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Heterochromatin Organization of a Natural Yeast Telomere

CHANGES OF NUCLEOSOME DISTRIBUTION DRIVEN BY THE ABSENCE OF Sir3p*

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We have defined the *in vivo* heterochromatin structure of the left telomere of *Saccharomyces cerevisiae* chromosome III (LIII). Analysis of heterochromatin of a single telomere was so far lacking, due to the difficulties intrinsic to the highly repetitive nature of telomeric sequences. In LIII, the terminal $(C_{1-3}A)_n$ repetitive sequences are followed by a complete *X* element and by the single copy Ty5-1 retrotransposon. Both the telosome and the *X* element exhibit overall resistance to micrococcal nuclease digestion reflecting their tight chromatin structure organization. The *X* element contains protein complexes and irregularly distributed but well localized nucleosomes. In contrast, a regular array of phased nucleosomes is associated with the promoter region of Ty5-1 and with the more centromere-proximal sequences. The lack of a structural component of yeast telomeres, the SIR3 protein, does not alter the overall tight organization of the *X* element but causes a nucleosome rearrangement within the promoter region of Ty5-1 and releases Ty5-1 silencing. Thus, Sir3p links the modification of the heterochromatin structure with loss of transcriptional silencing.

Telomeres play an essential role in cell biology in stabilizing chromosomes and facilitating complete replication of chromosomal termini. Telomeric DNA usually contains tandem repetitions of a short motif flanked by subtelomeric middle repetitive sequences (1). In yeast, telomeric sequences are composed of about 350 base pairs containing the $(C_{1-3}A)_n$ repeats and are followed by two main subtelomeric sequences: the *Y′* and *X* elements (2). *Y* elements are highly conserved and are found in about 70% of the telomeres (2–5). *X* elements are present in all telomeres and can exist in two main forms: a complete form containing the *X* core and the STR-A,B,C,D (6) elements or a short form containing essentially the *X* core or part of it (2, 4–6). The complete *X* is found in about 80% of the telomeres, whereas uncomplete forms are found in the remaining 20%.

Previous reports have referred to the chromatin structure of yeast telomeres as heterochromatin. This denomination is based on structural and functional similarities that yeast telomeres share with *Drosophila* heterochromatin (7–9). In *Saccharomyces cerevisiae* the terminal $(C_{1-3}A)_n$ repeats are organized into a nuclease-resistant structure called telosome (10, 11) that does not contain nucleosomes and is associated with the protein RAP1 (10–13). This protein binds to the repetitive $(C_{1-3}A)_n$ sequences (14, 15) and interacts with other proteins including RIF1, RIF2, SIR3, and SIR4 (16–18). The proteins SIR3 and SIR4 interact with each other, RAP1, and the amino terminus of the histones H3 and H4. Thus, the building of the telomeric heterochromatic structures in yeast involves complex homotypic and heterotypic interactions.

Although the chromatin structure of yeast telomeres is probably the best known among all eukaryotes, its specific organization is still poorly understood. It is known that both *Y′* and *X* elements contain nucleosomes (10). However, their distribution within both elements has not been described previously.

A previous report has described that the Ty5-1 retrotransposon, a subtelomeric transcriptional unit located in *S. cerevisiae* LIII, undergoes telomeric silencing (19). This retrotransposon is silenced in wild-type strains but is derepressed in a *sir3* mutant. We describe here the chromatin structure of LIII and its influence on the silencing of Ty5-1. A link between Ty5-1 silencing and a specific Sir3p-dependent heterochromatin structure is established.

EXPERIMENTAL PROCEDURES

Yeast Strains and Culture Conditions—*S. cerevisiae* wild-type strains LJY153 and PKY501 and mutant LJY155 (bearing deletion of the *SIR3* gene) (*sir3*) (32, 33) were grown at 28 °C on SC medium containing glucose to an A_{600} of about 1.0/ml.

