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Activator-dependent recruitment of SWI/SNF and INO80 during *INO1* activation

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Abstract

Transcriptional activation of yeast *INO1* requires SWI/SNF and INO80 for nucleosome disruption at the promoter. However, the cooperative interplay among remodelers and their recruitment dynamics in activation have thus far been vague. Here, we showed, using chromatin immunoprecipitation, that both SWI/SNF and INO80 are present at the promoter and are restricted to the promoter, indicating that they directly participate in localized *INO1* chromatin remodeling. Furthermore, both SWI/SNF and INO80 are absent at the *INO1* promoter in *ino2Δ* cells, suggesting that these are activator-dependent remodelers. We have also found that the presence of INO80 is required for SWI/SNF recruitment, indicating that INO80 arrives first at the promoter followed by SWI/SNF. In light of these findings, we proposed a model which describes the order of events in *INO1* activation.

Keywords

INO1; INO2; INO80; SNF2; SWI/SNF; ChIP. Chromatin Immunoprecipitation; Local Nucleosome Movement; Gene Activation

Introduction

Chromatin structure impedes many nuclear processes including transcription and recombination, which require access to the underlying DNA template. To overcome these repressive effects in transcription, chromatin structure must be remodeled at promoter regions prior to or during transcriptional activation [1]. This remodeling is due to histone modifying enzymes and chromatin remodeling complexes with the structural reconfiguration of chromatin for gene activation being a focus of vigorous research in recent years.

Many chromatin remodeling complexes have been isolated and characterized in budding yeast, including SWI/SNF [2] and INO80 [3,4]. These remodelers hydrolyze ATP to alter the contacts between histone octamers and DNA, resulting in nucleosome movement. The effects of individual remodelers on certain genes have been extensively characterized [5–8]. However,

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it is becoming clear that the regulation of some, if not all yeast genes, including *HIS3* [7] and *INO1* [8], requires more than one remodeler.

INO1 encodes a key enzyme required for the *de novo* synthesis of phosphatidylinositol, one of the major membrane phospholipids in dividing cells in yeast. Yeast adjusts its membrane lipid composition based on the availability of phospholipid precursors, namely inositol and choline. In their presence, transcription is repressed and in their absence, it is activated. This regulation is controlled by the *cis*-acting promoter element UAS_{INO} [9,10], which is the binding site for the heterodimeric transcriptional activator, Ino2p/Ino4p [11,12]. Despite the constitutive binding of Ino2p/Ino4p to UAS_{INO} [13,14], inositol suppresses transcriptional activation via Opi1p, which mediates repression through its activator interaction domain, by binding to the repressor interaction domain of Ino2p [15].

Several lines of evidence have demonstrated that SWI/SNF and INO80 are required for *INO1* activation [4,16,17]. However, these studies have only addressed the effect of individual remodelers in *INO1* activation. Presently, it is still unclear how these complexes cooperatively orchestrate the activation process at the promoter. In a study of *INO1* chromatin structure, we demonstrated that SWI/SNF and INO80 positively regulate *INO1* expression by creating a more accessible nucleosomal DNA and are both required for *INO1* chromatin reconfiguration and gene activation [8].

We were interested in further examining the structural consequences due to the interplay among remodelers. Here, we performed chromatin immunoprecipitation (ChIP) assays to determine how remodelers are recruited to the *INO1* promoter. We demonstrated that the Ino2p activator is essential for recruiting INO80 and that INO80 is required for recruiting SWI/SNF to the *INO1* promoter. Based on our findings, we have proposed a model which describes *INO1* activation.

Materials and Methods

Yeast strains and growth conditions

WT (wild-type) yeast strain BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*), *snf2Δ* strain (*snf2Δ, MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0 snf2Δ*), and *ino80Δ* strain (*ino80Δ, MATa ino80Δ::TRP1 his3Δ200 leu2Δ0 met15Δ0 trp1Δ63 ura3Δ0*) were used in this study. All yeast culture was grown at 30°C in SC media (synthetic complete media) containing 2% glucose (wt/vol) with 100 μM inositol (for repressing conditions; SC) or without (for inducing conditions; SC-ino) except *ino80Δ* which was grown in SC-trp (SC medium lacking tryptophan) or SC-trp-ino (SC medium lacking both tryptophan and inositol) (8).

