Histone H4 and the maintenance of genome integrity

Paul C. Megee,¹ Brian A. Morgan,² and M. Mitchell Smith³

Department of Microbiology, University of Virginia School of Medicine Charlottesville, Virginia 22908 USA

The normal progression of *Saccharomyces cerevisiae* through nuclear division requires the function of the amino-terminal domain of histone H4. Mutations that delete the domain, or alter 4 conserved lysine residues within the domain, cause a marked delay during the G₂+M phases of the cell cycle. Site-directed mutagenesis of single and multiple lysine residues failed to map this phenotype to any particular site; the defect was only observed when all four lysines were mutated. Starting with a quadruple lysine-to-glutamine substitution allele, the insertion of a tripeptide containing a single extra lysine residue suppressed the G₂+M cell cycle defect. Thus, the amino-terminal domain of histone H4 has novel genetic functions that depend on the presence of lysine per se, and not a specific primary peptide sequence. To determine the nature of this function, we examined H4 mutants that were also defective for G₂/M checkpoint pathways. Disruption of the mitotic spindle checkpoint pathway had no effect on the phenotype of the histone amino-terminal domain mutant. However, disruption of *RAD9*, which is part of the pathway that monitors DNA integrity, caused precocious progression of the H4 mutant through nuclear division and increased cell death. These results indicate that the lysine-dependent function of histone H4 is required for the maintenance of genome integrity, and that DNA damage resulting from the loss of this function activates the *RAD9*-dependent G₂/M checkpoint pathway.

[**Key Words:** Acetylation; DNA damage; chromatin structure; *Saccharomyces cerevisiae*; cell division cycle]

Received March 6, 1995; revised version accepted June 9, 1995.

The amino-terminal domains of the core histone proteins are highly charged basic polypeptide sequences that comprise approximately the first 25%–33% of each protein. They extend outward from the nucleosome core particle at intervals around its surface (Arents and Moudrianakis 1993), and function as distinct protein domains by numerous biochemical, biophysical, and genetic criteria (for reviews, see van Holde 1988; Wolffe 1992). The functions of the amino-terminal domains have been the focus of intense investigation, and current evidence suggests that they are responsible for many of the dynamic properties of eukaryotic chromatin (Grunstein 1990; Hansen and Ausio 1992). These regulated changes in chromatin structure and function may be mediated in part by a wide variety of transient post-translational modifications targeted to the amino-terminal domains (Allfrey 1964; van Holde 1988; Wolffe 1992). For example, the reversible acetylations of the ϵ -amino groups of conserved lysines within the amino-terminal domains have been correlated with changes in transcriptional potential (Lee et al. 1993; Hebbes et al. 1994; Juan et al. 1994; for recent reviews, see Turner 1991; Davie and

Hendzel 1994), DNA replication and chromatin assembly (Perry et al. 1993), and nuclear division (Megee et al. 1990).

One way in which amino-terminal modifications might mediate dynamic changes in chromatin function is by serving as signals for other *trans*-acting regulatory factors (Tordera et al. 1993; Turner 1993). For example, it is likely that Lys-16 in histone H4 must not be acetylated to assemble heterochromatin, perhaps by serving as a binding site for specific protein complexes (Johnson et al. 1990; Braunstein et al. 1993; Jeppesen and Turner 1993; Bone et al. 1994; Hecht et al. 1995). A second way in which the amino-terminal domains might regulate chromatin function is through changes in higher order folding and compaction (Hansen and Ausio 1992; Wolffe 1994). Nucleosomes reconstituted with core histones lacking their amino-terminal domains are unable to undergo normal chromatin compaction in vitro and the linker DNA between consecutive nucleosomes is apparently unable to bend, preventing the chain from folding (Allan et al. 1982; Garcia-Ramirez et al. 1992). The ability of the amino-terminal domains to facilitate compaction may be regulated by lysine acetylation as highly acetylated histones are less capable of supporting chromatin condensation (Marvin et al. 1990; Ridsdale et al. 1990; Perry and Annunziato 1991; Hong et al. 1993; Kraewski et al. 1993). The high positive charge of the

Present addresses: ¹Department of Embryology, The Carnegie Institute of Washington, Baltimore, Maryland 21210 USA; ²National Institutes for Medical Research, The Ridgeway, London NW7 1AA, UK.
³Corresponding author.

amino-terminal domains may be important in shielding the negatively charged DNA phosphate backbone during compaction (Manning 1978; Widom 1986; Clark and Kimura 1990). Hyperacetylation of histones H3 and H4 directly alters the linking number of circular minichromosomes reconstituted in vitro (Norton et al. 1989, 1990), likely through subtle changes in core particle shape (Bauer et al. 1994), and similar changes may be detected in vivo (Thomsen et al. 1991; but see also Lutter et al. 1992). Thus, dynamic alterations in histone acetylation states are expected to play critical roles in the remodeling of chromatin that must accompany many nuclear processes.

Recent genetic experiments in yeast have defined several functional roles for histone H4 in vivo. The amino-terminal domain of H4 can be divided into two functional subdomains (Durrin et al. 1991): (1) domain R, a sequence required for transcriptional repression; and (2) domain A, a region with pleiotropic roles in transcriptional activation, nuclear division, and sporulation. A summary of these two domains is shown in Figure 1. Domain R was first identified as a basic region spanning residues 15–19 required for the transcriptional repression of the silent mating type loci *HML* and *HMR* (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990). Subsequent detailed mutagenesis showed that the repressor domain extended through residues 21–29 (Johnson et al. 1992). In addition to transcriptional silencing of the mating type loci, domain R is also responsible for repressing telomere-proximal genes (Aparicio et al. 1991) and for directing nucleosome positioning adjacent to the $\alpha 2$ operator in response to the $\alpha 2$ re-

pressor (Roth et al. 1992). Single point mutations within the domain R are sufficient to eliminate repression. Thus, the molecular genetics of domain R are consistent with a signaling model involving specific protein-protein interactions (Johnson et al. 1990; Hecht et al. 1995).

Domain A comprises residues 1–16. These residues, and particularly the lysines at positions 5, 8, 12, and 16, have roles distinct from those of domain R. For example, the simultaneous replacement of these four lysines with neutral polar amino acid residues was found to cause a marked increase in the length of the $G_2 + M$ periods of the cell division cycle (Megee et al. 1990). Similar mutations were found to block sporulation in homozygous diploids (Park and Szostak 1990) and decrease transcriptional activation of *GAL1* and *PHO5* (Durrin et al. 1991). The mechanism of action of domain A is not clear. In all cases, single point mutations were relatively ineffective in disrupting function (Megee et al. 1990; Park and Szostak 1990; Durrin et al. 1991).

In the experiments reported here we have sought to better understand the role of domain A in chromosome dynamics. Our results indicate that, unlike domain R, the residues important for nuclear division cannot be mapped to a specific polypeptide sequence. Instead, the domain appears to function through lysine residues independent of primary amino acid sequence. Disruption of mitotic checkpoint pathways in domain A mutants revealed that the mitotic defect can be attributed to intrinsic DNA damage. These results define a novel function for H4 in the maintenance of genome integrity.

Figure 1. Summary of the amino-terminal domains of histone H4. The predicted sequence of the first 30 amino acid residues of histone H4 (*HHF1*) is shown in the center with the extents of domain A and domain R indicated by brackets. The amino acid substitutions in a selection of amino-terminal domain mutants are shown, compiled from this work and from the references cited. Allele designations are shown at left. Amino acid substitutions in individual alleles are grouped by underlining. Mutants that are defective for domain A are presented above the wild-type sequence. Those that are not defective for domain A are presented below. Amino acid substitutions that define mutants in domain R are shaded. For each domain, functions identified by one or more of the mutant alleles are listed. Function references are as follows: (1) Megee et al. 1990; (2) Durrin et al. 1991; (3) Park and Szostak 1990; (4) this study; (5) Megee et al. 1990; Park and Szostak 1990; Johnson et al. 1990, 1992; (6) Aparicio et al. 1991; (7) Roth et al. 1992; (8) this study.