Nuclease Sensitivity Analyses—Cells from 50-ml cultures were collected by centrifugation, treated with zymolyase, and digested with micrococcal nuclease (MNase)[1] or Dnase I as described previously (34). For digestions with DNase I, the incubation buffer was supplemented with 6 mM $MgCl_2$. After digestions of chromatin or naked DNA, the sensitivity to the enzymes was analyzed by the indirect end-labeling technique (20). The DNA samples were purified, digested with *Bam*HI, resolved in agarose gels, and transferred to nylon membranes. The cutting profiles generated by both enzymes were visualized after hybridization with an internal probe from Ty5-1. This probe abuts the *Bam*HI site selected for the analyses and extends from position 1495 to 1725 of *S. cerevisiae* chromosome (35). Nucleosomal spacing analyses were performed with MNase. After digestion of chromatin with MNase, the purified DNA samples were directly resolved in agarose gels generating a nucleosome ladder that could be visualized by staining with ethidium bromide. The DNA samples were then transferred to nylon membranes and hybridized with the probes as indicated in the legend to Fig. 2.

RESULTS

The Chromatin Organization of the STR, X-core, and Ty5-1 Sequences—The chromatin structure of *S. cerevisiae* LIII has been analyzed by using the enzymes MNase and DNase I. Permeabilized cells were digested with increasing concentra-

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[1] The abbreviation used is: MNase, micrococcal nuclease.

