

H2A.Z has a function reminiscent of an activator required for preferential binding to intergenic DNA

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H2A.Z has been shown to regulate transcription in yeast, and that function resides in its C-terminal region as the reciprocal portion of H2A cannot substitute for the latter. We show that fusion of a transcriptional activating region to the C-terminal region of H2A, which is substituted for that of H2A.Z, can allow the chimera to fulfil the special role of H2A.Z in positive gene regulation, as well as complement growth deficiencies of *htz1Δ* cells. We further show that the ‘transcription’ function of H2A.Z is linked to its ability to preferentially localize to certain intergenic DNA regions. Our results suggest that H2A.Z modulates functional interactions with transcription regulatory components, and thus increases its localization to promoters where it helps poise chromatin for gene activation.

Keywords: H2A.Z/PUR5/transcription/yeast

Introduction

Chromatin is known to present a formidable obstacle to many cellular processes including gene transcription. Thus it is generally believed that nucleosomes must be remodelled at certain regions of a given gene in order to trigger efficient gene induction. Current views suggest that there are three classes of chromatin remodelling mechanism. The first involves the action of proteins that hydrolyze ATP as a source of energy. Such complexes include, for example, the well-studied Swi/Snf complex (Vignali *et al.*, 2000; Havas *et al.*, 2001; Becker and Hörz, 2002). The second mechanism involves protein factors that chemically modify histone proteins, such as the SAGA histone acetyltransferase complex (Roth *et al.*, 2001). Both of the remodelling complexes mentioned above have been shown to be recruited to promoter regions, namely by the action of gene-specific transcriptional activators (Ütley *et al.*, 1998; Neely *et al.*, 1999; Yudkovski *et al.*, 1999; Kuo *et al.*, 2000; Brown *et al.*, 2001). The third mechanism involves the incorporation of histone variants that can presumably create ‘specialized’ regions of chromatin allowing it to become more permissive to the process of transcription. An example of this can be illustrated by histone variant H3.3 in *Drosophila*. H3.3 differs from regular H3 by only four amino acids, and it gradually replaces H3 in differentiated cells in a replication-independent fashion upon exiting the cell cycle (Ahmad and Henikoff, 2002). The replacement of H3 by H3.3

forces the remodelling of nucleosomes, and thus the quick removal of post-transcriptional modifications to histones. The outcome of this scenario gives rise to rapid induction of genes that were previously repressed by the chemical modification of histones (Ahmad and Henikoff, 2002). Another variant histone that could potentially create such specialized chromatin is H2A.Z.

Variant histone H2A.Z has been identified in such diverse eukaryotic organisms as humans (Hatch and Bonner, 1988), mice (Faast *et al.*, 2001), sea urchins (Ernst *et al.*, 1987), chickens (Harvey *et al.*, 1983), *Xenopus laevis* (Iouzaen *et al.*, 1996), *Drosophila* (van Daal *et al.*, 1988), *Tetrahymena* (White *et al.*, 1988) and yeasts (Carr *et al.*, 1994; Jackson *et al.*, 1996), and it has been found to be essential in many of these organisms including mice (Faast *et al.*, 2001). In *Tetrahymena*, H2A.Z has been shown to be associated with transcriptionally active chromatin (Stargell *et al.*, 1993). Experiments carried out in *Saccharomyces cerevisiae* have validated the importance of the H2A.Z variant histone in both positive and negative gene transcription (Dhillon and Kamakaka, 2000; Santisteban *et al.*, 2000; Adam *et al.*, 2001; Meneghini *et al.*, 2003). Importantly, H2A.Z has been shown to be essential for the recruitment of both RNA polymerase II (pol II) and the TATA-binding protein (TBP) to the *GALI-10* promoters (Adam *et al.*, 2001), and it also performs redundant functions with both the Swi/Snf and SAGA chromatin remodelling complexes (Santisteban *et al.*, 2000). A recent report has suggested that the function of H2A.Z in yeast is also redundant with H2B ubiquitination in the formation of active chromatin (Hwang *et al.*, 2003). An insightful study in *Drosophila* has revealed that the essential function of H2A.Z, compared with regular H2A, resides in its C-terminal region (Clarkson *et al.*, 1999). These authors have shown that substitution of H2A.Z amino acids for those of H2A in a short α -helix at the C-terminus was essential for fly viability. We have previously shown that substitution of the H2A.Z C-terminal region by that of H2A in yeast could not fulfil the role of H2A.Z in *GALI-10* gene induction in a yeast strain bearing a deletion of *HTZ1* (*htz1Δ*), the H2A.Z-encoding gene (Adam *et al.*, 2001). On the other hand, fusion of the H2A.Z C-terminal region to H2A could complement a *htz1Δ* mutation for *GALI-10* gene induction. These results suggest that the essential role of H2A.Z in *GAL* gene transcription resides in the C-terminal region of the variant histone and that this function of H2A.Z cannot be provided by regular H2A. Importantly, structure determination of an H2A.Z-containing nucleosome particle (Suto *et al.*, 2000) has revealed an altered surface that includes a metal ion. The authors of that study suggest that this surface could lead to changes in higher-order structure and/or could be involved in mediating interactions between H2A.Z and other nuclear proteins. In addition,

the H2A.Z C-terminal region, through its docking domain, is essential in creating a large interaction surface with (H3-H4)₂ tetramers. A recent report has shown that H2A.Z-containing nucleosome particles facilitate intra-nucleosomal interactions while simultaneously inhibiting intermolecular nucleosome associations (Fan *et al.*, 2002). In contrast to H2A, H2A.Z forms a non-uniform binding pattern on *Drosophila* polytene chromosomes (Leach *et al.*, 2000), and it preferentially binds promoter DNA of some, but not all genes in *S.cerevisiae* (Santisteban *et al.*, 2000; Meneghini *et al.*, 2003). Meneghini and colleagues (Meneghini *et al.*, 2003) have also shown that many H2A.Z-activated genes cluster near telomeres in yeast, and that variant histone protects euchromatin from telomeric silencing as reduced expression of those genes in *htz1Δ* cells is reversed by deletion of *SIR2*. These important results suggest that H2A.Z possesses an additional function that allows it to bind selectively to certain genomic regions, the significance of which is still unknown.

In order to study the mechanism by which H2A.Z can modulate positive gene transcription, we set out to study further the functional role of the C-terminal region of the variant histone. In this study, we show that transcriptional activation domains can provide the special function of the H2A.Z C-terminal region in gene induction, and they can also complement various growth deficiencies of *htz1Δ* cells. We also demonstrate that the H2A.Z C-terminal region allows H2A.Z to bind preferentially the promoter regions of the *PUR5* and *PHO5* genes. Finally, we show that both the 'locator' and 'transcription' functions of the H2A.Z C-terminal region are linked.

