



## Analysis of *Saccharomyces cerevisiae* histone H3 mutants reveals the role of the $\alpha$ N helix in nucleosome function

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### ABSTRACT

To understand the role of histone H3 sub-domains in chromatin function, 35 histone H3 tandem alanine mutants were generated and tested for their viability and sensitivity to DNA damaging agents. Among 13 non-viable H3 mutants, 6 were mapped around the  $\alpha$ N helix and preceding tail region. Mutants with individual alanine substitutions in this region were viable but developed multiple sensitivities to DNA damaging agents. The only viable triple mutant, REI49-50A, in the  $\alpha$ N helix region could not grow when combined with histone chaperone mutations, such as *asf1*  $\Delta$ , *cac1*  $\Delta$ , or *hir1*  $\Delta$ , suggesting that this particular region is important when the histone assembly/disassembly pathway is compromised. In addition, further analysis showed that T45, E50, or F54 of the  $\alpha$ N helix genetically interacted with a histone chaperone (*Asf1*) and transcription elongation factors (*Paf1* and *Hpr1*). These results suggest a specific role of the H3  $\alpha$ N helix in histone dynamics mediated by histone chaperones, which might be important during transcription elongation.

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The genomic DNA of eukaryotes forms a highly ordered structure called chromatin. The fundamental unit of chromatin is a nucleosome, which consists of 146 bp of supercoiled DNA and a histone octamer composed of two copies of each histone, H2A, H2B, H3, and H4 [1]. Each H2A–H2B dimer is attached on both sides of the (H3–H4)<sub>2</sub> tetramer within the core nucleosome. The (H3–H4)<sub>2</sub> binds the central 60 bp of nucleosomal DNA, and the two H2A–H2B dimers bind 30 bp of both DNA ends [1,2].

Histones contain two structural regions: the histone fold domain involved in histone–histone and histone–DNA interactions and the flexible tails. The N- and C-terminal tails of four histones protrude from the nucleosome core. Although tails do not contribute to the stability of the nucleosome itself [3], they regulate higher-ordered chromatin formation via interactions with DNA or histones of neighboring nucleosomes [4]. In addition, many residues of histone tails are covalently modified (by acetylation, methylation, phosphorylation, ubiquitination, etc.) and recognized by proteins with specific binding motifs, to affect chromatin function [5–8].

Each histone is composed of similar secondary structural elements: three  $\alpha$ -helices ( $\alpha$ 1,  $\alpha$ 2, and  $\alpha$ 3) and two loops (L1 and L2) connecting them [1]. Histone H3 and H2B each have an additional  $\alpha$ -helix called  $\alpha$ N and  $\alpha$ C, respectively, while H2A has very short  $\alpha$ N and  $\alpha$ C helices. Those structural elements are important for chromatin function in viability [9,10], telomeric gene silencing [9,11,12], and the DNA damage response [13,14]. Many functional histone residues have been elucidated by mutational, genetic, and biochemical approaches [10,15–18].

The bulk of the nucleosomes are assembled when the DNA is replicated in S phase through the replication-coupled (RC) pathway by histone H3/H4 chaperones, Chromatin assembly factor 1 (CAF-1) and *Asf1* (Anti-silencing function 1) [19,20]. Histone chaperones bind H3/H4 and assemble histones on newly synthesized DNA [21]. Histones are also deposited by the replication independent (RI) pathway by HIRA (HIR complex in *Saccharomyces cerevisiae*) and *Asf1* [22]. In yeast, HIR consists of *Hir1*, *Hir2*, *Hir3*, and *Hpc2*, which were originally identified as repressors of histone genes [23,24]. *Asf1* interacts with both CAF-1 and HIR, and affects both the RC and RI pathways [23,25,26].

In this study, we generated H3 alanine mutants via site-directed mutagenesis to understand the molecular structure of nucleosomes and the histone domains important for nucleosome function. H3 mutants were analyzed for their viability and sensitivity to the genotoxic agents, hydroxyurea (HU), methyl-methanesulfonate (MMS), and camptothecin (CPT). Amino acids encompassing the  $\alpha$ N domain were particularly sensitive to alanine substitution [10]. The genetic interaction study with histone chaperones further revealed a potential role of this region in chromatin dynamics. Our data suggest that the  $\alpha$ N helix of H3 is particularly important for the nucleosome assembly/disassembly pathways mediated by histone chaperones during transcription elongation.

