Exposing the core promoter is sufficient to activate transcription and alter coactivator requirement at *RNR3*

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Chromatin is a formidable barrier to transcription. Nucleosome density is lowest over the regulatory regions of active genes, and many repressed genes have a tightly positioned nucleosome over their core promoter. However, it has not been shown that nucleosome positioning is sufficient for repression or whether disrupting a core promoter nucleosome specifically can activate gene expression in the absence of activating signals. Here we show that disrupting the nucleosome over the core promoter of *RNR3* is sufficient to drive preinitiation complex assembly and activate transcription in the absence of activating signals. Remodeling of chromatin over the *RNR3* promoter requires the recruitment of the SWI/SNF complex by the general transcription factor TFIID. We found that disrupting the nucleosome over the *RNR3* core promoter relieves its dependence on TFIID and SWI/SNF, indicating a functional link between these two complexes. These results suggest that the specific function of TAF_{II}s is to direct the chromatin remodeling step through SWI/SNF recruitment, and not core promoter selectivity. Our results indicate that nucleosome placement plays a dominant role in repression and that the ability of the core promoter to position a nucleosome is a major determinant in TAF_{II} dependency of genes *in vivo*.

chromatin | TFIID | repression | TAF_{II}s | SWI/SNF

The incorporation of DNA into chromatin has a profound effect on the binding of various transcription factors to their sites *in vitro*, and it is a widely held belief that nucleosome positioning plays an essential role in transcriptional repression *in vivo*. Evidence linking nucleosomes to repression includes the observation that depleting core histones from the cell causes the derepression of many yeast genes (1, 2), nucleosomes are removed from activated promoters (3, 4), and promoters of active genes are depleted of nucleosomes (5–7). Furthermore, artificially disrupting chromatin reassembly by using FACT complex mutants causes the expression of repressed genes, production of cryptic transcripts, and increases in recombination *in vivo* (8–11). However, depleting core histones or disabling chromatin assembly factors can cause widespread disruptions in chromatin structure and affect promoter and coding regions. It was recently shown that disrupting chromatin reassembly can bypass the need for an activator to maintain ongoing transcription, but the method used did not target the core promoter directly and specifically addressed the need to reestablish repression of an activated promoter (10). Thus, it remains to be seen whether depleting the core promoter nucleosome specifically can cause preinitiation complex (PIC) formation in the absence of activating signals and whether it can overcome ongoing attempts by the repression machinery to silence gene expression.

Here we used the *RNR3* gene from *Saccharomyces cerevisiae* to address whether disrupting the core promoter nucleosome is sufficient to drive PIC formation and increase gene expression in the absence of activating signals. Nucleosomes are strongly positioned over the gene by the Ssn6–Tup1 corepressor complex, including one over the core promoter (12–14). Activation of this gene requires the release of the repressor complex and remodeling by the SWI/SNF complex (14, 15). *RNR3* employs a novel mechanism for chromatin remodeling and activation. Recruitment of the SWI/SNF complex and subsequent remodeling require the TAF_{II} subunits of TFIID (15). It is also possible that TFIID subunits may play a role in accessing the TATA box incorporated into a nucleosome by making direct contact with core promoter sequences or the nucleosome through the bromodomain-containing factors Bdf1 and Bdf2 (16–18). Here we address the mechanism of how TFIID recognizes the core promoter of *RNR3* and the contributions of nucleosome positioning in the TAF_{II} dependence of this gene.

Results

Polynucleotide Tracts (PNTs) Activate Transcription. A nucleosome is positioned over the TATA box of *RNR3* (13). We used *RNR3* to test whether excluding the nucleosome from the core promoter could lead to the activation of transcription and PIC formation independent of activating signals, DNA damage. The strategy used was to insert PNTs and a random sequence of equal length 15 bp upstream and 15 bp downstream of the TATA box (Fig. 1*A*). The altered promoter constructs were reintroduced into the natural chromosomal locus, and their expression was monitored under repressed and derepressed conditions [without or with 0.03% methyl methanethiosulfonate (MMS)]. We examined the expression of *HUG1*, a gene that responds similarly to *RNR3* (14, 19), as an internal control for the integrity of the DNA damage response. First we inserted 19- and 34-bp dA:dT tracts, and equally sized random sequence, upstream of the TATA box at −90. Insertion of the 19R insert had little effect on the level of transcription, but insertion of a 19A PNT caused a small, but significant, level of derepression (Fig. 1*B*). Next we examined whether increasing the length of the PNTs could cause further derepression by inserting one and two copies of a 34-bp PNT (34A) upstream of the TATA box. Increasing the length of PNTs significantly increases the level of derepression in these strains. The increased derepression is consistent with the observation that longer PNTs have a greater disruptive effect on nucleosome incorporation *in vitro* (20, 21). In addition, insertion of PNTs tracts caused enhanced activation of the promoters in MMS-treated cells, ≈1.5- to 2-fold. The enhanced activation suggests that PNTs antagonize ongoing attempts by the repression ma-

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Abbreviations: PNT, polynucleotide tract; MMS, methyl methanethiosulfonate; PIC, preinitiation complex; Pol II, RNA polymerase II; TBP, TATA box-binding protein.

