Chromatin structure mapping in Saccharomyces cerevisiae in vivo with DNase I

Xi Wang and Robert T. Simpson*

Department of Biochemistry and Molecular Biology, 308 Althouse Laboratory, The Pennsylvania State University, University Park, PA 16802, USA

Received December 13, 2000; Revised February 2, 2001; Accepted March 6, 2001

ABSTRACT

Most methods for assessment of chromatin structure involve chemical or nuclease damage to DNA followed by analysis of distribution and susceptibility of cutting sites. The agents used generally do not permeate cells, making nuclear isolation mandatory. In vivo mapping strategies might allow detection of labile constituents and/or structures that are lost when chromatin is swollen in isolated nuclei at low ionic strengths. DNase I has been the most widely used enzyme to detect chromatin sites where DNA is active in transcription, replication or recombination. We have introduced the bovine DNase I gene into yeast under control of a galactose-responsive promoter. Expression of the nuclease leads to DNA degradation and cell death. Shorter exposure to the active enzyme allows mapping of chromatin structure in whole cells without isolation of nuclei. The validity and efficacy of the strategy are demonstrated by footprinting a labile repressor bound to its operator. Investigation of the inter-nucleosome linker regions in several types of repressed domains has revealed different degrees of protection in cells, relative to isolated nuclei.

INTRODUCTION

Eukaryotic DNA transcription, replication, recombination and repair all take place in the context of chromatin, the complex of the nucleic acid with histones and other proteins. Increasingly, the relevance of structural features of chromatin to these functions of DNA is being appreciated (reviewed in 1,2). Analysis of chromatin structure is done mainly by determination of features of DNA structure using nucleases or chemicals that modify the nucleic acid. Patterns of modification are revealed by primer extension or indirect end-label methods and inter- modification site in native yeast DNA and the long times necessary for achieving levels of modification suitable for mapping experiments. In search of a more general reagent for mapping chromatin, we have turned to the first reagent used for this purpose with isolated nuclei, bovine pancreatic DNase I.

DNase I was used in the first definition of the general nuclease sensitivity that distinguishes active from inactive genes by Weintraub and Groudine (9). Wu and Gilbert used this same nuclease for description of nuclease hypersensitive sites that signaled a regulated promoter in the active state (10). Digestion of core particle DNA with a periodicity of ~10 nt leads to a distinctive pattern for rotationally positioned nucleosomes. Differential sensitivity of linker DNA allows DNase I mapping of nucleosome locations, although not with the precise resolution of micrococcal nuclease which cuts linker DNA almost exclusively (4,5). Most importantly, extension of the general rule that DNase I hypersensitivity marks the sites where the action is in chromatin has made hypersensitive sites synonymous with enhancers, promoters, replication origins or other features of DNA activity (11,12). In an indirect end-label study of DNase I digestion of ~50 kb of the left arm of yeast chromosome III, we have confirmed the correlation between these DNA elements and hypersensitivity to the nuclease (S.Ercan and R.T.Simpson, unpublished observation). For these reasons, we elected to attempt to establish DNase I as an in vivo chromatin probe.

Worrall and co-workers (13,14) synthesized and expressed a number of variants of the predicted DNA sequence for the DNase I protein. The different proteins encoded by these DNAs varied in specific enzymatic activity. We have cloned the DNA sequence corresponding to the native protein plus a nuclear localization signal (NLS) into a shuttle expression
vector. Fortuitous expression in bacteria required modification of the vector to eliminate this toxic activity. Expression in yeast under control of the GAL1 promoter was sufficiently controlled by dextrose repression to allow growth of transformed strains without differences from similar strains lacking the nuclease gene. When these strains were cultured in galactose, expression of the DNase I gene was lethal for long-term growth. Prior to cell death, chromatin mapping in vivo is possible using the nuclease to explore the DNase I susceptibility of the genome without perturbation of the normal nuclear environment of chromatin. In this report, in addition to documenting the methodology for use of DNase I as an in vivo probe for chromatin structure, we present evidence that suggests an unexpected higher order structure for some silenced or strongly repressed parts of the yeast chromosome.