### Materials and methods

**Yeast strains and plasmids.** Histone H3 mutants were constructed by transformation of WZY42 (*MATa*, *ura3-52*, *leu2*  $\Delta$ 1, *trp1*  $\Delta$ 63, *his3*  $\Delta$ 200, *lys2-801*, *ade2-101*, *hht1-hhf1*::pWZ405-F2F9-LEU2, *hht2-hhf2*::pWZ403-F4F10-HIS3, YCp50-copyII (*HHT2-HHF2*) from

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S. Dent) with various plasmids. DNA fragments encompassing *asf1Δ::KanMX4*, *cac1Δ::KanMX4*, *hir1Δ::KanMX4*, *hpr1Δ::KanMX4*, *paf1Δ::KanMX4*, or *ppr2Δ::KanMX4* (all from EUROSCARF) were amplified by PCR and introduced into WZY42 to obtain *asf1Δ* (YC247), *cac1Δ* (YC107), *hir1Δ* (YC106), *hpr1Δ* (YC240), *paf1Δ* (YC246), or *ppr2Δ* (YC241) in the histone shuffle background.

**PCR-mediated mutagenesis.** The histone H3 mutants were constructed by PCR-mediated site-directed mutagenesis using pWZ414-F13 (from S. Dent; [27]) as a template. PCR was performed with mutagenic primers shown in Supplementary Table S1. The PCR condition was: 1 min at 95 °C followed by 17 cycles of 30 s at 95 °C, 1 min at 42 or 50 °C, and 15 min at 68 °C. The final step of PCR was 1 min at 94 °C, 1 min at 55 °C, and 10 min at 72 °C. PCR-amplified DNA was treated with 10 U of *DpnI* (New England Biolabs) for 3 h at 37 °C to remove template DNA before *Escherichia coli* transformation. Each mutation was confirmed by DNA sequencing.

**Sensitivity assay.** WT, *cac1Δ*, and *hir1Δ* strains containing WT or each histone H3 mutant plasmid were cultured in YPD media overnight. They were diluted to OD<sub>600</sub> = 0.8, and 10-fold serial dilutions were spotted on 5 μg/mL CPT, 100 mM HU, or 0.03% MMS (all from Sigma) plates. Plates were incubated at 30 °C for 3–5 days.

## Results and discussion

### Generation of histone H3 alanine mutants

Recently, Horikoshi's group performed global analysis of functional residues located on the surface of the histone core, and revealed distinctive regions important for several chromatin-based pathways [10]. To understand nucleosome structure and function, we focused on the histone H3 subunit and independently generated 35 triple alanine substitution H3 mutants (intrinsic alanines were not changed) using site-directed mutagenesis (PCR primers

are listed in Table S1). Among them, 22 were viable, while 13 were non-viable (Fig. 1).

Alanine mutations that affected viability roughly fell into two regions: surrounding the αN helix and the histone fold domain (Fig. 1 and Supplementary Fig. S1). The first group of non-viable mutants was located around the nucleosome entry site along the αN helix. Five amino acids (H39 to P43) comprise the region that passes through the minor grooves of the DNA, letting out its N terminus. R40, Y41, K42, V46, and A47 are involved in the formation of hydrogen bonds with DNA at each DNA terminus [1]. Therefore, structural changes in this region lead to critical instability of nucleosomes probably by stripping off the DNA from the histone octamer. The REI49-51A mutant was viable, although a single-mutant I51A was reported to be essential [10]. Mutational effect between I51A and two additional A substitution preceding I51 (REI49-51A) might be different.

The second group of non-viable mutations was located in the histone-histone or histone-DNA interaction domains. The FQR67-69A mutant, located at the beginning of the α1 helix, was not viable. F67 and Q68 are close to I89 and Q93 of the H3 α2 helix. However the proximal configuration of FQR67-69 to α2 helix might not be absolutely required for viability, as the IG89-90A mutant was viable. The LRF82-84A mutant within L1 loop was not viable. R83 can form a hydrogen bond with H4-K79 and -V81 and extend into the DNA minor groove [2]. Several Irs (loss of rDNA silencing) mutants, such as L82S and R83A, were also located in this region [28]. Thus, histone-histone and histone-DNA interactions provided by LRF are important for nucleosome structure and viability. Likewise, IH112-113, which contributes to the H3-H3 interaction, is also essential, and KKD121-123A and IKL124-126A were non-viable. K121 and K125 form hydrogen bonds with H2B-T128 and -Q129, respectively [2]. Taken together, our results confirm that amino acids located in the histone-histone and histone-DNA interaction surfaces are important for maintenance