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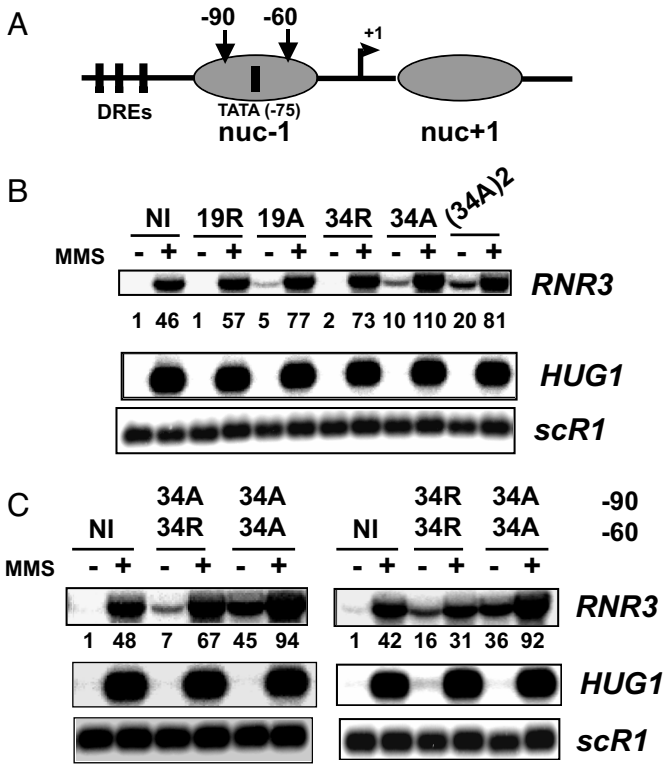

Fig. 1. Insertion of PNTs within *RNR3*. (*A*) A schematic of *RNR3* and its chromatin structure based on Li and Reese (13). (*B*) Random DNA sequence (R) or poly dA:dT (A) of differing lengths were inserted upstream (−90) of the TATA box, which is located at −75 relative to the start site of transcription (black box). Shown are Northern blots of *RNR3* and *HUG1* in strains containing the insertion indicated above the panel. Cells were treated with 0.03% MMS for 2.5 h (+) or were not treated (−). The signal in untreated wild-type cells containing the unmodified promoter was arbitrarily set to 1.0, normalized to *scR1*. (*C*) As in *B* except that insertions were made at −90 and −60.

chinery to reestablish nucleosome positioning under induced conditions.

Insertion of a PNT upstream of the TATA box caused relatively mild derepression compared with the level obtained by MMS treatment, likely because a single insertion upstream of the TATA box would provide only partial increased accessibility of the TATA box. Next, PNTs and random sequences were inserted upstream and downstream of the promoter at −90 and −60, respectively. Flanking the TATA box with 34-bp PNTs (34A/34A) greatly increased the level of derepression (Fig. 1*C*). In fact, the level of transcription from this construct equaled that of an unaltered *RNR3* promoter in MMS-treated cells [Fig. 1*C* and supporting information (SI) Fig. 6]. Inserting the random sequence at both positions also caused some depression because the random sequence is somewhat refractory to nucleosome deposition, which will be supported by data presented below. Given that our goal is to disrupt the nucleosome, and not to prove the efficacy of PNTs *per se*, this does not impact our conclusions. The effects of PNT insertions on transcription are highly reproducible (SI Fig. 6).

A possible explanation for the enhanced transcription from the promoter derivatives in the untreated cells is that PNTs enhance core promoter activity independent of changes in chromatin structure. To rule this out, we compared the level of transcripts produced from promoter derivatives in cells deleted of *CRT1*. Deletion of *CRT1* disrupts nucleosome positioning (13, 22) and thus would nullify the effects of nucleosome repression. We found that the level of transcript produced from the promoter containing PNTs upstream and downstream of the TATA box was very similar to that of the unmodified promoter in the Δ*crt1* background (SI Fig. 7). Thus, our data suggest that PNTs enhance transcription by disrupting chromatin over the core promoter.