Results

Transcriptional activating regions can confer the special function of H2A.Z in positive gene transcription and growth on hydroxyurea

Recent reports have shown that H2A.Z is important for gene expression in yeast, with possible roles in chromatin remodelling and transcriptional elongation (Santisteban *et al.*, 2000; Adam *et al.*, 2001; Krogan *et al.*, 2003). Importantly, H2A.Z is required for appropriate recruitment of RNA pol II at the yeast *GALI-10* genes under inducing conditions, and the C-terminal region of H2A.Z also interacts with RNA pol II components *in vitro* (Adam *et al.*, 2001). It is interesting to note that both of the above mentioned functions can also be elicited by a DNA-tethered transcriptional activating region (e.g. Ptashne and Gann, 1997). In addition, it has been suggested (Suto *et al.*, 2000) that within an H2A.Z-containing nucleosome particle, the latter variant histone has an acidic patch in its C-terminal region that extends to H2B. Based on all these facts, we hypothesized that one special function of H2A.Z in gene transcription, as compared with regular H2A, would be to contact components of the transcriptional machinery and/or chromatin remodelling components directly. According to this prediction, the H2A.Z C-terminal region should harbour a function reminiscent of a transcriptional activating region when incorporated into a nucleosomal particle. However, investigation of the specific function of the H2A.Z C-terminal region in gene transcription is difficult to assess because of its importance

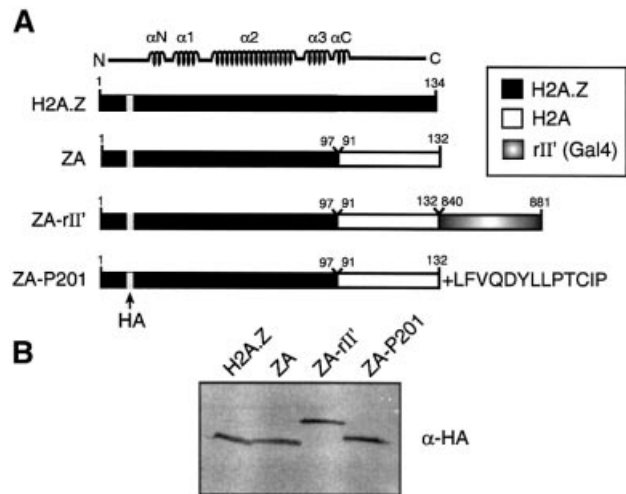


Fig. 1. Structure and expression of variant histone protein chimeras used in this study. (A) Schematic representation of the chimeras. The location of the HA tag is shown as a gray bar. (B) Immunoblot analysis of H2A.Z chimeras. Histone protein levels were determined by immunoblotting with an anti-HA antibody.

in nucleosome stability, namely in the docking domain (Suto *et al.*, 2000). Accordingly, we have found that deletion of the H2A.Z C-terminal region prevented stable expression of the latter in yeast (M.Larochelle and L.Gaudreau, unpublished data). Hence, in order to test our model directly, we first made use of the ZA fusion protein fused to the Gal4 acidic transcriptional activating region (ZA-rII') (see Figure 1A). ZA bears the N-terminal region as well as the core domain of H2A.Z, fused to the C-terminal region of H2A (Figure 1A). Such a fusion protein is expected to be able to assemble into nucleosome particles but would lack the special function of H2A.Z in positive gene transcription (Adam *et al.*, 2001). We reasoned that if ZA-rII' could complement the phenotypes of *htz1Δ* cells and rescue transcription defects, then the function of H2A.Z in positive gene transcription would overlap with that of a transcriptional activating region. We also wanted to test a non-acidic activating region that functions in yeast. Hence we made a chimera consisting of ZA fused to a synthetic activating region, P201 (Lu *et al.*, 2000) (Figure 1A). Western blotting experiments reveal that all fusion proteins used in our experiments were expressed at comparable levels (Figure 1B). Deletion of *HTZ1* negatively affects *GALI* and *GAL7* gene induction as previously reported (Figure 2A, lane 2) (Adam *et al.*, 2001). In addition, expression of the ZA fusion also negatively affects *GAL* gene induction (lane 3) to a further extent than the empty vector alone in *htz1Δ* cells. We have observed in certain experiments that expression of ZA downregulates *GAL* transcription to a greater extent than expressing no H2A.Z derivative (e.g. empty vector in the *htz1Δ* strain). Importantly, expression of ZA-rII' could efficiently restore full activation of the *GALI* and *GAL7* genes in *htz1Δ* cells (lane 4). ZA-P201 could reverse the negative effect of ZA on transcription but could not fully restore *GAL* transcription (lane 5).

In addition to their inability to grow on certain carbon sources, slow growth and thermosensitivity (ts) phenotype, we have also previously observed that *htz1Δ* cells are

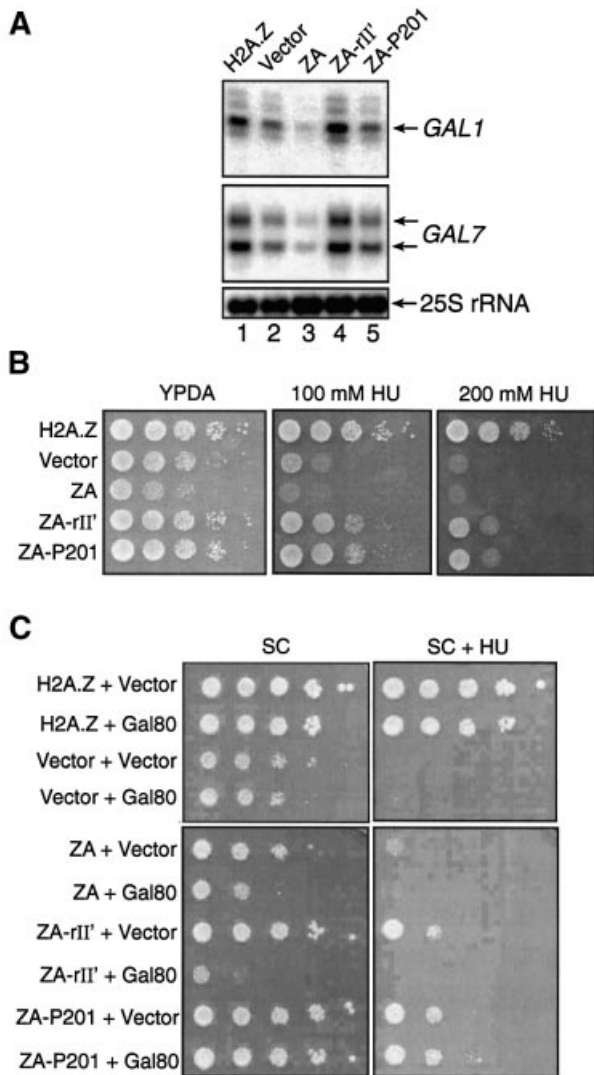


Fig. 2. Fusion of a transcriptional activating region to ZA is sufficient to restore *GAL1* and *GAL7* transcription, as well as the HU-sensitivity phenotype of *htz1Δ* yeast cells. (A) Ability of H2A.Z derivatives to induce *GAL1* and *GAL7* expression in *htz1Δ* cells. Primer extension analyses of *GAL1* and *GAL7* expression upon galactose induction. The 25S rRNA is shown at the bottom of the figure as a loading control. (B) Ability of variant H2A.Z derivatives to complement *htz1Δ*-mediated HU growth deficiency. Ten-fold serial dilutions of yeast cell suspensions were dropped on YPDA plates supplemented or not with either 100 or 200 mM HU. Plates were incubated at 30°C for 2–5 days. (C) Overexpression of Gal80 in $\Delta htz1$ cells expressing ZA-rII'. $\Delta htz1$ cells containing the indicated chimeras were transformed with either an empty vector or a Gal80 expression vector under the control of the *ACT1* promoter on a 2 μ plasmid. Serial dilutions of cells were spotted on plates without or with HU and grown for 3–6 days at 30°C.

highly sensitive to hydroxyurea (HU) (J.Brodeur and L.Gaudreau, unpublished data). HU is known to arrest cells in S phase, and previous reports have shown that a complete replication block can occur when yeast cells are treated with 200 mM HU (Allen *et al.*, 1994). Hence, we also tested if our ZA constructs fused to activating regions could rescue HU sensitivity of *htz1Δ* yeast cells. Figure 2B first shows that *htz1Δ* yeast cells bearing either an empty vector or expressing the ZA fusion have a marked growth deficiency on rich media containing HU. As expected, expression of wild-type (WT) H2A.Z rescues that defi-

ciency, as does the expression of both ZA-rII' and ZA-P201. Again, these results support the possibility that the H2A.Z C-terminal region harbours a function that resembles that of a transcriptional activating region since both types of activating region allow ZA to complement *htz1Δ*-associated defects.

