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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.

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, 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

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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. [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 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 (Fig. 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 (Fig. 1A, lane 7 and Fig. 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

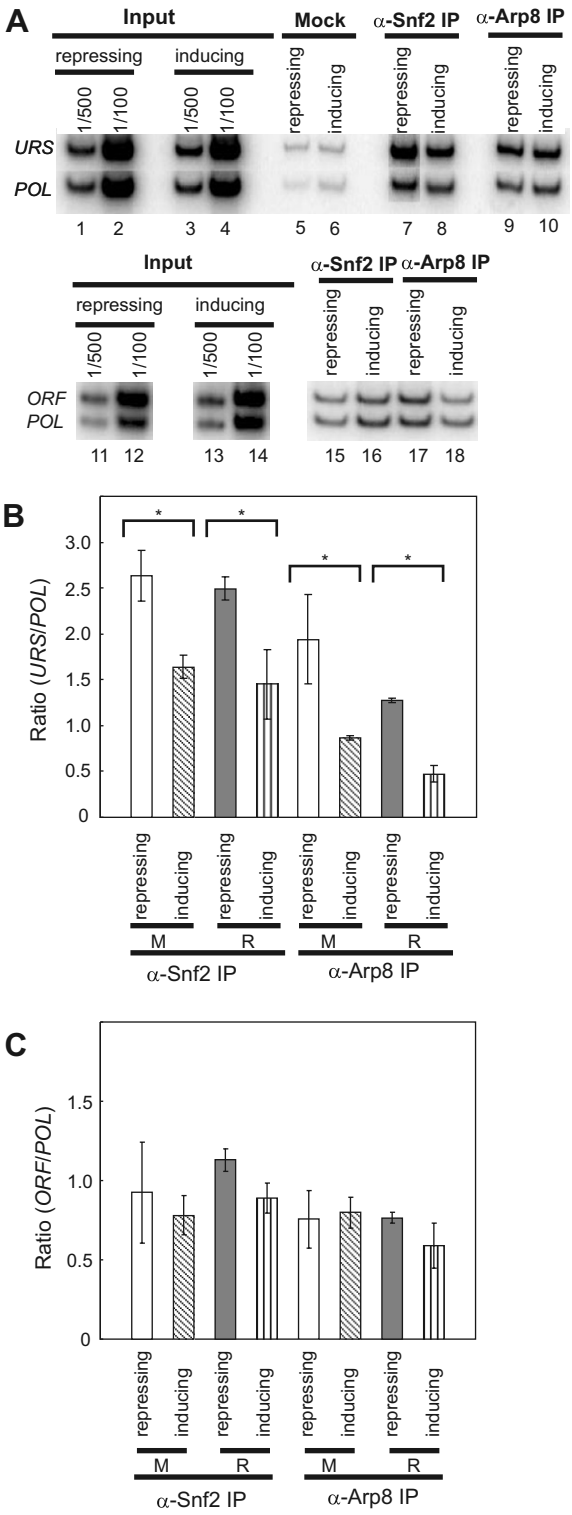

Fig. 1. Both SWI/SNF and INO80 are present at the *INO1* promoter. (A) Multiplex PCR analysis of DNA immunoprecipitated with antibodies against the Snf2p ATPase subunit of SWI/SNF (α-Snf2-IP) and the Arp8p subunit of INO80 (α-Arp8-IP) for WT cells. The amounts of DNA in the immunoprecipitates were measured 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 ± 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 ± 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.