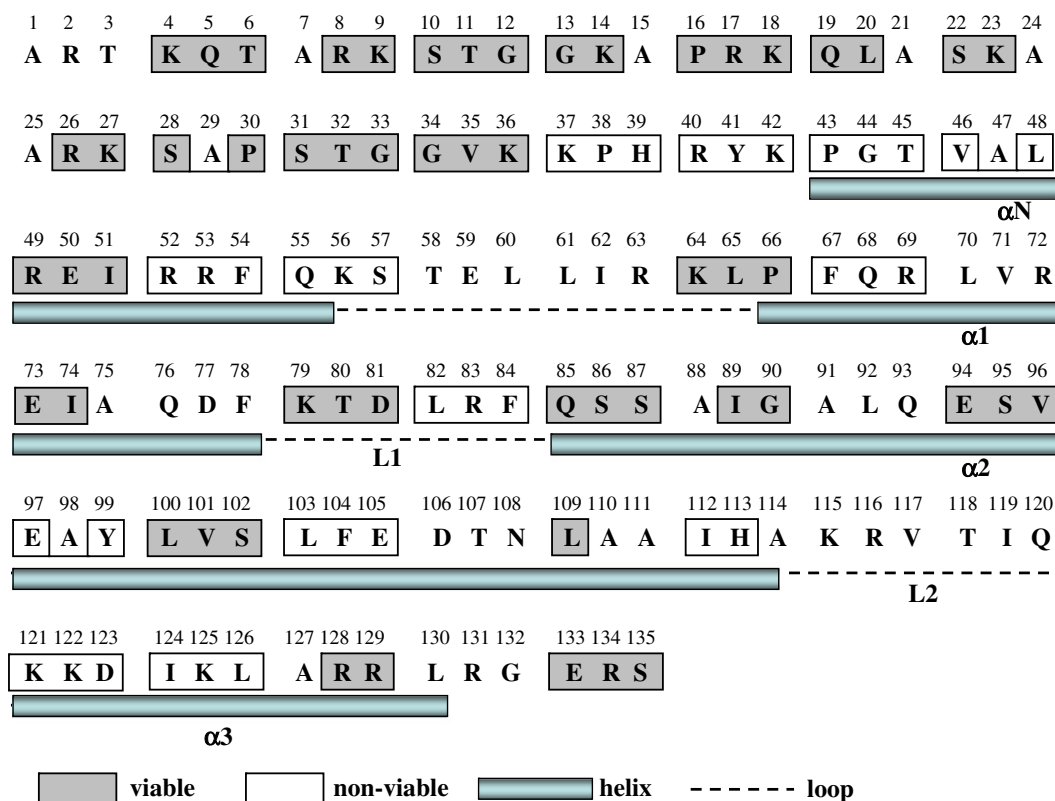


Fig. 1. Summary of the viability test for H3 alanine mutants. Viable and non-viable mutants are shown as filled and open boxes, respectively.

of nucleosome structure. In addition, our data show that amino acid residues aligned along the  $\alpha$ N helix and preceding tail region are as important for nucleosome function as histone fold domains.

#### Analysis of the various H3 mutants for sensitivity to HU and MMS

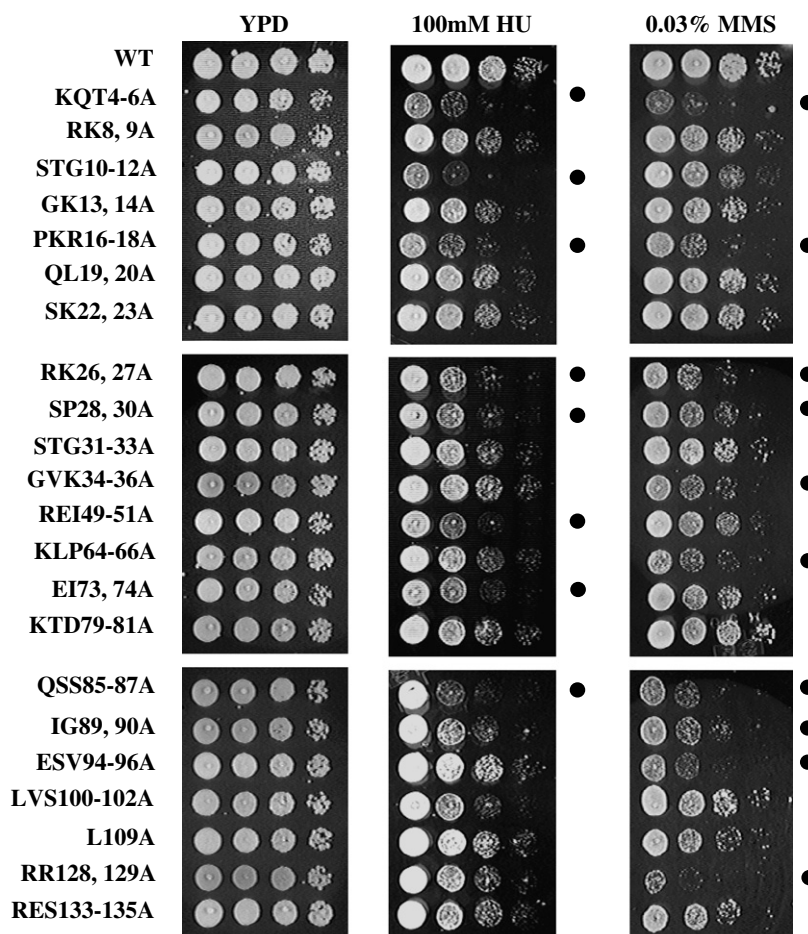
To analyze the roles of H3 residues in maintaining DNA integrity, the 22 viable H3 alanine mutants were spotted on YPD plates containing HU (a ribonucleotide reductase inhibitor) or MMS (a DNA alkylating agent) (Fig. 2). Eight mutants showed weak sensitivity to HU, whereas 10 mutants showed weak or strong sensitivity to MMS. Five out of eight HU-sensitive mutants also showed MMS sensitivity. H3 residues important for maintenance of DNA integrity during replication are also important for survival in the presence of DNA alkylating damage, indicating that the two pathways are closely related at the nucleosome level. In fact, DNA replication checkpoint signaling and DNA repair signaling pathways share many factors, such as Rad9, Rad53, and Mec1 [29]. Furthermore, DNA replication factors are involved in homologous recombination for repair of double strand breaks and DNA methylation [30,31]. Histones must be common components of the two pathways for maintenance of genome integrity.