GAL gene transcription is strongly induced by the Gal4 activator, which in turn can be negatively regulated by Gal80, a specific inhibitor that binds the Gal4 rII' activating region in the absence of galactose (Ma and Ptashne, 1987a; Bash and Lohr, 2001). In order to verify whether the action of the Gal4 activating region was indeed responsible for the observed effects with ZA-rII', we overexpressed Gal80 to ensure efficient titration of ZA-rII'. Thus we made use of the HU growth deficiency phenotype of *htz1Δ* cells to test the specificity of rII' in our assays. Figure 2C shows that ZA-rII' can reverse the slow growth and HU-sensitivity phenotypes of *htz1Δ* cells, and that overexpression of Gal80 can reverse the complementation effect of the rII' activating region. Gal80 has no effect on yeast cells expressing WT H2A.Z and ZA-P201, an anticipated result since Gal80 is not expected to bind either of these two proteins. Similar results were also obtained with the *ts* phenotype of *htz1Δ* cells (data not shown).

Replacement of the H2A.Z C-terminal region by the corresponding H2A region strongly inhibits *PUR5* induction

We have previously observed that *htz1Δ* cells are somewhat sensitive to 6-azauracil (6-AU) (M.Adam and L.Gaudreau, unpublished data), a result suggesting that H2A.Z can be important for transcriptional elongation since 6-AU is known to deplete GTP and UTP cellular pools (Shaw and Reines, 2000). This depletion makes RNA pol II more susceptible to pausing during elongation, thus making the role of elongation factors essential under those conditions. We thus decided to investigate further the behaviour of our ZA fusions with growth assays in the presence of 6-AU. Figure 3A shows that *htz1Δ* cells are significantly more sensitive to 6-AU when compared with either cells expressing WT H2A.Z or the ZA-rII' and ZA-P201 fusions. The figure further shows that expression of the ZA fusion can completely prevent yeast growth even at a concentration of 10 μ g/ml 6-AU, and is consistent with the results obtained with ZA in our *GAL* gene assays (e.g. Figure 2A). Since we observed a growth defect on 6-AU plates, especially with the ZA fusion, we decided to investigate the transcriptional activity of the *PUR5* gene with our different ZA fusion proteins. *PUR5* is a gene that encodes an IMP dehydrogenase that is activated by the presence of 6-AU (Exinger and Lacroute, 1992; Shaw and Reines, 2000; Escobar-Henriques and Daignan-Fornier, 2001). In the presence of 6-AU, yeast cells harbouring a defect in *PUR5* expression are usually defective in transcriptional elongation, but cells sensitive to 6-AU when *PUR5* is fully expressed are not (Shaw and Reines, 2000). Thus, to test if H2A.Z is important for *PUR5* induction, yeast cells were grown in synthetic complete (SC) medium lacking uracil and 6-AU was added to a final concentration of 75 μ g/ml to induce *PUR5* expression fully. Figure 3B shows primer extension analyses of *PUR5* transcription. Upon addition

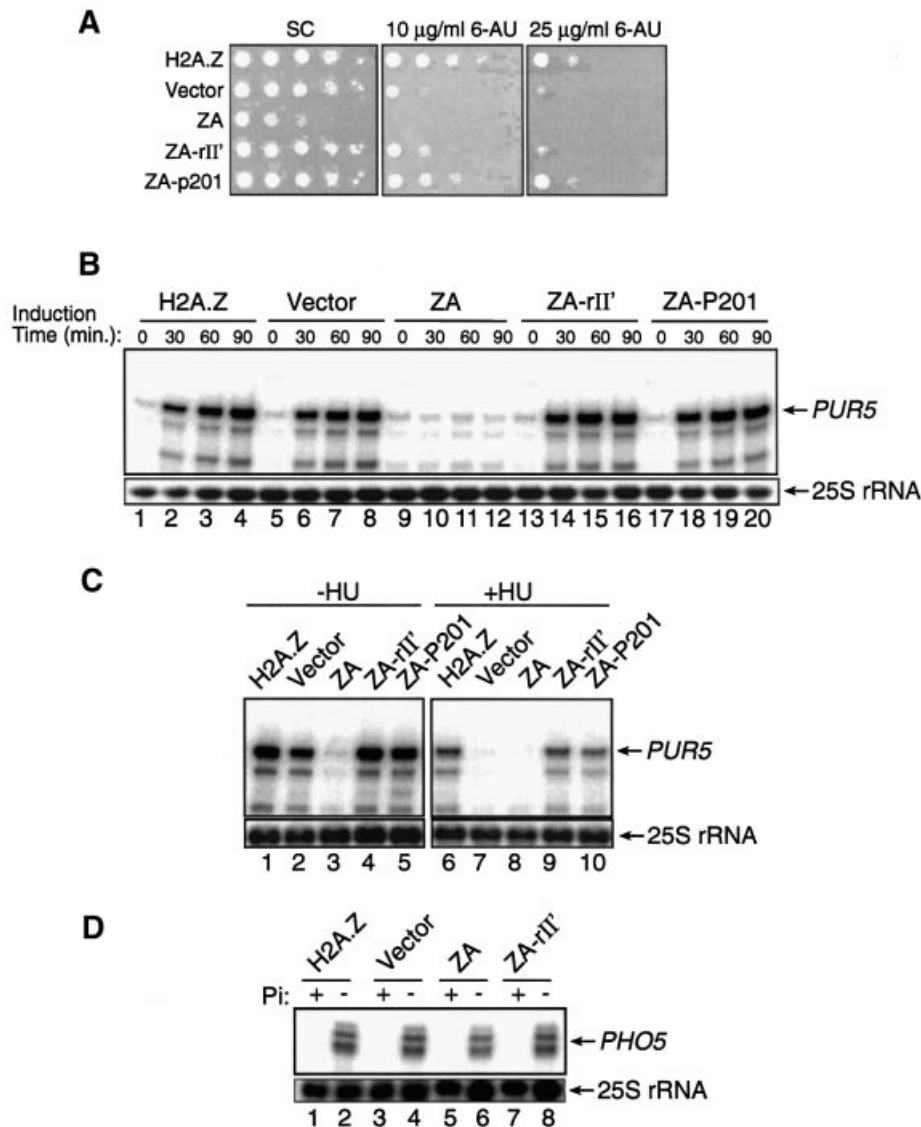


Fig. 3. Effect of H2A.Z derivatives on *PUR5* and *PHO5* transcription. (A) Growth of cells in the absence or presence of 6-AU. Cells were grown and then spotted onto plates containing no 6-AU, 10 $\mu\text{g/ml}$ 6-AU or 25 $\mu\text{g/ml}$ 6-AU. (B) Primer extension analysis of the *PUR5* gene in the presence of 6-AU. Induction time in the presence of 6-AU is shown in minutes. The 25S rRNA is shown at the bottom of the figure as a loading control. (C) Transcription of the *PUR5* gene in the presence of HU. Primer extension analysis of the *PUR5* gene after yeast cells were grown and incubated or not for 3.5 h with 200 mM HU. The 25S rRNA is shown at the bottom of the figure as a loading control. (D) Transcription of the *PHO5* gene. Primer extension analyses of the *PHO5* locus after yeast cells were grown in the presence (+) or absence (-) of phosphate (Pi). The 25S rRNA is shown at the bottom of the figure as a loading control.

