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A SWI/SNF- and INO80-dependent nucleosome movement at the *INO1* promoter

Jason Ford[a], **Oluwafemi Odeyale**[a], **Antonious Eskandar**[a], **Nafila Kouba**[a], and **Chang-Hui Shen**[a,b*]

a*Department of Biology, College of Staten Island, City University of New York, Staten Island, New York 10314, USA*

b*Institute for Macromolecular Assemblies, City University of New York, Staten Island, New York 10314, USA*

Abstract

Transcriptional activation in yeast *INO1* chromatin was studied using the indirect end-labeling technique. *INO1* chromatin is organized into an ordered, overlapping nucleosomal array under repressing conditions. Nucleosome positions were only disrupted at the promoter region under inducing conditions in the presence of SWI/SNF and INO80. Mutants lacking either remodeler demonstrated identical positioning patterns as the wild type under repressing conditions. This indicates that these two remodelers are responsible and essential for local nucleosomal mobilization at the *INO1* promoter. The area of local nucleosome movement is consistent with the previously identified region of histone deacetylation activity. In light of these findings, we suggest that nucleosomes subject to local mobilization are also targets for local histone modifications.

Keywords

INO1; SWI/SNF; INO80; indirect end-labeling; chromatin structure; local nucleosome movement

Introduction

Phospholipid structural genes play an important role in regulating yeast cellular processes. These genes encode enzymes responsible for phospholipid synthesis, which define the structural integrity of cells during mitosis. One of the major membrane phospholipids found in mitotically growing cells is phosphatidylinositol (PI). PI is a ubiquitous membrane lipid, which represents a significant portion of the total phospholipid composition in yeast. In addition to its role in membrane structural maintenance, PI also plays a part in many cellular activities such as mRNA export, signal transduction pathways and vesicular trafficking (1,2)

The *de novo* synthesis of PI from glucose-6-phosphate requires the structural gene *INO1*. *INO1* encodes inositol-1-phosphate synthase, which catalyzes the rate-limiting step in the synthesis of inositol. Its expression is transcriptionally regulated by inositol and choline, two precursor molecules in phospholipid biosynthesis. In their presence, transcription is repressed and in their absence, it is activated (3). This regulation is dictated by a *cis*-acting promoter element termed UAS_{INO}, known as the inositol sensitive upstream activation sequence; consensus: 5′ CATGTGAAAT 3′ (4,5). UAS_{INO} elements are binding sites for the

*To whom correspondence should be addressed. Phone: 718-982-3998. Fax: (718) 982-3852. E-mail: cshen@mail.csi.cuny.edu

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heterodimeric transcriptional activator, Ino2p/Ino4p, encoded by *INO2* and *INO4*, respectively. They possess basic helix-loop-helix DNA binding motifs, which activate transcription through interactions with UAS_{INO} (6–9). Sequence analysis reveals that there may be 6 potential UAS_{INO} elements within the *INO1* promoter. However, it has been shown that only two are bound by Ino2p/Ino4p, and that both are required to support transcriptional activation (4,6,9). Nonetheless, despite the continual binding of Ino2p/Ino4p to UAS_{INO}, inositol suppresses transcriptional activation via the Opi1p repressor. Opi1p mediates repression via its activator interaction domain, which binds to the repressor interaction domain of Ino2p (10–12).

INO1 expression is also regulated by chromatin remodeling activities. It has been shown that the histone acetylases Gcn5p and Esa1p acetylate histones H3 and H4, respectively, at the *INO1* promoter (13). Furthermore, the recruitment of the Gcn5p-SAGA complex is dependent on histone H3 Ser10 phosphorylation. This histone modification is catalyzed by Snf1 kinase, which is recruited by the Ino2p activation domain (14–16). On the other hand, as with other genes, histone deacetylation negatively regulates *INO1*. Much evidence suggests that Ume6p binds to a URS1 (upstream repressor sequence) element within the *INO1* promoter, and this interaction recruits the Sin3p-Rpd3p HDAC (histone deacetylase complex) complex to repress *INO1* expression (17–21).

Chromatin remodeling complexes provide a different means for gene activation. Several lines of evidence have shown that both SWI/SNF and INO80 are required for *INO1* activation (22–25), although ISWI has been shown to negatively regulate *INO1* expression (26,27). However, current studies have only demonstrated the influence of individual remodelers in *INO1* activation. It is still unclear how these complexes work cooperatively at the *INO1* promoter.

