The Histone Chaperone Asf1p Mediates Global Chromatin Disassembly in Vivo*

Received for publication, June 2, 2004, and in revised form, September 22, 2004
Published, JBC Papers in Press, September 26, 2004, DOI 10.1074/jbc.M406113200

Melissa W. Adkins and Jessica K. Tyler‡
From the Department of Biochemistry and Molecular Genetics, University of Colorado Health Sciences Center at Fitzsimons, Aurora, Colorado 80045

The packaging of the eukaryotic genome into chromatin is likely to be mediated by chromatin assembly factors, including histone chaperones. We investigated the function of the histone H3/H4 chaperones anti-silencing function 1 (Asf1p) and chromatin assembly factor 1 (CAF-1) in vivo. Analysis of chromatin structure by accessibility to micrococcal nuclease and DNase I digestion demonstrated that the chromatin from CAF-1 mutant yeast has increased accessibility to these enzymes. In agreement, the supercoiling of the endogenous 2μ plasmid is reduced in yeast lacking CAF-1. These results indicate that CAF-1 mutant yeast globally underassemble their genome into chromatin, consistent with a role for CAF-1 in chromatin assembly in vivo. By contrast, asf1 mutants globally overassemble their genome into chromatin, as suggested by decreased accessibility of their chromatin to micrococcal nuclease and DNase I digestion and increased supercoiling of the endogenous 2μ plasmid. Deletion of Asf1 causes a striking loss of acetylation on histone H3 lysine 9, but this is not responsible for the altered chromatin structure in asf1 mutants. These data indicate that Asf1p may have a global role in chromatin disassembly and an unexpected role in histone acetylation in vivo.

Chromatin assembly factor 1 (CAF-1) was discovered over a decade ago as a factor that is required to complement a crude extract in order to deposit H3 and H4 onto newly replicated DNA in vitro. The preference of CAF-1 for newly replicated DNA appears to be mediated via its interaction with the replication protein, proliferating cell nuclear antigen. A second H3 and H4 histone chaperone, termed anti-silencing function 1 (ASF1), was identified by fractionation of a crude extract. ASF1 can assemble unreplicated DNA into chromatin and is also required to facilitate CAF-1-mediated assembly of newly replicated DNA into chromatin in vivo.

Because of the artificial nature of biochemical chromatin assembly systems (reviewed in Ref. 2), it is imperative that the function of histone chaperones be examined in vivo. Recent evidence indicates that CAF-1 may well be a chromatin assembly factor in human cells. Removal of CAF-1 by short interfering RNA-mediated knockdown resulted in increased sensitivity of bulk chromatin to micrococcal nuclease digestion, indicating that the chromatin is less well packaged in the absence of CAF-1. However, it is not known whether ASF1 is a chromatin assembly factor in vivo, as global effects of ASF1 on chromatin structure in vivo have not been reported. ASF1 is highly conserved among eukaryotes, and its inactivation leads to a broad range of cellular defects, the molecular basis of which is unclear. By contrast to the in vitro role of Asf1 as a chromatin assembly factor, we have recently discovered that yeast ASF1 (Asf1p) mediates chromatin disassembly at the PHO5 and PHO8 promoters in vivo. Here, we provide evidence suggesting that Asf1p is a global chromatin disassembly factor in yeast, whereas CAF-1 is a global chromatin assembly factor in vivo. Furthermore, we have found that Asf1p plays an unexpected role in acetylation of histone H3 lysine 9.

EXPERIMENTAL PROCEDURES

Yeast Strains and Media—Yeast cultures and genetic manipulations were performed following standard methods. The genotypes of all strains used in this study are described in Table I. The H4K4Q strain carries substitutions of lysines 5, 8, 12, and 16 to glutamate in histone H4, and the H3tailΔ strain has the N terminus of histone H3 deleted (amino acids 1–28). Yeast strains were grown to a density of ~1 × 10^7 cells/ml in yeast extract-peptone-dextrose for all analyses.