Results

Conserved lysines in domain A are functionally redundant

The amino-terminal domain of histone H4 has been highly conserved during evolution and contains 4 invariant lysine residues at positions 5, 8, 12, and 16. We have shown previously that the amino-terminal domain of histone H4, and these conserved lysine residues, are necessary for normal cell cycle progression through nuclear division (Megee et al. 1990; Morgan et al. 1991). Both a deletion mutant lacking the complete amino-terminal domain of H4, and one in which the four conserved lysines are replaced by glutamines, grow significantly slower than wild-type cells because of an increase in the time spent traversing $G_2 + M$. This phenotype is illustrated in Figure 2. In this experiment exponentially growing cultures were analyzed by flow cytometry for their cell cycle distributions. From the DNA histograms shown in Figure 2, it can be seen that the quadruple lysine mutant (*hhf1-10*) has an abnormally high proportion of cells with a 2C DNA content. The percentage of cells in each phase of the cell division cycle was estimated from the DNA profiles, and an approximate measure of the lengths of each period was calculated using these percentages and the culture doubling times (Slater et al. 1977; Smith and Stirling 1988; Megee et al. 1990). A summary of the cell cycle data is shown in the diagram of Figure 3A. This analysis indicates that the slower growth of *hhf1-10* cells is predominantly the result of a $G_2 + M$ period that is nearly twice that of wild-type cells. This strong $G_2 + M$ phenotype of *hhf1-10* implies a novel

Figure 2. Cells with quadruple lysine-to-glutamine substitutions in H4 accumulate with a 2C DNA content. Isogenic strains expressing either wild-type H4 (left) or *hhf1-10* (right) were stained for DNA content with propidium iodide and analyzed by flow cytometry. Relative fluorescence versus cell number is plotted. The circles in each panel represent flow cytometry experimental data points. The solid lines show the fit of the data to a model of G_1 , S, and $G_2 + M$ phase cell populations. Below each plot the individual G_1 , S, and $G_2 + M$ model fits are shown at half-scale.

Figure 3. Cell division cycle profiles of lysine-to-glutamine substitution mutants. A summary of the relative times spent in the G_1 , S, and $G_2 + M$ phases of the cell division cycle is shown for wild-type H4 and *hhf1-10* (A), double glutamine substitution mutants (B), triple glutamine substitution mutants (C), lysine insertion mutants (D), and arginine replacement mutants (E). The lengths of each cell cycle period were approximated using culture doubling times and the percentage of cells in each phase of the cell division cycle estimated from DNA histograms such as those shown in Fig. 2. The cell cycle time estimates represent the means of three to seven independent measurements for each mutant. The profiles are aligned at the S/ $G_2 + M$ boundary for ease of comparison of the relative lengths of the $G_2 + M$ cell cycle periods. The identity of residues at positions 5, 8, 12, and 16 is indicated by the shade of the squares: black for lysine (LYS), gray for glutamine (GLN), or hatched for arginine (ARG). The relative locations of the lysine insertions are shown in D by the dashed lines. Culture doubling times are indicated in minutes.

role of the H4 amino-terminal domain in chromosome dynamics and nuclear division.

As a first step toward a better understanding of this function, we sought to determine which of the four lysines was responsible for the phenotype. Our model for these experiments was the successful genetic mapping of domain R to include residue Lys-16 (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990). Single and multiple substitutions in the conserved lysine residues at positions 5, 8, 12, and 16 were made by oligo-directed mutagenesis, changing individual lysine residues to neutral polar glutamine residues. Isogenic strains differing only in their H4 alleles were then obtained by integration of the mutant construct at the chromosomal copy-I

locus by one-step gene replacement (see Materials and methods). Cell cycle progression in these mutants was then analyzed by flow cytometry.

An initial analysis of several single point mutants did not detect any defect in $G_2 + M$ (data not shown). Therefore, we focused on a collection of multiple substitution mutants. The doubling times and cell cycle profiles of four double glutamine substitution mutants are summarized in Figure 3B. Each mutant had a doubling time comparable to an isogenic wild-type control strain and each exhibited a normal $G_2 + M$ cell division cycle period. The results for all four possible triple substitution mutants are shown in Figure 3C. All four of these also grew with approximately wild-type doubling times and showed normal progression through $G_2 + M$. Thus, unlike the silent mating type repressor function, the $G_2 + M$ delay phenotype of *hhf1-10* failed to map to a specific peptide region. These results indicated that any single lysine residue, regardless of its position within the amino-terminal domain, could provide the function necessary for normal cell cycle progression.

Domain A mutants are suppressed by lysine insertions

We reasoned that if the $G_2 + M$ function of domain A did not require a specific peptide sequence, then simply inserting a lysine residue into the quadruple glutamine substitution allele should revert the cell division cycle delay phenotype. To address this hypothesis, two new H4 alleles were constructed by oligo-directed mutagenesis. Each allele contains an insertion of the tripeptide G-K-G into the amino-terminal domain of *hhf1-10* either after position 3 (*hhf1-25*) or after position 13 (*hhf1-35*). A comparison of the predicted protein sequences of these two insertion alleles with those of wild-type H4 and *hhf1-10* is shown in Figure 4. Because the amino-terminal domain is anchored within the nucleosome by the structure of the carboxy-terminal histone fold (Arents and Moudrianakis 1993), the result of amino acid insertions into the domain is to shift the sequence in the

amino-terminal direction. In the case of *hhf1-25*, the insertion sequence places a lysine at a new position relatively more distant from the core than any lysine present in the wild-type protein. In the case of *hhf1-35*, the insertion places a lysine residue at the same relative position as Lys-12 in the wild-type nucleosome, but shifts the positions of the upstream amino acids, thus destroying any primary sequence alignment that might be necessary for function.

The results of cell cycle assays on these mutants are summarized in Figure 3D. Both insertions completely revert the $G_2 + M$ cell cycle delay phenotype of *hhf1-10*. The culture doubling times and cell division cycles of *hhf1-25* and *hhf1-35* mutants are essentially identical to those of the isogenic wild type H4 strain and the previous double and triple glutamine substitution mutants. In contrast, the lysine insertions do not suppress defects in domain R. Because of the glutamine substitution at position 16, *hhf1-10* is defective for silent mating-type repression and thus, is phenotypically sterile (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990). Both *hhf1-25* and *hhf1-35* remain sterile, indicating that they are both defective for the repressor function of domain R (see Fig. 1). Thus, the lysine insertion alleles provide a clear genetic separation of the functions of domain A and domain R.

An additional example of separate domain A and R functions became evident once all of the cell cycle data in Figure 3 were compiled. Although changes in the lengths of G_2/M are the most obvious differences seen among the mutants, there are also significant differences in the lengths of G_1 . An examination of the patterns revealed that a short G_1 period is correlated with a glutamine substitution at position 16, thus mapping the phenotype to domain R. This phenotype is independent of the G_2/M delay of domain A mutants. The function of domain R in G_1 can be seen by comparing the cell cycle profiles of *HHF1* with *hhf1-10* (Fig. 3A), *hhf1-32* with the other double substitutions (Fig. 3B), and *hhf1-22* with the other triple substitutions (Fig. 3C). It is also supported by the comparison of *hhf1-33* with *hhf1-34* (Fig. 3E), as Gln16 is mutant for domain R function, whereas Arg16 has only a slight effect (Johnson et al. 1990; Megee et al. 1990; Park and Szostak 1990); that is, *hhf1-33* is mutant for repression of the silent mating type loci and sterile, whereas *hhf1-34* is not. On the basis of its known role in gene repression, these results predict that domain R normally acts to repress the full expression of one or more genes involved in the completion of G_1 .