GVK34-36A was weakly sensitive to MMS, but not to HU. H3 K36 is the residue methylated by Set2 [32]. The *set2 $\Delta$*  mutant has a mild IR-sensitive phenotype [33] but not an HU-sensitive phenotype. Moreover, *set2 $\Delta$*  suppresses HU sensitivity caused by deletion of DNA replication genes [34]. These reports and our data suggest that H3 K36 plays a distinct role in DNA repair and replica-

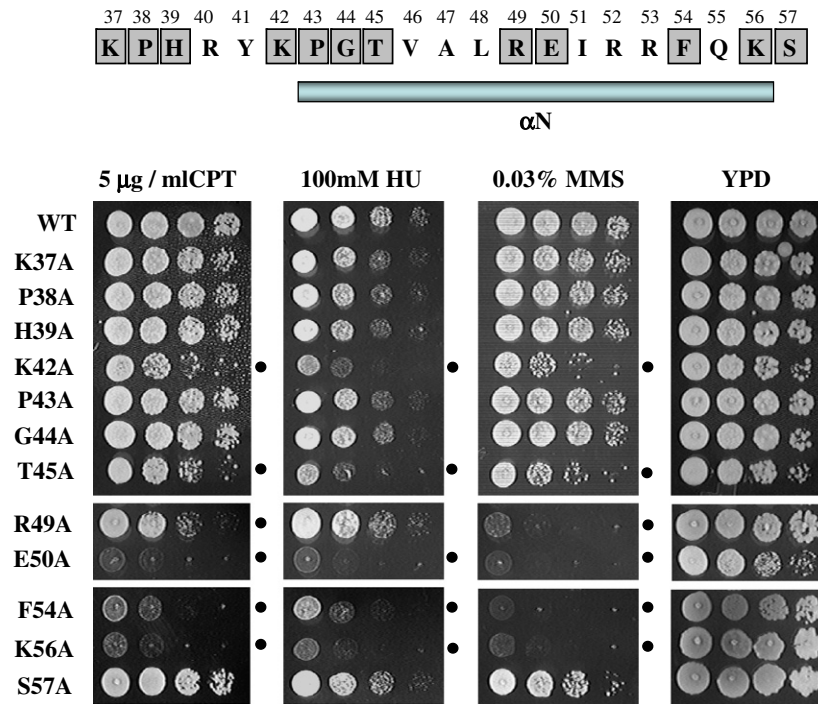
tion. QSS85-87 is located on the  $\alpha$ 2 helix and is very close to DNA, although not directly involved in hydrogen bond formation with DNA [1]. QSS85-87A was one of the most sensitive mutants to both HU and MMS in this study. Other MMS sensitive mutants, IG89-90A, ESV94-96A, and RR128-129A, all have mutations within the core domain. Among them, R129 is involved in binding the histone chaperone, Asf1 [35]. Depletion of Asf1 generally results in CPT, HU, and MMS sensitivity [13,35]. Thus, Asf1-nucleosome interaction via R129 is especially important for growth in the presence of MMS.