In the present study, we aimed to understand the structural consequences of the interplay between the SWI/SNF and INO80 remodeling complexes at the *INO1* promoter. To address this interest, we studied the effect of both remodelers on *INO1* chromatin structure. We compared nucleosome positions in *INO1* in repressing wild-type, inducing wild-type, inducing *snf2*Δ, and inducing *ino80*Δ chromatin. Our results suggest that nucleosome movement occurs at the promoter during gene activation and that both SWI/SNF and INO80 are equally important for this activity.

Materials and methods

Yeast strains and media

WT (wild-type) yeast strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), *snf2*Δ strain (*snf2Δ, MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf2Δ*), and *ino80*Δ strain (*ino80Δ, MATa ino80Δ::TRP1 his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0*) (26) were used in this study. All yeast culture was grown at 30°C in SC media (synthetic complete media) containing 2% glucose (wt/vol) with100 μM inositol (*myo*-inositol; Sigma I-7508)(for repressing conditions; SC) or without (for inducing conditions; SC-ino) except for *ino80*Δ, which was grown in SC-trp (SC medium lacking tryptophan) or SC-trp-ino (SC medium lacking both tryptophan and inositol) (3).

RNA preparation and Northern blot analysis

For WT and *snf2*Δ strains, cells from overnight cultures were harvested and washed three times with SC-ino. Subsequently, cells were inoculated into SC or SC-ino and 40 ml of culture were removed immediately and 1 h (hour) after inoculation for RNA preparation. For the *ino80*Δ strain, cells were washed in SC-trp-ino, and inoculated in either SC-trp or SC-trp-ino. Total RNA was prepared from collected cells as described previously (28). Equal amounts (3 μg) of

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RNA were used in the Northern blots as described previously (28). The *INO1* probe was a 459-bp PCR fragment (forward primer: TAATATTGCTCCAATCACCTCC; reverse primer: GCTTCGTATAGATCTGCGTTAT) and the *ACT1* probe was a 269-bp PCR fragment (forward primer: ATGGATTCTGGTATGTTCTAGC; reverse primer: CATGAGACTTAGTAACAGTAGC).

Indirect end-labeling analysis

Nuclei were prepared from yeast cells as described previously (28,29). Briefly, WT cells were grown to mid-log phase in SC-ino or SC, harvested, and washed twice with SC-ino or SC, and spheroplasts were prepared. Spheroplasts were incubated in SM (spheroplast medium, SC with 1M D-sorbitol) or SM-ino (spheroplast medium without inositol, SC-ino with 1M D-sorbitol) for 1 h under repressing or inducing conditions. Nuclei were released from spheroplasts and digested with MNase (*Micrococcal nuclease*). The DNA was purified, digested with *Blp*I and electrophoresed in long 1.2% (w/v) agarose gels. The gels were blotted and probed with an *INO1 Blp*I/*Sna*BI fragment labeled by random priming. All data were quantified using a phosphorimager. For inducing *snf2*Δ and *ino80*Δ cells, cells were grown to mid-log phase in SC or SC-trp, harvested, washed twice with SC-ino or SC-trp-ino, and spheroplasts were prepared as described above using SM-ino or SM-trp-ino. Spheroplasts were incubated in SM-ino or SM-trp-ino for 1 h under inducing conditions followed by nuclei preparation.

Results

Both INO80 and SNF2 are required for *INO1* transcriptional activation

It has been shown that *INO1* is expressed only during the logarithmic phase of growth in the absence of inositol (30,31). To study how INO80 regulates *INO1* expression, both WT and *ino80*Δ cells were grown in SC or SC-trp. When the O.D. reached 0.1~0.3, cells were harvested by centrifugation and washed twice with SC-ino or SC-trp-ino to remove inositol completely. For inducing conditions, cells were resuspended in SC-ino or SC-trp-ino and incubated at 30° C. Inositol was added to a final concentration of 100 μM as required for repressing conditions. Aliquots of culture were removed for O.D. measurement following inositol addition. The results showed that *ino80*Δ cells grew poorly in the absence of inositol (Figure 1A). Addition of inositol, as a result, rescues the inositol auxotrophy of the *ino80*Δ cells. Therefore, INO80 is required for *INO1* expression.