**MATERIALS AND METHODS**

**Plasmid construction**

A synthetic DNA designed to code for the native form of bovine pancreatic DNase I and cloned in M13 was kindly provided by Dr A.F.Worrall (13). The SV40 NLS (ATG CCA AAG AGA AAG GTT), flanked by an EcoRI site, was attached to the N-terminus using the polymerase chain reaction. In the same reaction, an Escherichia coli lac-UV5 constitutive promoter in antisense orientation and an XhoI site were added to the C-terminus of the coding sequence. The fragment was cloned into the polylinker of pYES2 (Invitrogen) for creating shuttle expression vector. Fortuitous expression in bacteria required modification of the N-terminus using the polymerase chain reaction. In the same reaction, an Escherichia coli lac-UV5 constitutive promoter in antisense orientation and an XhoI site were added to the C-terminus of the coding sequence. The fragment was cloned into the polylinker of pYES2 (Invitrogen). This vector is a high copy number shuttle expression vector containing a polylinker between the vector is a high copy number shuttle expression vector was cloned into the polylinker of pYES2 (Invitrogen). This added to the C-terminus of the coding sequence. The fragment Xba I-active promoter in antisense orientation and an XhoI site were added to the N-terminus using the polymerase chain reaction. In the same reaction, an Escherichia coli lac-UV5 constitutive promoter in antisense orientation and an XhoI site were added to the C-terminus of the coding sequence. The fragment was cloned into the polylinker of pYES2 (Invitrogen). This vector is a high copy number shuttle expression vector containing a polylinker between the GAL1 promoter and the CYC1 terminator, a URA3 selection marker and 2µ origin of replication, as well as the ampicillin resistance gene and colEl replication origin for selection/growth in E.coli. After growth in bacteria and confirmation of the construction by restriction endonuclease mapping and limited sequencing, the plasmid, pSUN-1, was transferred by electroporation to Saccharomyces cerevisiae YPH500 (MATα, ade2-101, his3-Δ200, leu2-1, lys2-801, trp1-Δ63, ura3-52) (15). As a control, the parental plasmid, pYES2, was transformed into the same strain in parallel.

**Cell growth**

Standard yeast media, both rich (YPD) and synthetic medium lacking uracil [CSM-Ura (Bio 101), 0.67% yeast nitrogen base without amino acids (Difco), and an appropriate carbon source (2% dextrose, 4% lactic acid or 2% galactose)] were used. Cells containing pSUN-1 or pYES2 were grown at 30°C to OD600 1.0 in CSM-Ura dextrose medium. Similar densities of cells were spread on CSM-Ura dextrose or CSM-Ura galactose plates to observe any effect of DNase I on cell viability. For chromatin structure studies in cells expressing DNase I, yeast cells transformed with pSUN-1 or pYES2 were grown at 30°C to OD600 0.8 in 10 ml CSM-Ura dextrose medium, changed to 10 ml CSM-Ura lactic acid medium and grown to OD600 1.0, and then switched to CSM-Ura galactose medium for periods of up to 12 h.

