to DNase I relative to the coding region of the *CIN2* gene, either before (−) or after (+) induction. The 3′-end of the *CIN2* gene, however, possesses nuclease hypersensitive sites (filled bars). In marked contrast, the *HSP82* gene of HSP(URA) cells lacks heightened DNase I sensitivity but exhibits a hypersensitive site at its 3′-end (Figure 2, arrow) either before (−) or after (+) heat-shock induction. In strain HSP(P2), the chromatin structure of the *HSP82* gene before heat shock (−) resembles that seen in HSP(URA) cells, while after heat shock (+) its structure parallels that seen in HSP(WT) (Figure 2). Thus, mutations that eliminate *HSP82* gene transcription abolish DNase I sensitivity in the corresponding chromatin. Furthermore, even the relatively weak, basal level transcription in HSP(WT) cells is linked to the nuclease sensitive state.

DNase I sensitivity is associated with a 'half-nucleosomal' cleavage periodicity

To examine alterations in the chromatin structure of the *HSP82* gene in more detail, we performed indirect end-labeling experiments at higher resolution. As shown in

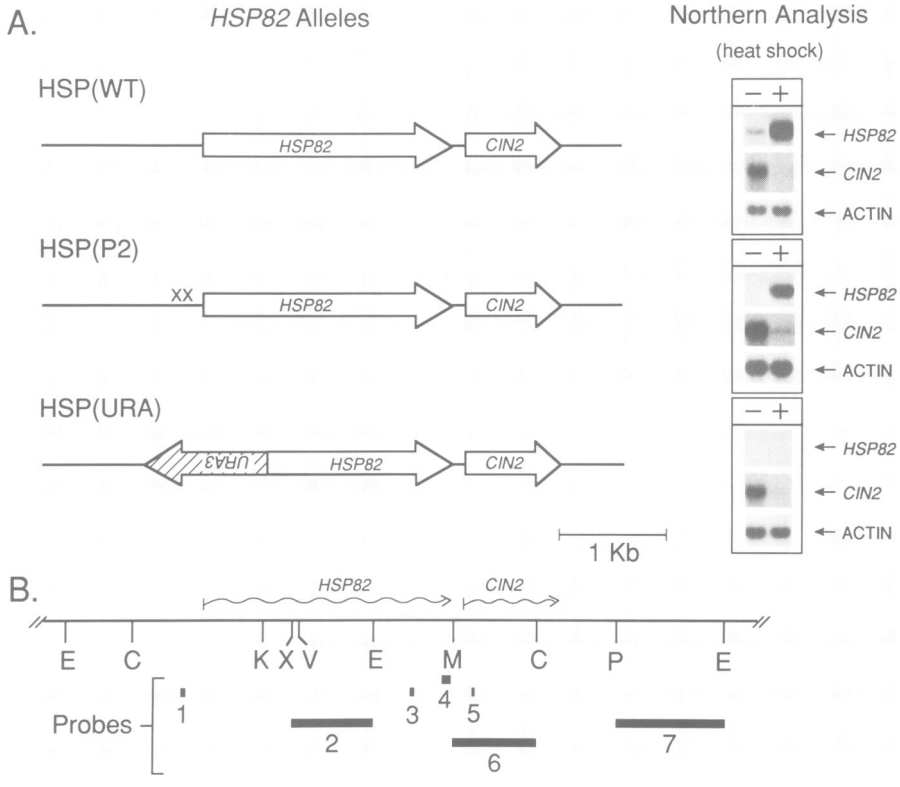

Fig. 1. Structure and expression of *HSP82* alleles. *Panel A*: Three *HSP82* alleles are schematically represented adjacent to the *CIN2* gene with the arrows depicting the transcription units. HSP(WT) is the wild type allele. HSP(P2) is a promoter point mutant where 2 bases were mutated in the HSE element closest to the TATA box (McDaniel *et al.*, 1989). HSP(URA) has the 5′-portion of the *HSP82* gene (−174 to +536) replaced by the *URA3* gene in inverse orientation (see Materials and methods). Northern analyses of the heat-shock response of strains carrying these alleles are shown immediately to the right of the corresponding allele diagrams. RNA samples isolated from control (−) and 10 min heat-shocked (+) cells were electrophoretically separated, transferred to membrane filters, and hybridized with probe 4 of Figure 1B to visualize 2.3 kb *HSP82* gene transcripts. [The 10 min time was selected because this is the point that yields the maximal response in HSP(WT) cells (see Figure 3C, McDaniel *et al.*, 1989)]. The same filters were sequentially hybridized with probe 6 of Figure 1B to detect 0.9 kb *CIN2* gene transcripts and an actin gene probe to detect 1.4 kb transcripts. Panel **B**: Pertinent restriction sites shown are *Eco*RI (E), *Cla*I (C), *Pst*I (P), *Mlu*I (M), *Kpn*I (K), *Eco*RV (V) and *Xmn*I (X). Shown below are probes used in various analyses: 1, a 35-mer spanning −269 to −235 (upper strand) used in Southern analysis to confirm site-directed integration of mutated sequences (data not shown); 2, a 753 bp *Xmn*I-*Eco*RI fragment spanning +848 to +1601 used for indirect end labeling (Figures 3 and 6C); 3, a 35-mer spanning +1974 to +2008 (upper strand) used to assess nucleosomal structure and phasing frames (Figures 4 and 5); 4, a 100 mer spanning +2190 to +2289 (lower strand) used in Northern analysis (Figure 1A); 5, a 30-mer spanning +2520 to +2549 (lower strand) used for primer extension to map the *CIN2* gene major start sites (data not shown); 6, a 774 bp *Mlu*I−*Cla*I fragment used for Northern analysis of *CIN2* gene transcripts (Figure 1A); and 7, a 1 kb *Pst*I−*Eco*RI fragment spanning ~+3900 to ~+4900 used for low resolution, indirect end-labeling (Figure 2).