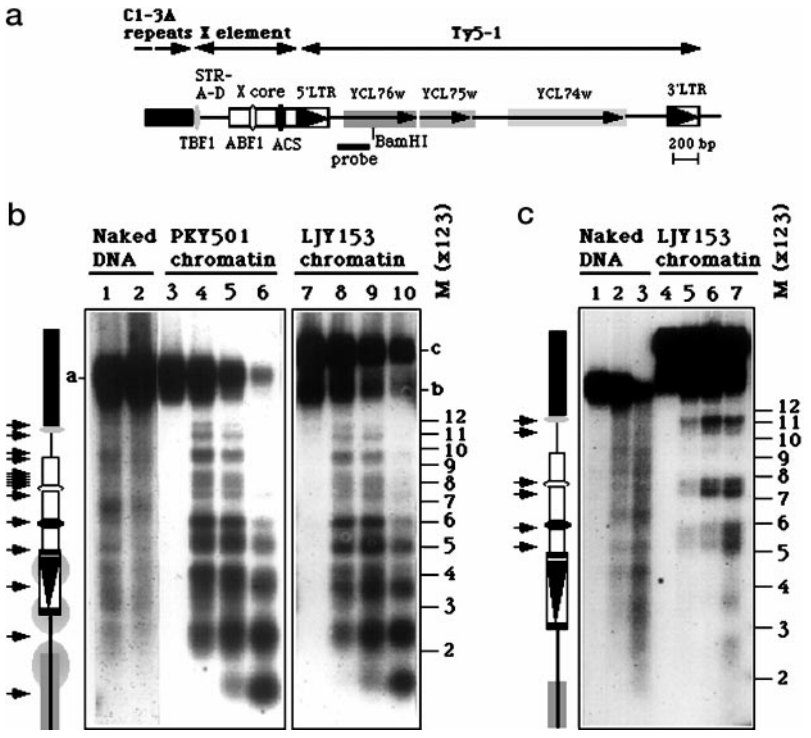

FIG. 1. **Nucleases sensitivity analyses reveal different kinds of heterochromatin within *S. cerevisiae* LIII.** *a*, representation of LIII. The position of the $(C_{1-3}A)_n$ repeats, the *X* element and the Ty5-1 retrotransposon are indicated as well as the TBF1, ABF1, and ACS binding sites, the STR-A-D elements and the *X* core inside the *X* subtelomeric element. The 5′ and 3′ long terminal repeats of Ty5-1 are also shown. YCL76w, 75w, and 74w refer to open reading frames previously defined by sequencing (35). The positions of the probe (*black bar*) and the restriction site used for indirect end-labeling are indicated at the *bottom*. *b*, sensitivity of the LIII to MNase. *S. cerevisiae* wild-type strains LJY153 and PKY501 (32, 33) were grown at 28 °C on SC medium containing glucose to an A_{600} of about 1.0 per ml. Naked DNA from strain PKY501 and chromatin samples from PKY501 and LJY153 were digested with increasing concentrations of MNase as described previously (34) and analyzed by the indirect end-labeling technique (20). The DNA samples were purified, digested with *Bam*HI, resolved in agarose gels, and transferred to nylon membranes. Then the cutting profiles were visualized after hybridization with an internal probe from Ty5-1. This probe abuts the *Bam*HI site selected for the analyses and extends from position 1495 to 1725 of *S. cerevisiae* chromosome III (35). Samples 1 and 2 were digested with 1.25 and 2.5 units/ml of MNase. Samples 3 and 7, 4 and 8, 5 and 9, and 6 and 10 were digested with 0, 4, 12, and 40 units/ml, respectively. The migration distances of commercial molecular size markers (123-base pair ladder from Life Technologies, Inc.) are indicated on the *right*. A scaled representation of the LIII is shown on the *left*. *Arrows* point to the main cuts detected in chromatin. *Gray circles* inside Ty5-1 represent translationally phased nucleosomes. *c*, sensitivity of the LIII to DNase I. Naked DNA from strain LJY155 and chromatin samples from LJY153 were digested with increasing concentrations of DNase I and analyzed by the indirect end-labeling technique. The incubation buffer for DNase I digestion was the same buffer used for MNase digestion supplemented with 6 mM $MgCl_2$. Samples 1, 2, and 3 were digested with 0, 1.2, and 4.5 ng/ml of DNase I. Samples 3–6 were digested with 0, 0.5, 2.5, and 12.5 μg/ml, respectively.

tions of MNase and the sensitivity to the enzyme analyzed by indirect end-labeling (20) of purified DNA using an internal probe from Ty5-1 (Fig. 1*a*). Fig. 1*b* shows the MNase digestion profiles of chromatin from two different wild-type strains (PKY501 and LJY153) and of naked DNA. One fuzzy telomeric band is observed in the lanes containing undigested naked DNA or chromatin from PKY501 (Fig. 1*b*, *band a*). In contrast, two bands are observed in the lane containing undigested chromatin from LJY153 (Fig. 1*b*, *bands b* and *c*). These bands correspond to two different subpopulations of telomeric fragments containing different average amounts of $(C_{1-3}A)_n$ repeats.[2]

Digestion of naked DNA with MNase yielded a rather uniform pattern (Fig. 1*b*, *lanes 1* and *2*). However, the digestion profiles of chromatin from both PKY501 and LJY153 are not uniform (Fig. 1*b*, *lanes 3–10*). The more relevant aspect of both digestion patterns is that the $(C_{1-3}A)_n$ repeats and most of the *X* subtelomeric element show overall protection against MNase reflecting a closed chromatin structure organization. Inside the *X* element, this protection extends from the STR-A sequences to the ACS binding site, a silenced origin of replication (6). The 5′ region of Ty5-1 is more sensitive to the enzyme (see the lower part of the gel). This higher sensitivity extends also to the region located at the right side of the *Bam*HI site (data not shown). Thus, the *X* subtelomeric element, like the telosome, exhibits an overall nuclease-resistant chromatin structure that does not extend into the Ty5-1 element.

Fig. 1*b* shows a regular MNase cutting pattern inside the 5′ region of Ty5-1 in both pKY501 and LJY153 (*lanes 3–10*). The enzyme cuts this region at specific sites indicating the presence of a regular array of translationally phased nucleosomes. Thus, the 5′ long terminal repeat of Ty5-1, where the promoter of the retrotransposon is located, accommodates nucleosomes that are associated with specific DNA sequences.