ChIP and Multiplex PCR analysis

ChIP experiments were based on the method of Eriksson *et al.* (2006) [18] with modifications. The preparation of cross-linked chromatin, Immunoprecipitation (IP) procedures, the analysis of DNA quantification in the IP samples by multiplex PCR with internal control, and PCR primers used in the multiplex PCR reaction are described in the supplementary materials. All experiments were repeated three times and all data were quantified with a phosphorimager.

Real-Time PCR analysis

All extracted DNAs analyzed by multiplex PCR were also analyzed by singleplex real-time PCR using the TaqMan probe-based method in a 7500 sequence detection system (Applied Biosystems). All real-time PCR primers and TaqMan MGB probes are listed in the supplementary materials. All amplicons are within the same region as those amplified above. The real-time PCR procedure is described in the supplementary materials. All experiments

were repeated twice and, in each experiment, PCR reactions were done in triplicate. Target DNA sequence quantities were estimated from the threshold amplification cycle number (C_T) using Sequence Detection System software (Applied Biosystems). A ΔC_T value was calculated for each sample by subtracting the C_T value for the IP sample from the C_T value for the corresponding input DNA to normalize the differences in ChIP aliquots. Each IP quantity was then calculated with the following formula: $2^{(-\Delta C_T)}$. Each DNA quantity (normalized to the input) was normalized to the *POL1* DNA quantity (normalized to the *POL1* input) by taking the ratios of *INO1* DNA to *POL1* DNA [19].

Results

SWI/SNF and INO80 are responsible for nucleosome movement at the *INO1* promoter

Prior studies have revealed that both SNF2 and INO80 are required for nucleosome mobilization at the *INO1* promoter under inducing conditions [4,8]. This suggests that both remodelers may work cooperatively on *INO1* chromatin. However, no evidence has shown that both remodelers work directly at the *INO1* promoter. To prove that both remodelers directly mobilize nucleosomes during *INO1* activation, ChIP assays were performed.

For inducing and repressing WT cells, the IP signals of the Snf2p subunit of SWI/SNF (Snf2-IP) and of Arp8p subunit of INO80 (Arp8-IP) were observed at the *INO1* upstream regulatory sequences (*URS*), and part of the coding region of the *POL1* gene (*POL*). *POL1* is often used as a control since it is relatively long and there should be no contributing signal fluctuation due to *POL1* [7,19]. The input signals come from DNA extracted directly from cross-linked cells. To confirm linear amplification, 1:100 and 1:500 dilutions of the materials used in the IP were used in the PCR. The input signals obtained from the 1:100 dilution were always approximately 5 times those obtained from the 1:500, demonstrating a linear amplification in the quantitative multiplex PCR (Figure 1A, compare lanes 1, 3, 11 and 13 with 2, 4, 12 and 14).

The relative IP value (*URS/POL*) represented the ratio of *INO1* IP DNA normalized to *INO1* input to *POL1* IP DNA normalized to *POL1* input [7,19]. The *URS/POL* ratio obtained from the Snf2-IP was 2.6 ± 0.3 for repressed chromatin, indicating that the amount of Snf2p at the *INO1* promoter is 2.6 times greater than the amount of Snf2p at the *POL1* coding region under repressing conditions (Figure 1A, lane 7; Figure 1B). Furthermore, the *URS/POL* ratio was 1.6 ± 0.1 for induced chromatin, suggesting that there is 1.6 times more Snf2p at the *INO1* promoter than at the *POL1* coding region under inducing conditions (Figure 1A, lanes 8; Figure 1B). Real-time PCR was also performed and showed very similar results (Figure 1B). Results from this quantitative analysis showed that the relative IP value obtained from the Snf2-IP were 2.5 ± 0.1 and 1.5 ± 0.4 for repressed and induced chromatin, respectively. Based on these ChIP data, we concluded that Snf2p is present at the promoter under both conditions. Nevertheless, there is a significant reduction in the IP signals after *INO1* induction (repressed chromatin vs. induced chromatin, p value = 0.00135 for multiplex PCR and p value = 0.03368 for real-time PCR). This indicates that a large amount of SWI/SNF was present at the *INO1* promoter prior to induction and only basal levels of SWI/SNF remained at the *INO1* promoter following induction. This reduction implies that it is unnecessary to maintain a large quantity of SWI/SNF at the promoter after induction. These findings illustrate that SWI/SNF is constitutively present at the *INO1* promoter but that almost half departs during induction.