**Nuclear and DNA preparation and analysis**

Yeast nuclei were prepared as described from cells grown at 30°C to OD600 of 1.0 in YPD (16). DNase I digestion, isolation of nuclear DNA and determination of the locations of DNase I cleavage sites by primer extension were all performed exactly as previously described (17). For isolation and analysis of DNA cut by DNase I in vivo, cells were harvested by centrifugation, broken by homogenization with glass beads in 100 mM Tris–HCl pH 8.0, 50 mM EDTA, 2% sodium dodecyl sulfate, and the DNA was extracted (18). Purification of DNA involved treatment with 100 ng/ml RNase A at 37°C for 1 h and then 100 ng/ml proteinase K in 2% Sarkosyl, 200 mM NaClO4 at 50°C for 2 h. DNA was further purified by extractions with equal volumes of phenol/chloroform/isoamyl alcohol (25:24:1) and chloroform/isoamyl alcohol (24:1), followed by ethanol precipitation. The DNA was dissolved in 20 μl 0.1× TE and passed through a 1 ml Sephadex G-50 spin column prior to analysis. Primer extension was carried out as previously described using the following primers: recombination enhancer (RE) region, B297 (17); HMRα region, p14 (19); and STE6 region, –373 to –352 (relative to transcription start site) 5’-CGTACCATTCCATTGGCTTTTC-3’ (20). In Figures 5–7, the schematic diagrams of nucleosome locations are based on previously published micrococcal nuclease primer extension maps (17,19,20) using the same primers and identical molecular weight standards to those employed in the present work. Analysis of cutting of the GAL control region of pSUN-1 from 0–12 h of induction of DNase I expression revealed similar cutting site patterns at all time points. Intensities of bands increased with time and shorter fragments became more prominent, as expected.

**Southern blots**

Yeast cells transformed with either pSUN-1 or pYES2 were grown sequentially in CSM-Ura with dextrose, lactic acid and galactose, as detailed above. At 0, 1 and 6 h after exposure to galactose, cells were harvested and DNA was recovered by the glass bead method (18). DNA from equal cell numbers (based on A600nm) was subjected to electrophoretic separation on a 1.2% agarose gel, transferred to Hybond-NX membrane (Amersham), crosslinked with UV light and hybridized as previously described (21). The pYES2 plasmid was random primer labeled with [α-32P]dATP for use as probe. Blots were exposed to film or a PhosphorImager screen and analyzed using Image Quant v.5.0 software (Molecular Dynamics).

**RESULTS**

**DNase I expression in vivo**

Others have successfully cloned and expressed native DNase I in bacterial systems (13,14). Our attempts to clone DNase I with a NLS into yeast shuttle expression vectors led to deletions or mutation of the active site histidyl residue (data not shown). Further analysis showed that these mutations occurred during bacterial manipulations. Apparently, expression from a surrogate promoter led to nuclelease toxicity and selection for inactive mutants. To block DNase I expression in bacteria, we inserted a constitutively active E.coli promoter, lac-UV5, downstream of the DNase I gene in the opposite transcriptional orientation. Inhibition of nuclease expression may involve antisense RNA, physical interference with polymerase movement, or another unknown mechanism. In any case, this approach allowed cloning of the intact DNase I gene in bacteria.
Yeast containing the DNase I plasmid, pSUN-1, grew on dextrose-containing medium identically to controls bearing an empty vector (Fig. 1). Plating the two strains on galactose led to striking differences in growth patterns. While the control strain, lacking DNase I, grew more slowly than it did on dextrose, colony numbers were similar. However, the strain containing the DNase I gene under GAL1 control failed to form any colonies when expression of the gene was induced (Fig. 1). Similarly, in suspension cultures the strain carrying pSUN-1 did not grow in 12 h after a medium change from CSM-Ura dextrose to CSM-Ura galactose medium (data not shown). It is likely that DNase I killed cells in which it was expressed by degradation of genomic DNA.