MNase and DNase I Analysis—Equal amounts of nuclei were digested with increasing concentrations of micrococcal nuclease MNase (Sigma) or DNase I (Worthington) as described previously. DNA was isolated and electrophoresed on 1.2% agarose gels and stained with ethidium bromide.

Supercoiling Analysis—30 ml of the indicated yeast strains were grown to a density of 2 × 10^7 cells/ml, sedimented, resuspended in 400 ml of resuspension buffer (50 mM Tris, pH 8, 50 mM EDTA, 10 mM

*This work was supported in part by National Institutes of Health Grant GM64475 (to J. K. T.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
‡Leukemia & Lymphoma Society scholar. To whom correspondence should be addressed. Tel.: 303-724-3224; Fax: 303-724-3221; E-mail: Jessica.tyler@uchsc.edu.
1The abbreviations used are: CAF-1, chromatin assembly factor 1; ASF1, anti-silencing function 1; MNase, micrococcal nuclease; WT, wild type.
is consistent with the observation that gross nucleosome loss results in inviability (14), whereas asf1 and CAF-1 mutants are viable. However, closer examination revealed that the chromatin from the cac1 mutant was slightly more accessible to digestion by MNase, as compared with the wild type chromatin. This is indicated by the slightly smaller DNA fragment sizes in the cac1Δ strain at any given MNase concentration when compared with the same MNase concentration in the wild type strain (compare the quantitation tracings of the MNase digestion of chromatin from WT and cac1Δ strains at 0.16 units/ml MNase) (Fig. 1). By contrast, chromatin from the asf1 mutant appeared to be slightly more resistant to digestion with MNase, as compared with the wild type chromatin. This was apparent by the larger DNA fragment sizes in the asf1Δ strain at any given MNase concentration when compared with the same MNase concentration in the wild type strain, even though there appears to be less DNA in the asf1Δ samples than in the wild type (compare quantitation tracings of the MNase digestion of chromatin from WT and asf1Δ strains at 0.16 units/ml MNase) (Fig. 1).

Loss of CAF-1 Leads to Increased Accessibility to DNase I, whereas Loss of Asf1p Leads to Decreased Accessibility to DNase I Digestion—Next, we examined the accessibility of the chromatin to digestion with DNase I, a more sensitive measure of chromatin structure than MNase digestion (15). Similar to the result obtained with MNase above, chromatin from the cac1Δ strain was more accessible to DNase I digestion than chromatin from the wild type strain, apparent from the smaller DNA fragments at lower nucleosome concentrations as compared with the wild type strain (compare quantitation tracings of the DNase I digestion of chromatin from WT and cac1Δ strains at 2 units/ml DNase I) (Fig. 2). Therefore, chromatin in yeast lacking CAF-1 is more open or less fully assembled into nucleosomes than normal, consistent with a role of CAF-1 as a chromatin assembly factor. Similar to the result obtained for MNase digestion above, chromatin from the asf1Δ strain was less accessible to DNase I digestion, apparent from the smaller DNA fragments in the wild type strain at lower nucleosome concentrations as compared with the asf1Δ strain (compare quantitation tracings of the DNase I digestion of chromatin from WT and asf1Δ strains at 4 units/ml DNase I) (Fig. 2). Therefore, chromatin in yeast lacking Asf1p appears to be less open or more assembled into nucleosomes than normal. These findings are in contrast to the in vitro function of Asf1p as a chromatin assembly factor and instead suggest that Asf1p may disassemble chromatin in a global manner in vivo.