The wild-type function of the amino-terminal insertion mutants was difficult to reconcile with our previous report that a quadruple lysine-to-asparagine H4 allele (*hhf1-11*) was lethal (Megee et al. 1990). A re-examination of that strain showed that it was an unintended frameshift mutant. The frameshift was caused by a rare base duplication in the specific oligonucleotide primer incorporated into that isolate during mutagenesis. Therefore, we reisolated the correct in-frame quadruple asparagine substitution allele and assayed its function. The phenotypes of cells expressing an authentic *hhf1-11*

Sequence Comparison of Histone H4 Lysine Insertion Mutants

H4 N-Terminal Sequence	Allele
S G R G K G G K G L G K G G A K R H R K	<i>HHF1</i>
S G R G Q G G Q G L G Q G G A Q R H R K	<i>hhf1-10</i>
S G R G K G Q G G Q G L G Q G G A Q R H R K	<i>hhf1-25</i>
S G R G Q G G Q G L G Q G G K G G A Q R H R K	<i>hhf1-35</i>

Figure 4. Sequence comparison of lysine insertion mutants. Sequences are aligned with respect to the histone fold within the carboxy-terminal domains (Arents et al. 1991), and the first 20–23 residues for each allele are shown. Lysine residues are highlighted in black, and glutamine substitutions are highlighted in gray. The three amino acid insertions are enclosed within a rectangle.

allele are identical to those expressing *hhf1-10*, the quadruple glutamine mutant (data not shown). Thus, *hhf1-11* is not lethal and its properties are consistent with those of the other domain A mutants.

Arginine cannot replace lysine for function

A simple model for the suppression of domain A mutants by *hhf1-25* and *hhf1-35* was that the inserted lysine merely restored a net positive charge above some threshold necessary for function. This model predicted that other positively charged amino acid residues might suppress the $G_2 + M$ division cycle defect. To address this question, additional H4 alleles were constructed in which three of the lysine residues were replaced by glutamine, whereas the fourth was replaced by arginine. In these constructs arginine was located at either position 5 (*hhf1-33*) or position 16 (*hhf1-34*). Measurements of doubling times of isogenic strains differing only in the position of the arginine substitution showed that these strains grow significantly more slowly than strains retaining a lysine residue (Fig. 3E). In both cases, cell division cycle analysis showed that the growth defect was attributable to an approximate doubling of the lengths of the $G_2 + M$ periods. Therefore, arginine cannot substitute for lysine in domain A and these results rule out a simple model in which positive charge is sufficient for H4 function.

Domain A mutants activate the DNA damage checkpoint control pathway

The G_2/M transition in *Saccharomyces cerevisiae* is regulated by at least two independent checkpoint pathways: one to monitor the integrity of the spindle apparatus (Hoyt et al. 1991; Li and Murray 1991), and one to monitor the integrity of the DNA (Weinert and Hartwell

1988, 1990; Hartwell and Weinert 1989; Weinert et al. 1994). If either the spindle or the DNA is damaged or incomplete, the appropriate checkpoint control pathway arrests cells before mitosis to allow repair before nuclear division. We reasoned that if expression of the *hhf1-10* allele caused defects in the mitotic apparatus, such as loss of sister chromatid cohesion or spindle attachment, then disruption of the spindle checkpoint control pathway would eliminate the mitotic delay, giving continued cycling and increased cell death. Similarly, disruption of the DNA damage checkpoint would give cycling and cell death if DNA integrity was compromised in *hhf1-10* mutants.

The *BUB2* gene product is a component of the spindle checkpoint control pathway, and is required to arrest cells in G_2 in response to defects in microtubule assembly (Hoyt et al. 1991). In a *bub2* mutant, cells with spindle defects are unable to delay in G_2 and undergo reinitiation of DNA replication and rapid cell death. As shown in Table 1, in the presence of wild-type histone H4 there is no effect of a *bub2* disruption on cell viability. This is the expected result, as *BUB2* function is not evident in the absence of spindle or microtubule damage. However, the *bub2* gene disruption also has little if any effect on the viability of an *hhf1-10* strain. The plating efficiency of the *hhf1-10 bub2* double mutant was $69 \pm 5\%$ compared to $76 \pm 5\%$ for the isogenic *hhf1-10* single mutant (Table 1). Furthermore, the DNA histograms of the *hhf1-10* mutant in the presence or absence of *BUB2* gene function are indistinguishable (Fig. 5). Thus, it is unlikely that the $G_2 + M$ cell division cycle delay is attributable to the activation of the spindle checkpoint pathway because of defects in the spindle apparatus.

A different result was obtained for the DNA damage pathway. *RAD9* is one of a group of genes that monitors the integrity of cellular DNA and arrests cells in G_2 in

Table 1. Plating efficiencies of checkpoint gene–H4 double mutants

H4 Allele	Mutation ^a	Checkpoint gene disruption	Plating efficiency (%)	Experiment no. ^b
<i>HHF1</i>	WT	WT	86 ± 9	6
<i>HHF1</i>	WT	$\Delta rad9::LEU2$	95 ± 8	4
<i>HHF1</i>	WT	$\Delta bub2::URA3$	92 ± 2	3
<i>hhf1-10</i>	K5Q,K8Q,K12Q,K16Q	WT	76 ± 5	3
<i>hhf1-10</i>	K5Q,K8Q,K12Q,K16Q	$\Delta rad9::LEU2$	48 ± 4	3
<i>hhf1-10</i>	K5Q,K8Q,K12Q,K16Q	$\Delta bub2::URA3$	69 ± 5	3
<i>hhf1-25</i>	K5Q,K8Q,K12Q,K16Q with GKG insertion at 3	WT	85 ± 14	3
<i>hhf1-25</i>	K5Q,K8Q,K12Q,K16Q with GKG insertion at 3	$\Delta rad9::LEU2$	96 ± 4	4
<i>hhf1-25</i>	K5Q,K8Q,K12Q,K16Q with GKG insertion at 3	$\Delta bub2::URA3$	93 ± 4	3

^a(WT) A wild-type checkpoint gene or histone H4 allele. Substitutions are indicated with the wild-type residue first, then the residue's position, followed by the designated substitution. Abbreviations for the amino acid residues are (G) glycine; (K) lysine; and (Q) glutamine.

^bNumber of independent measurements.

Figure 5. The $G_2 + M$ cell division cycle delay in *hhf1-10* is *RAD9*-dependent. Representative DNA histograms of isogenic *hhf1-10* (left), *hhf1-10 bub2::URA3* (center), and *hhf1-10 rad9::LEU2* (right) mutants are shown. Relative fluorescence versus cell number is plotted as described in the legend to Fig. 2.

response to DNA damage (Weinert and Hartwell 1988; Weinert et al. 1994) or incompletely replicated chromosomes (Hartwell and Weinert 1989). Disruption of *rad9* had no effect in a wild-type histone H4 background. As shown in Table 1, the plating efficiency of the *rad9* strain is similar to the isogenic wild-type strain and there was no change in DNA histograms (data not shown). In contrast, in the *hhf1-10* background, disruption of *rad9* clearly behaved as expected for the loss of active checkpoint regulation. A comparison of the DNA histograms of the *hhf1-10 rad9* double mutant with that of the *hhf1-10* single mutant shows that there is an increase in the proportion of cells with a 1C DNA content in the absence of the *RAD9* gene (Fig. 5). Measurements of plating efficiencies in the single and double mutants indicate that the viability of *hhf1-10* decreases in the absence of the *RAD9* checkpoint gene. As shown in Table 1, the number of viable cells in an exponentially growing culture decreased significantly from 76% for *hhf1-10* to ~48% for the isogenic *hhf1-10 rad9* double mutant ($P = 0.008$). These results show that the delay in cell division in *hhf1-10* mutants is mediated by the *RAD9* gene product and imply that there is a loss of genomic DNA integrity in the H4 mutant. In the absence of *RAD9* function, the cells continue in the division cycle in the presence of damage and the inviable products of nuclear division then accumulate in the population as cells with a 1C DNA content.