To investigate whether SWI/SNF is also required for *INO1* gene expression, we performed growth experiments with *snf2*Δ cells. We found that the growth of *snf2*Δ cells, as with *ino80*Δ cells, is severely inhibited in the absence of inositol (Figure 1A). The inositol auxotrophy is circumvented upon addition of inositol to the medium, indicating that SWI/SNF also plays a crucial role in *INO1* induction. Thus, the deletion of both *INO80* and *SNF2* led to inositol auxotrophy due to an inability to express *INO1*.

To further examine the role of these remodelers on *INO1* expression, *INO1* mRNA levels were measured by Northern blot analysis. The normalized expression ratio of *INO1/ACT1* increased 10 fold for WT cells after a 1 h induction period; the ratio was approximately 0.83 for WT cells under repressing conditions (Figure 1B). Thus, *INO1* is expressed within 1 h of induction under the given conditions. For both *ino80*Δ and *snf2*Δ cells, *INO1* mRNA did not significantly increase in either inducing or repressing conditions. The ratio of *INO1/ACT1* was approximately 1 for both strains under both conditions (Figure 1B). This suggests that both SWI/SNF and INO80 are required for *INO1* expression and that they regulate *INO1* induction at the transcriptional level.

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Analysis of the *INO1* Chromatin structure

Indirect end-labeling analysis coupled with MNase digestion of nuclei has been widely applied for studying the *in vivo* translational map of nucleosome positions (32–34). We employed this technique to investigate the *INO1* chromatin structure under repressing and inducing conditions. The WT cells from repressing and inducing conditions were compared and a complex but highly reproducible pattern was observed (Figure 2A, lanes 3 to 8 and lanes 11 to 14). Many bands of various intensities were observed; each band represents the upstream border of a nucleosome with respect to the *Blp*I restriction enzyme site downstream of the *INO1* transcription start site. The intensity indicated the distribution of nucleosomes in that particular position (Figure 2B). Nucleosome positions corresponding to the bands observed in Figure 2A are shown in detail in Figure 4. Theoretically, the area of interests including the *INO1* promoter and part of the *ORF* (−540 to +678 relative to the transcription start site; 1217 bp)(4) could accommodate a maximum of seven uniquely positioned nucleosomes if closely packed (Figure 4). However, there are many more than seven bands in repressed chromatin and the neighboring bands were significantly less than 147 bp apart. Based on our previous work, these bands probably indicate alternative overlapping nucleosome positions (28,29,35). Thus, our map revealed the presence of overlapping nucleosome positions on *INO1*. Furthermore, these positions are mutually exclusive as only one nucleosome can be present at any given moment.

Induced *INO1* chromatin displayed different patterns from repressed *INO1* chromatin, since some positions were disrupted under inducing conditions (Figure 2A, compare lane 7with lane 13; Figure 2B). For example, the upstream borders of Nucleosomes B, D, and E disappeared under inducing conditions, indicating that these three were remodeled or removed in the process of induction. The upstream borders of Nucleosome C became weaker during induction, suggesting that it has also been redistributed to other alternative positions or that it has been removed from its original position.

The disruption of both Nucleosomes B and C is very important. Nucleosome B covers UAS_{INO} 1, UAS_{INO} 2, UAS_{INO} 3, and URS1 sequences, while Nucleosome C covers UAS_{INO} 4, UAS_{INO} 5, and the TATA box (Figure 4). Both UAS_{INO} 3 and UAS_{INO} 4 have been confirmed as the target sites for the Ino2p/Ino4p heterodimer and appear to be functional in response to inositol deprivation (9). Thus, the removal of both nucleosomes provides the opportunity for the transcription machinery to access its binding sites. Interestingly, the TATA box element is occluded by both Nucleosomes C and D. However, Nucleosome C is not completely removed in induction. It is possible that the redistribution of nucleosomes within the alternative positions during induction allows RNA polymerase II to bind. This possibility is substantiated since Nucleosomes C, D, and E are within the same set of alternative overlapping positions (Figure 4). Nucleosomes C and D, and Nucleosomes D and E are mutually exclusive, but Nucleosomes C and E can be present simultaneously. We can only observe the average chromatin structure in a cell population under the current study. It is possible that nucleosomes are moving within these positions to provide accessibility for *trans*-acting factors.