#### A distinct role of the $\alpha$ N helix in maintaining DNA integrity

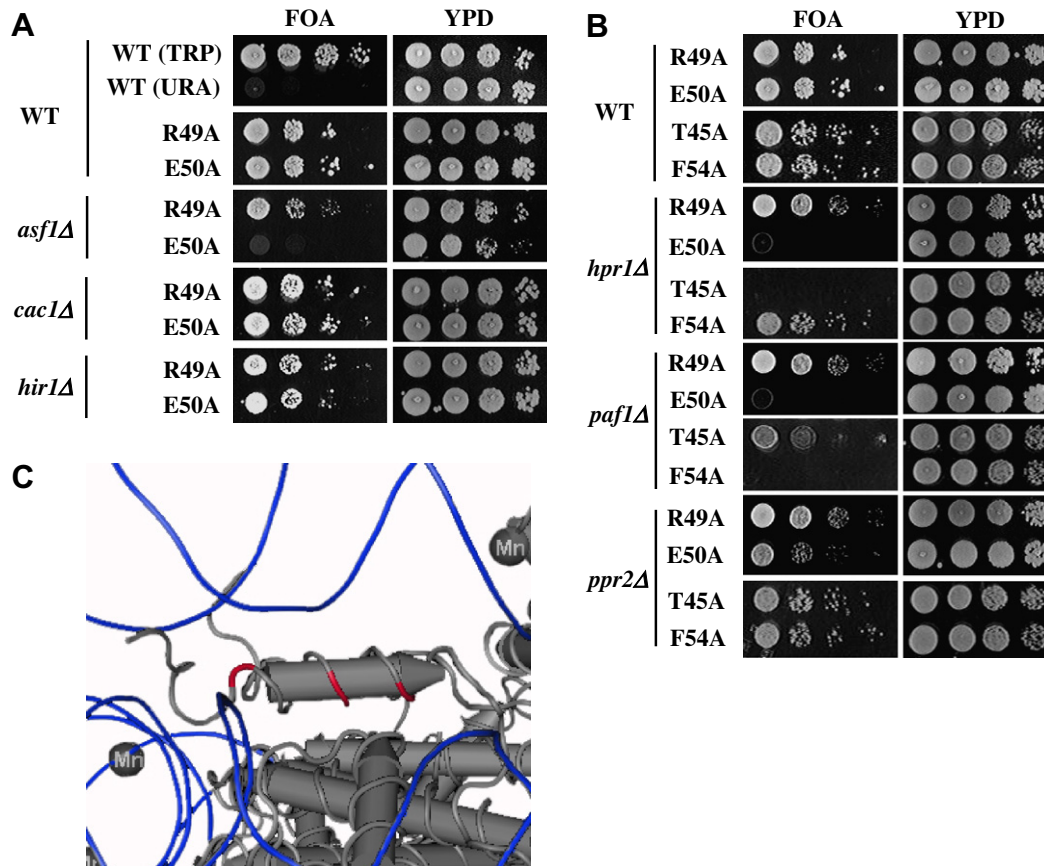
Many of the tandem alanine substitution mutations encompassing the  $\alpha$ N helix of H3 were lethal (Fig. 1). To further analyze the role of the  $\alpha$ N helix in chromatin function, we generated 12 individual alanine substitution mutants around the  $\alpha$ N helix (Fig. 3). These individual point mutants were viable. K42A, T45A, E50A, F54A, and K56A were sensitive to CPT (a topoisomerase I inhibitor), MMS, or HU, and R49A was sensitive to CPT and MMS, suggesting that this region is particularly important for resistance to genotoxic damage. Our data correlate with the report that H3 K42A, G44A, T45A, R49A, E50A, F54A, and K56A were sensitive to HU and MMS, and that H39A was sensitive to MMS [10], with slight differences in that the sensitivities of H39A and G44A to HU or MMS were negligible, and that R49A was sensitive to CPT and MMS rather than HU, as in this study (Fig. 3).



**Fig. 2.** Drug sensitivity of H3 mutations. WT and H3 mutants were serially diluted and spotted on 100 mM HU, 0.03% MMS, or YPD plates. Plates were incubated at 30 °C for 3–5 days. Dots indicate drug-sensitive mutants.



**Fig. 3.** Drug sensitivity of  $\alpha$ N helix mutants with individually substituted amino acids. Each alanine mutation is shown in the boxes (top panel). Drug sensitivity of individual alanine mutants (bottom panel).



**Fig. 4.** The  $\alpha$ N helix region is related to the histone chaperone activity. (A) Viability test of R49A and E50A in WT, *asf1Δ*, *cac1Δ*, and *hir1Δ* backgrounds. (B) Viability test of R49A, E50A, T45A, and F54A in WT, *hpr1Δ*, *paf1Δ*, and *ppr2Δ* backgrounds. (C) The position of H3 T45, E50, and F54 on the  $\alpha$ N helix. Blue ribbon represents DNA. H3 T45, E50, and F54 residues are indicated by red marks. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Yeast with *asf1Δ* develop sensitivity to multiple DNA damaging agents due to the loss of K56 acetylation by Rtt109 [13,35–38]. Although the  $\alpha$ N mutants developed similar sensitivities, in contrast to *asf1Δ*, K56 acetylation was normal in all mutants, indicating that the role of the  $\alpha$ N helix in maintenance of DNA integrity was independent of K56 acetylation (Supplementary Fig. S2).