Domain A mutants activate damage-inducible gene expression

The DNA damage model for the cell cycle delay in *hhf1-10* predicted that damage-inducible gene expression should be increased in the H4 mutant. To test this hypothesis, the levels of *UBI4* mRNA were determined in isogenic strains expressing different histone H4 alleles. *UBI4* encodes a polyubiquitin repeat and is under the control of the stress response regulatory network (Finley et al. 1987), including response to DNA damage (Treger et al. 1988). The results of these experiments are shown in Figure 6. As a positive control, wild-type cells were treated with the DNA-damaging agent 4-nitroquinoline-N-oxide (4-NQO) for increasing lengths of time, and total

RNA was analyzed for *UBI4* mRNA levels by Northern blot hybridization. There is a clear increase in *UBI4* mRNA levels in the cells with DNA damage (Fig. 6, lanes 5–8). As predicted, *hhf1-10* cells also have high levels of *UBI4* mRNA compared to wild-type controls (Fig. 6, lanes 1,3). Taken together, the genetic experiments and biochemical results suggests strongly that domain A function is required to maintain normal DNA integrity.

DNA integrity requires the lysine-specific function of domain A

The simplest interpretation of the mutant data is that the function of domain A in maintaining genome integrity is identical to the novel lysine-dependent function described earlier. There are two straightforward predictions of this model: (1) mutants expressing the lysine insertion alleles, such as *hhf1-25*, should not be affected by disruption of *RAD9*; and (2) these mutants should have low levels of *UBI4* mRNA. To test these predictions, we constructed an *hhf1-25 rad9* double mutant.

Figure 6. Northern blot analysis of *UBI4* expression. Total RNA (10 μ g) isolated from isogenic strains expressing the indicated H4 alleles was subjected to agarose gel electrophoresis, transferred to a nitrocellulose membrane, and probed with 32 P-labeled DNA encoding *UBI4*. RNA from a wild-type H4 (lane 1), *hhf1-25* (lane 2), and *hhf1-10* (lane 3) strains are shown. As a control for *UBI4* induction, wild-type cells were treated with the DNA-damaging agent 4-NQO for 0 (lane 4), 30 (lane 5), 60 (lane 6), 90 (lane 7), or 120 min (lane 8). The nitrocellulose filter was then stripped and reprobed with 32 P-labeled sequences encoding carboxypeptidase Y (*PRC1*) as a control for loading.

Plating efficiencies of the double mutant and the isogenic *hhf1-25* single mutant show that there is no loss in viability in the lysine insertion mutant in the absence of the *RAD9* gene (Table 1). Furthermore, the DNA histograms of the strains are indistinguishable (Fig. 7). These results show that the presence of a single lysine residue reverts both the cell division cycle delay and the activation of the *RAD9*-dependent checkpoint. We then examined the levels of *UBI4* mRNA in cells expressing the lysine insertion allele *hhf1-25*. As seen in Figure 6 (lanes 1–3), *hhf1-25* has low levels of *UBI4* mRNA comparable to those of the isogenic wild-type control strain. Thus, an artificial lysine insertion is also capable of suppressing the activation of a damage-inducible gene, consistent with the proposed role of the amino-terminal domain in maintaining DNA integrity.

hhf1-10 is not defective in DNA repair

In principle, the DNA damage that activates the *RAD9*-dependent checkpoint in *hhf1-10* could result from either an increase in intrinsic DNA damage, or a decrease in the repair of normal frequencies of damage. If *hhf1-10* were defective in repair, the single mutant would be expected to be hypersensitive to ultraviolet (UV) or γ -irradiation. In screening for the various *rad9* strains used in this study by radiation sensitivity we found that this was not the case (data not shown). However, to address this question more directly, we compared the ability of *HHF1* *RAD9* and *hhf1-10* *RAD9* cells to repair additional DNA damage induced by UV irradiation. Isogenic *HHF1* and *hhf1-10* cells were first blocked at metaphase by treatment with the microtubule inhibiting drug nocodazole (Jacobs et al. 1988). Samples were then washed free of drug, irradiated with different doses of UV light, and subsequently the cells were examined for completion of nuclear division. We reasoned that if *hhf1-10* were defective in DNA repair, then it would take longer for the mutant to repair the additional UV-induced damage, cancel its checkpoint arrest, and complete nuclear division. However, as illustrated in Figure 8, both wild-type and *hhf1-10* cells showed identical UV dose-dependent delays in completing nuclear division. These results sug-

Figure 8. Response to UV-induced DNA damage is normal in *hhf1-10*. Cells were arrested at the microtubule-dependent step of mitosis by growth for 3 hr in the presence of nocodazole. After the block, cells were washed free of drug, irradiated with UV light to induce DNA damage, and returned to growth. The kinetics of exit from mitosis was assayed by following the percentage of cells with a large bud and a single nucleus. (Top) The results for *HHF1*; (bottom) the results for *hhf1-10*. The UV exposures for each set were as follows: (○) No UV treatment; (□) low UV treatment (15 sec, 18 in); (◇) higher UV treatment (15 sec, 12 in).

gest that in cycling *hhf1-10* cells the *RAD9*-dependent G_2/M delay is caused by an increase in intrinsic DNA damage and not by a decrease in ability to carry out DNA repair.

Figure 7. A lysine insertion suppresses the accumulation of cells with a 2C DNA content and precocious nuclear division. Representative DNA histograms of isogenic *hhf1-10* (left), *hhf1-25* (center), and *hhf1-25 rad9::LEU2* (right) mutants are shown. Relative fluorescence versus cell number is plotted as described in the legend to Fig. 2.

Discussion

The maintenance of genome integrity is essential for the transmission of genetic information and cells have evolved sophisticated mechanisms both to monitor and to repair DNA damage (Hartwell and Weinert 1989; Prakash et al. 1993; Wevrick and Buchwald 1993). The results of the experiments reported here define a new role for histone H4 in maintaining the fidelity of genomic DNA. The evidence for this role derives from several observations. First, *hhf1-10* activates the RAD9-dependent G₂ checkpoint pathway, a pathway known to respond specifically to DNA damage (Weinert and Hartwell 1990; Weinert et al. 1994). The decreased viability of *hhf1-10 rad9* double mutants indicates that these cells experience actual damage. Thus, it is unlikely that *hhf1-10* is initiating a signal and activating the RAD9 pathway in the absence of any real DNA damage. This conclusion is supported by the activation of *UBI4* in *hhf1-10* cells. Although it is possible that this is a direct effect of the H4 mutation on *UBI4* transcription, and not an indirect result of damage, this is unlikely. The known transcriptional functions defined for domain A are in gene activation and not gene repression; that is, mutants like *hhf1-10* fail to activate transcription of certain regulated genes (Durrin et al. 1991). Thus, the induction of *UBI4* expression in *hhf1-10* is opposite to the effect expected for a direct transcriptional effect.

