Chromatin remodeler Ino80C acts independently of H2A.Z to evict promoter nucleosomes and stimulate transcription of highly expressed genes in yeast

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ABSTRACT

The chromatin remodelers SWI/SNF and RSC function in evicting promoter nucleosomes at highly expressed yeast genes, particularly those activated by transcription factor Gcn4. Ino80 remodeling complex (Ino80C) can establish nucleosome-depleted regions (NDRs) in reconstituted chromatin, and was implicated in removing histone variant H2A.Z from the −1 and +1 nucleosomes flanking NDRs; however, Ino80C’s function in transcriptional activation in vivo is not well understood. Analyzing the cohort of Gcn4-induced genes in ino80Δ mutants has uncovered a role for Ino80C on par with SWI/SNF in evicting promoter nucleosomes and transcriptional activation. Compared to SWI/SNF, Ino80C generally functions over a wider region, spanning the −1 and +1 nucleosomes, NDR and proximal genic nucleosomes, at genes highly dependent on its function. Defects in nucleosome eviction in ino80Δ cells are frequently accompanied by reduced promoter occupancies of TBP, and diminished transcription; and Ino80 is enriched at genes requiring its remodeler activity. Importantly, nuclear depletion of Ino80 impairs promoter nucleosome eviction even in a mutant lacking H2A.Z. Thus, Ino80C acts widely in the yeast genome together with RSC and SWI/SNF in evicting promoter nucleosomes and enhancing transcription, all in a manner at least partly independent of H2A.Z editing.

INTRODUCTION

In the yeast Saccharomyces cerevisiae, most genes transcribed by RNA Polymerase II (Pol II) contain a nucleosome-depleted region (NDR) of ∼120 bp situated upstream of the coding sequences (CDS) flanked by highly positioned ‘−1’ and ‘+1’ nucleosomes, with the transcription start site (TSS) frequently located within the +1 nucleosome (henceforth +1_Nuc). Given its proximity to the TSS and adjacent upstream promoter elements, the +1_Nuc can interfere with formation of the preinitiation complex (PIC) and initiation of transcription. Accordingly, the +1_Nuc is frequently evicted (1–3) or shifted in the 3′ direction (4–6) in the course of promoter activation. Certain highly regulated genes, in their repressed states, also exhibit appreciable nucleosome occupancies within NDRs, that may occlude transcription factor binding sites in upstream activation sequences (UASs) as well as promoter elements (7–9). The yeast ATP-dependent chromatin remodeling (CR) complexes SWI/SNF and RSC, capable of sliding or evicting nucleosomes in vitro, function to eliminate nucleosomes from NDRs, and also to reposition the +1 and −1 nucleosomes to maintain proper NDR widths. While RSC is important for establishing proper NDR widths at the majority of yeast genes (10–12,8,9), SWI/SNF partners with RSC in performing this function, and also stimulating transcription, at the subset of most highly expressed genes, including those activated by the starvation-induced transcription factor Gcn4 (3,6). Furthermore, both RSC and SWI/SNF are enriched at locations both 5′ and 3′ of NDRs and at promoter-proximal genic nucleosomes throughout the yeast genome (13,14); and both CRs are enriched at the most highly expressed genes in yeast (6,15).

Whereas RSC functions broadly to exclude the −1 and +1 nucleosomes from NDRs, the CRs ISW1 and CHD1

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have overlapping functions in establishing the regular spacing and phasing of genic nucleosomes in the CDS at most genes (16–18). In accordance with this, one or more ISWI complexes were required in addition to RSC to partially reconstitute the formation of NDRs and assembly of phased nucleosome arrays on genomic DNA in vitro (19). Interestingly, the CR Ino80C was capable on its own of reconstituting a nucleosomal organization approximating the native state (19); even though deletion of \textit{INO80}, encoding the catalytic subunit of Ino80C, did not confer a strong genome-wide defect in NDR formation or nucleosome phasing in cells. In fact, on average, the +1\_Nuc was found to shift downstream in \textit{ino80Δ} cells (17), in contrast to the widespread upstream-shift of +1\_Nucs and attendant NDR narrowing observed in RSC mutants. Certain genes did display upstream-shifts of the +1\_Nuc in \textit{ino80Δ} cells, which in a few cases was associated with reduced transcription (17); however, Ino80C did not appear to act broadly to evict or slide +1\_Nucs away from NDRs or promoters in otherwise WT cells. More recently, it was shown that Ino80C acts to restore, rather than remove, promoter nucleosomes following their rapid eviction in response to osmotic stress, and thereby prevent prolonged transcriptional activation of certain stress-induced genes (20).

Ino80C has also been implicated in nucleosome editing, catalyzing replacement of the histone variant H2A.Z (encoded by \textit{HTZ1}) with canonical H2A by replacing an H2A.Z:H2B dimer in an assembled nucleosome with an H2A:H2B dimer (21–23). The +1\_Nuc is enriched for H2A.Z and appears to be preferentially evicted from promoters compared to H2A-containing +1\_Nucs during gene activation (24–26). H2A.Z is required for wild-type transcriptional activation of a subset of yeast genes (27), including induced \textit{GAL} genes (24,28) and various genes induced by heat-shock (25). These findings, plus the fact that H2A.Z is present at low levels at genes expressed at high constitutive levels (25,29) led to a model that the presence of H2A.Z enhances eviction of the +1\_Nuc and thereby poises the promoter for rapid activation. It has been suggested that removal of an H2A.Z:H2B dimer by Ino80C would render the remaining partially disassembled nucleosome more susceptible to complete eviction from the DNA (30,31), thereby enhancing eviction of +1\_Nucs. Consistent with this model, a defect in eviction of H2A.Z-containing +1\_Nucs was observed at the induced \textit{KAR4} promoter in \textit{ino80Δ} cells (21). Moreover, eliminating the Arp5 subunit of Ino80C conferred a strong increase in H2A.Z at the +1\_Nuc (and regions upstream) and a concomitant decrease in turnover of the +1\_Nuc (30); and reduced eviction of H2A.Z was observed in an \textit{ino80Δ�900} truncation mutant at the induced \textit{GAL1, GAL7 and PHO5} genes (32).