Loss of CAF-1 Leads to Reduced Supercoiling of the Endogenous 2μ Plasmid, whereas Loss of Asf1p Leads to Increased 2μ
Supercoiling—To further investigate chromatin structure in asf1 and CAF-1 mutants, we measured supercoiling of the endogenous 2 μ/H9262 plasmid. Because the assembly of each nucleosome onto a closed DNA circle results in the incorporation of one negative supercoil (16), the extent of supercoiling is a measure of nucleosome assembly. Comparison of the topoisomer distributions of the 2 μ/H9262 plasmid demonstrated that the cac2 Δ/H9004 strain has approximately two supercoils less than the wild type strain, corresponding to the loss of two nucleosomes per plasmid in CAF-1 mutants (Fig. 3). This supercoiling result is consistent with the reduced accessibility of MNase and DNase I to chromosomal DNA in the asf1 mutant (Figs. 1 and 2), demonstrating that the loss of Asf1p results in a more compact chromatin structure.

We also examined the effect of deleting ASF1 in strains that have reduced 2 μ/H9262 supercoiling. Comparison of the asf1 Δ/H9004 cac2 Δ/H9004 and cac2 Δ/H9004 strains demonstrated an increase in supercoiling corresponding to a gain of approximately one nucleosome per plasmid upon deletion of ASF1 (Fig. 3). These results show that the deletion of ASF1 increases the number of supercoils on the endogenous 2 μ plasmid, corresponding to increased numbers of nucleosomes in the absence of Asf1p.

Loss of Asf1p Causes a Striking Decrease in Acetylation of Histone H3 on Lysine 9—To provide further evidence that the supercoiling changes in the asf1 and CAF-1 mutants are due to altered nucleosome density, we examined other factors that can influence plasmid supercoiling. It is well known that changes in the activity of topoisomerases in vivo can result in altered DNA supercoiling (17). Because CAF-1 and Asf1p influence chromatin structure (as shown above), it is possible that their...
can affect 2
been suggested in the field that mutations in the explain the change in supercoiling that we observed. It has also
minor (1.56-fold and 2-fold respectively) increases in expres-
creased 2
supercoiling, where reduced amounts of histones lead to de-
CAF-1. The amount of bulk histones is known to alter plasmid
levels of histone acetylation is altered upon loss of Asf1p or
result in altered supercoiling of the 2μ plasmid (18). To address whether this could explain the altered supercoiling in the asf1 and CAF-1 mutants, we measured transcript levels of the 2μ-encoded genes. We found that there was no change in the transcript levels from the FLP1 gene, whereas transcript levels increased for REP1, REP2, and RAF1 in the asf1 mutant as compared with wild type cells (Table II). However, increased transcription is known to result in decreased supercoiling (18); therefore, the increased supercoiling seen in the asf1 mutant cells cannot be because of the increased expression of the 2μ-encoded genes. Comparison of the levels of the 2μ-encoded gene transcripts between cac2Δ and wild type strains showed no change in expression of REP1 or REP2 and minor (1.56-fold and 2-fold respectively) increases in expres-
sion from the FLP1 and the RAF1 transcript (Table II). However,
this slight increase in transcription is not sufficient to explain the change in supercoiling that we observed. It has also been suggested in the field that mutations in the ADE2 gene can affect 2μ supercoiling. However, changes in ADE2 cannot explain the altered supercoiling that we observed with asf1 and CAF-1 mutants because we obtained the same supercoiling results in strains auxotrophic or wild type for ADE2 (Fig. 3; data not shown).

Next, we addressed whether the amount of bulk histones or levels of histone acetylation is altered upon loss of Asf1p or CAF-1. The amount of bulk histones is known to alter plasmid supercoiling, where reduced amounts of histones lead to decreased 2μ supercoiling in vivo (14). Therefore, we compared the total amounts of histones H3, H4, H2A, and H2B in strains lacking Asf1p or CAF-1. This was particularly important to examine for Asf1p, as it has been reported that lack of Asf1p alters gene expression of the histone genes (9). We found no difference in the total amount of the core histones between wild type, asf1Δ, or cac1Δ strains (Fig. 4A). Therefore, the altered supercoiling in cells lacking Asf1p and CAF-1 is not because of altered levels of histone proteins. To investigate the possibility that deletion of ASF1 or CAC1 may lead to accumulation of histones in the cytoplasm, which would result in altered extents of chromatin assembly, we examined the cellular distribution of core histones. Having determined that the total levels of core histones were similar between the wild type, asf1Δ, or cac1Δ strains, we compared the levels of core histones in nu-
clear extracts. We found that the levels of core histones were indistinguishable between wild type, asf1Δ, or cac1Δ strains, (Fig. 4B; data not shown). Therefore, the altered supercoiling in cells lacking Asf1p and CAF-1 is not due to altered cellular distribution of core histone proteins.