Figure 3 (filled circles), a DNase I cutting interval of ~80 bp is observed within the *HSP82* gene of HSP(WT) cells, both before (−) and after (+) heat-shock induction. Interestingly, this cleavage periodicity is approximately half of the nucleosomal repeat length of yeast chromatin (Thomas and Furber, 1976; Lohr and Van Holde, 1979). Under optimal conditions, 10 such 'half-nucleosomal' cleavage sites have been mapped in the 3′-region. In marked contrast, neither similar sites nor heightened DNase I sensitivity are visualized when the chromatin structure of the 5′-half of the transcription unit is analyzed in the same samples (see Figure 2A) of Szent-Gyorgyi *et al.*, 1987; data not shown). The 'half-nucleosomal' cleavage sites in the 3′-region reflect a specific chromatin structure since they are not observed when naked DNA is digested as a control (Figure 3, DNA). Furthermore, the same region in HSP(URA) cells exhibits only a 'whole-nucleosomal' cleavage periodicity of ~160 bp (Figure 3, open circles), either before (−) or after (+) heat-shock treatment. In strain HSP(P2), the chromatin structure of the *HSP82* gene before heat shock (−) resembles that seen in HSP(URA) cells, while after heat shock (+) its structure closely parallels that seen in HSP(WT) (Figure 3). On the other hand, the chromatin structure of the *CIN2* gene exhibits an irregularly spaced, whole-nucleosomal cleavage periodicity except upon heat-shock (+) in HSP(WT) cells where it becomes non-discrete (Figure 3). In conclusion, even relatively weak transcription causes the *HSP82* gene 3′-region to exhibit a half-nucleosomal cleavage periodicity.

Transcription causes nucleosomes to split

To demonstrate that the transcription-induced conversion in DNase I cutting patterns indeed reflects the splitting of *bona fide* nucleosomes, we utilized micrococcal nuclease (MNase) to dissect further the chromatin structure. As shown in Figure 4, MNase-generated nucleosomal ladders with repeat lengths of ~160 bp are visualized using a probe specific for a single nucleosome within the 3′-region of the *HSP82* gene, both before and after heat-shock induction of strain HSP(P2). In the induced state, the *HSP82* gene nucleosomal ladder exhibits a higher interband background relative to that exhibited by bulk DNA. Importantly, however, no half-nucleosomal band (Figure 4, 0.5 N) or corresponding higher multiples (eg. 1.5 N) were detected in the heat-shocked sample, raising the following two possibilities: (i) transcription does not cause nucleosomes to split, but instead induces nucleosomes to shift positions along DNA. The half-nucleosomal cleavage periodicity detected by DNase I could be the result of two different whole-nucleosomal phasing frames within a mixed population of yeast cells, which just happen to differ by ~80 bp; or (ii) transcription indeed may cause nucleosomes to split, but unlike DNase I, MNase may still have a strong preference to cut in the linker regions between the split nucleosomes. We describe below an experiment that distinguishes between these possibilities.

To determine directly the phasing frame(s) of the nucleosome arrays in the 3′-region of the *HSP82* gene, we mapped the positions of MNase cleavage sites both before and after heat-shock induction of HSP(P2) cells. For this purpose, we determined the lengths of MNase-generated mono- and dinucleosomal DNA fragments after digestion with *Taq*I using a probe specific for a single nucleosome within the 3′-region of the *HSP82* gene. As shown schematically in Figure 5A, if nucleosomes maintain a single phasing frame

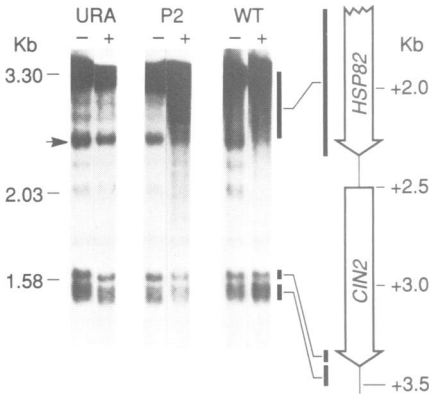

Fig. 2. DNase I sensitivity within the *HSP82* gene is abolished by promoter mutations that eliminate transcription. Nuclei isolated from control (−) and heat-shocked (+) cells [10 min for WT and URA, 15 min for P2 (the time points of maximal response)] were digested with DNase I. Resulting purified DNA samples were digested with *Eco*RI, separated by electrophoresis, and transferred to membrane filters. The region subsequently indirectly end-labeled corresponds to a 3.3 kb *Eco*RI fragment, from +1601 to ~+4900, with the probe spanning ~+3900 to ~+4900 (probe 7 of Figure 1B). The open vertical arrows depict the *HSP82* and *CIN2* gene transcription units. Calibration on the left is absolute DNA length and on the right is map position on a linear scale with respect to the *HSP82* gene transcription start site. Filled vertical bars and the arrow depict hypersensitive regions.