Since DNase I preferentially cuts one strand of duplex DNA, a highly sensitive assay for its activity in living cells is to measure conversion of a plasmid from closed circular form to a nicked, relaxed form; a single DNase I cut per plasmid molecule is measured by this assay. We examined the topological state of the pSUN-1 plasmid in yeast at various times after transferring cells to galactose. At the time of medium change, 10% of the isolated plasmid DNA was nicked, probably due to damage during preparation. Within 1 h of growth in galactose, ~35% of plasmid DNA was in the nicked form and this fraction increased to ~45% after 6 h exposure (Fig. 2A and B). There is a decrease in total plasmid DNA during expression of DNase I; this is likely due to cutting of the nuclease sensitive replication origin in the minichromosome. Control cells, either containing the DNase I plasmid and grown in dextrose or containing the plasmid backbone without the nuclease gene and grown in galactose, had a constant content of episomal DNA with ~10% nicked plasmid circles (data not shown). Examination of genomic DNA on native agarose gel electrophoresis revealed mostly high molecular weight material with some smearing to smaller fragment sizes, most >1500 bp. The amount of digestion was insufficient to generate a nucleosomal ladder. Attempts to use samples digested in vivo for indirect end label mapping experiments failed due to the low levels of DNase I digestion.

The time course of DNase I cutting of plasmid DNA was examined over a 12 h period (Fig. 3). Undigested or zero time samples were all high molecular weight. Patterns of cutting at 3 and 6 h were similar with a large amount of DNA of relatively high molecular weight and similar cutting sites. More material was present for cutting sites closer to the primer in the 6 h sample than the 3 h, as expected. After 12 h of DNase I expression, most of the plasmid DNA had been degraded to sizes <500 nt and some changes in cutting site patterns appeared. Clearly, the 3 and 6 h samples were in the range of single hit kinetics for fragments <1 kb in length, making these times appropriate for analysis of DNase I susceptibility. The 6 h sample gave a higher yield of fragments in the mapping size range; this expression time was therefore used for all the experiments presented below.

Primer extension analysis of the GAL control region DNA in the pSUN-1 plasmid showed that most of the nuclease cutting sites were similar to those cut in naked DNA by pure DNase I in vitro (data not shown). This finding, together with data presented below (Fig. 7), demonstrates that chromatin
mapping in vivo reflects expression of the bovine DNase I gene and not artefactually induced, unknown yeast nucleases. The three observations, nicking of the plasmid, plasmid DNA degradation, and the similarity of cutting sites on the plasmid to naked DNA, confirm DNase I expression under GAL control and its activity in yeast cells. They validate use of DNase I digestion and not artefactually induced, unknown yeast nucleases. The primer was located 222 bp upstream of the TATA box. Numbers to the left of the gel are coordinates relative to the start of the DNase I coding sequence. The rectangular box denotes the position of the TATA box.

DNase I footprints a labile repressor in vivo

In α-cells, Matα2p binds as a homodimer together with a homodimer of Mcm1p to a 31 bp operator DNA sequence to repress expression of α-cell-specific genes (22–24). The repressor is one of the shortest-lived proteins in yeast, with a half-life of <5 min. Consequently, it is absent from its cognate binding sites in isolated nuclear preparations. It is likely that the protein is gone well before spheroplasting, a mandatory prerequisite for nuclear isolation, is finished (25). We previously mapped features of interactions of Matα2p with DNA by using a very rapid method for isolation of crude nuclei in a yeast strain that overproduced the protein and lacked several ubiquitination enzymes (6). It was also possible to demonstrate Matα2p binding to its operator sequence in living cells using the SssI methyltransferase; a single CpG site was blocked by interactions of one monomer with DNA (26). The structure of the DNA binding domains of Matα2p and Mcm1p in a ternary complex with α2 operator DNA is known at 2.25 Å resolution from X-ray crystal studies (27). Taken together, the in vitro structural information and the in vivo difficulties in studying binding of Matα2p and Mcm1p to the operator made this system a good test of the possibility of mapping chromatin structures of regulatory protein complexes with DNA using DNase I expressed in living cells. There are nine functional Matα2p/Mcm1p binding sites in the yeast genome (22,28). Seven of these reside upstream of α-cell-specific genes and the other two are located in the recombination enhancer and help to control directionality of mating type interconversion (29–31). We have mapped chromatin structure of four of these Matα2p/Mcm1p binding sites in vivo using DNase I.