Unlike the 5′ region of the Ty5-1 retrotransposon, the *X* element has an irregular chromatin structure. To further analyze the chromatin structure of the *X* element, its sensitivity to DNase I was also tested. Fig. 1*c* shows the resulting digestion profiles. Three pairs of hypersensitive sites are clearly detected in chromatin but not in naked DNA. These sites are found inside the *X* element where the STR-A, B elements, the ABF1 and the ACS binding sites are located (6, 21). These results imply that these binding sites are associated with DNA-binding proteins.

Since the indirect end-labeling analyses shown in Fig. 1 do not provide data on the presence of nucleosomes inside the *X* element, a nucleosomal spacing analysis of this region was performed. Chromatin from the wild-type strain PKY501 was

[2] M. A. Vega-Palas, S. Venditti, and E. Di Mauro, unpublished observations.

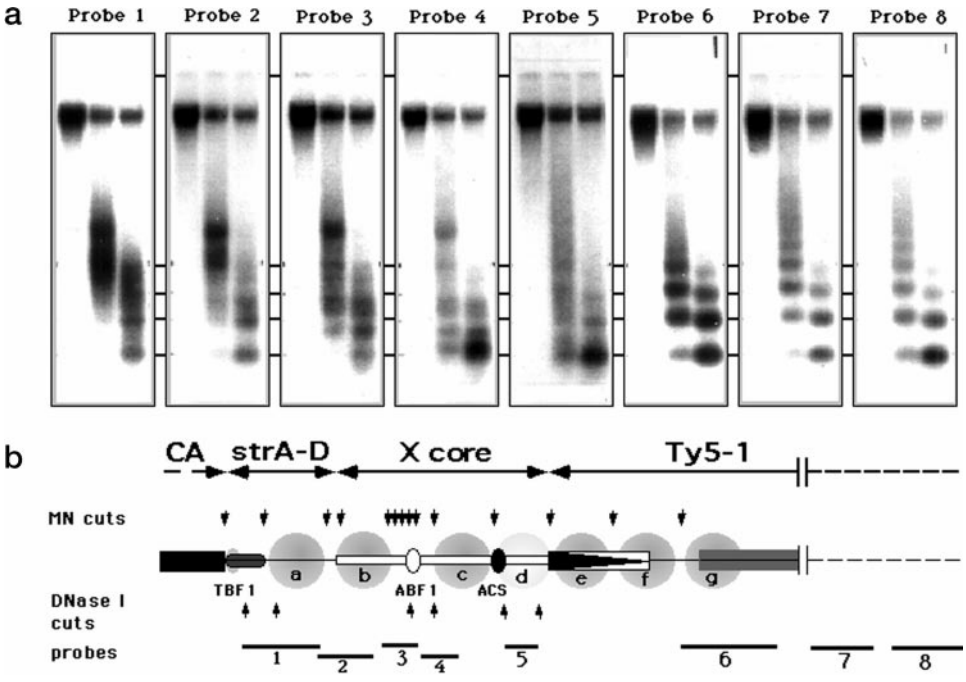

FIG. 2. **Irregular distribution of nucleosomes inside *X* subtelomeric elements.** *a*, nucleosomal spacing analysis. After digestion of chromatin from the wild-type strain PKY501 with increasing concentrations of MNase (0, 20, and 40 units/ml), the purified DNA samples were directly resolved in agarose gels generating a nucleosome ladder that could be visualized after staining with ethidium bromide. The DNA samples were then transferred to nylon membranes and successively hybridized with 5 probes for the *X* core and 3 probes for Ty5-1. Probes 1–5 extend from positions 405 to 589, 592 to 728, 752 to 837, 851 to 943, and 1057 to 1138 of chromosome III, respectively (35). Probes 6–8 extend from positions 1496 to 1726, 2131 to 2349, and 2761 to 2942, respectively. The migration distances of mono-, di-, tri-, and tetranucleosomic particles are indicated by horizontal lines. *b*, distribution of nucleosomes and protein complexes in the LIII. The position of the $(C_{1-3}A)_n$ repeats, the STR-A-D elements, the *X* core, and the Ty5-1 retrotransposon are indicated as well as the location of the TBF1, ABF1, and ACS binding sites. The 5′ long terminal repeat of Ty5-1 and part of YCL76w are also represented. The sites of MNase and DNase I cleavage were detected by indirect end-labeling and are indicated by *arrows* that correspond to the arrows depicted in Fig. 1 (*panels b* and *c*). The probes used for hybridization of the nucleosome ladder are represented at the *bottom* of the figure by *black bars*. *Filled circles* represent nucleosomes, whereas the *black ellipse* that contains the TBF1 binding site represents a protein-DNA complex that associates with the STR-A,B elements (see text).