The increase in DNA damage in *hhf1-10* is consistent with observed defects in several global parameters of genome stability. First, we have reported previously that cultures of *hhf1-10* contain ~10%–15% of cells that show aberrant cell and nuclear morphologies (Megee et al. 1990). These include fragmented and polymorphonuclear structures, and multiple nuclei per cell. These unusual morphologies are not observed in other histone mutants that retain wild-type domain A function. Second, populations of *hhf1-10* show an increase in the proportion of aneuploid cells by flow cytometry. This is evident in the trail of cells with greater than 2C DNA content in the histograms of *hhf1-10* (Figs. 2, 5, and 7). As with other domain A phenotypes, this aneuploidy is suppressed by the lysine insertion mutants (Fig. 7).

Domain A acts to maintain genome integrity through a novel lysine-dependent mechanism. The mutational analysis argues against a conventional signaling mechanism involving specific protein–protein recognitions. The 4 lysine residues in domain A are in different local contexts; the sequence around Lys-5, -8, and -12 is G-K-G, whereas around Lys-16 it is A-K-R. Nevertheless, each of these lysines is individually sufficient for function. Furthermore, artificial lysine insertions at abnormal positions are able to restore function to *hhf1-10* despite the disruption of the primary amino acid sequence. This contrasts with domain R in which amino acid insertions and deletions are not tolerated (Johnson et al. 1992). The finding that arginine cannot replace lysine for function makes a simple positive charge model unlikely, and suggests that domain A relies on properties specific to lysine. An attractive candidate for this property is the

reversible acetylation known to be targeted to these lysines and that results in alternate positively charged and uncharged states. Because arginine cannot be acetylated, substitution mutations are permanently charged, whereas glutamine and other neutral substitution mutations are permanently uncharged. Thus, unlike lysines, neither would be able to mediate functions that require charge modulation. At present there is no direct evidence to implicate acetylation in domain A function, and a test of this model must await the identification of the relevant histone acetyl transferase and deacetylase genes.

The results reported here are most consistent with a model in which the lysines in domain A determine dynamic alterations in chromatin structure (Manning 1978; Allan et al. 1982; Widom 1986; Clark and Kimura 1990; Garcia-Ramirez et al. 1992). Mutations affecting these structural determinants could affect DNA integrity indirectly through aberrant gene transcription. For example, if the *hhf1-10* mutant failed to fully activate expression of genes necessary for DNA replication, this could lead to increased DNA damage and induction of the RAD9-dependent checkpoint pathway. However, perhaps the simplest model is one in which defective chromatin structure contributes directly to the loss of DNA integrity. One step that might be particularly vulnerable to domain A mutations is chromatin assembly. The assembly of nucleosomes is concomitant with DNA replication, and both in vivo and in vitro experiments have shown that newly synthesized histone H4 is diacetylated when deposited on replicated DNA (Ruiz-Carrillo et al. 1975; Jackson et al. 1976; Allis et al. 1985; Smith and Stillman 1991; Sobel et al. 1995). Shortly after deposition, the acetyl groups on H4 are removed (Allis et al. 1985; Lin et al. 1989). If H4 deacetylation is prevented by the addition of sodium butyrate, the nascent chromatin remains sensitive to attack by nucleases and has a reduced capacity for the formation of higher order chromatin structures (Perry and Annunziato 1991). Thus, if mutants in domain A are unable to form mature nucleosomes efficiently, the resulting chromatin may be more susceptible to endogenous nucleases or to damage from torsional stresses. This direct structural model makes several predictions. If domain A acts to modulate higher order chromatin structure, then mutants should exhibit defects in the dynamics of chromatin compaction. Moreover, if the damage-sensitive steps occur during chromatin assembly, then the DNA damage induced in *hhf1-10* should be coupled to nucleosome deposition and DNA replication. Further experimentation to test these predictions should help expand our understanding of how the histones function to maintain genome integrity.

Materials and methods

Bacterial and yeast strains

Escherichia coli host strains JM83 [*ara* Δ(*lac-proAB*) *rpsL* (Φ80*dlac* Δ(*lacZ*)M15)] (Yanisch-Perron et al. 1985) and DH5α [*F'**endA1 hsdR17* (*r*)*xu*[−] *m*⁺*supE44 thi-1 recA1 gyrA relA1* Δ(*lacZYA-argF*) *U169* (Φ80*dlac* Δ(*lacZ*)M15)] were used for bac-

terial transformations. Recombinant M13 phage were transfected into host strain JM101 [$\Delta(lac-proAB)$ *thi-1 supE/F'* *traD36 proAB lacI'* Z Δ M15] (Yanisch-Perron et al. 1985). Uracil-containing single-stranded M13 phage DNA for oligo-directed mutagenesis was isolated from infected host strains BW313 [*dut ung thi-1 relA1 spoT1/F' lysA*] (Kunkel 1985) or CJ236 [*dut1 ung1 thi-1 relA1/pC*]105] (Kunkel et al. 1987).

The yeast strain MX4-22A [*MAT α ura3-52 leu2-3,112 lys2 Δ 201 $\Delta(HHT1-HHF1)$ $\Delta(HHT2-HHF2)$ pMS329*] (Megee et al. 1990) was the host strain used in the integration protocol described below.

Reagents and media

The media used for bacterial and yeast growth have been described previously (Sherman et al. 1979). Restriction endonucleases were purchased from New England Biolabs (Beverly, MA), Bethesda Research Laboratories (Gaithersburg, MD), and Boehringer Mannheim Biochemicals (Indianapolis, IN). Vent polymerase was purchased from New England Biolabs. 5-Fluoro-orotic acid (5-FOA) was purchased from PCR Incorporated (Gainesville, FL).

Oligo-directed mutagenesis and DNA sequencing

Oligo-directed mutagenesis was performed by the method of Kunkel (1985). A 476-bp *RsaI* restriction endonuclease fragment encoding the copy-I H4 gene was cloned into the *HincII* restriction site of M13mp7 to provide a source of single-stranded template for oligo-directed mutagenesis. Derivatives containing the desired base-pair substitutions were identified by direct DNA sequencing of individual phage isolates. DNA sequencing was done by the dideoxy method described previously (Sanger et al. 1977) using Sequenase enzyme purchased from U.S. Biochemical (Cleveland, OH).

Integration of histone mutants

The yeast *S. cerevisiae* contains two nonallelic histone H3 and H4 loci referred to as copy-I and copy-II. Each locus is composed of one H3 gene and one H4 gene (Smith and Andrsson 1983). The copy-I genes are designated *HHT1* and *HHF1* for histone H3 and H4, respectively, whereas the copy-II genes are designated *HHT2* and *HHF2*. Deletion of either gene pair results in viable cells, but the deletion of both gene pairs is lethal (Smith and Stirling 1988). The host strain MX4-22A used in the strain constructions is deleted for both the copy-I and copy-II gene pairs and is rescued for the deletions with pMS329, a plasmid carrying the wild-type copy-I H3 and H4 genes, the yeast selectable marker *URA3*, a centromere (*CEN4*), and an autonomously replicating sequence (*ARS*) (Megee et al. 1990). To provide homologous DNA sequences for targeted integration of histone H3 and mutant H4 genes, the polymerase chain reaction (PCR) was used to amplify 1-kb sequences flanking the chromosomal deletion end points at the copy-I locus (Smith and Stirling 1988). *HHT1-hhf1* cassettes generated by oligo-directed mutagenesis were then cloned between the two regions of homology. MX4-22A was cotransformed (Ito et al. 1983; Gietz and Schiestl 1991; Gietz et al. 1992) with DNA-encoding reconstituted mutant copy-I loci and pRS315 (Sikorski and Hieter 1989) as a means for selecting transformants. The transformants were then replica plated to synthetic media containing 5-FOA to screen for cells that had lost pMS329, which carries wild-type H3 and H4 genes (Boeke et al. 1984). Proper integrants were identified by hybridization (Southern 1975) of a 6.7-kb *HindIII* fragment encoding

the copy-I locus (Smith and Murray 1983) to genomic DNA prepared from 5-FOA-resistant colonies.