For INO80, the relative IP values obtained from the Arp8-IP were 1.9 ± 0.5 and 0.9 ± 0.1 for repressed and induced chromatin, respectively (Figure 1A, lanes 9 & 10; Figure 1B). The results from the real-time PCR analysis showed that the relative IP values obtained from the Arp8-IP were 1.3 ± 0.1 and 0.5 ± 0.1 for repressed and induced chromatin, respectively. This indicates that INO80 is also present at the *INO1* promoter prior to induction but to a lesser extent relative to SWI/SNF. The IP signal again decreases significantly following induction (repressed

chromatin vs. induced chromatin, p value = 0.03572 for multiplex PCR and p value = 0.01475 for real-time PCR), indicating that INO80 leaves the promoter after induction. It also suggests that INO80 is not present at the promoter after induction. As a result, we concluded that INO80 is also present at the *INO1* promoter but that all depart from the promoter during induction.

We then wanted to examine whether these remodelers are present on the *INO1* coding region. Consequently, ChIP assays were performed for the *INO1 ORF*. Our results demonstrate that the relative IP values (*ORF/POL*) for Snf2-IP were 0.9 ± 0.3 and 0.8 ± 0.1 for both repressed and induced chromatin (Figure 1A, lanes 15 & 16; Figure 1C), and the relative IP values obtained from the Arp8-IP were 0.8 ± 0.2 and 0.8 ± 0.1 for repressed and induced chromatin, respectively (Figure 1A, lanes 17 & 18; Figure 1C), indicating that both remodelers are absent at the *INO1 ORF* region under both conditions. All real-time PCR data were well corroborated with the multiplex PCR data (Figure 1C). These results suggest that both remodelers are confined to the promoter region, and the localized presence of both remodelers can only mobilize nucleosomes in the promoter region. Indeed, a recent study showed a local nucleosome movement at the promoter which is in accord with our observations [8].

Ino2p is required to recruit SWI/SNF and INO80

We have demonstrated that SWI/SNF and INO80 are present at the *INO1* promoter before induction (Figure 1). Since previous studies have also shown that Ino2p/Ino4p is present at the promoter under repressing conditions [13,14], we next wanted to examine whether SWI/SNF and INO80 are activator-dependent chromatin remodelers. To address this, we used an *ino2Δ* strain for ChIP assays to determine if the remodelers are present or absent under inducing conditions. If the remodelers are absent, it suggests that the Ino2p activator plays an essential role in the remodeler recruitment pathway.

For INO80, the *URS/POL* ratios obtained from the Arp8-IP were 0.9 ± 0.3 and 0.9 ± 0.1 for repressed and induced chromatin, respectively. This indicates that INO80 is absent at the *INO1* promoter in the absence of Ino2p (Figure 2A, lanes 7 & 8; Figure 2B). For SWI/SNF, the *URS/POL* ratios obtained from the Snf2-IP were 0.9 ± 0.1 and 0.9 ± 0.1 for repressed and induced chromatin, respectively. Likewise, this indicates that SWI/SNF is absent at the *INO1* promoter in the absence of Ino2p (Figure 2A, lanes 9 & 10; Figure 2B). The real-time PCR analysis showed that the *URS/POL* ratios were 1.0 ± 0.1 and 0.9 ± 0.1 for Arp8-IP under repressing and inducing conditions, respectively, and 1.0 ± 0.1 for Snf2-IP under both conditions (Figure 2B). As such, both SWI/SNF and INO80 are absent from the *INO1* promoter in the *ino2Δ* strain. Since SWI/SNF and INO80 are present at the *INO1* promoter in WT cells (Figure 1), these results strongly suggest that SWI/SNF and INO80 are activator-dependent chromatin remodelers.

INO80 is required to recruit SWI/SNF

We next wanted to determine if the presence of SWI/SNF and INO80 depend on each other or if they are recruited directly by the Ino2p activator. In *snf2Δ* cells, the *URS/POL* ratios obtained from the Arp8-IP were 1.6 ± 0.3 and 0.8 ± 0.1 from cells under repressing and inducing conditions, respectively (Figure 3A, lanes 3 & 4; Figure 3B). The relative ratios from the real-time PCR analysis were 1.6 ± 0.5 and 0.7 ± 0.1 for repressing and inducing conditions, respectively (Figure 3B). These results are identical to our observations in WT cells (Figure 1). This suggests that INO80 is present at the *INO1* promoter and is unaffected by the absence of SWI/SNF under repressing conditions. Therefore, we concluded that INO80 is directly recruited by the Ino2p activator and is independent of SWI/SNF.