*The  $\alpha$ N helix is functionally correlated with the histone chaperone activity of Asf1*

REI49-51A was the only viable  $\alpha$ N helix mutant. Based on the nucleosome structure, this region could be particularly important for nucleosome assembly and disassembly, as it is located at the nucleosome entry site of DNA [1]. To examine potential connections of the  $\alpha$ N helix to nucleosome stability catalyzed by histone chaperones, the growth of  $\alpha$ N mutants was analyzed in the genetically modified Asf1, CAF-1, or HIR backgrounds. We noticed that REI49-51A was viable only in the wild-type chaperone background, and not in *asf1Δ*, *cac1Δ* (CAF-1 component), or *hir1Δ* (HIR component), suggesting that this region is important in histone mobility and stability (Supplementary Fig. S3). To further identify the critical residues for genetic interaction with histone chaperones, we tested R49 and E50 mutants for their growth in the same genetic backgrounds. As shown in Fig. 4, R49A was viable in all genetic backgrounds tested. The growth of E50A was not affected by loss of CAF-1 (*cac1Δ*, *cac2Δ*, *cac3Δ*) or HIR (*hir1Δ*, *hir2Δ*, *hir3Δ*, *hpc2Δ*) (Fig. 4A and data not shown). However, E50A was lethal when combined with *asf1Δ*, suggesting that E50 of the  $\alpha$ N helix is critical when Asf1 activity is compromised. Overall, R49, E50, and I51 might additively contribute to the histone chaperone pathway, with E50 being critical in the absence of Asf1. The functional correlation between the  $\alpha$ N helix and Asf1 was further confirmed by finding two more mutants, T45A and F54A, which exhibited synthetic lethality with *asf1Δ* (Supplementary Fig. S4).

Dynamic changes in nucleosome structure and composition occur during transcription [22,39–42]. Therefore, we further analyzed the  $\alpha$ N helix mutants to understand its role in transcription. Their genetic interaction was examined with transcription elongation factors, such as Paf1 (a component of the Paf1 transcription elongation complex), Hpr1 (a component of the THO/TREX complex that coordinates transcription elongation and mRNA export), and Ppr2 (TFIIS). Among them, E50A and F54A were lethal in the presence of *paf1Δ*, and T45A and E50A were lethal in *hpr1Δ*, which are involved in transcription elongation on a chromatin template, but they were not greatly affected by *ppr2Δ*, a transcription elongation factor that facilitates the polymerase to overcome intrinsic arrest sites (Fig. 4B).

Asf1 is at the promoters and in the coding regions of highly transcribed genes [42,43], where it mediates histone eviction/deposition and histone exchange during transcription [22]. In both transcription-coupled and replication-coupled pathways, Asf1 mediates nucleosome assembly and disassembly and allows H3 K56 acetylation, which probably involves partial unfolding of nucleosomes [44]. As the  $\alpha$ N helix binds both ends of DNA [1], this region may be a target for Asf1 to initiate nucleosome disassembly or to complete nucleosome reassembly. T45, E50, and F54 are located in the space between DNAs facing outward within the nucleosome structure [1,2] (Fig. 4C). Although the recent H3/H4-Asf1 structure does not include the  $\alpha$ N region [35], this region is likely to be important for the reversible transition of nucleosome structure mediated by histone chaperones like Asf1. Further studies are needed to understand the functional role of the  $\alpha$ N helix during nucleosome mobility.

In conclusion, we showed that the  $\alpha$ N helix is important in dynamic pathways involving nucleosomes and Asf1, Paf1 or Hpr1, as well as viability and resistance against genotoxic agents. Based on

the configuration within the nucleosome structure, the  $\alpha$ N helix could serve as an important nucleation site for assembly and disassembly of nucleosomes, which might be important for transcription.

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## Appendix. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2008.07.084.