PNTs Disrupt Nucleosome Incorporation over the Promoter. Next we analyzed nucleosome occupancy at the promoters by measuring the level of histone H3 cross-linking with the ChIP assay. Chromatin was extensively sheared down to an average size of 100–200 bp, and short PCR probes were designed across the promoter region (Fig. 2*A*). PCR fragments A and C amplify regions that are predicted, based on our previous nucleosome mapping studies, to have a low probability of containing a nucleosome because they span regions that are hypersensitive to nuclease digestion (13, 22). PCR fragments B and D amplify a fragment predicted to be within nuc − 1 and nuc + 1, respectively (Fig. 2*A*). As expected, H3 cross-linking was higher over regions amplified by PCR probes B and D in untreated cells. Significantly, H3 cross-linking over probes B and D was reduced ≈2-fold in MMS-treated cells, suggesting nucleosome eviction. Next we examined the nucleosome density over the promoters containing 34A and 34R insertions at −90 (Fig. 2*B*) and at −90 and −60 (Fig. 2*C*). Insertion of 34R upstream of the TATA box had no effect on the nucleosome density, and the levels of H3 cross-linking over all probes were similar to that of wild type. On the other hand, inserting 34A at this position caused a significant reduction in cross-linking over probe B, with no effect on cross-linking at other locations (Fig. 2*B*). Histone H3 cross-linking was more strongly affected when the promoters contained insertions at −90 and at −60. Furthermore, as observed with the single-insertion mutants, cross-linking was preferentially reduced over nuc − 1, the site of the insertion. Comparing the level of cross-linking between the different promoter constructs revealed that the 34A/34A construct was more disruptive to nucleosome formation than the 34R/34R, which correlates well with the level of transcription produced from these promoters. In addition, insertion of random sequence (34R/34R) upstream and downstream caused some derepression (Fig. 1), and the ChIP data indicate reduced nucleosome occupancy. The destabilization of a nucleosome by the insertion of random sequence is likely caused by the disruption of natural nucleosome positioning sequences over the promoter. Computational analysis indicates that the promoter of *RNR3* has a repeated AT/AA dinucleotide pair that is favorable for nucleosome positioning (23, 24). Thus, it is not surprising that inserting random sequences caused some disruption in nucleosome positioning. In addition, the reduction in H3 cross-linking caused by MMS treatment was significantly stronger at the 34A/34A construct over nuc − 1, suggesting that these sequences are antagonizing ongoing attempts to reestablish repression.

It is possible that the inflexibility of the homopolymeric tracts disrupts the contact of DNA with histones, reducing cross-linking specifically, giving the impression that the nucleosome is excluded over the core promoter. To rule this out we used another method that does not rely on cross-linking agents, probing micrococcal nuclease-digested chromatin with a nucleosome-specific probe (Fig. 2*D*). The nuclei from MMS-treated and untreated cells were digested down to predominantly mononucleosome-sized DNA and analyzed by Southern blotting using a probe hybridizing across nuc − 1 (B in Fig. 2*A*). The *RNR3* signal was normalized to that obtained by using a probe that spans the tightly positioned nucleosome (nuc − 2) of the *PHO5* gene (25). The results shown in Fig. 2*E* indicate that inserting 34A at −90 had a weak effect on nucleosome placement over the promoter, whereas inserting it −90 and −60 had a very strong effect on nucleosome placement over this region. Thus, the reduced H3 cross-linking we observe is most likely due to