In *ino80Δ* cells, the *URS/POL* ratios obtained from Snf2-IP were 0.9 ± 0.1 and 1.0 ± 0.2 from cells under repressing and inducing conditions, respectively (Figure 3A, lanes 1 & 2; Figure

3B). The relative ratios from the real-time PCR analysis were 1.0 ± 0.2 and 0.8 ± 0.2 for repressing and inducing conditions, respectively (Figure 3B). As shown, the ratios were similar to those obtained from *ino2Δ* cells (Figure 2). This indicates that SWI/SNF is absent at the *INO1* promoter in the absence of INO80. Therefore, the Ino2p-dependent recruitment of INO80 is the key event for SWI/SNF recruitment. This implies that INO80 arrives prior to SWI/SNF at the *INO1* promoter and that INO80 is necessary for recruiting SWI/SNF.

Discussion

We have shown that SWI/SNF is constitutively present at the promoter and a significant amount departs from the *INO1* promoter following induction (Figure 1). INO80 is also present at the *INO1* promoter and it completely departs from the *INO1* promoter after induction. Since our previous study demonstrated that INO80 and SWI/SNF are required for localized nucleosome movement during *INO1* induction [8], our current findings clearly prove that both INO80 and SWI/SNF directly mobilize nucleosomes at the *INO1* promoter. Furthermore, we observed that both remodelers are absent at the *INO1* promoter under both conditions in the *ino2Δ* strain (Figure 2), suggesting that the Ino2p activator plays an essential role in recruiting both remodelers. Thus, INO80 and SWI/SNF are clearly activator-dependent chromatin remodelers.

SWI/SNF and INO80 are present at the *INO1* promoter and are absent on the *INO1 ORF* (Figure 1), suggesting that nucleosomes in the *ORF* are not subject to remodeling and only nucleosomes in the promoter are remodeled. Consistent with these findings, previous chromatin structure analysis demonstrated localized nucleosome movement relegated to the *INO1* promoter region ranging from -389 to +76 relative to the transcription start site, which includes Nucleosomes B, C, D, and E [8]. Since the primers we used for the *URS* sequence encompass Nucleosomes B and C and part of Nucleosome D (ranging from -365 to -122 relative to the transcription start site), our observations provide direct evidence that INO80 and SWI/SNF contribute to local nucleosome mobilization at the *INO1* promoter.

Our data showed that the presence of INO80 is essential for SWI/SNF recruitment but that INO80 recruitment is SWI/SNF-independent (Figure 3). Previous microarray analysis has shown that deletion of *INO80* did not affect *Snf2p* expression [20]. As such, the absence of SWI/SNF at the promoter observed in *ino80Δ* strains is not due to the decreased expression of SWI/SNF, but is instead due to the loss of INO80. Thus, INO80 is recruited to the promoter followed by SWI/SNF recruitment. Based on these results, it is possible that SWI/SNF may be recruited by INO80 or in concert with Ino2p/Ino4p.

From our work and that of others, we propose a model to describe how several factors act on *INO1* chromatin during gene activation (Figure 4). Under repressing conditions, Opi1p, Ino2p/Ino4p and Ume6p-Sin3p-Rpd3p complex are present at the promoter [13,14,21,22]. The histone tails of Nucleosomes B, C, D, and E are deacetylated by the Ume6p-Sin3p-Rpd3p complex, a corepressor-deacetylase complex [8,21,22]. Meanwhile, both INO80 and SWI/SNF are also present at the promoter. Although the transcriptional activator and chromatin remodelers are present at the promoter under repressing conditions, the transcriptional activator is quiescent in the presence of Opi1p [15]. The remodelers remain inactive and the chromatin is in a static state. Under inducing conditions, the Ume6p-Sin3p-Rpd3p complex and Opi1p depart from the promoter [23], the Ino2p/Ino4p activator becomes functional and the histone modifying enzymes are recruited to the promoter via either the activator or remodelers. Subsequently, the histone tails of Nucleosomes B, C, D, and E are subject to histone acetylation. Meanwhile, the activity of SWI/SNF and INO80 is stimulated by the functional Ino2p/Ino4p, resulting in the targeted movement of Nucleosomes B, C, D, and E. As a result, chromatin remodeling occurs at the promoter region. Once the movement has commenced, INO80 and some SWI/SNF are no longer needed and depart from the promoter before *INO1* induction is

completed. This dynamic nucleosomal flux allows RNA polymerase II access to its binding site and transcription is initiated. It is possible that the remaining SWI/SNF is responsible for moving Nucleosomes B, C, D, and E back to their original positions when the system returns to repressing conditions.