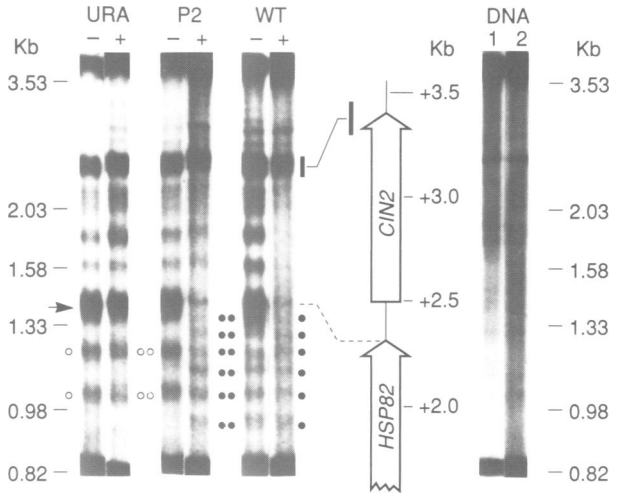

Fig. 3. Promoter mutations convert the DNase I cleavage patterns within the *HSP82* gene from a half- to whole-nucleosomal interval. **Left**: Nuclei isolated from control (−), and heat-shocked (+) cells (10 min for WT and URA, 15 min for P2) were digested with DNase I. Resulting purified DNA samples were cleaved with *Kpn*I and *Eco*RV, separated by electrophoresis, and transferred to membrane filters. The region subsequently indirectly end-labeled corresponds to a 3.6 kb fragment, from +906 to ~+4500, with the probe spanning +848 to +1601 (probe 2 of Figure 1B). *Kpn*I and *Eco*RV digestion reduce cross-hybridizing heat-shock cognate gene sequences (*HSC82*) to a fragment size of 819 bp, the fastest migrating band in the figure (see Szent-Gyorgyi *et al.*, 1987; Borkovich *et al.*, 1989). For the naked DNA controls (DNA), DNA was isolated from nuclei of heat shocked cells prior to different extents of DNase I digestion. The open vertical arrows depict the *HSP82* and *CIN2* gene transcription units. Calibrations shown reflect absolute DNA length and map position on a linear scale with respect to the *HSP82* gene transcription start site. The filled vertical bars and arrow depict hypersensitive regions, open circles refer to internucleosomal linker cleavage sites, and closed circles depict half-nucleosomal cleavage sites.

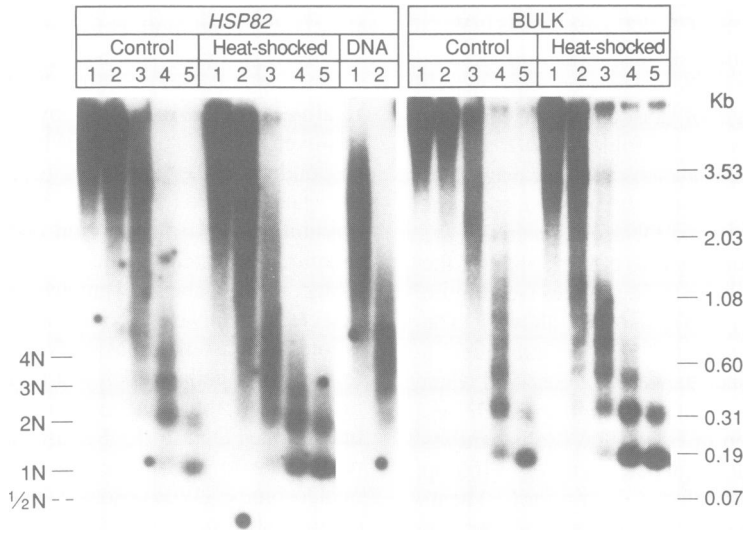

Fig. 4. The *HSP82* gene exhibits a nucleosomal organization. Nuclei isolated from control and 15 min heat-shocked HSP(P2) cells were digested with serial concentrations of MNase (lanes 1–5). Resulting purified DNA was separated by electrophoresis, and the transfer was hybridized with a 35-mer probe from +1974 to +2008 specific to the 3′-region of the *HSP82* gene (probe 3 of Figure 1B). The same filter was rehybridized with total labeled genomic DNA (Bulk). For the naked DNA control, purified genomic DNA was digested with MNase to varying extents and processed as described above. *Eco*RI and *Hin*dIII-restricted lambda DNA and *Hae*III-restricted ϕX174 were used as molecular size markers. To confirm specific hybridization to the *HSP82* gene, *Eco*RI and *Mlu*I-digested genomic DNA and *Eco*RI-restricted pUTX203 plasmid were used as positive and negative controls (data not shown). N-mers represent various oligomeric nucleosomal DNA fragments.