Figure 4 shows the DNase I cutting patterns at the recombination enhancer operon α2#1 operator, located 29194–29224 bp from the left end of chromosome III. Naked DNA from this region was susceptible to DNase I at a number of sites throughout the α2/MCM1 operator and its flanking sequences. DNase I cutting was severely restricted in both isolated nuclei and in vivo digested chromatin samples, relative to the protein-free DNA pattern. In isolated nuclei, where Matα2p is expected to be absent, four prominent nuclease-susceptible sites were present. Two sites flanked the operator and two were located within the operator. Mcm1p is known to bind to the 11 bp central region of the operator, while Matα2p binds to the 10 bp on either side of the central segment. The two susceptible sites in the operator in isolated nuclei likely resulted from Mcm1p-induced bending of the DNA with consequent widening of the minor
groove (27). DNase I is known to approach and cut DNA in the minor groove and the slightly bent, partially open minor groove resembles the geometry of DNA in complex with the enzyme (32). These two sites were inaccessible when the analysis was performed with DNase I expressed in vivo. A large region between the two operator-flanking hypersensitive sites was blocked from digestion by the nuclease (Fig. 4). Footprints for the α2 operator in nuclei and in vivo for the recombination enhancer α2#2 site and for the transcriptional repressor α2 operators upstream of the STE2 and STE6 genes in α-cells were essentially identical to those shown in Figure 4 (data not shown). In α-cells, which lack Matα2p, in vivo and nuclear footprints were indistinguishable and closely resembled the pattern observed for α-cell nuclei. We presume that binding of Matα2p precludes nuclease access to operator DNA in α-cells in vivo.

Different nucleosome linker accessibilities in repressed domains in vivo

In yeast chromatin, a striking example of DNase I susceptibility of a precisely positioned nucleosome was found near the α2#1 operator in the recombination enhancer at 29403–29560 map units (m.u.) (17). In isolated nuclei, digestion was highest in linkers and at the ends of the core particle and diminished symmetrically to a low point at the center of the nucleosome. While these accessible linkers may characterize some yeast chromatin (see below), use of DNase I expressed in whole cells has revealed nucleosomes with protected linker chromatin in some repressed domains in vivo. One of these is the RE nucleosome at 29403–29560 m.u.

We confirmed the DNase I digestion pattern for this nucleosome in isolated nuclei. The linker and edges of the core particle were accessible to DNase I digestion. The lowest levels of cutting were in the center of the core particle (Fig. 5). In contrast, for the sample cut by DNase I in vivo, the cutting site susceptibilities were almost reversed. Sites near the center of the nucleosome core particle were cut in vivo more frequently than sites near the ends of the nucleosomes and the intervening short linkers. This observation was confirmed for DNase I cuts in the other strand through this same region. Furthermore, the short linkers between several other pairs of positioned nucleosomes in the RE in α-cells also were differentially digested by DNase I between isolated nuclei and in vivo samples (data not shown).

Another type of repressed domain with organized chromatin in α-cells encompasses the α-cell-specific genes, specifically STE6 (20,33). A characteristic feature of these chromatin domains is the presence of closely packed dinucleosomes with a short (<10 bp) linker. Longer linkers, in the range of 40–45 bp, connect these dinucleosomes to one another (34). Both linkers of the first nucleosome, adjacent to the α2 operator, were protected in vivo (Fig. 6 and data not shown). Cutting in nuclei was observed at a single site in the short linker between the first and second nucleosomes. Within the second nucleosome, cutting in nuclei was concentrated towards the ends of the core particle while cutting in vivo was relatively greater towards the center of the nucleosome. Significant differences were observed between nuclei and in vivo nuclease sites in the long linker following the second nucleosome (Fig. 6). The strong nuclear site that marks the edge of the second nucleosome at the long linker was absent in vivo while the site at the other end of the linker was present in both samples. Three DNase I cutting sites were present for the long linker in the in vivo sample. In nuclei, one of these was also DNase I sensitive, the other two were weakly cut and two different sites were strongly cut. It is apparent that significant features of this linker region, in terms of proteins bound or packaging, are altered during the preparation of nuclei.