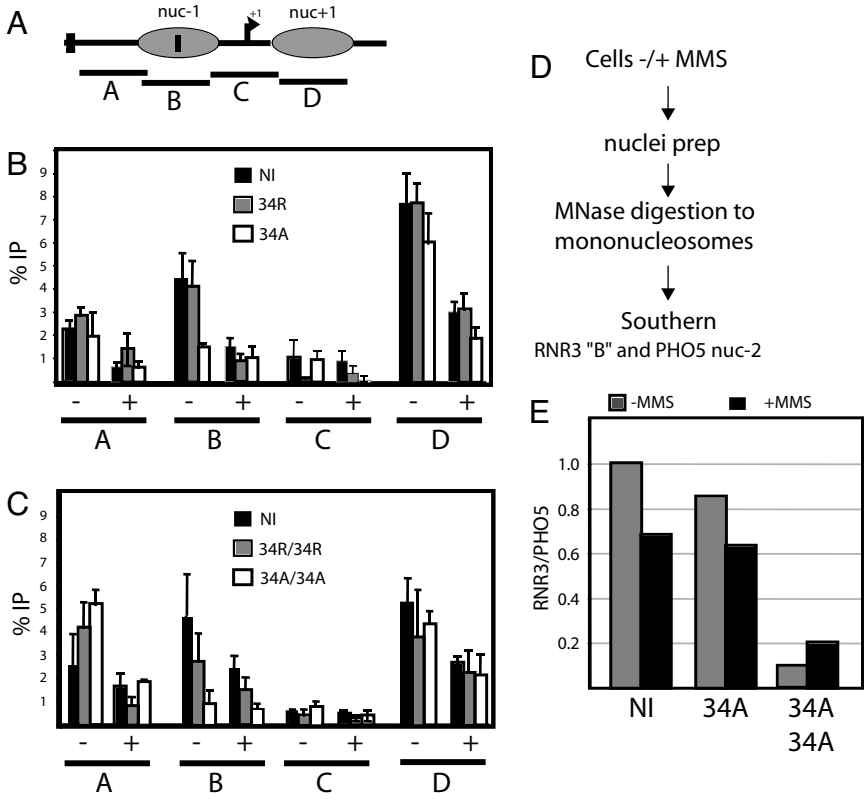

Fig. 2. PNTs disrupt nucleosome incorporation *in vivo*. (*A*) Schematic of the *RNR3* promoter with the location of primers used in the amplification of DNA. The black vertical line within nuc − 1 represents the TATA box, whereas the line upstream indicates the proximal DRE. (*B*) ChIP with antibodies to the core domain of H3. Cells were treated with 0.03% MMS for 2.5 h where indicated. Wild-type (NI) results are from an unaltered promoter. The data labeled 34A and 34R have inserts at −90. (*C*) As in *B* except strains containing 34A/34A and 34R/34R inserts at both −90 and −60. (*D*) Experimental design of the mononucleosome assay. (*E*) Quantification of the mononucleosome signals with a probe to *RNR3* (probe B in *A*) corrected for the signal of a nucleosome probe to the *PHO5* gene.

changes in nucleosome placement, not cross-linking. The reduction in the signal caused by treating cells with MMS was less than that observed in the H3 ChIP assay, ≈1.5-fold by using the mononucleosome assay versus 2- to 2.5-fold with ChIP. This difference may be caused by the sliding of nucleosomes from a downstream position to the promoter region during the nuclei preparation and digestion procedure in a fraction of the cells. In the case of the 34A/34A construct, the sequence is refractory to becoming incorporated into a nucleosome preventing a sliding of the nucleosome into this position. The disruption of nucleosome positioning in the mutants was also confirmed by partial micrococcal nuclease digestion and indirect end-labeling (SI Fig. 8). Collectively, the data indicate that insertion of PNTs disrupts nucleosome placement over the promoter, and the extent of disruption correlates with the levels of transcription observed.

Increased Accessibility Causes PIC Formation. Next we examined whether increased transcription correlates with increased PIC formation by measuring TATA box-binding protein (TBP) and RNA polymerase II (Pol II) recruitment. The results in Fig. 3*A* show that TBP and Pol II cross-linking increased at promoters containing 34A at −90, ≈2-fold for both proteins, but was less than the level observed in MMS-treated cells. The cross-linking of both TBP and Pol II was dramatically increased at promoters in untreated cells containing insertions at both −90 and −60. In fact, TBP cross-linking was equal in treated and untreated cells containing 34A at −90 and −60. On the other hand, the higher level of Pol II cross-linking can be further enhanced by MMS treatment. This suggests that Pol II requires a larger region of the promoter to be free of nucleosomes to bind, perhaps requiring the mobilization of nuc + 1. Insertion of PNTs had only a small effect on the incorporation of a nuc + 1 compared with nuc − 1 (Fig. 2*C*). Our Northern blotting experiments indicate that inserting PNTs enhanced the level of activated transcription under conditions of DNA damage (Fig. 1). Consistent with this, constructs that showed enhanced mRNA levels in MMS-treated cells also displayed higher levels of PIC formation, as judged by TBP and Pol II cross-linking. Thus, the level of transcription correlates very well with TBP and Pol II recruitment, suggesting that PNTs increase transcription by disrupting nucleosome placement and enhancing PIC formation.

Crt1 and Tup1 are recruited to the upstream repression sequences in the absence of DNA damage and repress transcription of *RNR3* (13, 14, 22). So we next examined the cross-linking of Crt1 and Tup1 to the upstream repression sequences of the *RNR3* promoter derivatives showing the highest level of derepression. Fig. 3*B* shows that inserting PNTs both upstream and downstream of the TATA box did not significantly affect Crt1 cross-linking in untreated cells. Furthermore, the normal derepression mechanism is intact at these promoters because Crt1 cross-linking was reduced by MMS treatment to a level equal to that observed at the unmodified promoter. The level of Tup1 cross-linking at the 34R/34R and 34A/34A constructs was reduced somewhat compared with wild-type promoter in the absence of DNA damage. However, this cannot fully explain the increased transcription and reduced nucleosome occupancy. The reduction in Tup1 cross-linking does not correlate well with the levels of derepression observed in each construct. For instance, Tup1 cross-linking to the 34R/34R-modified promoter is less than that of the 34A/34A promoter, yet transcription is