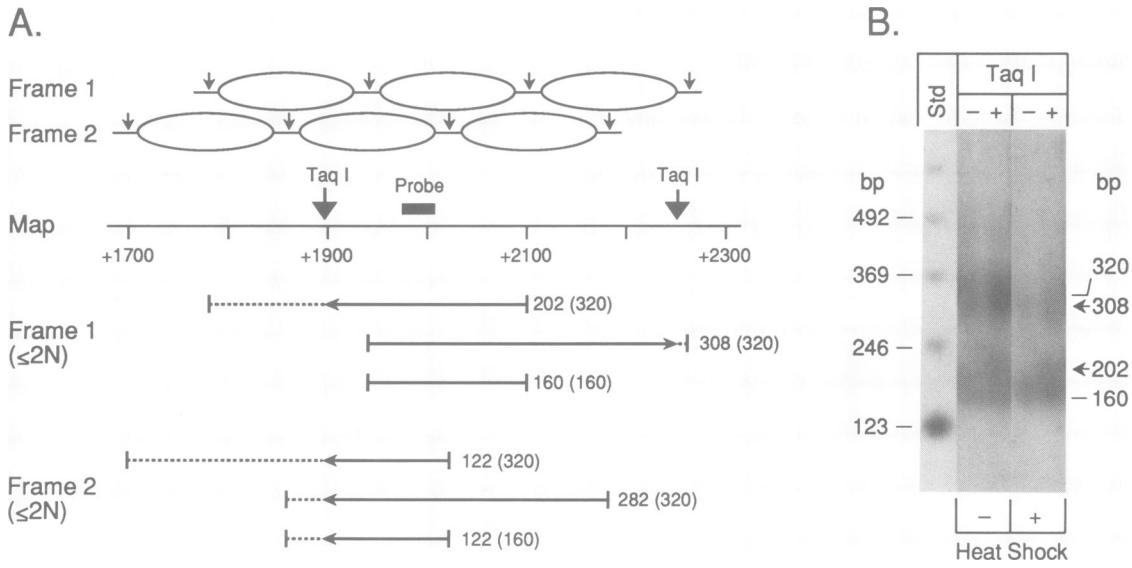

Fig. 5. *HSP82* gene nucleosomes possess a single phasing frame. Panel **A**: The schematic diagram depicts nucleosomes as ovals in two possible phasing frames. Frame 1 is that deduced from the DNase I whole-nucleosomal cutting intervals of HSP(URA) and non-heat-shocked HSP(P2) cells. Frame 2 is hypothetical. Below the map are shown solid lines and their corresponding sizes predicted for MNase-generated mono- and dinucleosomal DNA (≤2N) fragments hybridizing with the probe (probe 3 of Figure 1B), after *Taq*I digestion. Before *Taq*I digestion, the fragments extend as shown by the dashed line and possess the sizes shown in parentheses. Panel **B**: Nuclei isolated from control and 15 min heat-shocked HSP(P2) cells were digested with MNase to achieve primarily mono- and dinucleosomal products (1N and 2N). Purified DNA was separated by electrophoresis before (−) or after (+) treatment with *Taq*I restriction endonuclease as indicated. The transfer was hybridized with the 35-mer probe indicated in Panel A. The standard is an end-labeled 123 bp DNA ladder (BRL).

both before and after heat-shock induction (Frame 1), then dinucleosomal DNA would be converted upon *Taq*I digestion from a mean size of 320 bp (±10 bp due to MNase end-trimming) to 308 and 202 bp mean-sized fragments, whereas mononucleosomal DNA would remain uncut. Since these lengths are very close to those of intact mono- and dinucleosomal DNA fragments, the net effect of *Taq*I digestion on the fragment pattern would appear almost insignificant for phasing Frame 1. On the other hand, if transcription causes the nucleosome phasing frame to shift in a fraction of the cells (Frame 2), then 282 and 122 bp mean-sized fragments would result after *Taq*I digestion. As shown in Figure 5B, after *Taq*I digestion, no 122 bp fragments were detected, the 160 bp monomer bands per-

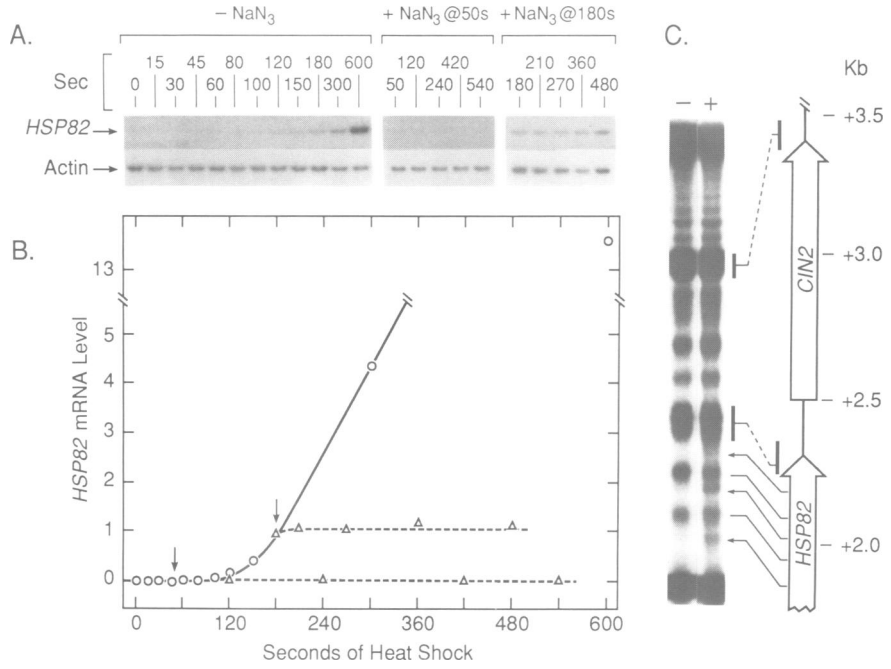

Fig. 6. Nucleosomes split during the first round of induced transcription. Panel **A**: RNA was isolated from HSP(P2) cells at the indicated times after heat shock, before or after addition of 20 mM sodium azide to the cultures. Northern analyses were performed as described in Figure 1. Panel **B**: Autoradiograms were scanned and quantified densitometrically. The *HSP82* mRNA levels represented have been normalized to those of actin to correct for slight differences in RNA loads between samples. The arrows indicate that 20 mM sodium azide was added to subcultures of the original cells at either 50 or 180 s. The dashed lines and triangles indicate the mRNA levels in the cultures maintained in the presence of the added sodium azide. The trace of basal level transcription exhibited by HSP(P2) is ~50-fold reduced from that observed in HSP(WT) (McDaniel *et al.*, 1989). This background has been subtracted from the analysis. Panel **C**: Nuclei were isolated from sodium azide-treated control and 50 s heat-shocked HSP(P2) cells. After DNase I digestion, the chromatin structure was analyzed by indirect end-labeling as described in Figure 3. Half-nucleosomal cutting sites are shown by the arrows.

sisted, and 308 and 202 bp products appeared for samples prepared either before (−) or after (+) heat-shock treatment. We conclude that the corresponding nucleosomes are organized in one and the same single phasing frame (Frame 1), both before and after heat-shock induction. This conclusion has been verified by performing a similar *TaqI* digestion experiment on isolated dinucleosomal DNA prepared from heat-shocked cells (data not shown). Taken together with the above analyses (Figures 3 and 4), we conclude that the 3′-region of the *HSP82* gene is organized with phased nucleosomes that split and become DNase I sensitive upon gene transcription, but that the split nucleosomes resist internal cleavage by MNase.