Both INO80 and SWI/SNF direct the localized disruption of nucleosomal arrays at the *INO1* promoter

Results obtained from growth experiments and Northern blot analysis suggested that both the Snf2p and Ino80p subunits of SWI/SNF and INO80, respectively, are required for *INO1* expression. Chromatin structural analysis also demonstrated the mobilization of nucleosomes upon induction. It is possible that these two chromatin remodelers are directly responsible for the nucleosome movement. The remodeler involved would presumably be capable of mobilizing nucleosomes on a DNA template *in vitro*. To date, only Ino80p has been tested on

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reconstituted chromatin containing an *INO1* promoter fragment (24). SWI/SNF has not yet demonstrated similar *in vitro* remodeling activity for *INO1* chromatin.

To confirm that both remodelers can directly mobilize nucleosomes at the *INO1* promoter *in vivo*, the *INO1* chromatin structure was analyzed using nuclei derived from *ino80*Δ and *snf2*Δ cells grown under inducing conditions. Chromatin structures were compared with that of WT *INO1* chromatin. The *ino80*Δ and *snf2*Δ chromatin were very similar to repressing WT chromatin but not to inducing WT chromatin (Figure 3A, compare lane 6 and 10 with Figure 2A lanes 7 and 13, Figure 3B). This suggests that the presence of either subunit of the chromatin remodeling complexes is insufficient for disruption of *INO1* chromatin. Consequently, both SWI/SNF and INO80 are required for nucleosome mobilization under inducing conditions.

Discussion

We have determined the *INO1* chromatin structure under repressing conditions. The *INO1* ORF and its promoter are organized into a set of well-positioned overlapping nucleosomes during repressing conditions. Such overlapping chromatin structures were also observed in other genes such as *CUP1* (28,35) and *HIS3* (36). It is interesting to observe overlapping positions under repressing conditions. We speculate that such arrangements provide alternative choices for nucleosomes in response to various cellular activities, such as replication, transcription and repair, each may require different positions.

During induction, the orderly positioned nucleosomes were disrupted in *INO1* chromatin. The disruption of nucleosomal organization strongly suggests the presence of remodeling activity at the promoter under inducing conditions. However, nucleosome movement is limited to the promoter region. These nucleosomes cover all regulatory sequences including the UAS, the URS1, and the TATA box (Figure 4). Such local remodeling is necessary for *trans*-acting factors to access *cis*-acting sequences for transcriptional activation. For instance, the TATA box is occluded by Nucleosome C and D, and the movement of both nucleosomes makes it accessible to TBP, which facilitates transcriptional activation.

In identifying the remodelers that are involved in *INO1* activation, both *ino80*Δ and *snf2*Δ strains were used for indirect end-labeling analysis. Unexpectedly, we did not observe any nucleosome movement in either mutant under inducing conditions (Figure 3). This strongly suggests that both INO80 and SWI/SNF are required for remodeling during induction. Furthermore, they did not demonstrate additive effects and must be present simultaneously for nucleosome movement. This differs from previous observations in other genes. For example, in *HIS*3, both SWI/SNF and ISW1 act synergistically in induction (36). The ISW1 complex plays a more subtle role in nucleosome mobilization over *HIS*3, favoring positions different from those preferred by SWI/SNF. As a result, *INO1* chromatin represents a unique transcriptional activation model.

We observed nucleosome movement only at the *INO1* regulatory region ranging from −389 to +76 relative to the transcription start site, which includes Nucleosomes B, C, D, and E (Figure 4). A previous study has shown that nucleosomes in this area are subject to histone deacetylation by Rpd3p (37). In yeast, Rpd3p is recruited by the transcriptional repressor Ume6p, through an interaction with the Rpd3p-associated corepressor Sin3p. Under repressing conditions, recruitment of Rpd3p leads to histone deacetylation surrounding the URS1 motif (21). This region matches the positions of Nucleosomes B through E. Under inducing conditions, the Ume6p-Sin3p-Rpd3p complex is no longer present at the promoter, and these nucleosomes are subject to localized remodeling activities, such as histone acetylation and nucleosome movement. Therefore, Nucleosomes B, C, D and E are not only targets for nucleosome movement but also for histone modifications.