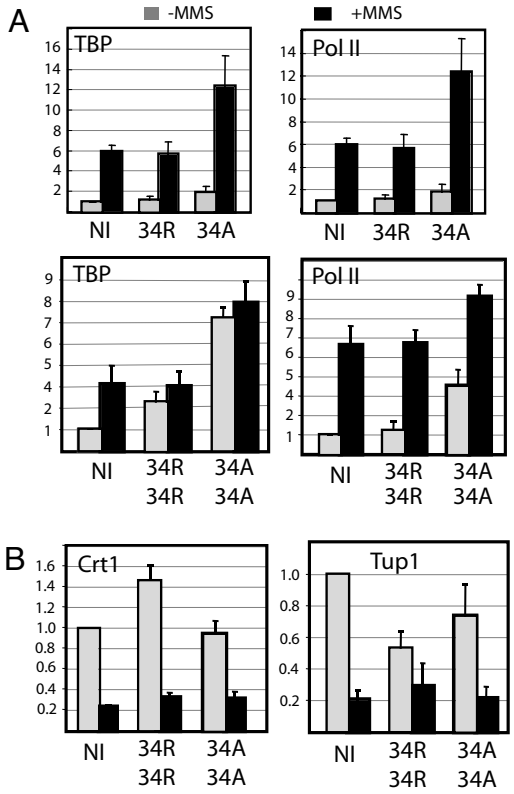

Fig. 3. Disruption of nuc − 1 causes PIC formation in the absence of activation signals. (*A*) ChIP assay monitoring TBP and Pol II recruitment over the *RNR3* promoter. Promoters containing inserts at −90 (34R and 34A) and at both −60 and −90 (34R/34R and 34A/34A) are shown. NI indicates data from cells without inserts in *RNR3*. Results from untreated cells (gray bars) and cells treated with 0.03% MMS (black bars) are displayed. Data are expressed as relative cross-linking compared with untreated cells containing no inserts, which was set to 1.0. (*B*) As in *A*, except that the cross-linking of Crt1 and Tup1 was examined over the DRE region of *RNR3*.

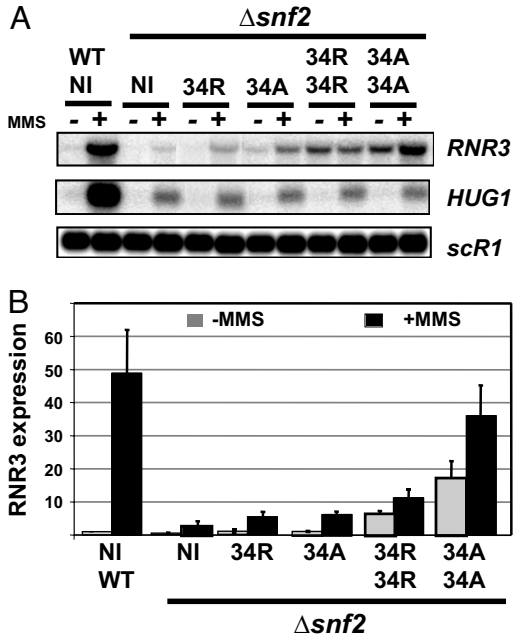

Fig. 4. Disruption of nuc − 1 suppresses the requirement for SWI/SNF. (*A*) Representative Northern blot of *RNR3* and *HUG1* expression levels. (*B*) Results from three experiments analyzing the expression of *RNR3* derivatives in the Δ*snf2* background. Northern blot signals were normalized to the *scR1* control. Expression in wild-type cells (*SNF2*) containing an unaltered promoter is shown on the far left (WT).

higher in the latter. Because Tup1 contacts the tails of histones (26), destabilizing the adjacent nucleosome may partially weaken cross-linking to *RNR3*, underestimating its association with the promoter.

Disrupting the Promoter Nucleosome Represses the SWI/SNF and TAF1 Requirement. Despite many years of research on the subject, the exact mechanism of how SWI/SNF affects chromatin structure *in vivo* is unclear. SWI/SNF remodels nucleosomes adjacent to the activator binding sites and promoter of genes; however, others hypothesize that it has additional functions such as in elongation and domain-wide remodeling (27–31). If the former is true, excluding the nucleosome over the core promoter of *RNR3* should suppress its requirement for SWI/SNF. We addressed this question by analyzing the expression of the *RNR3* promoter derivatives in a Δ*snf2* background. Consistent with the idea that the major function of SWI/SNF is to remodel the core promoter nucleosome, we found that the SWI/SNF dependence of the promoter derivatives decreased proportionally to the extent the insertions destabilized nuc − 1 over the TATA box (Fig. 4). In fact, the level of MMS-induced transcription of the promoter containing 34A upstream and downstream of the TATA box (34A/34A) was ≈80% of that observed from the unmodified *RNR3* promoter in wild-type cells. In contrast, induction of *HUG1* remained low in the same cells. PNTs cannot completely suppress the SWI/SNF requirement, because the 34A/34A construct showed an induction of ≈90-fold in wild-type cells, whereas the induction was ≈40-fold in the Δ*snf2* background (compare Fig. 1 with Fig. 4*B*). The failure to achieve 100% suppression is not surprising and can be explained by formation of a nucleosome over a fraction of the promoters in a population, or that remodeling of the downstream (nuc + 1) requires SWI/SNF. Thus, the predominant role of SWI/SNF at *RNR3* is to expose the core promoter, and it becomes dispensable when the core promoter is exposed.