Nucleosomes split during the first round of induced transcription

What is the mechanism for transcription-induced nucleosome splitting? Transcription might cause nucleosomes to split when they are traversed by RNA polymerase simply by physical disruption. On the other hand, splitting might be induced by signals that transiently spread down the chromatin fiber ahead of the transcription complex, such as waves of positive supercoils (see Discussion).

In an attempt to distinguish between the above mechanisms we looked for nucleosome splitting before RNA polymerase traversal. For this purpose we took advantage of strain HSP(P2), which lacks significant basal-level transcription, and utilized sodium azide, a potent inhibitor of ATP generating systems (Hewitt and Nicholas, 1963), to 'freeze' newly initiated transcription complexes (C.Szent-Gyorgyi and W.T.Garrard, unpublished results; see below). The Northern analysis shown in Figure 6A and B reveals that the first detectable newly made *HSP82* mRNA species appear after about 100 s of heat-shock treatment. [The Northern assay employed can detect one mRNA molecule per cell (data not shown)]. Significantly, addition of 20 mM sodium azide to cultures after 50 s of heat-shock prevents the appearance of new transcripts (dashed line). Furthermore, as an additional control, we found that addition of azide to cultures after 180 s of heat-shock arrested further transcription within a few seconds (Figure 6A and B, dashed line). As shown in Figure 6C (arrows), nucleosome splitting at the 3′-end of the *HSP82* gene could be readily detected when azide was added after 50 s of heat-shock induction to block further rounds of induced transcription. We conclude that nucleosomes split apparently even before they are traversed by newly initiated RNA polymerase II molecules.

Discussion

Transcription-induced DNase I sensitivity is associated with a split nucleosomal structure

As schematically summarized in Figure 7, we have demonstrated that nucleosomes split within the 3′-region of the *HSP82* gene by observing a transcription-associated change from a whole- to a half-nucleosomal DNase I cleavage periodicity with the same phasing frame (Figures 3, 5 and 6). Mutational analysis demonstrates a one-to-one correspondence between DNase I sensitivity, split nucleosomes, and transcription (Figures 2, 3 and 6). Our results

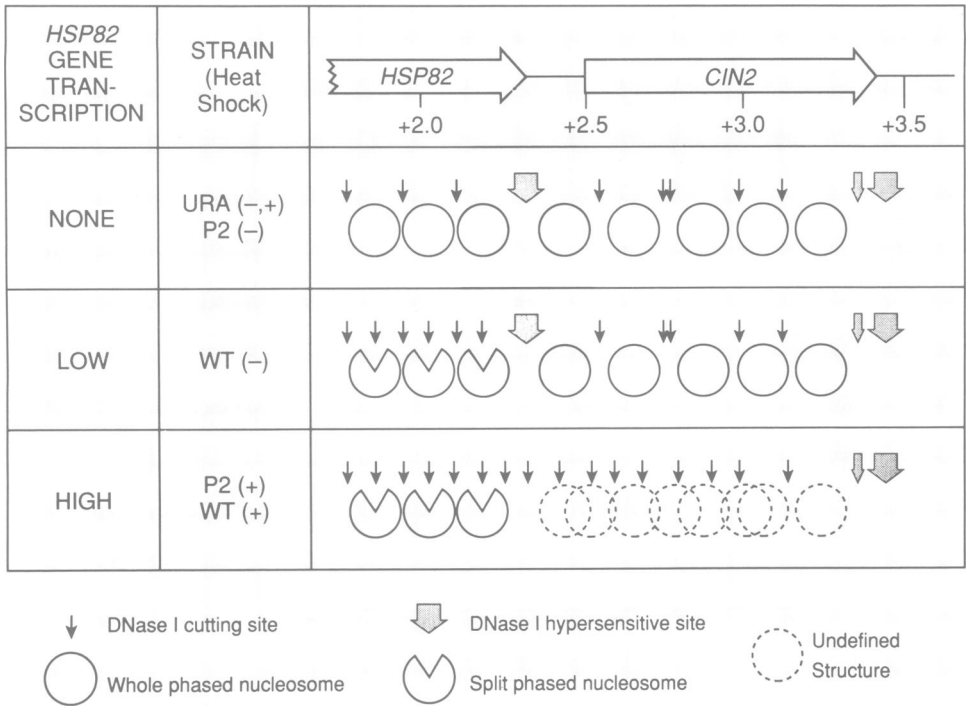

Figure 7. Chromatin structure of the yeast *HSP82* and *CIN2* genes. Schematic diagram summarizing the results of the chromatin structure analyses. The fourth downstream nucleosome in the *CIN2* gene is more readily observed upon indirect end-labeling from the opposite direction (Szent-Gyorgyi *et al.*, 1987; data not shown).

lead us to suggest that a split nucleosomal structure represents one of the underlying conformations responsible for the preferential DNase I sensitivity associated with active genes (Weintraub and Groudine, 1976). Why haven't split nucleosomes widely been seen previously?