digested with increasing concentrations of MNase. The purified DNA was resolved on agarose gel, blotted, and successively hybridized with five small probes for the *X* element and three for Ty5-1. This experimental approach indicates whether the DNA used as a probe is associated with nucleosomal particles and/or with protein-DNA complexes of a different nature, thus complementing the data obtained by indirect end-labeling. The sequences of the Ty5-1 probes are unique in PKY501 and, therefore, only displayed the nucleosomal array inside Ty5-1. On the contrary, *X* elements are found at all telomeres. Since 80% of the *X* elements are complete and highly conserved, *X* element probes provided information on the average chromatin structure of all these elements.

The profiles resulting from hybridization of the nucleosome ladder with all the probes are shown in Fig. 2*a*. Whereas Ty5-1 probes (*probes 6, 7*, and *8*) display a normal nucleosomal array, none of the probes from the *X* element (*probes 1–5*) does so. These results are consistent with the data obtained by indirect end-labeling for LIII and highlight the special chromatin structure of *X* subtelomeric elements. A model that integrates the indirect end-labeling and the nucleosomal spacing analyses is shown in Fig. 2*b*. This model represents the chromatin structure of the left telomeric region of chromosome III. A protein-DNA complex covering about 100 base pairs is located immediately adjacent to the $(C_{1-3}A)_n$ repeats. This complex is associated with the STR-A,B sequences where several iterations of the TTAGGG repeat are located. The TTAGGG repeat is the binding site for the TBF1 protein and the most widespread terminal telomeric repeat, present also in humans. Two translationally positioned nucleosomes are located after the first complex: nucleosome *a* in the STR-C,D elements and nucleosome *b* in the *X* core. These nucleosomes are followed by a second complex located within a region of the *X* core that contains the ABF1 binding site, which is highly conserved among all of the *X* cores. After this complex, a third nucleosome (*c*) is also translationally positioned. This nucleosome is followed by a third complex that encompasses the sequences of the ACS binding site. The ACS binding site is also conserved among *Saccharomyces X* cores and is known to be bound by the origin recognition complex. Since probe 5 detects a smeared mononucleosomal particle, we assume that the third complex is in the context of a fourth nucleosome (nucleosome *d*), possibly characterized by an open configuration, as indicated by its accessibility to DNase I. Finally, three additional positioned nucleosomes are located inside Ty5-1 (nucleosomes *e*, *f*, and *g*).

In summary, in LIII the telosome is followed by the *X* element that has a closed overall chromatin structure and contains protein complexes and irregularly distributed translationally positioned nucleosomes. Centrally to the *X* element, the Ty5-1 retrotransposon is organized into translationally positioned nucleosomes that follow a regular array.