Next, we asked whether histone acetylation levels are altered upon the loss of Asf1p or CAF-1, because increased histone acetylation is known to cause reduced 2μ supercoiling, whereas decreased histone acetylation leads to increased 2μ supercoiling (19). To address this question, we used antibodies specific for acetylated lysines on histones H3 and H4 (Fig. 4C). For the yeast lacking CAF-1, we found no change in the levels of histone acetylation on lysine 9, 14, 18, 23, or 27 of histone H3 and no change in either overall acetylation of histone H4 or acetylation on lysine 8 or 12 of H4 (Fig. 4C). Similarly, we found no change in the levels of acetylation of lysines 18, 23, or 27 of histone H3 nor lysines 8, 12, or overall acetylation of

| Gene | Average | S.D. | Call by Affymetrix |
|------|---------|------|--------------------|
| FLP1 | 0.10    | 0.99 | NC                 |
| REP1 | 1.43    | 0.50 | I                  |
| REP2 | 1.67    | 0.12 | I                  |
| RAF1 | 1.53    | 0.76 | I                  |
| TOP1 | 0.53    | 0.70 | NC                 |
| TOP2 | 0.64    | 0.29 | MI                 |
| FLP1 | 0.67    | 0.64 | MI                 |
| REP1 | 0.33    | 0.42 | NC                 |
| RAF1 | 1.00    | 0.60 | I                  |
| TOP1 | 0.60    | 1.11 | NC                 |
| TOP2 | 0.23    | 0.53 | NC                 |

FIG. 4. Loss of histone H3 lysine 9 acetylation in asf1 mutants. Equal amounts of whole cell extracts (A and C) and nuclei (B) from ROY1172 (WT), ROY1170 (asf1Δ), and ROY1171 (cac1Δ) strains were electrophoresed on a 15% SDS gel and transferred to nitrocellulose. The membrane was cut in half, and the lower half was probed with the indicated anti-histone antibodies. The top half of the membrane was probed for tubulin (A and C) or NUP-1 (B) as a loading control.

Microarray analysis of 2μ-encoded and topoisomerase genes in asf1 and CAF-1 mutants

Microarray data were analyzed with Affymetrix and GeneSpring software; shown are the log average and standard deviation for three independent experiments. The “call by Affymetrix” used gene-specific cutoffs that were designated as significant by Wilcoxon’s signed rank test in combination with noise filtering and only considering genes in which the changes were reproducible over three independent experiments. Topoisomerase (TOP1/TOP2) levels did not significantly change in asf1Δ and cac2Δ strains by Affymetrix microarray analyses. NC, no change; I, increase; MI, marginal increase.
histone H4 in asf1Δ strains compared with wild type (Fig. 4C). By contrast, we observed an approximate 50% reduction in acetylation using an antibody that recognizes both acetylated Lys-9 and Lys-14 of histone H3 upon loss of Asf1p. Using an antibody specific for acetylation of Lys-9 of histone H3, we observed a striking absence of detectable acetylation upon loss of Asf1p as compared with wild type (Fig. 4C). We were unable to test levels of Lys-14 acetylation alone, as a specific antibody for acetylated Lys-14 of histone H3 was not available. However, because of the striking loss of Lys-9 acetylation in asf1 mutants, we assume that the majority of the reduced signal from the antisera that recognized either acetylated Lys-9 or Lys-14 is due to the loss of Lys-9 acetylation. These results show that histone acetylation levels are not altered upon loss of CAF-1, whereas acetylation of H3 lysine 9 is drastically reduced upon loss of Asf1p.