In many cases the requirement for TAF_{II}s in transcription is dictated by the core promoter sequence (32, 33). One hypothesis is that the promoter recognition functions of TAF_{II}s help select the correct core promoter through specific DNA contacts and deliver TBP to the promoter. If TAF_{II}s are required for core promoter recognition *per se*, we expect that disrupting the nucleosome would not suppress the requirement of the TFIID-specific TAF1. We incorporated the promoter derivatives into a strain containing the temperature-sensitive *taf1-2* allele (34) and analyzed their expression after shifting the cells to the restrictive temperature. As reported previously (15), shifting the *taf1-2* cells to 37°C greatly reduced the expression of *RNR3* (Fig. 5*A*; NI, no insert). Disrupting nucleosome formation over the TATA box effectively suppressed the activation defect, and the strength of suppression by the different insertions correlated well with the effects of these inserts on the stability of nuc − 1 (Fig. 2 *B* and *C*). In fact, inserting PNTs upstream and downstream of the TATA box led to transcription levels equal to 80% of wild-type cells (Fig. 5*B*). Interestingly, PNTs were equally effective at suppressing the TAF1 requirement as they were at suppressing the SWI/SNF requirement. Thus, the data suggest that the TAF_{II} dependency is related to the chromatin structure at the promoter, and core promoter selectivity through specific TAF_{II}–DNA contacts does not account for the TAF1 dependency of this gene.

Discussion

It is believed that positioning a nucleosome over the core promoter of genes is a common repression mechanism; however,

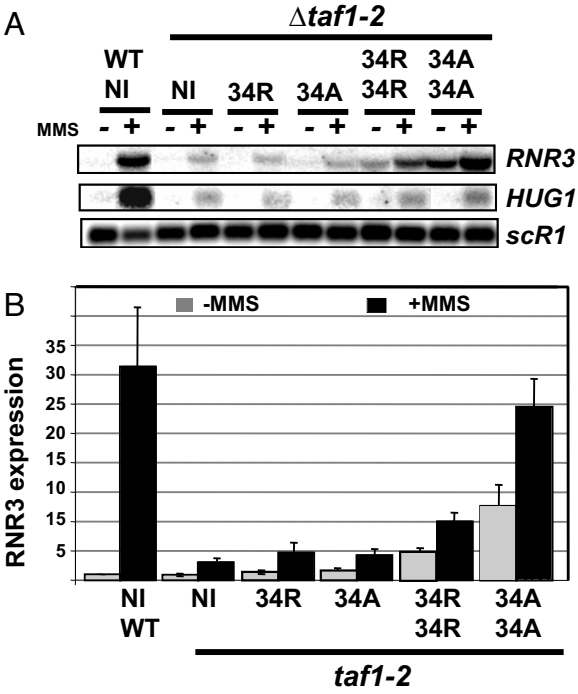

Fig. 5. Chromatin structure specifies the TAF1 dependence of *RNR3*. (*A*) A representative Northern blot of the expression of *RNR3* derivatives in a *taf1-2* temperature-sensitive mutant. Cells were shifted to 37°C for 15 min and then treated, or not, with 0.03% MMS for 2.5 h. (*B*) Quantification of three separate experiments. Northern blot signals were normalized to the *scR1* control. NI, a promoter with no insertions (control). Expression in wild-type cells (*TAF1*) containing an unaltered promoter is shown on the far left (WT).

the evidence so far has only correlated positioning and repression. An unresolved issue is whether exposing the core promoter is sufficient to activate transcription. PNTs are often found in nucleosome-free regions of yeast promoters (5, 6), and previous studies suggest that they can disrupt nucleosome positioning *in vivo* and enhance transcription by allowing activator binding (35–38). However, it has not been established that they exclude nucleosome placement (versus depositioning), and the enhanced transcription observed in some of these systems was activator-dependent. Finally, whether inserting PNTs can directly cause PIC formation was not addressed.

We provided three lines of evidence that inserting PNTs within the core promoter of *RNR3* excluded the incorporation of a nucleosome over this region. More importantly, we show that disruption of nuc − 1 results in PIC formation and activation of transcription in the absence of activating signals. Crt1 functions as a dual activator–repressor of *RNR3* and is required for TFIID and SWI/SNF recruitment (14). Crt1 requires DNA damage signals to convert it to an activator; thus, the disruption of the core promoter is not affecting Crt1 activity. An alternative interpretation of our results is that excluding nuc − 1 allows for the binding of an unidentified activator just upstream of the core promoter, and it directs the remodeling and recruitment events. We feel that this is unlikely because deleting sequences between the TATA box and the first Crt1–Tup1 binding site within the upstream repression sequences failed to disrupt the activation of *RNR3* (Y. Kim and J.C.R., unpublished observations). We cannot rule out that an activator is contacting the transcription machinery from a long distance; however, studies have shown that activators must be in close proximity to the core promoter to activate transcription (39). Moreover, because PNTs disrupt nuc − 1 and activate transcription independent of SWI/SNF, the likelihood that the mechanism involves the binding of an activator and subsequent recruitment of remodeling machinery is low.