## References

- [1] K. Luger, A.W. Mader, R.K. Richmond, D.F. Sargent, T.J. Richmond, Crystal structure of the nucleosome core particle at 2.8 Å resolution, *Nature* 389 (1997) 251–260.
- [2] C.L. White, R.K. Suto, C. Luger, Structure of the yeast nucleosome core particle reveals fundamental changes in internucleosome interactions, *EMBO J.* 20 (2001) 5207–5218.
- [3] K. Luger, T.J. Rechsteiner, A.J. Flaus, M.M. Waye, T.J. Richmond, Characterization of nucleosome core particles containing histone proteins made in bacteria, *J. Mol. Biol.* 272 (1997) 301–311.
- [4] K. Luger, T.J. Richmond, The histone tails of the nucleosome, *Curr. Opin. Genet. Dev.* 8 (1998) 140–146.
- [5] E.J. Cho, RNA polymerase II carboxy-terminal domain with multiple connections, *Exp. Mol. Med.* 30 (2007) 247–254.
- [6] A. Groth, W. Rocha, A. Verreault, G. Almouzni, Chromatin challenges during DNA replication and repair, *Cell* 128 (2007) 721–733.
- [7] T. Kouzarides, Chromatin modifications and their function, *Cell* 128 (2007) 683–705.
- [8] C.L. Peterson, M.A. Laniel, Histones and histone modifications, *Curr. Biol.* 14 (2004) R546–R551.
- [9] E.M. Hyland, M.S. Cosgrove, H. Molina, D. Wang, A. Pandey, R.J. Cottee, J.D. Boeke, Insights into the role of histone H3 and histone H4 core modifiable residues in *Saccharomyces cerevisiae*, *Mol. Cell. Biol.* 25 (2005) 10060–10070.
- [10] K. Matsubara, N. Sano, T. Umehara, M. Horikoshi, Global analysis of functional surfaces of core histones with comprehensive point mutants, *Genes Cells* 12 (2007) 13–33.
- [11] F. van Leeuwen, P.R. Gafken, D.E. Gottschling, Dot1p modulates silencing in yeast by methylation of the nucleosome core, *Cell* 109 (2002) 745–756.
- [12] H.H. Ng, Q. Feng, H. Wang, H. Erdjument-Bromage, P. Tempst, Y. Zhang, K. Struhl, Lysine methylation within the globular domain of histone H3 by Dot1 is important for telomeric silencing and Sir protein association, *Genes Dev.* 16 (2002) 1518–1527.
- [13] J. Recht, T. Tsubota, J.C. Tanny, R.L. Diaz, J.M. Berger, X. Zhang, B.A. Garcia, J. Shabanowitz, A.L. Burlingame, D.F. Hunt, P.D. Kaufman, C.D. Allis, Histone chaperone Asf1 is required for histone H3 lysine 56 acetylation, a modification associated with S phase in mitosis and meiosis, *Proc. Natl. Acad. Sci. USA* 103 (2006) 6988–6993.
- [14] R. Wysocki, A. Javaheri, S. Allard, F. Sha, J. Cote, S.J. Kron, Role of Dot1-dependent histone H3 methylation in G1 and S phase DNA damage checkpoint functions of Rad9, *Mol. Cell. Biol.* 25 (2005) 8430–8443.
- [15] M.J. Carrozza, B. Li, L. Florens, T. Saganuma, S.K. Swanson, K.K. Lee, W.J. Shia, S. Anderson, J. Yates, M.P. Washburn, J.L. Workman, Histone H3 methylation by Set2 directs deacetylation of coding regions by Rpd3S to suppress spurious intragenic transcription, *Cell* 123 (2005) 581–592.
- [16] A. Crampton, F. Chang, D.L. Pappas Jr., R.L. Frisch, M. Weinreich, An ARS element inhibits DNA replication through a SIR2-dependent mechanism, *Mol. Cell* 30 (2008) 156–166.
- [17] W. Fischle, B.S. Tseng, H.L. Dormann, B.M. Ueberheide, B.A. Garcia, J. Shabanowitz, D.F. Hunt, H. Funabiki, C.D. Allis, Regulation of HP1-chromatin binding by histone H3 methylation and phosphorylation, *Nature* 438 (2005) 1116–1122.
- [18] H. Santos-Rosa, R. Schneider, A.J. Bannister, J. Sherriff, B.E. Bernstein, N.C. Emre, S.L. Schreiber, J. Mellor, T. Kouzarides, Active genes are tri-methylated at K4 of histone H3, *Nature* 419 (2002) 407–411.
- [19] A. Verreault, De novo nucleosome assembly: new pieces in an old puzzle, *Genes Dev.* 14 (2000) 1430–1438.
- [20] A. Loyola, G. Almouzni, Histone chaperones, a supporting role in the limelight, *Biochim. Biophys. Acta* 1677 (2004) 3–11.
- [21] L. Glowczewski, J.H. Waterborg, J.G. Berman, Yeast chromatin assembly complex 1 protein excludes nonacetylatable forms of histone H4 from chromatin and the nucleus, *Mol. Cell. Biol.* 24 (2004) 10180–10192.