**Decreased Supercoiling in asf1 Mutants Is Not a Consequence of Loss of Lysine 9 Acetylation**—Having discovered that there are differences in histone acetylation between yeast with and without Asf1p, it was essential to establish that this was not responsible for the altered chromatin structure that we had observed in asf1 mutants. To address this question, we constructed an asf1Δ strain that also lacked the acetylatable N terminus of histone H3, "asf1ΔH3tailΔ" to prevent any effect of histone H3 acetylation on 2μ supercoiling. As a control, we also included a strain "H4K4Q" that has all of the acetylatable lysines of histone H4 altered to glutamic acid and therefore cannot be acetylated. The histone mutant strains H3tailΔ and H4K4Q cause a reduction of one or two supercoils, respectively, in the endogenous 2μ plasmid as compared with wild type2 (Fig. 5). Comparison between the H3tailΔ and H3tailΔasf1Δ strains showed an increase of ~1 supercoil/plasmid upon deletion of ASF1 (Fig. 5). This result demonstrates that the increased supercoiling that occurs upon loss of Asf1p is not due to loss of histone H3 lysine 9 acetylation, as it still occurs in strains lacking the H3 N-terminal tail. Similarly, comparison of the H4K4Q and H4K4Qasf1Δ strains showed an increase of ~2–3 supercoils/plasmid upon deletion of ASF1 (Fig. 5). This provides another example of where deletion of ASF1 results in increased 2μ supercoiling, as was seen above in Fig. 3. In summary, the increased plasmid supercoiling observed upon deletion of ASF1 is independent of any role of histone acetylation on plasmid supercoiling. Taking all of our results together, these data indicate that loss of Asf1p results in global over-assembly of the genome into chromatin, suggesting that Asf1p has a broad role as a chromatin disassembly factor in vivo.

**DISCUSSION**

**CAF-1 Is a Global Chromatin Assembly Factor**—It cannot be assumed that molecules that assemble chromatin in vitro actually perform this function in vivo. In fact, almost any negatively charged molecule can act as a histone chaperone to assemble chromatin in vitro (2), but there is little direct evidence that histone chaperones identified biochemically can assemble chromatin in vivo. The best previous evidence to hint that CAF-1 may assemble chromatin in yeast came from its transcriptional effects on the four silent loci of yeast (13), which are known to be sensitive to chromatin structure. Here we show by MNase and DNase I accessibility analyses and by supercoiling analysis of the endogenous 2μ plasmid that there are global changes in the bulk chromatin structure upon disruption of CAF-1, corresponding to nucleosome loss from the genome. This is consistent with the recently reported increased accessibility to MNase that results from short interfering RNA-mediated knockdown of CAF-1 in HeLa cells (6). As such, the biochemical role of CAF-1 as a chromatin assembly factor indeed appears to reflect its function as a chromatin assembly factor in vivo. Furthermore, the biochemical role of CAF-1 in the assembly of chromatin during DNA replication is likely also to be the case in vivo, because CAF-1 was recently identified in a complex with the histone variant H3.1 that is specifically assembled during DNA replication (20).

**Asf1p Appears To Be a Global Chromatin Disassembly Factor**—In biochemical chromatin assembly assays, ASF1 can mediate the assembly of chromatin (5, 21–23). However, our data strongly suggest that yeast Asf1p is a global chromatin disassembly factor. Specifically, we found changes in the bulk chromatin structure in yeast disrupted for ASF1 that correspond to a gain of nucleosomes over the genome by MNase, DNase I, and 2μ supercoiling analyses. Our findings contradict a recent report of a loss of plasmid supercoiling upon deletion of ASF1 (10). It is possible that the change in supercoiling they observed was in fact an increase upon deletion of ASF1, as there were no controls for the direction of the mobility of the topoisomers. In fact, their MNase accessibility analysis, which was reported as showing no difference between asf1 mutants and wild type, showed that the chromatin from their asf1Δ strain was more resistant to MNase digestion than their wild type chromatin upon limited digestion. Therefore, we interpret the data in this previous report (10) as being consistent with our findings, which indicate that Asf1p is involved in chromatin disassembly in vivo.