Split nucleosomes are probably of common occurrence but difficult to detect experimentally. To visualize these structures by indirect end-labeling, the half-nucleosomal cleavage sites must be in a single phasing frame along the DNA sequence. Either nucleosome sliding or multiple phasing frames would rule out detecting these structures. Although a previous report exists describing an MNase-generated half-nucleosome product from active 5S gene chromatin (Ryoji and Worcel, 1985), this component had a wide distribution of DNA lengths and no 1.5-mer intermediate could be detected. To the contrary, based on results here and elsewhere, neither MNase nor methidium-propyl-EDTA·iron(II) can be used routinely as the cleavage reagents to detect half-nucleosomal structures (Figure 4) (Cartwright and Elgin, 1986; Szent-Gyorgyi *et al.*, 1987). However, in the cases where DNase I has been used, apparent split nucleosomes have been seen along transcribing *Drosophila hsp22* and *26* genes (Cartwright and Elgin, 1986), the yeast *SUC2* gene (Perez-Ortin *et al.*, 1987), and the chicken β-globin gene (Kukushkin *et al.*, 1988). Although we have not detected split nucleosomes within the 3′-region of the expressed *CIN2* gene, this gene is transcribed at a much lower frequency than the basally-transcribed *HSP82* gene (unpublished results) and therefore, the *CIN2* gene probably lacks traversing RNA polymerase II molecules in the major fraction of cells. Furthermore previous studies have detected a weak half-nucleosomal repeat in DNase I digests of bulk chromatin isolated from yeast cells, chicken erythrocytes and HeLa cells (Lohr and Van Holde , 1979; Kukushkin *et al.*, 1988). Thus, taken together with our results, it seems likely that split nucleosomes are of common occurrence and may represent one of the underlying structures of DNase I sensitive chromatin (Weintraub and Groudine, 1976).

Structure of split nucleosomes

Clearly, our definition of nucleosome splitting is operational, and the actual structure of split nucleosomes is not known. The path of DNA about the histone octamer exhibits a dyad axis of symmetry (Sollner-Webb *et al.*, 1978; Richmond *et al.*, 1984), and even minor conformational alterations might generate DNase I cleavage sites near this dyad, as has been observed for the apparent behavior of DNase II (Altenburger *et al.*, 1976). However, major structural changes occur within nucleosomes associated with active genes (Prior *et al.*, 1983; Chen *et al.*, 1990; Solomon *et al.*, 1988; Nacheva *et al.*, 1989), and 'half-nucleosomes' have been observed directly by electron microscopy under specialized conditions (Oudet *et al.*, 1977). Symmetrical nucleosome splitting into pairs of heterotypic tetramers of the core histones during transcription and replication has been proposed previously (Weintraub *et al.*, 1976). However, the histone composition between split nucleosome halves may be different, since heterotypic interactions between the core histones do not appear stable (McGhee and Felsenfeld, 1980), whereas homotypic tetramers of histones H3 and H4 are conservatively inherited (Leffak *et al.*, 1977; Prior *et al.*, 1980; Jackson, 1988).

Short-term versus long-term memory in chromatin

The transcription-induced chromatin structures associated with the *HSP82* gene exhibit short-, but not long-term memory. Integration of upstream altered promoter sequences that inactivate transcription reverses the downstream DNase I sensitivity and nucleosome splitting (Figures 2 and 3) as

does growth of previously heat-shocked HSP(P2) cells under non-heat shock conditions (data not shown). However, once formed, these structures are not lost upon nuclear isolation, which requires as a prelude a 1 h incubation at 30°C during spheroplast formation (in the presence of sodium azide). Neither over-expressing topoisomerase I nor pre-nicking DNA with MNase prior to DNase I digestion of nuclei reverses the split nucleosomal structures (data not shown), indicating that maintenance of the structures does not require torsional stress. Perhaps split nucleosomes are stabilized by post-translational modifications of the histones, and/or by certain non-histone chromosomal proteins, like the high mobility group (HMG) proteins (see Gross and Garrard, 1987). The insoluble chromatin associated with active genes may be composed of a split nucleosome structure (see Xu *et al.*, 1986). Reversion of these structures to whole nucleosomes may normally require DNA replication. Furthermore, reversal of preferential nuclease sensitivity in chicken red cell nuclei requires a 37°C recovery period (Villeponteau and Martinson, 1987). Nevertheless, DNase I sensitivity and half-nucleosomal cutting patterns have proven difficult to preserve upon mononucleosome isolation (Lohr and Van Holde, 1979; Goodwin *et al.*, 1985). Subtle conformational differences may be maintained in isolated core particles, however, that may be responsible for memory of preferential HMG protein binding (see Brotherton *et al.*, 1990).

A primary role for transcription in mediating conformational changes in chromatin

We demonstrate here that even the relatively weak, basal level transcription can result in striking chromatin alterations (Figures 2 and 3). Furthermore, transcription-mediated alterations in chromatin structure are known to play crucial functional roles both in stimulating DNA repair (Smerdon and Thoma, 1990) and triggering recombination (Yancopoulos and Alt, 1985; Blackwell *et al.*, 1986; Voelkel-Meiman *et al.*, 1987; Lutzker *et al.*, 1988; Schlissel and Baltimore, 1989). Although earlier literature has sometimes speculated that DNase I sensitivity in chromatin may precede gene transcription, this idea has not been proven and in cases favoring this notion it is difficult to rule out the possibility that localized weak transcription had occurred along the corresponding region prior to or even immediately before the time of experimental analysis. Indeed, although the DNase I sensitive state appears to be propagated to daughter cells in higher eukaryotes in the absence of continued transcription (see Weintraub, 1985), we argue here that this state may be established primarily by the process of transcription itself. Clearly, if 'first-round' transcription is all that is necessary to trigger a series of events in chromatin that lead to quasi-stable conformational alterations along a locus, then the crucial regulatory event for switching chromatin becomes that of promoter recognition, which in turn may revolve around setting up a corresponding nuclease-hypersensitive site (see Gross and Garrard, 1988).