We have proposed the mechanism of *INO1* induction. Our model demonstrates how a functional transcriptional activator stimulates chromatin remodelers in the absence of a repressor. A similar finding has also been observed for *HIS3*, which involves the constitutive presence of two different chromatin remodeling complexes (7). Perhaps such an activation pathway is quite general. However, the current model leaves several questions unanswered. For example, what recruits the histone acetylase is still unclear. Further analysis is necessary to uncover the specific interplay between the transcriptional activator, remodelers, and histone modifying enzymes, which will provide key insight into the specific order of events in gene activation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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Figure 1. Both SWI/SNF and INO80 are present at the *INO1* promoter

(A) Multiplex PCR analysis of DNA immunoprecipitated with antibodies against the Snf2p ATPase subunit of SWI/SNF (α -Snf2 IP) and the Arp8p subunit of INO80 (α -Arp8 IP) for WT cells. The amounts of DNA in the immunoprecipitates were measured by multiplex PCR with end-labeled PCR primers. The URS was a 245-bp *INO1* promoter fragment, and the POL was a 180-bp *POL1* ORF fragment. (B) Quantification of multiplex and real-time PCR. For multiplex PCR, the amounts of DNA in the IP were measured using a phosphorimager. The ratios for the *INO1* promoter to *POL1* ORF are graphed as mean $\pm$ standard deviation. M: multiplex PCR. R: real-time PCR. (C) SWI/SNF and INO80 are not present at the *INO1* ORF. The ratios for the *INO1* ORF to *POL1* ORF are shown as mean $\pm$ standard deviation for

both α -Snf2 IP and α -Arp8 IP under repressing and inducing conditions. The *INO1 ORF* was a 207-bp *INO1* coding region fragment. *: $p < 0.05$, Student's t test.

Figure 2. The Ino2p activator is required for the recruitment of both SWI/SNF and INO80 at the *INO1* promoter

(A) Multiplex PCR analysis of DNA immunoprecipitated by antibodies against Snf2p (α -Snf2 IP) and Arp8p (α -Arp8 IP) for the *ino2Δ* cells. (B) Quantification of multiplex and real-time PCR analysis. Annotation was as described in the legend of Figure 2.

Figure 3. INO80 is required for SWI/SNF recruitment

(A) Multiplex PCR analysis of DNA immunoprecipitated using antibodies against the Snf2p ATPase subunit of SWI/SNF (α -Snf2 IP) for the *ino80Δ* strain. Likewise, antibodies against the Arp8p subunit of INO80 (α -Arp8 IP) were used for the *snf2Δ* strain. (B) Quantification of multiplex and real-time PCR analysis. Annotation was as described in the legend of Figure 2.

Figure 4. A working model for transcriptional activation of *INO1* chromatin

Under repressing conditions, Opi1p interacts with Ino2p/Ino4p, which is bound to the UAS_{INO} element. Ume6p-Sin3p-Rpd3p complex is also present at the promoter and Nucleosomes B, C, D, and E are deacetylated. Both SWI/SNF and INO80 are present at the promoter, but their activity is minimized as they are not stimulated by the non-functional activator. Under inducing conditions, Opi1p dissociates from Ino2p activator, and departs from the promoter (23). The Ino2p/Ino4p activator becomes functional, stimulating the activity of SWI/SNF and INO80, resulting in nucleosome movement from Nucleosomes B through E (indicated by the arrows) (8). After the initiation of the mobilization, INO80 and part of SWI/SNF depart from the promoter. Meanwhile, the Ume6p-Sin3p-Rpd3p complex departs from

the promoter and the histone modifying enzymes are recruited. The histone tails of Nucleosomes B through E are acetylated. The resulting nucleosomal flux would allow the transcription initiation complex to access its binding site on the DNA template. As a result, *INO1* becomes activated.