of 6-AU, the *PUR5* gene can be strongly induced when WT H2A.Z is present (Figure 3B, lanes 1–4). The *PUR5* gene can still be efficiently induced even in the absence of any H2A.Z derivative (Figure 3B, lanes 5–8), whereas the expression of ZA completely prevented *PUR5* induction (Figure 3B, lanes 9–12). Finally, expression of either ZA-II' (Figure 3B, lanes 13–16) or ZA-P201 (Figure 3B, lanes 17–20) rescued *PUR5* induction as compared with ZA. These results suggest that the 6-AU sensitivity of *htz1* Δ cells is not solely due to a *PUR5* transcriptional defect, but perhaps to other genes inhibited by 6-AU such as those encoding for an orotidylate decarboxylase (Shaw and Reines, 2000).

We next wanted to determine if preventing DNA replication would affect *PUR5* induction in *htz1* Δ cells.

We reasoned that if H2A.Z has a redundant function with other cellular factors possibly involved in transcription, then generally crippling DNA synthesis-dependent chromatin remodelling might make *PUR5* transcription dependent on H2A.Z. In order to block DNA replication, we made use of HU as in Figure 2B. Thus Figure 3C shows similar *PUR5* primer extension analyses to those in Figure 3B but all are under activated conditions. In the absence of HU, *PUR5* induction is only slightly decreased in *htz1* Δ cells (compare lanes 1 and 2), whereas expression of ZA prevents *PUR5* induction as previously observed (compare lanes 1 and 3). Lanes 4 and 5 show transcription levels at *PUR5* when ZA-rII' and ZA-P201 are expressed. When HU is added to yeast cultures to arrest DNA synthesis, *PUR5* induction is dramatically affected in

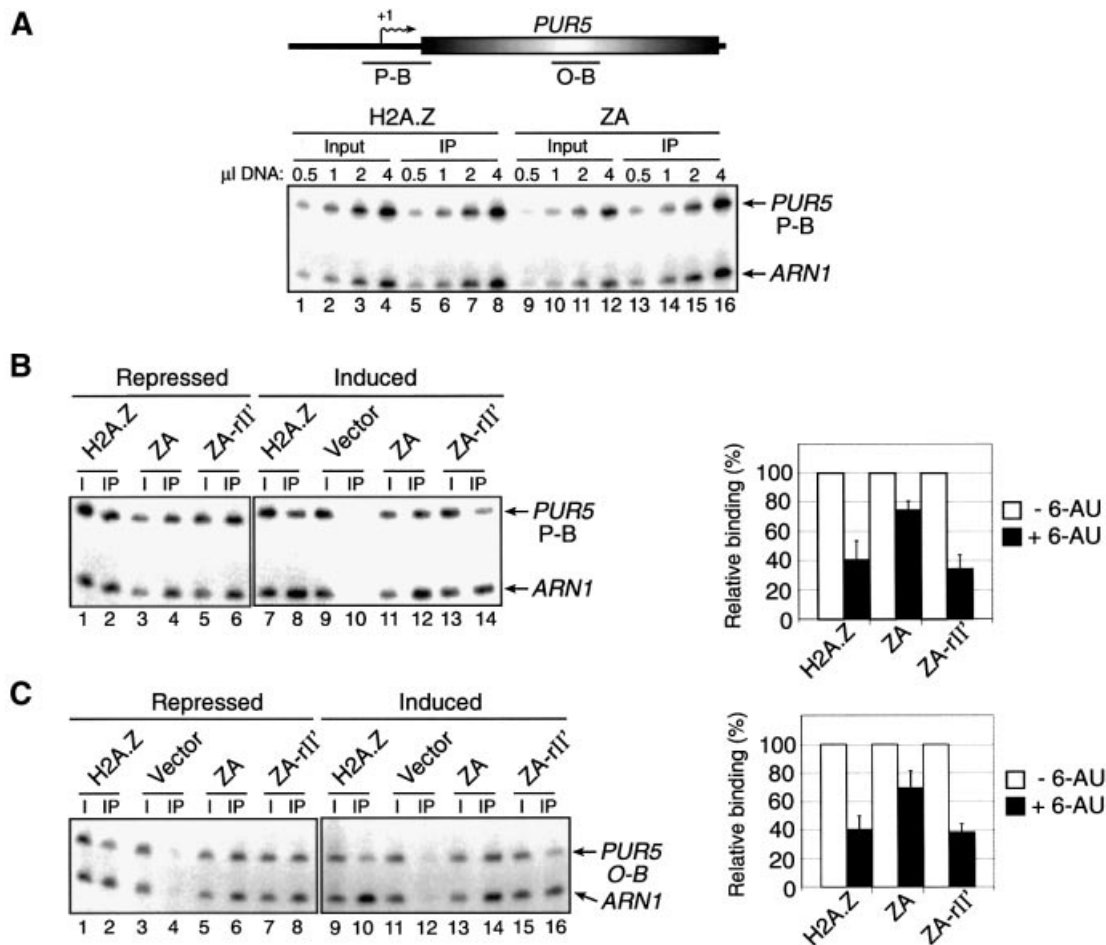


Fig. 4. Binding of H2A.Z derivatives at the *PUR5* gene. ChIP experiments were performed using anti-HA antibodies to monitor the binding of H2A.Z, ZA and ZA-rII' to *PUR5* under repressed (–6-AU) or induced (+6-AU) conditions. All PCRs contain *ARN1* primers used here as an internal control to normalize signals for each lane. (A) Top: representation of the *PUR5* locus. Transcription start site (arrows with +1), open reading frame (open shaded rectangle) and regions amplified by PCR (black bars) are represented. Bottom: PCR titration of input (lanes 1–4 and 9–12) and immunoprecipitated material (lanes 5–8 and 13–16) for H2A.Z (lanes 1–8) and ZA (lanes 9–16) at the *PUR5* promoter. (B) ChIP analysis of the binding of H2A.Z derivatives at the *PUR5* promoter. Binding of the derivatives under repressed (–6-AU, lanes 1–6) and induced (+6-AU, lanes 7–14) conditions (left panel). The right part of the figure depicts a quantification of that experiment. Binding of each H2A.Z derivative under repressed conditions is normalized to 100%. (C) Same experiment as in (B) with the exception that the *PUR5* region analyzed was in the open reading frame.

htz1Δ cells (compare lanes 6 and 7) as well as when ZA is expressed (lane 8). Expression of ZA-rII' and ZA-P201 is able to recover *PUR5* transcription levels fully (lanes 9 and 10). These results suggest that H2A.Z function is essential for *PUR5* induction in the absence of DNA replication.