The Heterochromatin Structure in the Absence of Sir3p—The chromatin structure of *S. cerevisiae* LIII has been also analyzed in a *sir3* mutant (LJY155). Fig. 3 shows the MNase and DNase I digestion profiles of the mutant and of its isogenic wild-type strain (LJY153). As in the wild-type strain, the *X* element shows overall protection against MNase in the *sir3* mutant (Fig. 3*a*). Thus, SIR3 is not necessary to keep the closed overall organization of the *X* element. In addition, the digestion pattern of the *X* element is quite similar in both strains suggesting that the lack of Sir3p does not cause major changes in the specific chromatin structure of the *X* element.

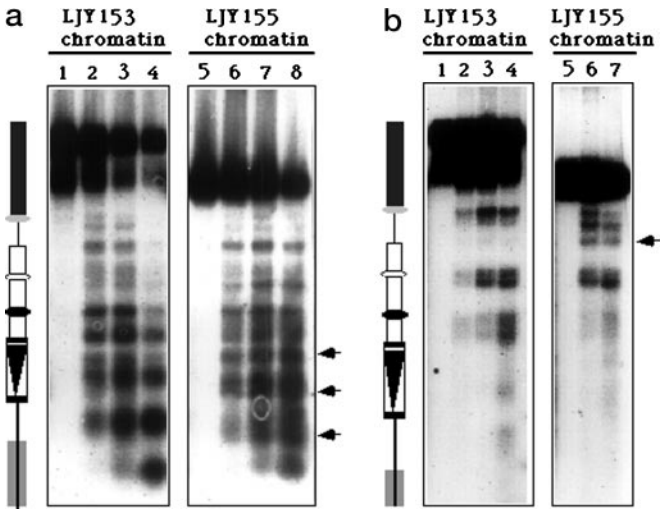

FIG. 3. **The chromatin structure of the LIII is altered in a *sir3* mutant.** *a*, altered sensitivity to MNase. Chromatin samples from two isogenic strains, LJY153 (wild-type) and LJY155 (*sir3* mutant), were digested with increasing concentrations of MNase. Then, the sensitivity of LIII was analyzed by indirect end-labeling. Samples 1 and 5, 2 and 6, 3 and 7, and 4 and 8 were digested with 0, 4, 12, and 40 units/ml, respectively. A scaled representation of LIII is shown on the *left*. *Arrows* point to the additional cuts detected inside Ty5-1 in the *sir3* mutant. *b*, altered sensitivity to DNase I. Chromatin samples from LJY153 and LJY155 were digested with increasing concentrations of DNase I. The sensitivity of LIII was analyzed in both strains by indirect end-labeling. Samples 1 and 5, 2, 3 and 6, and 4 and 7 were digested with 0, 0.5, 2.5, and 12.5 μg/ml, respectively. The *arrow* on the *right side* of the *panel* points to an additional DNase I hypersensitive band detected in the *sir3* mutant.

Fig. 3*b* shows the DNase I sensitivity analyses of LJY153 and LJY155. Two major differences are observed between the two strains. An additional hypersensitive band is clearly detected in the *sir3* mutant between nucleosomes *a* and *b* (indicated by an *arrow* in Fig. 3*b*). In addition, the hypersensitive bands associated with nucleosome *d* are less pronounced in the mutant than in the wild-type strain. Thus, although the basic chromatin structure of the *X* element is conserved in the *sir3* mutant, the lack of Sir3p affects specific protein-DNA interactions inside this element.

The digestion pattern generated by MNase inside the *X* element is in general more smeared in the *sir3* mutant than in the wild-type strain (Fig. 3*a*). This result could be explained assuming that most of the nucleosomes and protein complexes found within wild-type *X* elements are also present in the *X* elements of the *sir3* mutant but in a more relaxed conformation. The digestion pattern generated by MNase in the promoter region of Ty5-1 is altered in the *sir3* mutant. Three additional bands are observed (Fig. 3*a*, *arrows*) which correlate the absence of Sir3p with a change of nucleosome distribution in the promoter region of Ty5-1.