Mechanism of nucleosome splitting: a proposed role for positive supercoiling

Nucleosome splitting could be caused by direct physical disruption of the particles during traversal by RNA polymerase II and/or by transient positive supercoiling downstream of the transcription complex (Liu and Wang, 1987; Giaever and Wang, 1988). Split nucleosomes exist within the basally-transcribed *HSP82* gene when it can be estimated that only a few RNA polymerase II molecules are likely to be engaged in transcription (Figure 3) (Osheim *et al.*, 1985). Thus, split nucleosomes theoretically could be present at distances up to 1 kb away from the transcription apparatus. Splitting also occurs during the first round of induced transcription, apparently even before the downstream nucleosomes are traversed by the first newly initiated RNA polymerase II molecules (Figure 6). Interestingly, preferential DNase I sensitivity and nucleosome splitting exhibit a gradient that is maximal at the 3′-end of the *HSP82* gene (Szent-Gyorgyi *et al.*, 1987; data not shown), in the region expected to have the highest density of positive superhelical stress (Liu and Wang, 1987), assuming that a topological boundary exists at the 3′-end of the *HSP82* gene (see below). Taken together, we therefore suggest that positive supercoils generated transiently by RNA polymerase II movement may cause chromatin decondensation and nucleosome splitting, as outlined schematically in Figure 8. Currently, we are testing this hypothesis using topoisomerase mutants (Giaever and Wang, 1989).

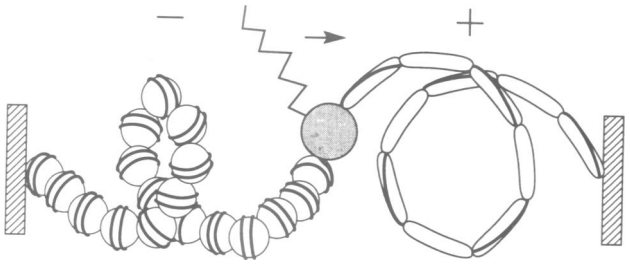

Fig. 8. Positive supercoils downstream of the transcription complex clear the path for RNA polymerase II. The schematic diagram depicts a chromosomal loop with anchored ends, composed of a polynucleosomal array that is being traversed from left to right by RNA polymerase II. The upstream nucleosomes become tightly packed and the downstream nucleosomes split as a consequence of the twin-domain model of DNA supercoiling of Liu and Wang (1987). Topoisomerases will offset these processes.

A presumptive topological boundary at the 3′-end of the HSP82 gene

It is striking that there is an abrupt transition from a DNase I sensitive, split- to a relatively DNase I resistant, whole-nucleosomal configuration immediately downstream of the basally-transcribed *HSP82* gene (Figures 2 and 3). Furthermore, *CIN2* gene transcripts initiate only 182 bp beyond the 3′-end of the *HSP82* gene (data not shown). This suggests that several *cis-acting* elements are localized very near each other: a transcription termination element for the *HSP82* gene, a promoter element for the *CIN2* gene, and possibly a topological boundary that prevents spreading of *HSP82* gene torsional stress into the *CIN2* gene. When the *HSP82* gene is heavily transcribed, the chromatin structure of the *CIN2* gene becomes non-discrete (Figure 3), suggesting that the putative topological boundary becomes transiently disrupted, possibly leading to nucleosome sliding and/or splitting along the *CIN2* gene.

Studies in other systems sometimes reveal sharp boundaries in DNase I sensitivity in chromatin (Flint and Weintraub, 1977), and increased sensitivity toward the 3′-end of genes (Bellard *et al.*, 1986). On the other hand, DNase I sensitive domains can spread long distances both upstream and downstream of transcribed regions (eg. Jantzen

et al., 1986). If caused by torsional stress, this spreading would be predicted to occur until topological barriers are reached. We have previously identified a class of DNA sequences termed *MARs* (matrix association regions), which we have proposed act as topological anchorage elements for chromosomal loops (Cockerill and Garrard, 1986). Interestingly, the *HSP82/CIN2* gene boundary region contains numerous *MAR* consensus sequences (data not shown), and *MARs* flank the DNase I sensitive domain surrounding the chicken lysozyme gene (Phi-Van and Stratling, 1988). In the future, it will be of interest to assess directly the role of topological anchorage in chromatin structure and gene expression within the *HSP82* and other loci by targeting sites for *in vivo* template linearization utilizing the HO endonuclease system (Jensen and Herskowitz, 1984).

Materials and methods

Yeast strains and mutant construction

The haploid strain W303-1B (*MAT*a *ade2-1 ura3-1 his3-11,15 leu2-3,112 can1-100*) of *S.cerevisiae* was used in this study. This strain is designated HSP(WT) and served as the parent for construction of the promoter mutants designated HSP(URA) and HSP(P2). To generate HSP(URA), the 2.9 kb *Eco*RI fragment encompassing the position of −1309 to +1601 relative to the *HSP82* gene transcription unit was subcloned into the *Eco*RI site of pBR322. [See Farrelly and Finkelstein, 1984 for the *HSP82* gene sequence (formerly termed *HSP90*)]. An internal *Xba*I fragment (−174 to +536) was then deleted and substituted with a 1.2 kb *Xba*I fragment of the *URA3* gene in inverse orientiation relative to the *HSP82* transcription unit. To replace the wild-type *HSP82* gene with the mutant construct (Rothstein, 1983), the resulting plasmid was cleaved with *Eco*RI, yeast cells were transformed (Ito *et al.*, 1983) and plated on medium to select for *URA3* expression. Transformants were screened and a site-directed integration mutant was identified by genomic Southern analysis. Because of the *Pst*I site in the introduced *URA3* gene (Rose *et al.*, 1984), *Pst*I and *Eco*RI digested HSP(URA) genomic DNA exhibits a 2 kb DNA fragment after hybridizing with an end-labeled 35-mer probe (probe 1 of Figure 1B), while HSP(WT) yields a 2.9 kb fragment. The HSP(P2) mutant has been described elsewhere (McDaniel *et al.*, 1989). For RNA and chromatin analysis, cells were grown to mid-log phase in YPD medium [1% yeast extract (Difco), 2% bacto peptone (Difco), 2% dextrose (Sigma)] at 30°C in a shaking water bath. For genomic Southern analysis, cells were grown similarly but to stationary phase. Heat-shock was performed as described below.