Excluding the core promoter nucleosome activates transcription even in the presence of the repression machinery, in this case Crt1–Ssn6–Tup1. This reinforces the view that nucleosomes play a dominant role in Tup1 repression at *RNR3*. We previously reported that deleting *ISW2* disrupted nucleosome positioning without significant derepression of *RNR3* (19, 22); this finding could be seen as contradictory to our results reported here, but it is not. The difference is attributed to the nature of the change in the chromatin during activation (MMS or insertion of PNTs) versus those caused by deleting *ISW2*. Treating cells with MMS or inserting PNTs lead to nucleosome eviction (this study), whereas our more recent data indicate that deleting *ISW2* causes disruption of positioning specifically (H.Z. and J.C.R., unpublished observations). This was not evident from previous studies because we did not employ the higher-resolution ChIP method described here. Nucleosomes are randomized over the promoter of *RNR3* in the Δ*isw2* mutant but are present nonetheless. This suggests that Tup1 requires the presence of a nucleosome to carry out most of its repression functions at *RNR3*, but nucleosomes do not need to be precisely positioned.

Shifting the positioning of a nucleosome over the *PHO5* promoter makes it dependent on Bdf1 (17). However, it is unclear whether this is caused by changes in TFIID function or whether the effects are on transcription initiation *per se*. Bdf1 is found in multiple transcription factor complexes and is part of the SWR-C complex that is required for Htz1 deposition (40). Htz1 is present at *PHO5* and regulates its expression (41). We provide strong evidence that TAF_{II}s are required for the access of the transcription machinery to the promoter when it is incorporated into a nucleosome. The fact that both TAF1 and SWI/SNF dependence can be suppressed by excluding nuc − 1 suggests that one of the functions of the TAF_{II} subunits is to mediate SWI/SNF-dependent remodeling, possibly by recruitment or retention of the complex at the promoter (15).

The core promoter of *RNR3* is not TAF_{II}-dependent and has a canonical TATA box, making it unique among all TAF_{II}-dependent genes characterized in yeast (42). Interestingly, even though the *RNR3* core promoter is not TAF_{II}-sensitive when taken out of its natural context, our results suggest that the core promoter does factor into the TAF_{II} dependency of the gene by a novel mechanism. The TAF_{II} dependency is not mediated, at least exclusively, through specific core promoter elements, such as initiator or downstream promoter element, but by the propensity of the core promoter to be incorporated into a nucleosome. Thus, the regulation of *RNR3* and recognition of its core promoter by TFIID is novel. Altering the location of the core promoter nucleosome can affect the levels of gene expression and transcription factor requirements (refs. 17 and 43 and this study). The discovery that many genes contain sequences that direct nucleosome formation over the core promoter and, more striking, that the locations of these signals are conserved in related yeast species suggests a selective pressure to preserve this feature (23, 24). These observations and our results support the idea that nucleosome positioning sequences and natural PNTs evolved to confer specific coactivator and corepressor requirements on genes. The role of these sequences is not simply to enhance transcription or expose activator binding sites, but also perhaps to play a crucial role in programming the regulatory circuitry of the genome.

Materials and Methods

Yeast Strains and Northern Blotting. *S. cerevisiae* strains used in this study are described in SI Table 1. Cells were grown at 30°C in 1% yeast extract/2% peptone/2% dextrose medium supplemented with 0.05 mg/ml adenine. MMS was added to a final concentration of 0.03% for 2.5 h where indicated. Temperature-shift

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experiments, RNA isolation, and Northern blotting were carried out as described in previous publications (15, 44). Promoter derivatives were constructed in a plasmid containing *RNR3* with the *LEU2* gene inserted downstream of the stop codon and before the regulatory region of the downstream gene, pBS-RNR3-LEU2. The cassette was liberated by NdeI and SacI digestion and transformed into cells. Restriction sites were introduced at −90 and/or −60 relative to the start site of transcription, and oligonucleotides containing dA::dT or random sequence were inserted at these sites. Details of construction are available upon request. Oligonucleotides used in this study are listed in SI Table 2.

Chromatin Mapping. Yeast nuclei were isolated by differential centrifugation and digested with micrococcal nuclease with a procedure described (45). The DNA was purified and digested with PstI, and the products were detected by Southern blotting using a 200-bp probe corresponding to the end of the PstI fragment (13). A derivation of this procedure was used in Fig. 2 *D* and *E* where nuclei were extensively digested down to predominantly mononucleosome-sized fragments with micrococcal nuclease. The Southern blot was probed with a nuc − 1-specific probe and then reprobed with the nuc − 2 probe from *PHO5* (25). Cells containing a deletion of the *RNR3* promoter (Δ*rnr3*) were analyzed in parallel to determine that the signal is not due to cross-hybridization with nonspecific targets.

ChIP and Nucleosome Density Analysis. The ChIP assay was performed essentially as described (15, 22). Chromatin was sheared into fragments averaging 400 bp in size by using a Bioruptor (Diagenode, Philadelphia, PA), with the exception of the experiments in Fig. 2 where chromatin was extensively sheared to 100–200 bp. Chromatin was precipitated with antibodies against core H3 (Abcam, Cambridge MA), Pol II (8WG16; Covance, Berkeley CA), Crt1, TBP, or Tup1. Noncommercial antibodies have been described (13, 14). The immunoprecipitated DNA and input DNA were analyzed by semiquantitative PCR with primers directed toward *RNR3* (see SI Table 2). The results shown are the means and standard deviations of at least three chromatin preparations.