In conclusion, the left telomeric region of *S. cerevisiae* chromosome III shows a major architectural feature: the *X* subtelomeric element is organized into a non-canonical closed chromatin structure that does not extend into the adjacent Ty5-1 retrotransposon. This kind of heterochromatic structure does not necessarily associate with the sequences located in the immediate proximity of the telosomes. In fact, it was not observed in a telomere in which a reporter *URA3* gene was placed adjacent to terminal $(C_{1-3}A)_n$ telomeric repeats (data not shown and Ref. 10). Thus, this special chromatin organization is associated with the specific DNA sequence of the *X* element.

DISCUSSION

The URA3 reporter gene and other reporter genes placed immediately adjacent to terminal $(C_{1-3}A)_n$ repeats in the absence of subtelomeric sequences are silenced (22). Therefore, the special heterochromatic organization of the *X* elements is not required for the spreading of transcriptional telomeric silencing.

Yeast telomeres repress the expression of adjacent genes (7). This repression, referred to as telomere position effect, requires the integrity of telomeric proteins like RAP1, SIR2, SIR3, SIR4, and the amino termini of histones H3 and H4 (23–25). SIR3 and SIR4 interact among them, with RAP1 and with the amino terminus of the core histones. Thus, they have been proposed to physically connect telosomes and the nucleosomes located in the telomeric regions that undergo silencing (17, 26–28). Genetic and biochemical data support this notion. SIR3 and SIR4 require RAP1 and the amino termini of histones H3 and H4 to silence telomeric genes (23–25). SIR3, SIR4, and RAP1 localize by immunofluorescence to a number of foci near the nuclear periphery, being the amino termini of histones H3 and H4 required for the perinuclear localization of SIR3 and SIR4. The positioning of these foci inside the nuclei is coincident with hybridization signals of subtelomeric repeats (26, 29, 30). In addition, SIR3, SIR4, RAP1, and histone proteins coimmunoprecipitate and are found at the same distance from the telomeres (27, 31).

We have shown in this report that the lack of Sir3p affects the chromatin structure of the *X* element and of the adjacent Ty5-1 retrotransposon in LIII. These results provide evidence for *in vivo* interactions between Sir3p and nucleosomes in the *X* element and in the Ty5-1 retrotransposon.

Ty5-1 has been previously described to be silenced in its natural context in a *SIR3* dependent manner (19). The promoter region of the retrotransposon is located at about 1.3 kilobases from the end of LIII. This result is in agreement with previous studies showing that Sir3p can be immunolocalized up to 2–3 kilobases from the right telomere of *S. cerevisiae* chromosome VI (27, 31). Since the chromatin structure of the Ty5-1 promoter region is altered in a *sir3* mutant, Sir3p establishes a link between chromatin remodeling and transcriptional telomeric silencing. The SIR complex could function as a stapler joining nucleosomes from the *X* element and from the Ty5-1 retrotransposon and impair the access of the transcriptional machinery to the Ty5-1 promoter.

In wild-type strains, the 5′ region of Ty5-1 shows overall sensitivity to MNase similar to the sensitivity of bulk DNA (data not shown) and contains a normal array of nucleosomes. Thus, this heterochromatic region does not show specific structural features. However, according to previous results (27), the 5′ region of Ty5-1 should associate with proteins like SIR2, SIR3, or SIR4. Therefore, these proteins should interact among them and with the amino termini of histones H3 and H4 without affecting the accessibility of MNase to the linker internucleosomal regions.