The silent mating type loci, HMLα and HMRα, are perhaps the best-characterized repressed domains in the yeast genome (19,35,36). The chromatin structure of the regions between the E and I silencers has been determined by mapping with micrococcal nuclease. HMRα is the smaller and simpler of the two loci, having 12 precisely positioned nucleosomes between the silencer elements. These are arranged as six pairs of closely packed dinucleosomes with ~20 bp linker between the pairs (19). Figure 7 shows the results of mapping DNase I cutting sites in nuclei and in vivo for the nucleosome that is closest to the E silencer. The linkers flanking the nucleosome were significantly more susceptible to DNase I than the central region of the core particle, both in vivo and in isolated nuclei. For both samples, cutting sites within the core particle were spaced at ~10 nt intervals. However, several strong cutting sites...
were present in nuclei at the E end of the nucleosome and in the silencer, but absent in whole cells. This observation suggests that certain proteins, e.g. silent information regulator proteins (SIRs), involved in establishing or stabilizing the repressive domain, might protect this region in living cells.

**DISCUSSION**

Aspects of chromatin structure can be inferred because of a distinctive component, DNA, which can be assayed by enzymes and chemicals that specifically probe its structure. Based on known features of the reagent and permutations of the nucleoprotein environment of the chromatin segment under study, we can infer features of chromatin structure such as presence or absence of nucleosomes, their organization and possible positioning, binding of regulatory proteins, unusual DNA geometries, etc. Almost all such studies are carried out in the context of isolated nuclei, since reagent access to the nuclear contents is blocked in most cases by the plasma membrane and/or cell wall. Here we have surmounted this limitation by controlled intracellular expression of a nuclease and targeting the enzyme to the nucleus, where it can map chromatin organization without disruption of cell structures.

The nuclease of choice is DNase I. Micrococcal nuclease, the other relatively non-specific enzyme used widely for chromatin structure studies, cuts linker regions preferentially and is therefore used for mapping nucleosome locations. It has been less useful in revealing features of interactions of regulatory proteins with DNA (4). Of greater concern, however, is the fact that micrococcal nuclease is a general nuclease, with a strong preference for degrading single-stranded nucleic acids. Thus, it will likely be cytotoxic by extensive degradation of RNA. Such activity might well kill cells before the desired levels of DNA cutting for a mapping experiment were achieved.
DNase I, on the other hand, has no ribonuclease activity, is active with micromolar concentrations of calcium or magnesium and makes single strand nicks in double-stranded DNA as its preferential mechanism of action. A crystal structure of the enzyme as a complex with a DNA substrate is available (32). This structure shows both the N- and C-termini of the enzyme to be on the opposite side of the protein from the active site; with this knowledge, we could modify the N-terminus of the enzyme with the NLS, confident that it would not alter DNase I enzymatic activity or specificity. DNase I is a good probe for chromatin structure, cutting within the nucleosome at ~10 nt intervals as well as cutting linker DNA. Additionally, DNase I has been the benchmark enzyme for defining sites of DNA function in chromatin, marking interactions of regulatory proteins with DNA, for over two decades (4,9,10,37).