All mutants described in this report were also analyzed by a plasmid shuffle protocol described previously (Boeke et al. 1987; Megee et al. 1990). In every case the cell cycle progression phenotypes in both the plasmid-bearing and integrated strains were essentially identical. The plasmid was reisolated from each shuffle strain and the H4 gene was resequenced to confirm the presence of the desired base substitutions and the absence of any other change.

RAD9 and BUB2 disruptions

RAD9 and *BUB2* gene disruptions were constructed using a one-step gene disruption (Orr-Weaver et al. 1981; Rothstein 1983). To construct *RAD9* disruptions, strains were transformed with *NotI*-digested pTW031 DNA ($\Delta rad9::LEU2$) (Weinert and Hartwell 1990). Leucine prototrophs were then scored for their sensitivity to a 64.5-krad exposure from a ^{137}Cs source (Neff and Burke 1992). In the case of a *rad9* disruption constructed in the *hhf1-10* strain, a *URA3 CEN/ARS* plasmid carrying the wild-type copy-II H4 gene (pMS339) (Chen et al. 1991) was introduced into the strain before the disruption was made in the event that the disruption produced a synthetic lethality. Radiation-sensitive isolates were then grown on 5-FOA to screen for cells that had lost the *URA3* plasmid carrying the wild-type H4 gene. The presence of a *rad9* disruption in radiation-sensitive transformants (and the absence of pMS339 in the case of the *hhf1-10* strain) was confirmed by Southern blot analysis of genomic DNA.

To construct *BUB2* disruptions, strains were transformed with *SacI*, *Clal*-digested pTR24 (*bub2::URA3*) (Hoyt et al. 1991). Uracil prototrophs were then scored for their recovery from a 20-hr exposure to 70 $\mu\text{g}/\text{ml}$ of benomyl as compared to recovery from a 20-hr incubation on an agar-only substrate (Hoyt et al. 1991). Similarly, in the case of a *bub2* disruption constructed in the *hhf1-10* strain, a *LEU2 CEN/ARS* plasmid carrying wild-type copy-I H3 and H4 genes (pMS337) (Megee et al. 1990) was introduced into the strain before the disruption was made for the same reason given above. Uracil prototrophs were then grown on rich media and colonies were screened for leucine auxotrophy, indicating that pMS337, carrying wild-type histones H3 and H4, was lost. The presence of a *bub2* disruption in benomyl-sensitive strains (and the absence of pMS337 in the case of the *hhf1-10* strain) was confirmed by Southern blot analysis of genomic DNA.

Isolation of RNA and Northern blot analysis

Total RNA was isolated from exponentially growing strains as described (Keleher et al. 1992), resolved on 1% formaldehyde-agarose gels, and transferred to nitrocellulose by capillary blotting. Radiolabeled probes were prepared by the method of Feinberg and Vogelstein (1983). Ubiquitin-specific sequences were isolated as a 240-bp *EcoRI*-*HindIII* fragment of *UBI4* from pUB1 (zkaynak et al. 1984). As a control for *UBI4* induction, cells were treated with 4-NQO (Sigma) at a final concentration of 1 $\mu\text{g}/\text{ml}$ for the time periods indicated. As a control for equal loading, a 609-bp fragment of the carboxypeptidase Y (*PRC1*) gene was generated by PCR using the forward oligonucleotide primer 5'-ACTGTCGCCGCTGGTAAG-3' and the reverse primer 5'-CTTCATCCAATCACCCGC-3'.

Growth analyses and flow cytometry

The growth of cells in liquid media was monitored by cell counts using a Coulter model ZM particle counter. To measure

plating efficiencies, liquid cultures were grown at 28°C to $\sim 1 \times 10^7$ cells/ml in YPD media, sonicated to generate single-cell suspensions, and counted using the particle counter. Serial 1:10 dilutions were made to 1×10^3 cells/ml, and aliquots were plated in triplicate on YPD, and incubated at 28°C. Colonies were counted after a 2- to 3-day incubation. Flow cytometry was performed on cells fixed in ethanol and stained with propidium iodide as described previously (Megee et al. 1990) using a FAC-Scan fluorescence-activated cell sorter (Becton and Dickinson). The propidium iodide was excited with a 15-mW laser source at 488 nm.

UV damage response

Exponentially growing cultures of isogenic *HHF1* and *hhf1-10* strains were adjusted to 1×10^7 cells/ml in YPD. Cells were blocked at nuclear division by 3 hr of incubation at 24°C in the presence of nocodazole at a concentration of 15 μ g/ml (Jacobs et al. 1988). After arrest, cells were washed twice in ice-cold sterile water, resuspended in water at 1.7×10^7 cells/ml, and kept on ice. For UV treatment, aliquots of 10 ml were placed in 90-mm plastic Petri dishes and exposed using a Sylvania G8T5 germicidal lamp placed 12 or 18 inches above the cells. Pairs of dishes, one for *HHF1* and one for *hhf1-10*, were exposed side-by-side for 15 sec with continuous agitation. Treated cells were kept in the dark, pelleted, resuspended in YPD medium, and incubated at 28°C. The cultures were sampled at time intervals, stained with DAPI (Smith 1991), and scored for the percentage of cells arrested as large budded cells with a single nucleus.