We next attempted to test the effect of ZA on *PHO5* transcription in order to determine if the dominant negative effect of the latter molecule was manifested at all genes that we tested. *PHO5* expression is repressed in the presence of inorganic phosphate but is strongly induced when starved of the latter (Svaren and Hörz, 1997). Thus, Figure 3D shows primer extension analyses of the *PHO5* locus from yeast cells expressing various H2A.Z derivatives under repressed and induced conditions. As previously reported (Santisteban *et al.*, 2000), *PHO5* induction is not significantly affected in *htz1Δ* cells when compared with WT cells or cells expressing ZA-rII' (compare lanes 2, 4 and 8). Moreover, expression of ZA also had little or no effect on *PHO5* induction (lane 6). In addition, the results demonstrate that expression of ZA-rII'

does not derepress *PHO5* transcription (compare lanes 1, 3 and 7). Similar results were obtained with the *PHO8* gene (data not shown). These results first suggest that fusion of an activating region to ZA does not generally derepress gene transcription (see also Figure 3B for *PUR5*) and, secondly, that the dominant negative effect of ZA is somewhat gene specific.

H2A.Z derivatives are able to bind the *PUR5* gene

Previous reports have shown that H2A.Z binding is gradually lost upon gene induction (Santisteban *et al.*, 2000; Adam *et al.*, 2001) implying that the variant histone is remodelled prior to mRNA synthesis. In order to ensure that our ZA, ZA-rII' and ZA-P201 fusion proteins can associate with chromatin, we tested their ability to bind the *PUR5* gene using chromatin immunoprecipitation (ChIP) experiments, followed by quantitative PCR analysis. For our ChIP assays, we made use of the HA tag at the N-terminal end of the H2A.Z derivatives for specific immunoprecipitation. As an internal control for our ChIP experiments, we made use of the *ARN1* gene as we found

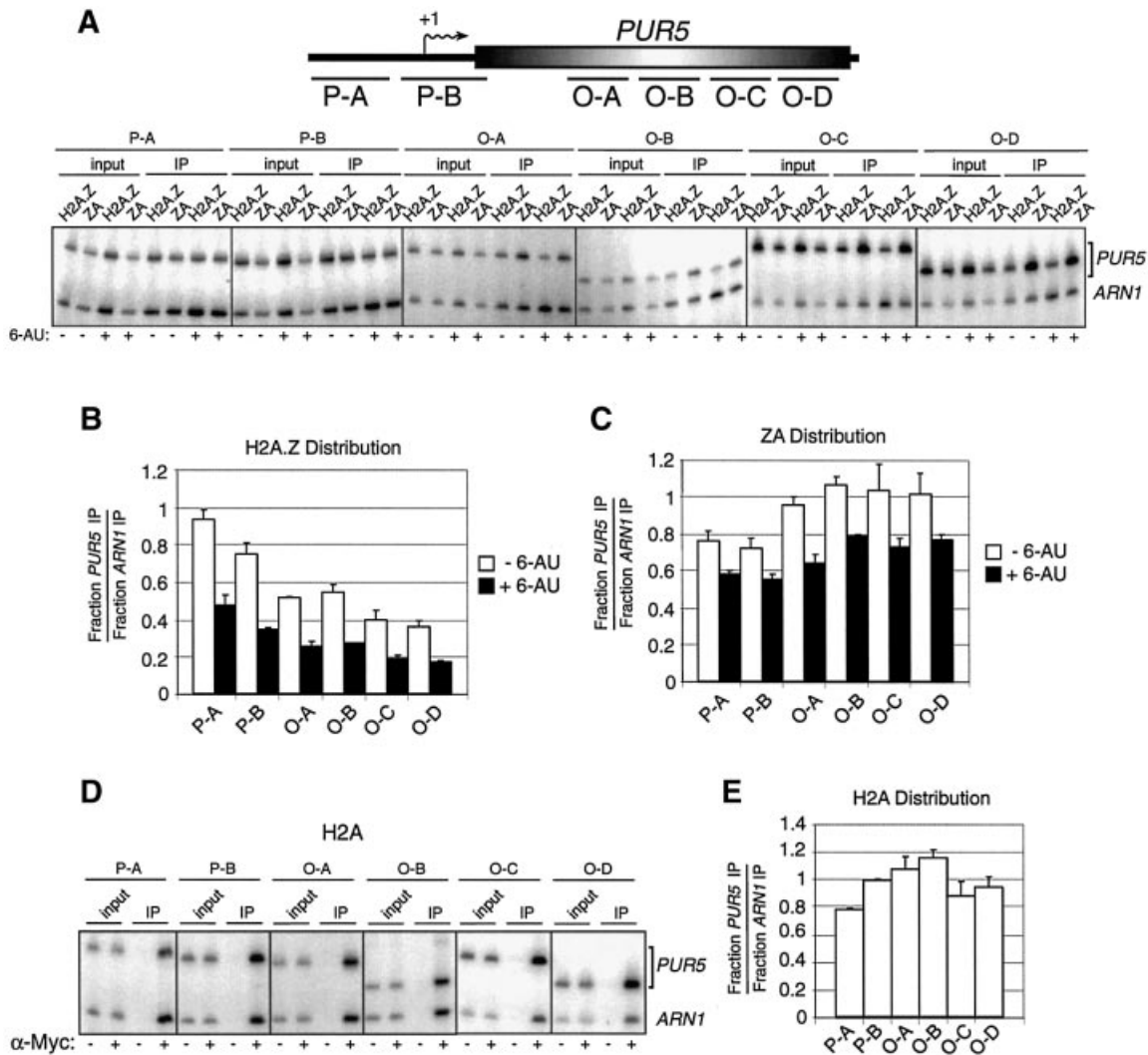


Fig. 5. Localization of H2A.Z, ZA and H2A at the *PUR5* locus. (A) Top: representation of the *PUR5* locus. Regions amplified by PCR in the promoter region (P-A and P-B) or in the open reading frame (O-A to O-D) are represented. Bottom: ChIP analysis of the binding of H2A.Z and ZA at the *PUR5* locus. All PCRs contain *ARN1* primers used here as an internal control to normalize signals for each lane. (B and C) Distribution of H2A.Z and ZA at the *PUR5* locus. Quantification of (B) H2A.Z or (C) ZA under repressed (–6-AU, open bar) and induced (+6-AU, black bar) conditions. (D) ChIP analysis of the binding of Myc-H2A at the *PUR5* locus. Cells were grown under repressed condition and chromatin was immunoprecipitated with (+) or without (–) an anti-Myc antibody. (E) Distribution of H2A over the *PUR5* locus.

that its expression was not affected by H2A.Z nor induced in our yeast culture conditions. The *ARN1* region analyzed by PCR lies within the promoter region and is constant throughout our experiments. The lower part of Figure 4A shows a PCR titration of input and immunoprecipitated material at the *PUR5* promoter. The results show that both H2A.Z and ZA can efficiently bind the *PUR5* promoter region, and also that our PCR amplifications are within linear range. Such titrations have been carried out for all the ChIP experiments presented in this paper (data not shown). Figure 4B (left panel) shows PCR analyses from a ChIP experiment designed to assess the presence of H2A.Z, ZA and ZA-rII' over a *PUR5* promoter region under repressed and induced conditions. In this experiment, *ARN1* serves as an internal control to normalize signals for each lane. The right panel of Figure 4B shows a graphic representation of the binding efficiency at a region of the *PUR5* promoter of the H2A.Z derivatives. The

results indicate that all three derivatives can efficiently bind that promoter region under repressed conditions and, upon induction, H2A.Z and ZA-rII' binding is significantly reduced compared with ZA binding. Figure 4C shows a similar experiment but with PCR analyses carried out in the coding region of *PUR5*. We have also been able to locate ZA-P201 at the *PUR5* locus as well as all the H2A.Z derivatives at the *GALI* locus (data not shown). Taken together, these results show that all our H2A.Z derivatives are able to bind the *PUR5* gene and are thus likely to incorporate into nucleosomes.