The nuclease was outfitted with an NLS and placed in yeast under GAL control. Culture of cells in galactose induced expression of DNase I leading to nicking and degradation of plasmid DNA. Cytotoxic effects of DNase I expression led to cell death, presumably due to damage to genomic DNA in excess of the capacity of repair systems. Nuclease activity footprinted binding of regulatory proteins and in a critical test detected the interactions of a labile repressor with its cognate binding site, interactions which are absent in isolated nuclei. The nuclease susceptibility of the Matα2p/Mcm1p operator is completely consistent with the structure of the ternary complex of the DNA binding domains of the two proteins with DNA (27) and the observation that, in vitro, full-length Matα2p is bulky enough to protect the entire operator from a nuclease (24). While methyltransferases also provide the ability to detect binding of regulatory proteins in living cells, the resolution of studies using these enzymes is less than for DNase I, due to the relative infrequency of modification sites for the methyltransferases compared to susceptible sites for the nuclease (26). Another limitation to use of some methyltransferases is the occurrence of endogenous cytosine methylation in many eukaryotic species. Use of DNase I expressed in whole cells as a general probe for chromatin structure thus offers many advantages over currently available technologies for investigation of interaction of regulatory proteins with DNA.

Features of chromatin structure involving histone DNA interactions, higher order structure, and arrangements of linker DNA and perhaps H1 histones are also amendable to study using in vivo expressed DNase I. A striking and unexpected set of observations about the organization of chromatin has emerged from this study. For several repressed yeast chromatin domains, differences in DNase I digestion patterns between samples digested with the enzyme in vivo and those analyzed with exogenous enzyme in isolated nuclei have been found. Unique features of the DNase I digestion pattern span a spectrum from minor changes in living cells versus isolated nuclei to wholesale alterations in the relative nuclease susceptibilities of linkers and core particle nucleosome segments in the two situations. Higher order chromatin structure in larger eukaryotes is stabilized by the linker histone, H1 (38,39). The yeast ortholog, Hho1p, differs from usual lysine rich histones by having two globular domains and lacking highly basic N- and C-terminal tails (40,41). Given this composition, we anticipate that binding of Hho1p to DNA might be more labile than typical H1 histones. The possible role of Hho1p in the differences between chromatin features detected by DNase I in isolated nuclei and in vivo remains to be evaluated. Similarly, a contribution to the observed results of differential repair in particular chromatin regions cannot be dismissed out of hand. Contributions of endogenous nucleases to the results seem less likely and if present must reflect a yeast nuclease with similar properties to bovine pancreatic DNase I.

Each of the three types of repressed domains, RE (17), a-cell-specific genes (20) or HM locus (19), has a distinctive organization of positioned nucleosomes. Chromatin structure of each is dependent on a corepressor, Tup1p or Sir3p, that has been shown to interact in vitro with the basic N-terminal regions of histones H3 and H4 (42,43). For the two domains that depend on Tup1p for repression, RE and a-cell-specific genes, there are significant differences in linker susceptibility between chromatin in cells and chromatin in isolated nuclei (Figs 5 and 6). Protection from DNase I cutting in vivo suggests that some degree of higher order structure and/or protection of linker regions by interaction of histone tails with the corepressor is present in vivo. In the case of the a-cell-specific genes, this suggestion is strongly supported by electron microscopic images of repressed chromatin domains in isolated minichromosomes. A portion of the repressed STE6 gene exists as a loop or hairpin of highly condensed chromatin (C.L.Woodcock, C.E.Ducker and R.T.Simpson, unpublished observations).

Somewhat surprising was the observation that the chromatin structure of the HMHa locus was similar in nuclei and in vivo. The simple pattern with linker susceptibility greater than that in the center of the core particle is presumed to be characteristic of extended or zigzag strings of nucleosomes. Since the HM loci are the prime examples of silencing thought to be based in chromatin architecture, one anticipated some indication of higher order structure for these elements. Instead, the sole indication of additional protein interactions is protection of sensitive sites near the nucleosome–E silencer border in vivo. Direct investigation of the extent of interaction of the HM domains with corepressors and their morphology when isolated and visualized in the electron microscope is of high interest.

ACKNOWLEDGEMENTS

We are indebted to members of the Simpson and Workman laboratories for criticism and support. We particularly thank Dr John Diller for comments on the manuscript. These studies were supported by grant R01 GM-52908 from the National Institute of General Medical Sciences.

REFERENCES