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REFERENCES

1. Zakian, V. (1989) *Annu. Rev. Genet.* **23,** 579–604
2. Louis, E. (1995) *Yeast* **11,** 1553–1573
3. Walmsley, R., Chan, C., Tye, B. & Petes, T. (1984) *Nature* **310,** 157–160
4. Chan, C. & Tye, B. (1983) *J. Mol. Biol.* **168,** 505–523
5. Chan, C. & Tye, B.-K. (1983) *Cell* **33,** 563–573
6. Louis, E., Naumova, E., Lee, A., Naumov, G. & Haber, J. (1994) *Genetics* **136,** 789–802
7. Laurenson, P. & Rine, J. (1992) *Microbiol. Rev.* **56,** 543–560
8. Weiler, K. & Wakimoto, B. (1995) *Annu. Rev. Genet.* **29,** 577–605
9. Henikoff, S.(1990) *Trends Genet.* **6,** 422–426
10. Wright, J., Gottschling, D. & Zakian, V. (1992) *Genes Dev.* **6,** 197–210
11. Wright, J. & Zakian, V. (1995) *Nucleic Acids Res.* **23,** 1454–1460
12. Klein, F., Laroche, T., Cardenas, M., Hofman, J., Schweizer, D. & Gasser, S. (1992) *J. Cell Biol.* **117,** 935–948
13. Conrad, M., Wright, J., Wolf, A. & Zakian, V. (1990) *Cell* **63,** 739–750

14. Longtime, M., Wilson, N., Petracek, M. & Berman, J. (1989) *Curr. Genet.* **16,** 225–240
15. Buchman, A., Kimmerly, W., Rine, J. & Kornberg, R. (1988) *Mol. Cell. Biol.* **8,** 210–225
16. Wotton, D. & Shore, D. (1997) *Genes Dev.* **11,** 748–760
17. Moretti, P., Freeman, K., Coodly, L. & Shore, D. (1994) *Genes Dev.* **8,** 2257–2269
18. Hardy, C., Sussel, L. & Shore, D. (1992) *Genes Dev.* **6,** 801–814
19. Vega-Palas, M., Venditti, S. & Di Mauro, E. (1997) *Nat. Genet.* **15,** 232–233
20. Wu, C. (1980) *Nature* **286,** 854–860
21. Pryde, F., Huckle, T. & Louis, E. (1995) *Yeast* **11,** 371–382
22. Gottschling, D., Aparicio, O., Billington, B. & Zakian, V. (1990) *Cell* **63,** 751–762
23. Kyrion, G., Boakye, K. & Lustig, A. (1992) *Mol. Cell. Biol.* **12,** 5159–5173
24. Thompson, J., Ling, X. & Grunstein, M. (1994) *Nature* **369,** 245–247
25. Aparicio, O., Billington, B. & Gottschling, D. (1991) *Cell* **66,** 1279–1287
26. Hecht, A., Laroche, T., Strahl-Bolsinger, S., Gasser, S. & Grunstein, M. (1995) *Cell* **80,** 583–592
27. Hecht, A., Strahl-Bolsinger, S. & Grunstein, M. (1996) *Nature* **383,** 92–96
28. Moazed, D., Kistler, A., Axelrod, A., Rine, J. & Johnson, A. (1997) *Proc. Natl. Acad. Sci. U. S. A.* **94,** 2186–2191
29. Gotta, M., Laroche, T., Formenton, A., Maillet, L., Shertan, H. & Gasser, S. (1996) *J. Cell Biol.* **134,** 1349–1363
30. Palladino, F., Palladino, F., Laroche, T., Gilson, E., Axelrod, A., Pillus, L. & Gasser, S. (1993) *Cell* **75,** 543–555
31. Strahl-Bolsinger, S., Hecht, A., Luo, K. & Grunstein, M. (1997) *Genes Dev.* **11,** 83–93
32. Johnson, L., Kayne, P., Kahn, E. & Grunstein, M. (1990) *Proc. Natl. Acad. Sci. U. S. A.* **87,** 6286–6290
33. Kayne, P., Kim, U.-J., Han, M., Mullen, J., Yoshizaki, F. & Grunstein, M. (1988) *Cell* **55,** 27–39
34. Venditti, S. & Camilloni, G. (1994) *Mol. Gen. Genet.* **242,** 3811–3812
35. Oliver, S. *et al.* (1992) *Nature* **357,** 38–46

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