The localization patterns of H2A.Z and ZA are different at the *PUR5* gene

Santisteban *et al.* (2000) have previously demonstrated that H2A.Z is preferentially associated with intergenic regions as compared with open reading frames at the *PHO5* and *GALI* loci. To test if the H2A.Z C-terminal

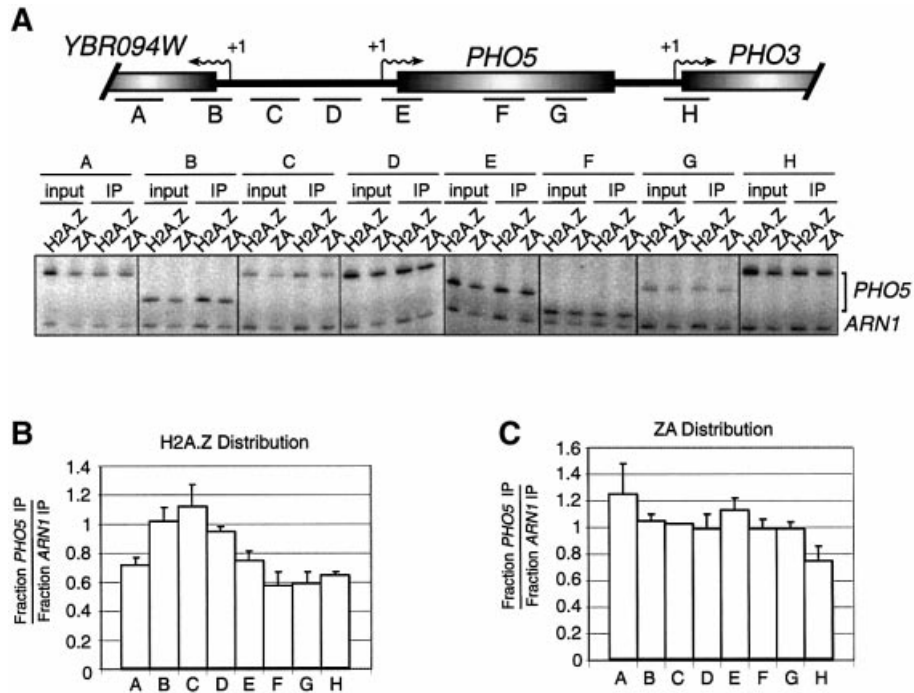


Fig. 6. Distribution of H2A.Z and ZA at the *PHO5* genomic region. (A) Top: representation of a 4.3 kb genomic region spanning the *PHO5* gene. The transcription start sites (arrows with +1), open reading frames (open shaded rectangle) and regions amplified by PCR (black bars) are represented. Bottom: ChIP analysis of H2A.Z and ZA binding over the *PHO5* genomic region. Cells were grown under *PHO5* repression conditions. All PCRs contain *ARN1* primers used here as an internal control to normalize signals for each lane. (B and C) Distribution of (B) H2A.Z and (C) ZA over the *PHO5* genomic region.

region is important for proper localization of the variant histone on DNA, we used ChIP experiments to compare the binding of H2A.Z and ZA at the *PUR5* gene under repressed and induced conditions. The upper part of Figure 5A depicts the regions of *PUR5* that were analyzed by PCR. Figure 5A shows the PCR analyses of the different *PUR5* regions. Figure 5B shows a graphic representation of the WT H2A.Z distribution over the *PUR5* gene under either repressed or induced conditions. The results suggest that H2A.Z is located more abundantly in the promoter region and significantly less in the coding region. However, we observe a totally different distribution pattern with ZA (Figure 5C) in that it is found to be less abundant in the promoter region than in the coding region. In addition, it appears that H2A.Z binding throughout the *PUR5* locus is more strongly diminished upon induction of *PUR5* as compared with ZA.

We next wanted to compare the distribution of H2A over the *PUR5* gene and assess whether it would be similar to that of H2A.Z or ZA. For this purpose, we made use of a yeast strain in which one H2A-encoding gene has been Myc tagged (Adam *et al.*, 2001). Thus, Figure 5D shows PCR analyses of a ChIP experiment using anti-Myc antibodies to assess the distribution of H2A over the *PUR5* locus under repressed conditions. Figure 5E shows a quantification of the H2A distribution over the *PUR5* locus. The results clearly indicate that the latter's distribution closely resembles that of ZA. Taken together, these results suggest that H2A.Z is more abundant in the *PUR5* promoter region than in the coding region, whereas the distribution of ZA and H2A is spread out more uniformly

and is even slightly enriched in the coding region compared with the promoter region.

The localization patterns of H2A.Z and ZA are different at the *PHO5* gene

Since it has been shown (Santisteban *et al.*, 2000) that H2A.Z preferentially localizes to the *PHO5* intergenic region and that the distribution of H2A is rather uniform, we decided to investigate the distribution of our ZA fusion at that genomic region. As can be seen in Figure 6B, the distribution of H2A.Z over that locus is very similar to the previous report (Santisteban *et al.*, 2000), i.e. H2A.Z is preferentially localized at the *PHO5* promoter region compared with the coding region. However, as shown in Figure 6C, the distribution of ZA is more uniform and certainly not preferentially located to the intergenic region, a result similar to the previously published distribution of H2A at that locus (Santisteban *et al.*, 2000). We have also obtained a similar distribution for H2A at *PHO5* (data not shown) to that reported previously (Santisteban *et al.*, 2000). These results further support our observations at the *PUR5* gene and suggest that the H2A.Z C-terminal region is also responsible for the preferential binding to the *PHO5* intergenic region.

The ZA-rII' fusion has a distribution similar to H2A.Z over the *PUR5* and *PHO5* genes

In order to test if the 'locator' function of the H2A.Z C-terminal region can be functionally separable from its function in transcription, we first carried out a ChIP experiment to determine the distribution of ZA-rII' over the *PUR5* gene under repressed conditions. We reasoned