Although our proposed role for Asf1p as a chromatin disassembly factor may appear to conflict with the function of Asf1p as a chromatin assembly factor in vitro (5), Asf1p may have a chromatin assembly function in vivo that is not apparent in these studies of the static state of chromatin in yeast lacking Asf1p. Alternatively, the biochemical function of ASF1 as a chromatin assembly factor may be an in vitro artifact. This is unlikely to be the case as we originally isolated *Drosophila* ASF1 in a complex with histones that were strictly acetylated at the residues found specifically on newly synthesized histones (5). If the function of ASF1 were only to disassemble chromatin, then it would not have been isolated with the newly synthesized form of histones. Furthermore, ASF1 was recently isolated from human cells in a soluble complex with the histone variant H3.1 that is only assembled into chromatin during DNA replication and in a complex with the histone variant H3.3 that is only assembled into chromatin during transcription (20). This suggests that ASF1 is involved broadly in chromatin assembly.
We propose that Asf1p functions as both an assembly and disassembly factor in vivo and that the balance is more in favor of chromatin disassembly during transcriptional regulation, as suggested by the more compact chromatin structure in asf1 mutants. Consistent with our proposed role for Asf1p as a chromatin disassembly factor during transcriptional activation, we have recently shown that yeast Asf1p mediates the disassembly of chromatin from the promoters of the PHO5 and PHO8 genes during transcriptional activation (12).

The changes in chromatin structure upon disruption of ASF1 or Caf-1 are modest. This suggests that either additional chromatin assembly/disassembly factors remain to be discovered or that budding yeast has some ability to spontaneously assemble/disassemble chromatin structures. On this note, it worth mentioning that deletion of ASF1 from fission yeast (24) or fruit flies (8) is lethal, whereas budding yeast can tolerate loss of Asf1p. It will be interesting to determine in the future whether the inability of higher eukaryotes lacking ASF1 is a consequence of more profound defects in chromatin structure or whether higher eukaryotes are more sensitive to subtle changes in chromatin structure.

Loss of Histone H3 Lys-9 Acetylation in Yeast Lacking Asf1p—In this study we performed many controls to verify that the altered chromatin structure in yeast lacking Asf1p or Caf-1 is indeed due to increased and decreased numbers of nucleosomes, respectively, on the genome. The only other change that we found that could possibly cause altered chromatin structure was a drastic reduction in levels of acetylation on histone H3 lysine 9 in yeast lacking Asf1p. This loss of histone acetylation on lysine 9 in asf1 mutants is quite striking and is the subject of another study. However, we ruled out the possibility that this altered histone acetylation was responsible for the altered chromatin structure in yeast lacking Asf1p by deleting the acetylable N-terminal tail from the only copy of histone H3 in these cells. The additional deletion of ASF1 still resulted in changes to the chromatin structure, indicating that this loss of H3 Lys-9 acetylation, although interesting, is not responsible for the altered chromatin structure in asf1 mutants. As such, we interpret our supercoiling results as reflecting altered numbers of nucleosomes. Although it is difficult to disprove, it remains a possibility that the altered supercoiling we observed reflects an altered path of the DNA around the nucleosomes. How the loss of histone chaperones may result in changes to the DNA-histone interactions within nucleosomes is difficult to envision. In conclusion, these studies have revealed that ASF-1 is a genome-wide chromatin assembly factor, whereas Asf1p appears to be a genome-wide chromatin disassembly factor.