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1. Han M, Grunstein M (1988) *Cell* 55:1137–1145.
2. Wyrick JJ, Holstege FC, Jennings EG, Causton HC, Shore D, Grunstein M, Lander ES, Young RA (1999) *Nature* 402:418–421.
3. Reinke H, Horz W (2003) *Mol Cell* 11:1599–1607.
4. Boeger H, Griesenbeck J, Strattan JS, Kornberg RD (2003) *Mol Cell* 11:1587–1598.
5. Lee CK, Shibata Y, Rao B, Strahl BD, Lieb JD (2004) *Nat Genet* 36:900–905.
6. Yuan GC, Liu YJ, Dion MF, Slack MD, Wu LF, Altschuler SJ, Rando OJ (2005) *Science* 309:626–630.
7. Sekinger EA, Moqtaderi Z, Struhl K (2005) *Mol Cell* 18:735–748.
8. Malagon F, Aguilera A (2001) *Genetics* 158:597–611.
9. Kaplan CD, Laprade L, Winston F (2003) *Science* 301:1096–1099.
10. Adkins MW, Tyler JK (2006) *Mol Cell* 21:405–416.
11. Mason PB, Struhl K (2003) *Mol Cell Biol* 23:8323–8333.
12. Huang M, Zhou Z, Elledge SJ (1998) *Cell* 94:595–605.
13. Li B, Reese JC (2001) *J Biol Chem* 276:33788–33797.
14. Zhang Z, Reese JC (2005) *Mol Cell Biol* 25:7399–7411.
15. Sharma VM, Li B, Reese JC (2003) *Genes Dev* 17:502–515.
16. Ladurner AG, Inouye C, Jain R, Tjian R (2003) *Mol Cell* 11:365–376.
17. Martinez-Campa C, Politis P, Moreau JL, Kent N, Goodall J, Mellor J, Goding CR (2004) *Mol Cell* 15:69–81.
18. Matangkasombut O, Buratowski S (2003) *Mol Cell* 11:353–363.
19. Zhang Z, Reese JC (2004) *J Biol Chem* 279:39240–39250.
20. Prunell A (1982) *EMBO J* 1:173–179.
21. Widom J (1998) *Annu Rev Biophys Biomol Struct* 27:285–327.
22. Zhang Z, Reese JC (2004) *EMBO J* 23:2246–2257.
23. Ioshikhes IP, Albert I, Zanton SJ, Pugh BF (2006) *Nat Genet* 38:1210–1215.
24. Segal E, Fondufe-Mittendorf Y, Chen L, Thastrom A, Field Y, Moore IK, Wang JP, Widom J (2006) *Nature* 442:772–778.
25. Gregory PD, Horz W (1999) *Methods Enzymol* 304:365–376.
26. Edmondson DG, Smith MM, Roth SY (1996) *Genes Dev* 10:1247–1259.
27. Corey LL, Weirich CS, Benjamin IJ, Kingston RE (2003) *Genes Dev* 17:1392–1401.
28. Kim Y, Clark DJ (2002) *Proc Natl Acad Sci USA* 99:15381–15386.
29. Peterson CL, Workman JL (2000) *Curr Opin Genet Dev* 10:187–192.
30. Sudarsanam P, Winston F (2000) *Trends Genet* 16:345–351.
31. Hassan AH, Neely KE, Vignali M, Reese JC, Workman JL (2001) *Front Biosci* 6:1054–1064.
32. Green MR (2000) *Trends Biochem Sci* 25:59–63.
33. Verrijzer CP, Tjian R (1996) *Trends Biochem Sci* 21:338–342.
34. Walker SS, Reese JC, Apone LM, Green MR (1996) *Nature* 383:185–188.
35. Morohashi N, Yamamoto Y, Kuwana S, Morita W, Shindo H, Mitchell AP, Shimizu M (2006) *Eukaryotic Cell* 5:1925–1933.
36. Shimizu M, Mori T, Sakurai T, Shindo H (2000) *EMBO J* 19:3358–3365.
37. Iyer V, Struhl K (1995) *EMBO J* 14:2570–2579.
38. Koch KA, Thiele DJ (1999) *J Biol Chem* 274:23752–23760.
39. de Bruin D, Zaman Z, Liberatore RA, Ptashne M (2001) *Nature* 409:109–113.
40. Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V, *et al.* (2003) *Mol Cell* 12:1565–1576.
41. Millar CB, Xu F, Zhang K, Grunstein M (2006) *Genes Dev* 20:711–722.
42. Li B, Reese JC (2000) *EMBO J* 19:4091–4100.
43. Lomvardas S, Thanos D (2002) *Cell* 110:261–271.
44. Reese JC, Green MR (2003) *Methods Enzymol* 370:415–430.
45. Zhang Z, Reese JC (2005) *Methods Mol Biol* 313:245–256.