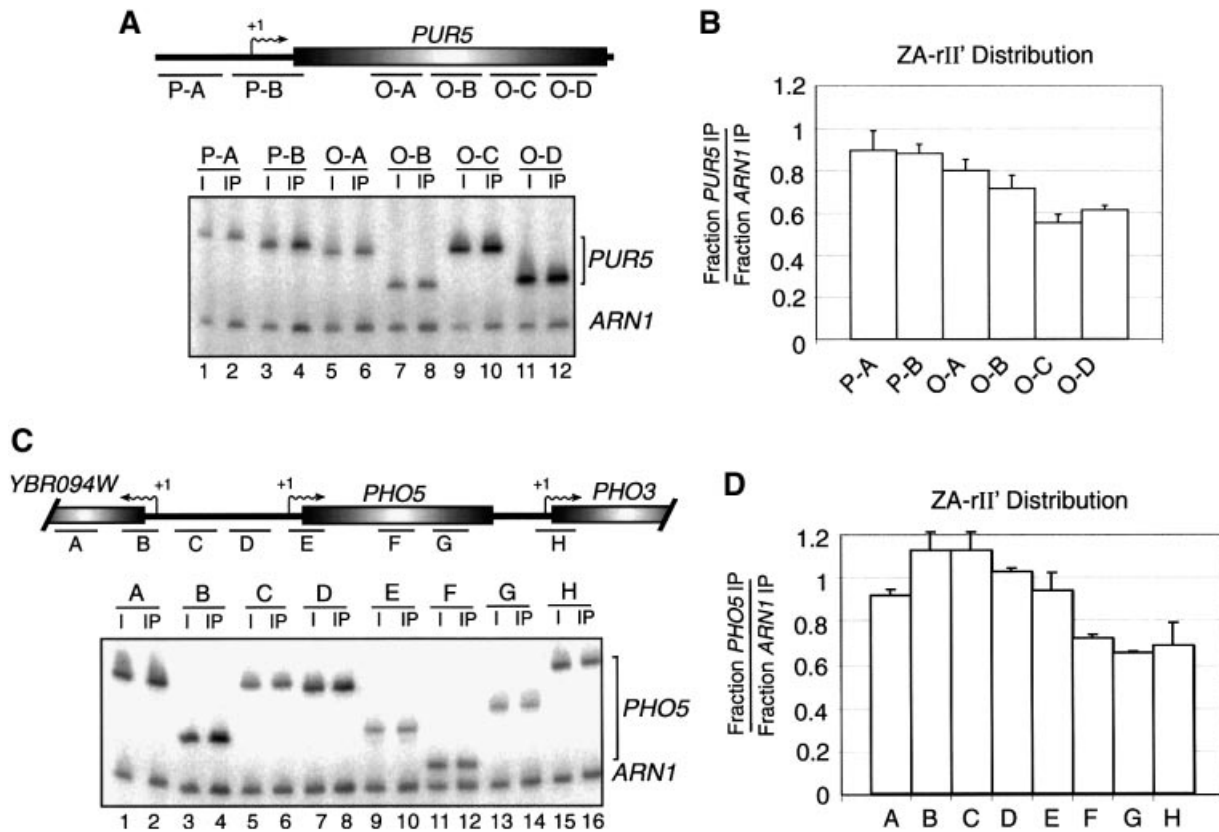


Fig. 7. Distribution of the ZA-rII' fusion over the *PUR5* and *PHO5* genomic regions. (A) Binding of ZA-rII' at *PUR5* gene under repressed conditions. (B) Distribution of ZA-rII' at the *PUR5* gene. (C) Same experiment as in (A) but the *PHO5* genomic region was analyzed. (D) Distribution of ZA-rII' at the *PHO5* genomic region. All PCRs contain *ARN1* primers used as an internal control to normalize signals for each lane.

that if the locator function of the H2A.Z C-terminal region can be dissociated from its function in transcription, then the ZA-rII' distribution should be similar to that of ZA and H2A. Figure 7A depicts PCR analyses of the ZA-rII' ChIP over the *PUR5* locus. Figure 7B shows a graphical representation of the ZA-rII' distribution over that locus. Figure 7C and D shows a similar experiment, but at the *PHO5* genomic region. At both genomic regions investigated, the results show that the distribution of the ZA-rII' chimera is similar to that of WT H2A.Z since it is more abundant over the promoter regions than the coding regions of both *PUR5* and *PHO5*.

Discussion

In this work, we have shown that addition of two different transcriptional activating regions to ZA, one taken from a natural activator (rII') and one synthetic (P201), can allow the latter to complement efficiently most transcription and growth deficiencies of *htz1Δ* cells that we have tested so far. In addition, at least in the case of ZA-rII', the activating region alone specifically confers to the C-terminal region of H2A the missing function found in H2A.Z since this activity can be specifically inhibited by Gal80. Thus we suggest that the H2A.Z C-terminal region is different from the reciprocal H2A region in that it harbours an 'activating region-like surface', but only when it is incorporated into a nucleosome particle. In fact, the crystal structure of a H2A.Z-containing nucleosome

reveals an uninterrupted acidic patch at the surface of the nucleosome that starts at the H2A.Z C-terminus and extends to H2B, thus creating a potential surface that could mediate novel protein-protein interactions (Suto *et al.*, 2000). In contrast, an H2A-containing nucleosome does not bear such a surface. Thus it is likely that this acidic patch might serve as a specialized activating region whose integrity would be dependent on the formation of a nucleosome. The primary mechanism by which transcriptional activating regions are believed to stimulate transcription, at least in yeast, is by recruitment of the transcriptional machinery to a target promoter (e.g. Ptashne and Gann, 1997; Barberis and Gaudreau, 1998; Keaveney and Struhl, 1998). The Gal4 activating region (rII') has been shown to interact with numerous transcription factors including TBP and TFIIB (Wu *et al.*, 1996), Srb4 (Koh *et al.*, 1998), the SAGA histone acetyltransferase complex (Melcher and Johnston, 1995; Bhaumik and Green, 2001; Brown *et al.*, 2001; Larschan and Winston, 2001) and the NuA4 acetyltransferase complex (Brown *et al.*, 2001). The P201 synthetic activating peptide has been shown to interact with the Gal11 mediator component (Lu *et al.*, 2002). It thus seems likely that activating regions can interact with a plethora of different factors involved in the process of gene transcription. The fact that both activating regions fused to ZA can complement *htz1Δ* defects to a different extent may reflect their intrinsic ability to recruit appropriate factors for a given gene. Thus we imagine that H2A.Z could recruit certain

transcription factors that would, for example, help poise promoter chromatin for gene activation once an appropriate induction signal is triggered. Importantly, in the absence of such a gene induction signal, H2A.Z would not be expected to derepress genes since expression of ZA-rII' does not derepress the *PUR5*, *PHO5* or *PHO8* genes prior to their activation.

We have also shown that the function of the H2A.Z C-terminal region may act in concert with other nuclear factors for *PUR5* gene induction. Support for this claim is based on the fact that the ZA fusion strongly prevents *PUR5* induction while the absence of any H2A.Z derivative does not significantly affect *PUR5* induction. However, preventing DNA replication, a condition that makes transcription-dependent chromatin remodelling necessary, renders *htz1Δ* cells incapable of efficiently inducing *PUR5* transcription. Similarly, *PHO5* gene induction has been shown to be independent of H2A.Z as well as the Swi/Snf chromatin remodelling complex, but mutation of a Swi/Snf subunit renders H2A.Z essential for *PHO5* induction (Santisteban *et al.*, 2000). Thus it seems likely that the function of H2A.Z in transcription will become essential under a variety of conditions that may either compact chromatin, such as in mitosis, or even prevent DNA replication. Intriguingly, *GAL1* gene induction, which is normally independent of Swi/Snf function (Gaudreau *et al.*, 1997) becomes highly dependent on the latter complex when cells are arrested in mitosis (Krebs *et al.*, 2000). Also, the function of H2A.Z could be essential where a number of transcription factors, including chromatin remodelling complexes, are crippled.