RNA analysis

For the control and heat-shock induction, HSP(WT) and mutant cells were treated as described previously (McDaniel *et al.*, 1989). For assay of the rapidity of transcriptional arrest by sodium azide, inhibitor solution (prewarmed to 39°C) was added after 50 s or 3 min heat induction and the culture was maintained at 39°C. Cells were withdrawn at the indicated times and transferred to Eppendorf tubes on ice. RNA was isolated as escribed elsewhere (Kurtz and Lindquist, 1984), maintaining 20 mM sodium azide in samples until lysis. Resulting purified total RNA was assayed colorimetrically by the orcinol procedure. Ten microgram samples were treated with 50% formamide, incubated at 65°C for 3 min, and separated on 1.4% agarose−formaldehyde gels. RNA was transferred to a Zeta-Probe membrane (BioRad), and hybridized with a radiolabeled 100-mer probe as described elsewhere (probe 4 of Figure 1B) (McDaniel *et al.*, 1989). Autoradiography was performed by exposing pre-flashed Kodak XAR-5 film with intensifying screens (Dupont Cronex) at −70°C for 1−4 days (Laskey, 1980). The same filters were hybridized subsequently with a radiolabeled *CIN2* gene probe (probe 6 of Figure 1B) or an anti-sense RNA probe transcribed *in vitro* from pGem-actin, a recombinant plasmid containing a 600 bp yeast actin gene fragment (Parikh *et al.*, 1989). Hybridization signals were scanned and quantified densitometrically. The levels of *HSP82* gene transcripts were normalized relative to those of actin.

Nuclei isolation and nuclease digestion

For the control state, one liter of wild-type mutant cells were grown in YPD medium at 30°C and cold sodium azide was rapidly added while shaking the cultures (20 mM final concentration). Cultures were chilled on ice until harvesting of the cells. For the heat-shocked state, the temperature was shifted rapidly from 30°C to 39°C by addition of an equal volume of medium prewarmed to 51°C; the resulting cultures were maintained at 39°C in a shaking water bath. At various times of heat-shock induction as indicated in the text, cultures were treated with sodium azide as described above. Nuclei were isolated as reported elsewhere (McDaniel *et al.*, 1989) with the following modification. Cells were incubated with oxalyticase [800 U/ml (Enzogenetics)] in spheroblast buffer (1.4 M sorbitol, 40 mM HEPES pH 7.5, 0.5 mM $MgCl_2$ 20 mM sodium azide) for 60 min at 30°C. All solutions contained 20 mM sodium azide up to the nuclear pelleting stage. Nuclei or naked DNA controls were digested with either DNase I or MNase and DNA was purified as described elsewhere (Szent-Gyorgyi *et al.*, 1987; McDaniel *et al.*, 1989). For naked controls, genomic DNA was purified from nuclei of heat-shocked cells.

Chromatin analysis

For indirect end-labeling, 20 μg DNA samples were digested with *Eco*RI or *Kpn*I and *Eco*RV (as indicated in the figure legends), and separated on a 1.5 or 2% agarose gel in 1× TPE buffer (Sambrook *et al.*, 1989). To prepare internal size marker standards, genomic DNA was digested with *Eco*RI and *Mlu*I or *Kpn*I, *Eco*RV and *Mlu*I. *Eco*RI and *Hin*dIII-digested lambda DNA fragments were used further for gel calibrations. DNA was transferred to Zeta-Probe membranes and filters were prehybridized for 2 h, hybridized with a radiolabeled 753 bp *Xmn*I−*Eco*RI fragment (probe 2 of Figure 1B) or a 1 kb *Pst*I−*Eco*RI fragment (probe 7 of Figure 1B) for 24 h at 65°C as described elsewhere (Reed and Mann, 1985). Both restriction fragments were isolated from recombinant plasmid pUTX5 (Farrelly and Finkelstein, 1984). The probes were labeled with [^{32}P]dCTP by random primer synthesis, using an oligo-labeling kit (Pharmacia), and were purified by the cetylpyridinium bromide precipitation method (Sambrook *et al.*, 1989). Filters were washed once at room temperature for 10 min and three times, 20 min each, at 65°C with washing buffer (0.1× SSC, 20 mM sodium phosphate buffer, pH 7.0, 2% sodium dodecyl sulfate). Autoradiograms were exposed with an intensifying screen at −70°C for 12−36 h. For nucleosomal structure analyses, 20 μg, DNA samples prepared from nuclei were separated on 1.5% agarose gels (Figure 4) or 2% NuSieve GTG agarose (FMC)−0.7% agarose composite gels (Figure 5). *Eco*RI and *Mlu*-digested genomic DNA and *Eco*RI-digested recombinant plasmid pUTX203 (Borkovich *et al.*, 1989) were used for positive and negative controls. *Eco*RI and *Hin*dIII-digested lambda and *Hae*III-digested φX174 DNA fragments or an end-labeled 123 bp DNA ladder (BRL) were used as molecular size markers. DNA was transferred to Zeta-Probe membranes, prehybridized for 4 h, hybridized with an ^{32}-end labeled 35-mer probe (probe 3 of Figure 1B) at 37°C for 48 h, and washed four times, 30 min each, at 37°C with washing buffer containing 2× SSC. An autoradiogram was exposed with an intensifying screen at −70°C for two weeks. For the bulk DNA control, the same filter was subsequently hybridized with radiolabeled total genomic DNA for 5 h, washed at 65°C with buffer containing 0.1× SSC, and an autoradiogram was exposed at room temperature for 1 h.

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