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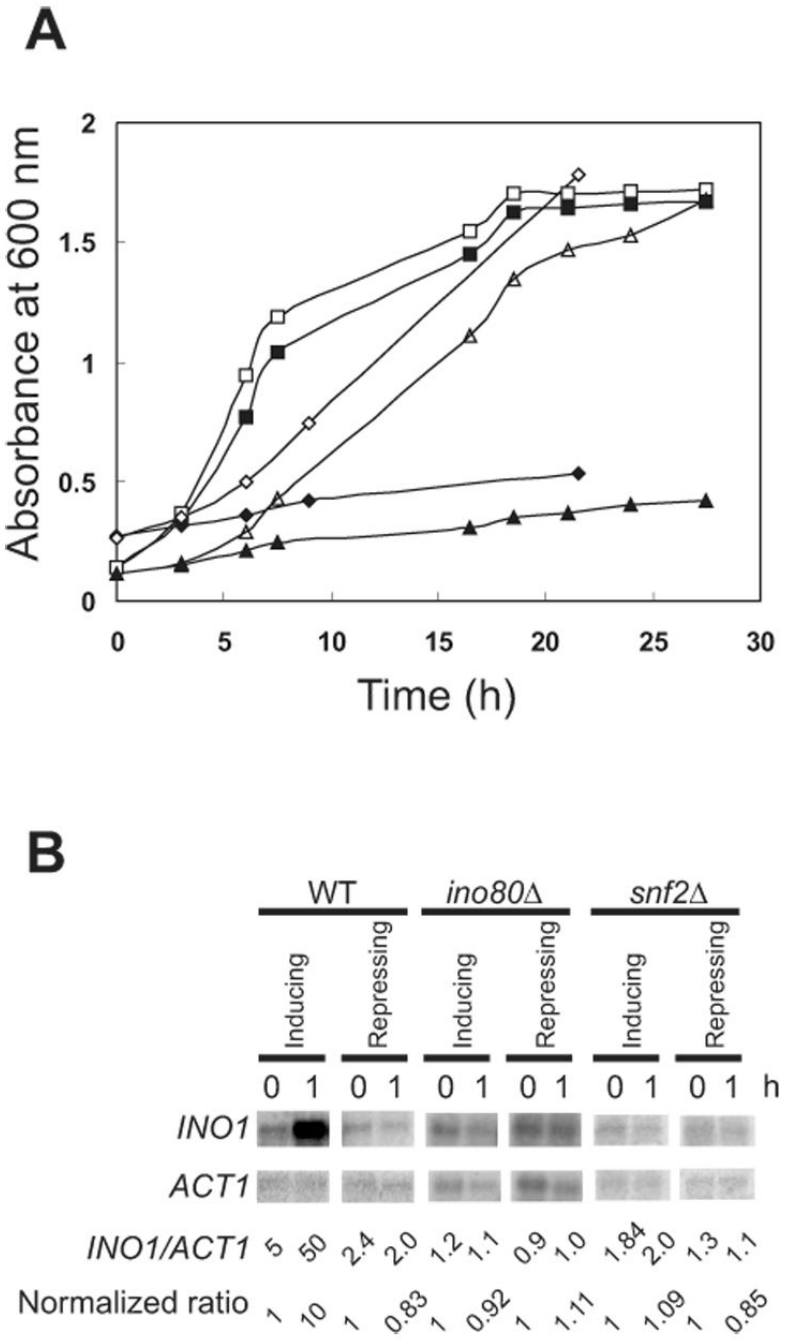

Figure 1.

Both INO80 and SWI/SNF remodeling complexes are required for *INO1* expression. (A) INO80 and SWI/SNF rescue cells from inositol auxotrophy. Yeast cell growth was monitored by A_{600}. Cells from overnight cultures were grown in SC/SC-trp and washed three times in media without inositol. Subsequently, cells were inoculated into media with or without 100 μM inositol. WT: (□) SC + inositol, (■) SC −inositol. Mutant *ino80*Δ: (△) SC – trp + inositol, (▲)SC – trp − inositol. Mutant *snf2*Δ: (◇) SC + inositol, (◆) SC − inositol. (B) Induction of *INO1* in WT, *ino80*Δ and *snf2*Δ cells. *INO1* and *ACT1* mRNA were detected by Northern blot

hybridization with *INO1* and *ACT1* probes, respectively. Cells were collected immediately and 1 h after inoculation in repressing or inducing media.