- [22] S. Henikoff, K. Ahmad, Assembly of variant histones into chromatin, *Annu. Rev. Cell Dev. Biol.* 21 (2005) 133–153.
- [23] E.M. Green, A.J. Antczak, A.O. Bailey, A.A. Franco, K.J. Wu, J.R. Yates 3rd, P.D. Kaufman, Replication-independent histone deposition by the HIR complex and Asf1, *Curr. Biol.* 15 (2005) 2044–2049.
- [24] M.S. Spector, A. Raff, H. DeSilva, K. Lee, M.A. Osley, Hir1p and Hir2p function as transcriptional corepressors to regulate histone gene transcription in the *Saccharomyces cerevisiae* cell cycle, *Mol. Cell. Biol.* 17 (1997) 545–552.
- [25] D.C. Krawitz, T. Kama, P.D. Kaufman, Chromatin assembly factor I mutants defective for PCNA binding require Asf1/Hir proteins for silencing, *Mol. Cell. Biol.* 22 (2002) 614–625.
- [26] H.J. Kim, J.H. Seol, J.W. Han, H.D. Youn, E.J. Cho, Histone chaperones regulate histone exchange during transcription, *EMBO J.* 26 (2007) 4467–4474.
- [27] W. Zhang, J.R. Bone, D.G. Edmondson, B.M. Turner, S.Y. Roth, Essential and redundant functions of histone acetylation revealed by mutation of target lysines and loss of the Gcn5p acetyltransferase, *EMBO J.* 17 (1998) 3155–3167.
- [28] J.H. Park, M.S. Cosgrove, E. Youngman, C. Wolberger, J.D. Boeke, A core nucleosome surface crucial for transcriptional silencing, *Nat. Genet.* 32 (2002) 273–279.
- [29] D. Branzei, M. Foiani, The Rad53 signal transduction pathway: replication fork stabilization, DNA repair, and adaptation, *Exp. Cell Res.* 312 (2006) 2654–2659.
- [30] B. Sedgwick, Repairing DNA-methylation damage, *Nat. Rev. Mol. Cell Biol.* 5 (2004) 148–157.
- [31] C. Wyman, R. Kanaar, DNA double-strand break repair: all's well that ends well, *Annu. Rev. Genet.* 40 (2006) 363–383.
- [32] B.D. Strahl, P.A. Grant, S.D. Briggs, Z.W. Sun, J.R. Bone, J.A. Caldwell, S. Mollah, R.G. Cook, J. Shabanowitz, D.F. Hunt, C.D. Allis, Set2 is a nucleosomal histone H3-selective methyltransferase that mediates transcriptional repression, *Mol. Cell. Biol.* 22 (2002) 1298–1306.
- [33] J.C. Game, M.S. Williamson, T. Spicakova, J.M. Brown, The RAD6/BRE1 histone modification pathway in *Saccharomyces* confers radiation resistance through a RAD51-dependent process that is independent of RAD18, *Genetics* 173 (2006) 1951–1968.
- [34] D. Biswas, S. Takahata, H. Xin, R. Dutta-Biswas, Y. Yu, T. Formosa, D.J. Stillman, A role for Chd1 and Set2 in negatively regulating DNA replication in *Saccharomyces cerevisiae*, *Genetics* 178 (2008) 649–659.
- [35] C.M. English, M.W. Adkins, J.J. Carson, M.E. Churchill, J.K. Tyler, Structural basis for the histone chaperone activity of Asf1, *Cell* 127 (2006) 495–508.
- [36] R. Driscoll, A. Hudson, S.P. Jackson, Yeast Rtt109 promotes genome stability by acetylating histone H3 on lysine 56, *Science* 315 (2007) 649–652.
- [37] J. Han, H. Zhou, B. Horazdovsky, K. Zhang, R.M. Xu, Z. Zhang, Rtt109 acetylates histone H3 lysine 56 and functions in DNA replication, *Science* 315 (2007) 653–655.
- [38] T. Tsubota, C.E. Berndsen, J.A. Erkmann, C.L. Smith, L. Yang, M.A. Freitas, J.M. Denu, P.D. Kaufman, Histone H3-K56 acetylation is catalyzed by histone chaperone-dependent complexes, *Mol. Cell* 25 (2007) 703–712.
- [39] M.L. Kireeva, W. Walter, V. Tchernajenko, V. Bondarenko, M. Kashlev, V.M. Studitsky, Nucleosome remodeling induced by RNA polymerase II: loss of the H2A/H2B dimer during transcription, *Mol. Cell* 9 (2002) 541–552.
- [40] A. Kristjuhan, J.Q. Svejstrup, Evidence for distinct mechanisms facilitating transcript elongation through chromatin *in vivo*, *EMBO J.* 23 (2004) 4243–4252.
- [41] C.K. Lee, Y. Shibata, B. Rao, B.D. Strahl, J.D. Lieb, Evidence for nucleosome depletion at active regulatory regions genome-wide, *Nat. Genet.* 36 (2004) 900–905.
- [42] M.A. Schwabish, K. Struhl, Asf1 mediates histone eviction and deposition during elongation by RNA polymerase II, *Mol. Cell* 22 (2006) 415–422.
- [43] J.K. Tyler, C.R. Adams, S.R. Chen, R. Kobayashi, R.T. Kamakaka, J.T. Kadonaga, The RCAF complex mediates chromatin assembly during DNA replication and repair, *Nature* 402 (1999) 555–560.
- [44] H. Masumoto, D. Hawke, R. Kobayashi, A. Verreault, *Nature* 436 (2005) 294–298.