Acknowledgments—We thank Paul Megee for helpful discussions and Mitch Jr. Smith for yeast strains. We thank Jeff Linger for critical reading of the manuscript. We are also grateful to Tara Dobson and Susan Howar for assistance with setting up earlier versions of the plasmid supercoiling analyses.

REFERENCES

1. Lugner, K., Mader, A. W., Richmond, R. K., Sargent, D. F., and Richmond, T. J. (1997) Nature 388, 251–260
2. Tyler, J. K. (2002) Eur. J. Biochem. 269, 2288–2274
3. Smith, S., and Stillman, B. (1989) Cell 58, 15–25
4. Shibahara, K., and Stillman, B. (1999) Cell 96, 575–585
5. Tyler, J. K., Adams, C. R., Chen, S. B., Kobayashi, R., Kamakaka, R. T., and Kadonaga, J. T. (1999) Nature 402, 555–560
6. Nahatiyan, A., and Krude, T. (2004) Mol. Cell. Biol. 24, 2853–2862
7. Singer, M. S., Kahana, A., Wolf, A. J., Meisinger, L. L., Peterson, S. E., Goggin, C., Mahowald, M., and Gottesman, D. E. (1998) Genetics 150, 613–632
8. Moshkin, Y. M., Armstrong, J. A., Maeda, R. K., Tamkun, J. W., Verrijzer, P., Kennison, J. A., and Karch, F. (2002) Genes Dev. 16, 2621–2626
9. Sutton, A., Bucaria, J., Osley, M. A., and Sternaglitz, R. (2003) Genetics 158, 587–596
10. Prado, F., Cortes-Ledesma, F., and Aguilar, A. (2004) EMBO Rep. 5, 497–502
11. Chimura, T., Kuzuhara, T., and Horikoshi, M. (2002) Proc. Natl. Acad. Sci. U. S. A. 99, 9334–9339
12. Adkins, M. W., Howar, S. R., and Tyler, J. K. (2004) Mol. Cell 14, 657–666
13. Kaufman, P. D., Kobayashi, R., and Stillman, B. (1997) Genes Dev. 11, 345–357
14. Kim, U. J., Han, M., Kayne, P., and Grunstein, M. (1988) EMBO J. 7, 2211–2219
15. Hebbes, T. R., Clayton, A. L., Thorne, A. W., and Crane-Robinson, C. (1994) EMBO J. 13, 1823–1830
16. Wang, J. C. (1982) Cell 29, 724–726
17. Pruss, G. J. (1985) J. Mol. Biol. 185, 51–63
18. Wang, J. C., and Lynch, A. S. (1993) Curr. Opin. Genet. Dev. 3, 764–768
19. Lutter, L. C., Judis, L., and Paretti, R. F. (1992) Mol. Cell. Biol. 12, 5004–5014
20. Tagami, H., Ray-Gallet, D., Almouzni, G., and Nakatani, Y. (2004) Cell 116, 51–61
21. Munakata, T., Adachi, N., Yokoyama, N., Kuzuhara, T., and Horikoshi, M. (2000) Genes Cells 5, 221–233
22. Sharp, J. A., Fouts, E. T., Krawitz, D. C., and Kaufman, P. D. (2001) Curr. Biol. 11, 463–473
23. Mello, C. A., Sillje, H. H., Roche, D. M., Kirschner, D. B., Nigg, E. A., and Almouzni, G. (2002) EMBO Rep. 3, 329–334
24. Umehara, T., Chimura, T., Ichikawa, N., and Horikoshi, M. (2002) Genes Cells 7, 59–73
25. Adams, A. G., Gotteschling, D. E., and Kaiser, C. A. (1997) Methods in Yeast Genetics, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
26. Gregory, P. D., and Horz, W. (1999) Methods Enzymol. 306, 365–376

M. Adkins and J. Tyler, manuscript in preparation.