Acknowledgments

We thank our colleagues for helpful discussions during the course of this work, T. Weinert and M.A. Hoyt for the gifts of *RAD9* and *BUB2* disruption plasmids, respectively, D. Burke for the gift of benomyl, and Chris Eichman and William Ross for expert technical assistance with the flow cytometry. This work was supported in part by an Achievement for Research College Scientists Foundation fellowship, Metropolitan Washington chapter, to P.C.M., and by National Institutes of Health grant GM28920 to M.M.S.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Allan, J., N. Harborne, D.C. Rau, and H. Gould. 1982. Participation of core histone "tails" in the stabilization of the chromatin solenoid. *J. Cell Biol.* **93**: 285–297.
- Allfrey, V.G. 1964. Structural modifications of histones and their possible role in the regulation of ribonucleic acid synthesis. *Proc. Can. Cancer Res. Conf.* **6**: 313–335.
- Allis, C.D., L.G. Chicoine, R. Richman, and I.G. Schulman. 1985. Deposition-related histone acetylation in micronuclei of conjugating *Tetrahymena*. *Proc. Natl. Acad. Sci.* **82**: 8048–8052.
- Aparicio, O.M., B.L. Billington, and D.E. Gottschling. 1991. Modifiers of position effect are shared between telomeric and silent mating-type loci in *S. cerevisiae*. *Cell* **66**: 1279–1287.
- Arents, G. and E.N. Moudrianakis. 1993. Topography of the histone octamer surface: Repeating structural motifs utilized in the docking of nucleosomal DNA. *Proc. Natl. Acad. Sci.* **90**: 10489–10493.
- Arents, G., R.W. Burlingame, B. Wang, W.E. Love, and E.N. Moudrianakis. 1991. The nucleosomal core histone octamer at 3.1 Å resolution: A tripartite protein assembly and a left-handed superhelix. *Proc. Natl. Acad. Sci.* **88**: 10148–10152.
- Bauer, W.R., J.J. Hayes, J.H. White, and A.P. Wolffe. 1994. Nucleosome structural changes due to acetylation. *J. Mol. Biol.* **236**: 685–690.
- Boeke, J.D., F. LaCroute, and G.R. Fink. 1984. A positive selection for mutants lacking orotidine-5'-phosphate decarboxylase activity in yeast: 5-fluoro-orotic acid resistance. *Mol. Gen. Genet.* **197**: 345–346.
- Boeke, J.D., J. Trueheart, B. Natsoulis, and G.R. Fink. 1987. 5-Fluoroorotic acid as a selective agent in yeast molecular genetics. *Methods Enzymol.* **154**: 164–175.
- Bone, J.R., J. Lavender, R. Richman, M.J. Palmer, B.M. Turner, and M.I. Kuroda. 1994. Acetylated histone H4 on the male X chromosome is associated with dosage compensation in *Drosophila*. *Genes & Dev.* **8**: 96–104.
- Braunstein, M., A.B. Rose, S.G. Holmes, C.D. Allis, and J.R. Broach. 1993. Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes & Dev.* **7**: 592–604.
- Chen, T.A., M.M. Smith, S. Le, R. Sternglanz, and V.G. Allfrey. 1991. Nucleosome fractionation by mercury affinity chromatography. Contrasting distribution of transcriptionally active DNA sequences and acetylated histones in nucleosome fractions of wild-type yeast cells and cells expressing a histone H3 gene altered to encode a cysteine-110 residue. *J. Biol. Chem.* **266**: 6489–6498.
- Clark, D.J. and T. Kimura. 1990. Electrostatic mechanism of chromatin folding. *J. Mol. Biol.* **211**: 883–896.
- Davie, J.R. and M.J. Hendzel. 1994. Multiple functions of dynamic histone acetylation. *J. Cell. Biochem.* **55**: 98–105.
- Durrin, L.K., R.K. Mann, P.S. Kayne, and M. Grunstein. 1991. Yeast histone H4 N-terminal sequence is required for promoter activation in vivo. *Cell* **65**: 1023–1031.
- Feinberg, A. and B. Vogelstein. 1983. A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**: 6–13.
- Finley, D., E. Özkaynak, and A. Varshavsky. 1987. The yeast polyubiquitin gene is essential for resistance to high temperatures, starvation, and other stresses. *Cell* **48**: 1035–1046.
- Garcia-Ramirez, M., F. Dong, and J. Ausio. 1992. The role of the histone "tails" in the folding of oligonucleosomes depleted of histone H1. *J. Biol. Chem.* **267**: 19587–19595.
- Gietz, D., A. St. Jean, R.A. Woods, and R.H. Schiestl. 1992. Improved method for high efficiency transformation of intact yeast cells. *Nucleic Acids Res.* **20**: 1425.
- Gietz, R.D. and R.H. Schiestl. 1991. Applications of high efficiency lithium acetate transformation of intact yeast cells using single-stranded nucleic acids as carrier. *Yeast* **7**: 253–263.
- Grunstein, M. 1990. Histone function in transcription. *Annu. Rev. Cell Biol.* **6**: 643–678.
- Hansen, J.C. and J. Ausio. 1992. Chromatin dynamics and the modulation of genetic activity. *Trends Biochem. Sci.* **17**: 187–191.
- Hartwell, L.H. and T.A. Weinert. 1989. Checkpoints: Controls that ensure the order of cell cycle events. *Science* **246**: 629–634.
- Hebbes, T.R., A.L. Clayton, A.W. Thorne, and C. Crane-Robinson. 1994. Core histone hyperacetylation co-maps with generalized DNase I sensitivity in the chicken beta-globin chromosomal domain. *EMBO J.* **13**: 1823–1830.

- Hecht, A., T. Laroche, S. Strahl-Bolsinger, S.M. Gasser, and M. Grunstein. 1995. Histone H3 and H4 N-termini interact with SIR3 and SIR4 proteins: A molecular model for the formation of heterochromatin in yeast. *Cell* **80**: 583–592.
- Hong, L., G.P. Schroth, H.R. Matthews, P. Yau, and E.M. Bradbury. 1993. Studies of the DNA binding properties of histone H4 amino terminus. Thermal denaturation studies reveal that acetylation markedly reduces the binding constant of the H4 "tail" to DNA. *J. Biol. Chem.* **268**: 305–314.
- Hoyt, M.A., L. Totis, and B.T. Roberts. 1991. *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**: 507–517.
- Ito, H., Y. Fukada, K. Murata, and A. Kimura. 1983. Transformation of intact yeast cells with alkali cations. *J. Bacteriol.* **153**: 163–168.
- Jackson, V., A. Shires, N. Tanphaichitr, and R. Chalkley. 1976. Modifications to histones immediately after synthesis. *J. Mol. Biol.* **104**: 471–483.
- Jacobs, C.W., A.E. Adams, P.J. Szaniszló, and J.R. Pringle. 1988. Functions of microtubules in the *S. cerevisiae* cell cycle. *J. Cell Biol.* **107**: 1409–1426.
- Jeppesen, P. and B.M. Turner. 1993. The inactive X chromosome in female mammals is distinguished by a lack of histone H4 acetylation, a cytogenetic marker for gene expression. *Cell* **74**: 281–289.
- Johnson, L.M., G. Fisher-Adams, and M. Grunstein. 1992. Identification of a non-basic domain in the histone H4 N-terminus required for repression of the yeast silent mating loci. *EMBO J.* **11**: 2201–2209.
- Johnson, L.M., P.S. Kayne, E.S. Kahn, and M. Grunstein. 1990. Genetic evidence for an interaction between SIR3 and histone H4 in the repression of the silent mating loci in *Saccharomyces cerevisiae*. *Proc. Natl. Acad. Sci.* **87**: 6286–6290.
- Juan, L., R.T. Utley, C.C. Adams, M. Vettee-Dadey, and J.L. Workman. 1994. Differential repression of transcription factor binding by histone H1 is regulated by the core histone amino termini. *EMBO J.* **13**: 6031–6040.
- Keleher, C.A., M.J. Redd, J. Schultz, M. Carlson, and A.D. Johnson. 1992. Ssn6–Tup1 is a general repressor of transcription in yeast. *Cell* **68**: 709–719.
- Krajewski, W.A., V.M. Panin, and S.V. Razin. 1993. Acetylation of core histones causes the unfolding of 30 nm chromatin fiber: Analysis by agarose gel electrophoresis. *Biochem. Biophys. Res. Comm.* **196**: 455–460.
- Kunkel, T.A. 1985. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci.* **82**: 488–492.
- Kunkel, T.A., J.D. Roberts, and R.A. Zakour. 1987. Rapid and efficient site-specific mutagenesis without phenotypic selection. *Methods Enzymol.* **154**: 367–382.
- Lee, D.Y., J.J. Hayes, D. Pruss, and A.P. Wolffe. 1993. A positive role for histone acetylation in transcription factor access to nucleosomal DNA. *Cell* **72**: 73–84.
- Li, R. and A.W. Murray. 1991. Feedback control of mitosis in budding yeast. *Cell* **66**: 519–531.
- Lin, R., J.W. Leone, R.G. Cook, and C.D. Allis. 1989. Antibodies specific to acetylated histones document the existence of deposition-related histone acetylation in *Tetrahymena*. *J. Cell Biol.* **108**: 1577–1588.
- Lutter, L.C., L. Judis, and R.F. Paretti. 1992. Effects of histone acetylation on chromatin topology in vivo. *Mol. Cell. Biol.* **12**: 5004–5014.
- Manning, G.S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.* **11**: 179–246.
- Marvin, K.W., P. Yau, and E.M. Bradbury. 1990. Isolation and characterization of acetylated histones H3 and H4 and their assembly into nucleosomes. *J. Biol. Chem.* **265**: 19839–19847.
- Megee, P.C., B.A. Morgan, B.A. Mittman, and M.M. Smith. 1990. Genetic analysis of histone H4: Essential role of lysines subject to reversible acetylation. *Science* **247**: 841–845.
- Morgan, B.A., B.A. Mittman, and M.M. Smith. 1991. The highly conserved N-terminal domains of histones H3 and H4 are required for normal cell cycle progression. *Mol. Cell. Biol.* **11**: 4111–4120.
- Neff, M.W. and D.J. Burke. 1992. A delay in the *Saccharomyces cerevisiae* cell cycle that is induced by a dicentric chromosome and dependent upon mitotic checkpoints. *Mol. Cell Biol.* **12**: 3857–3864.
- Norton, V.G., B.S. Imai, P. Yau, and E.M. Bradbury. 1989. Histone acetylation reduces nucleosome core particle linking number change. *Cell* **57**: 449–457.
- Norton, V.G., K.W. Marvin, P. Yau, and E.M. Bradbury. 1990. Nucleosome linking number change controlled by acetylation of histones H3 and H4. *J. Biol. Chem.* **265**: 19848–19852.
- Özkaynak, E., D. Finley, and A. Varshavsky. 1984. The yeast ubiquitin gene: Head-to-tail repeats encoding a polyubiquitin precursor protein. *Nature* **312**: 663–666.
- Orr-Weaver, T.L., J.W. Szostak, and R.J. Rothstein. 1981. Yeast transformation: A model system for the study of recombination. *Proc. Natl. Acad. Sci.* **78**: 6354–6358.
- Park, E. and J.W. Szostak. 1990. Point mutations in the yeast histone H4 gene prevent silencing of the silent mating type locus *HML*. *Mol. Cell. Biol.* **10**: 4932–4934.
- Perry, C.A. and A.T. Annunziato. 1991. Histone acetylation reduces H1-mediated nucleosome interactions during chromatin assembly. *Exp. Cell Res.* **196**: 337–345.
- Perry, C.A., C.A. Dadd, C.D. Allis, and A.T. Annunziato. 1993. Analysis of nucleosome assembly and histone exchange using antibodies specific for acetylated H4. *Biochemistry* **32**: 13605–13614.
- Prakash, S., P. Sung, and L. Prakash. 1993. DNA repair genes and proteins of *Saccharomyces cerevisiae*. *Annu. Rev. Genet.* **27**: 33–70.
- Ridsdale, J.A., M.J. Hendzel, G.P. Delcuve, and J.R. Davie. 1990. Histone acetylation alters the capacity of the H1 histones to condense transcriptionally active/competent chromatin. *J. Biol. Chem.* **265**: 5150–5156.
- Roth, S.Y., M. Shimizu, L. Johnson, M. Grunstein, and R.T. Simpson. 1992. Stable nucleosome positioning and complete repression by the yeast $\alpha 2$ repressor are disrupted by amino-terminal mutations in histone H4. *Genes & Dev.* **6**: 411–425.
- Rothstein, R.J. 1983. One-step gene disruption in yeast. *Methods Enzymol.* **101**: 202–211.
- Ruiz-Carrillo, A., L.J. Wangh, and V.G. Allfrey. 1975. Processing of newly synthesized histone molecules. *Science* **190**: 117–128.
- Sanger, F., S. Nicklen, and A.R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci.* **74**: 5463–5467.
- Sherman, F., G.R. Fink, and C.W. Lawrence. 1979. *Methods in yeast genetics*. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
- Sikorski, R.S. and P. Hieter. 1989. A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. *Genetics* **122**: 19–27.
- Slater, M.L., S.O. Sharrow, and J.J. Gart. 1977. Cell cycle of