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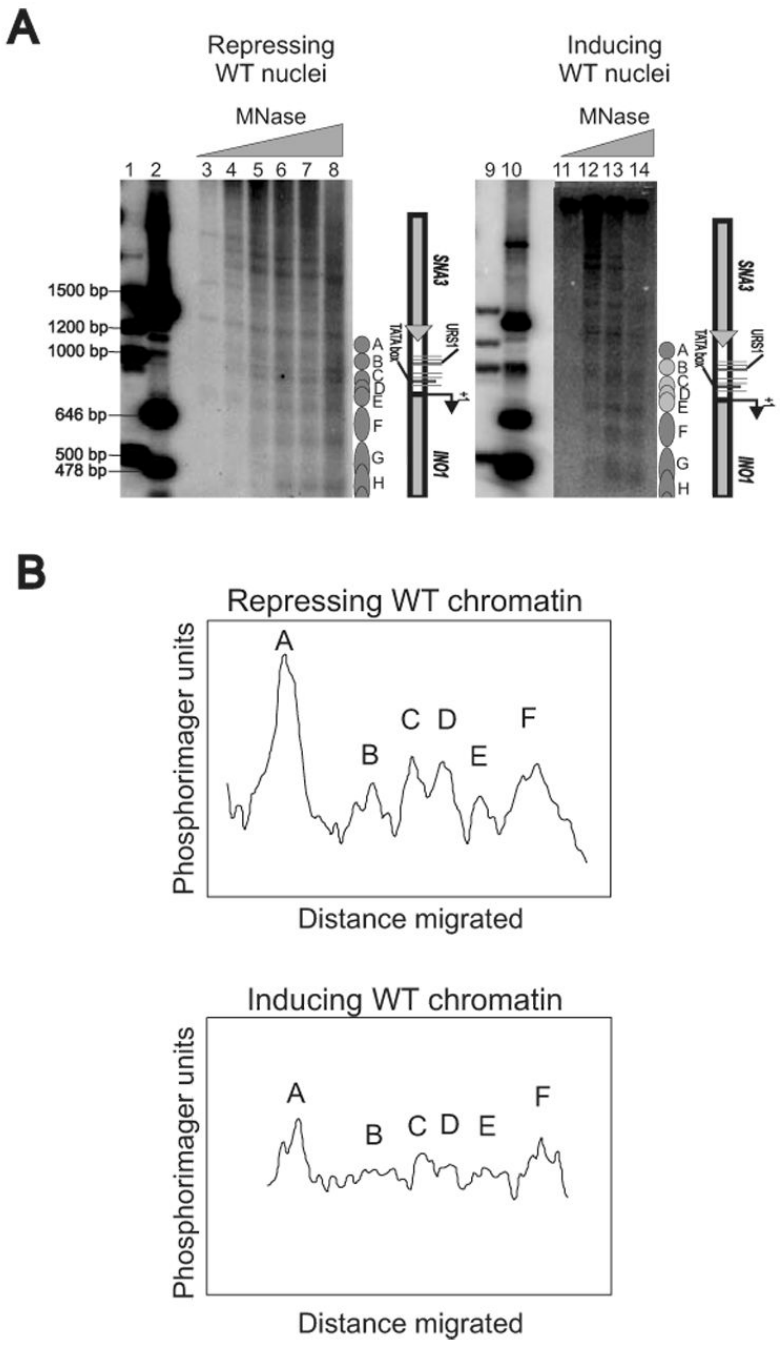

Figure 2.

INO1 chromatin is organized into an overlapping nucleosomal array. (A) Nucleosomal organization of the *INO1* chromatin in repressing WT and inducing WT cells. Nuclei were prepared from different strains and digested with increasing amounts of MNase; purified DNA was analyzed in a 1.2% agarose gel. The Southern blot was probed with a radiolabeled *Blp*I/*Sna*BI fragment. Lanes 1, 2, 9, and 10 are size markers. (B) Scan of the indirect end-labeling maps shown in panel A. The lanes corresponding to repressing WT chromatin (lane 6) and inducing WT chromatin (lane 13), were scanned using a phosphorimager.