Our results also suggest that once H2A.Z performs its positive role in gene activation, it must then be remodelled for efficient gene induction to occur. In addition, our results suggest that, at least for the *PUR5* gene, H2A.Z-bearing nucleosomes are more stable than H2A-bearing nucleosomes. This possibility is highlighted by the fact that ZA exerts a strong dominant negative effect at *PUR5*. In addition, a recent report has shown that H2A.Z-containing chromatin is more stably folded than H2A-containing chromatin (Fan *et al.*, 2002), a result that may explain the requirement for more localized alterations caused by chromatin remodelling complexes (Santisteban *et al.*, 2000). It is conceivable that H2A.Z-poised chromatin, by virtue of its C-terminal region, would be easier to remodel than ZA-containing chromatin given possible interactions with chromatin remodelling components. We propose that without its 'activating region' function, H2A.Z could become particularly repressive at certain genes. However, this dominant negative effect of ZA is not general since there is no significant effect at the *PHO5* and *PHO8* genes, and ZA has a more modest effect at *GAL* genes. We have also shown that H2A.Z binding is strongly reduced upon gene induction as compared with the ZA fusion, a result again suggesting that H2A.Z is more readily remodelled than ZA at *PUR5*. These results also correlate with the transcriptional activity of yeast cells expressing these fusion proteins. At this point, we do not know if the role of H2A.Z at *PUR5* is exerted at the level of transcription initiation or elongation, or at both.

In *Tetrahymena*, H2A.Z has been shown to be associated with active chromatin, a result that further implies the

importance of the variant histone in gene transcription, but also the fact that it has a distinct distribution pattern (Stargell *et al.*, 1993). Experiments in *Drosophila* (Leach *et al.*, 2000) and yeast (Santisteban *et al.*, 2000; Meneghini *et al.*, 2003) have shown that H2A.Z has a non-uniform binding pattern. In addition to its important role in gene induction, we have shown that the H2A.Z C-terminal region was also necessary to preferentially locate this variant histone to the *PUR5* and *PHO5* promoter regions as the distribution of the ZA fusion more closely resembled that of H2A. Importantly, the distribution of the ZA-rII' chimera had more similarity to that of H2A.Z than ZA, suggesting that this 'locator' function of the C-terminus is linked to its role in gene induction. Thus we propose a model in which H2A.Z would first poise chromatin at an inactive gene by interaction with a transcription-related factor, or chromatin remodelling component, that might have certain gene-specific determinants. The fact that H2A.Z interacts with such a factor(s) would also allow it to preferentially localize to promoter regions, and upon an appropriate gene induction signal H2A.Z would become remodelled by virtue of the same interaction(s). The H2A.Z-poised chromatin would allow a gene to be remodelled and activated quickly. It is also conceivable that the so-called transcription-related factors, or chromatin remodelling factors, might be recruited to promoter regions because local chromatin architecture might readily accommodate these factors at promoter regions, but not in other regions of the genome, thus providing the required specificity function. This last suggestion may be more plausible since H2A.Z does not preferentially localize to the promoter regions of all genes (Meneghini *et al.*, 2003). Consistent with our model is the fact that acetylation levels of H3 (lysine 9) and H4 (lysine 12) are predominant over the *PHO5* promoter region, as compared with its coding region, and surprisingly under gene repression conditions (Vogelauer *et al.*, 2000). This acetylation pattern remarkably resembles the distribution of H2A.Z over the same locus. In addition, the H3–H4 acetylation state at the *PHO5* promoter region does not increase upon gene activation (Vogelauer *et al.*, 2000), and these authors suggest that this preacetylated state of chromatin at *PHO5* might also poise the promoter for gene activation. It is tempting to speculate that different chromatin-modifying enzymes could also share a similar distribution over promoter regions of inactive genes. Of course, our model does not exclude the possibility that H2A.Z-containing chromatin itself would be more permissive to transcription, for example by mimicking an acetylated state of chromatin as suggested by the *in vitro* studies of Fan *et al.* (2002).

Materials and methods

Strains and plasmids

The *htz1Δ* and Myc-H2A strains used in this study are as described (Adam *et al.*, 2001). All H2A.Z derivatives bear an HA tag inserted at the *Bgl*III site of the *HTZ1* gene and were expressed from the *ACT1* promoter. The ZA fusion was made by fusing amino acids 91–132 of H2A to the C-terminal part of amino acids 1–97 of H2A.Z (Adam *et al.*, 2001). ZA-rII' was generated by adding amino acids 840–881 of Gal4 to the C-terminal end of ZA. ZA-P201 was constructed by adding the amino acid sequence LfVQDYLLPTCIP (Lu *et al.*, 2000) to the C-terminus of ZA. The Gal80 expression plasmid was constructed by cloning its open

reading frame in a high copy number vector (YEplac181, *LEU2*) under the control of the *ACT1* promoter.

Phenotypic assays

For the HU-sensitivity growth assays, yeast cells were spotted on rich media (YPDA) with or without 100 or 200 mM HU, except for the Gal80 experiment where cells were spotted onto plates containing SC media lacking uracil and leucine. For the 6-AU-sensitivity growth assays, yeast cells were grown overnight to logarithmic phase in SC media lacking uracil and containing 2% glucose. Cells were washed, serially diluted and spotted on plates containing SC media lacking uracil but containing 2% glucose or 2% glucose plus 10 or 25 µg/ml 6-AU. Plates were incubated for 3–7 days at 30°C or as indicated.

Gene induction conditions and primer extension assays

PUR5 expression was induced essentially as described (Lee *et al.*, 2002). Cells were grown to an OD₆₀₀ of 0.6 and 6-AU was added to a final concentration of 75 µg/ml for 60 min. For *GAL1–7* transcription, cells were grown to an OD₆₀₀ of 0.6 in SC media containing 2% raffinose and galactose was then added to a final concentration of 5% for 60 min. In order to monitor *PUR5* expression upon HU treatment, yeast cells were grown as above and subsequently washed with water. Cells were then blocked in S phase by incubating cultures in SC media lacking uracil with the indicated concentration of HU for 3.5 h at 30°C. After incubation, cultures were washed again in water and resuspended in SC media lacking uracil with 2% glucose containing HU and 75 µg/ml 6-AU for *PUR5* induction. All inductions were carried out for 60 min. For *PHO5* transcription, cells were grown to an OD₆₀₀ of 0.6–1.0, washed with sterile water and resuspended in SC media lacking phosphate. Cells were then further incubated for 6 h. For primer extensions, 25 µg of RNA was used and the assays were carried out as previously described (Ma and Ptashne, 1987b). For RNA loading controls, 15 µg of total RNA was loaded onto agarose–formaldehyde gels.

ChIP experiments

ChIP experiments were performed essentially as described previously (Adam *et al.*, 2001) with some modifications. Briefly, cells were grown to an OD₆₀₀ of 0.6 in SC medium lacking uracil and containing 2% glucose. Then, 6-AU was added or not to a final concentration of 75 µg/ml for 60 min to induce *PUR5*. For the *PHO5* ChIP, cells were grown in SC medium lacking uracil and containing 2% glucose and 500 µl of whole-cell extract was incubated with anti-HA antibodies (12CA5; Roche) coupled to magnetic beads (Dynal). Two microliters of DNA were used for the PCRs. ChIP experiments performed with anti-Myc antibodies were as previously described (Adam *et al.*, 2001). All the ChIP data were quantified by phosphorImaging (PhosphorImager, Molecular Dynamics). Reactions with antibodies in untagged strains were also carried for all experiments but are not shown here since they were negative. The sequence of the oligonucleotides used for all of the experiments is available upon request. All experiments were carried out at least twice and gave similar results.

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