- Saccharomyces cerevisiae* in populations growing at different rates. *Proc. Natl. Acad. Sci.* **74**: 3850–3854.
- Smith, M.M. 1991. Mutations that affect chromosomal proteins in yeast. *Methods Cell Biol.* **35**: 485–523.
- Smith, M.M. and Ó.S. Andrésson. 1983. DNA sequences of yeast H3 and H4 histone genes from two non-allelic gene sets encode identical H3 and H4 proteins. *J. Mol. Biol.* **169**: 663–690.
- Smith, M.M. and K. Murray. 1983. Yeast H3 and H4 histone messenger RNAs are transcribed from two non-allelic gene sets. *J. Mol. Biol.* **169**: 641–661.
- Smith, M.M. and V.B. Stirling. 1988. Histone H3 and H4 gene deletions in *Saccharomyces cerevisiae*. *J. Cell Biol.* **106**: 557–566.
- Smith, S. and B. Stillman. 1991. Stepwise assembly of chromatin during DNA replication *in vitro*. *EMBO J.* **10**: 971–980.
- Sobel, R.E., R.G. Cook, C.A. Perry, A.T. Annunziato, and C.D. Allis. 1995. Conservation of deposition-related acetylation sites in newly synthesized histones H3 and H4. *Proc. Natl. Acad. Sci.* **92**: 1237–1241.
- Southern, E. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**: 503–517.
- Thomsen, B., C. Bendixen, and O. Westergaard. 1991. Histone hyperacetylation is accompanied by changes in DNA topology *in vivo*. *Eur. J. Biochem.* **201**: 107–111.
- Tordera, V., R. Sendra, and J.E. Pérez-Ortín. 1993. The role of histones and their modifications in the informative content of chromatin. *Experientia* **49**: 780–788.
- Treger, J.M., K.A. Heichman, and K. McEntee. 1988. Expression of the yeast *UBI4* gene increases in response to DNA-damaging agents and in meiosis. *Mol. Cell. Biol.* **8**: 1132–1136.
- Turner, B.M. 1991. Histone acetylation and control of gene expression. *J. Cell Sci.* **99**: 13–20.
- . 1993. Decoding the nucleosome. *Cell* **75**: 5–8.
- van Holde, K. 1988. *Chromatin*. Springer-Verlag, New York.
- Weinert, T.A. and L.H. Hartwell. 1988. The *RAD9* gene controls the cell cycle response to DNA damage in *Saccharomyces cerevisiae*. *Science* **241**: 317–322.
- . 1990. Characterization of *RAD9* of *Saccharomyces cerevisiae* and evidence that its function acts posttranslationally in cell cycle arrest after DNA damage. *Mol. Cell. Biol.* **10**: 6554–6564.
- Weinert, T.A., G.L. Kiser, and L.H. Hartwell. 1994. Mitotic checkpoint genes in budding yeast and the dependence of mitosis on DNA replication and repair. *Genes & Dev.* **8**: 652–665.
- Wevrick, R. and M. Buchwald. 1993. Mammalian DNA-repair genes. *Curr. Opin. Genet. Dev.* **3**: 470–474.
- Widom, J. 1986. Physicochemical studies of the folding of the 100Å nucleosome filament into the 300Å filament. *J. Mol. Biol.* **190**: 411–424.
- Wolffe, A.P. 1992. *Chromatin structure and function*. Academic Press, San Diego, CA.
- . 1994. Transcription: In tune with the histones. *Cell* **77**: 13–16.
- Yanisch-Perron, C., J. Vieira, and J. Messing. 1985. Improved M13 phage cloning vectors and host strains: Nucleotide sequences of the M13mp18 and pUC19 vectors. *Gene* **33**: 103–119.

Histone H4 and the maintenance of genome integrity.

P C Megee, B A Morgan and M M Smith

Genes Dev. 1995, **9**:

Access the most recent version at doi:[10.1101/gad.9.14.1716](https://doi.org/10.1101/gad.9.14.1716)

References

This article cites 81 articles, 36 of which can be accessed free at:
<http://genesdev.cshlp.org/content/9/14/1716.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).