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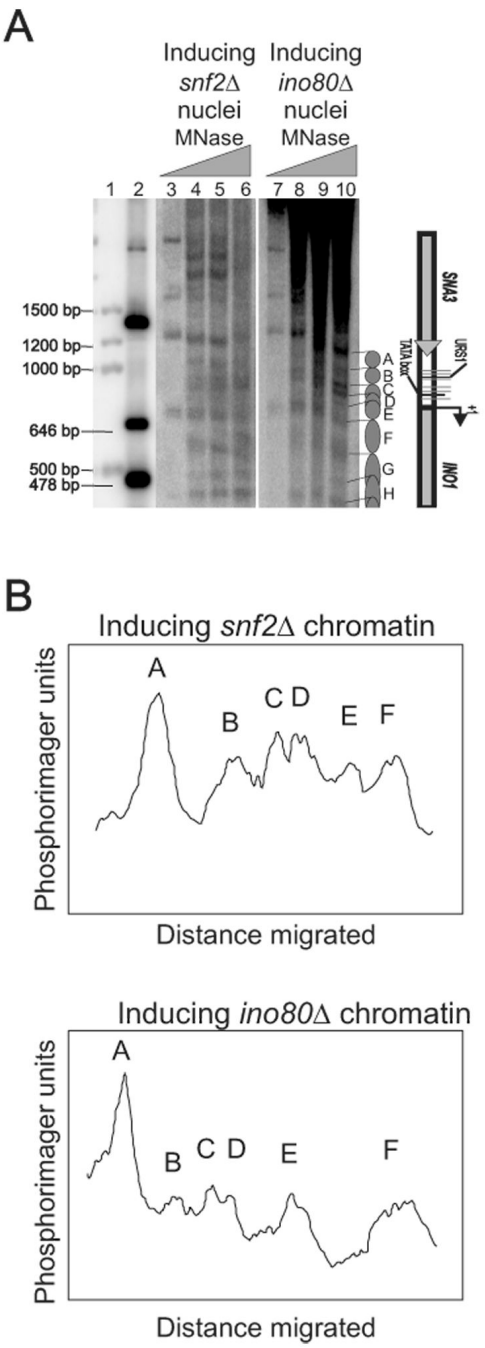

Figure 3.

Both SWI/SNF and INO80 are required for nucleosome movement. (A) Nucleosomal organization of the *INO1* chromatin in inducing *snf2*Δ and inducing *ino80*Δ cells. Samples were prepared as described in Figure 2. Lanes 1 and 2 are size markers. (B) Scan of the indirect end-labeling maps shown in panel A. The lanes corresponding to inducing *snf2*Δ chromatin (lane 4) and inducing *ino80*Δ chromatin (lane 10) were scanned using a phosphorimager.

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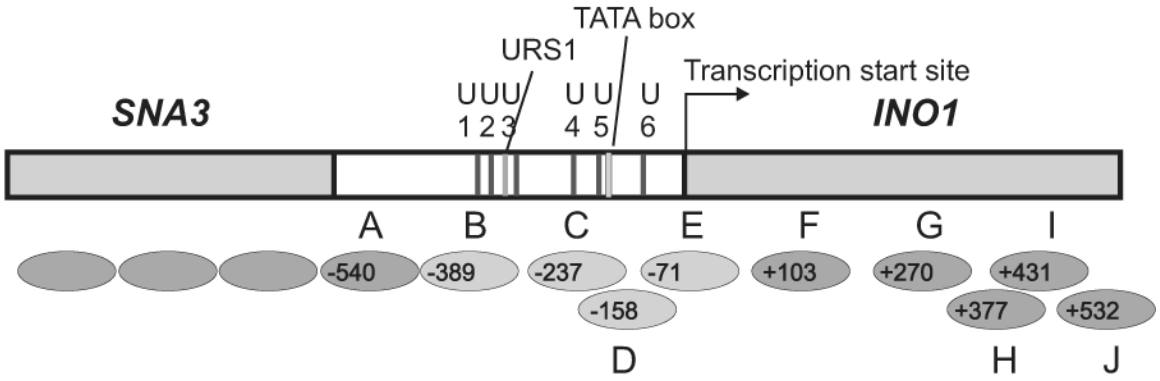

Figure 4.

Arrays of positioned nucleosomes on *INO1*. The nucleosome positions mapped by indirect end-labeling in Figure 2 and Figure 3 are given with upstream borders mapped with respect to the *Blp*I site. *INO1* coordinates are given with respect to the transcription start site at nucleotide +1 (6)(indicated by the arrow). The most important sequence elements in the *INO1* promoter are indicated by the labeled lines: the TATA box, the URS1 element, and the potential $UAS_{INO}1$ to $UAS_{INO}6$ (U1 to U6). Positioned nucleosomes are shown as ovals labeled A to J. Nucleosomes shown as light gray ovals are nucleosomes subject to remodeling in induction.