Other evidence however does not support the model that Ino80C is responsible for specifically disassembling and evicting H2A.Z-containing +1\_Nucs. This nucleosome editing activity was not observed in a purified system in which the ability of the related SWR1 complex to exchange H2A—H2B dimers for H2A.Z—H2B dimers was reconstituted (33). In addition, H2A.Z occupancies at promoters were found to be unchanged genome-wide both in an \textit{ino80Δ} mutant (34), or when Ino80 was depleted from the nucleus (29). The latter study also indicated that eviction of H2A.Z-containing +1\_Nucs and their replacement with H2A-containing nucleosomes occurs normally in the course of PIC assembly or transcription initiation (29). In addition, the defect in \textit{GAL1} and \textit{GAL7} induction conferred by the \textit{ino80Δ�900} mutation was greater than that given by \textit{htz1Δ}, suggesting an H2A.Z-independent role for Ino80C in remodeling promoter nucleosomes containing H2A (32). The latter possibility is also consistent with the limited overlap (∼10%) between sets of genes exhibiting reduced expression in \textit{ino80Δ} versus \textit{htz1Δ} cells (27). Interestingly, Ino80 also functions in coordination with the Mot1 and NC2 factors to suppress non-coding transcripts initiating from many Pol II promoters (35).

Despite conflicting evidence concerning the role of Ino80C in evicting H2A.Z from +1\_Nucs, we were intrigued by the similar genome-wide occupancy patterns observed for Ino80C, RSC and SWI/SNF, with enrichment over the −1, +1, and promoter-proximal genic nucleosomes (13); and also by studies implicating Ino80C in chromatin remodeling and activation of \textit{PHO5} (36) and \textit{GAL} genes (32). Accordingly, we wondered whether Ino80C cooperates with RSC and SWI/SNF in evicting promoter nucleosomes at the cohort of genes induced by Gcn4. Production of Gcn4 is up-regulated at the translational level by amino acid starvation and induces transcription of hundreds of genes, including nearly all amino acid biosynthetic genes (37). A subset of genes induced by Gcn4 during starvation for isoleucine and valine (Ile/Val) were shown to exhibit substantial eviction of promoter nucleosomes, as well as repositioning of the −1 and +1 nucleosomes with attendant NDR widening. The eviction of promoter nucleosomes on Gcn4 induction is stimulated by SWI/SNF and RSC, as double mutants lacking both CRs exhibit more pronounced increases in promoter nucleosome occupancies than do the corresponding single mutants. Additive reductions in transcriptional activation by Gcn4 also occurred in the CR double mutant, particularly for the genes most highly induced and remodeled in WT cells. Similar functional cooperation between SWI/SNF and RSC was observed for the subset of constitutively transcribed genes with the highest Pol II occupancies, which like Gcn4-induced genes, also show enrichment for both CRs compared to all genes (6).

In this report, we provide evidence that Ino80C is required, in addition to SWI/SNF and RSC, for efficient promoter nucleosome eviction in the Gcn4 transcriptome, and at a group of several hundred genes that are unusually dependent on Ino80C for promoter nucleosome remodeling. We also obtained evidence that Ino80C functions more broadly than SWI/SNF and RSC in nucleosome eviction across the interval spanning the −1\_Nuc, NDR, and +1\_Nuc; and we show that defects in nucleosome eviction in cells lacking Ino80C are frequently associated with reduced TBP occupancies and, hence, PIC assembly in promoters. Finally, we demonstrate that Ino80C enhances promoter nucleosome eviction in cells lacking H2A.Z, indicating that Ino80C can function analogously to SWI/SNF and RSC in evicting promoter nucleosomes containing canonical H2A apart from its proposed role in H2A.Z editing.
MATERIALS AND METHODS

Yeast strain constructions

Yeast strains used in this study are listed in Table 2. WT strain BY4741 and kanMX4-marked deletion derivatives were described previously (38) and purchased from Research Genetics, and all deletions in these strains were verified by PCR analysis of genomic DNA. YR092 (ino80ΔΔ) was generated by tetrad dissection from heterozygous inO80/inO80Δ::kanMX strain Y24517 purchased from EUROscarf. Myc13-tagged INO80 was generated by tetrad dissection from heterozygous inO80 in HQY1687 was constructed by a PCR-based method for tagging chromosomal genes by yeast transformation (39), using plasmid pFA6a-13Myc-His3MX6 DNA as template. To construct the anchor away strains, the tor1–1 allele was first introduced into BY4741 by transformation with the tor1–1 URA3 integrating plasmid pHQ2135 (constructed as described below) after linearizing at position +5748 in the tor1–1 CDS by digestion with NruI to direct integration into the TOR1 locus. The resulting transformants were cultured on medium containing 5-FOA to select for the Ura− segregant HQY1693 in which the URA3 sequences were lost and TOR1 was replaced by tor1–1. The presence of the tor1–1 mutation in HQY1693 encoding substitution S1972I as well as a second, conservative substitution K2414R also encoded in pHQ2135 was confirmed by DNA sequencing of a PCR fragment amplified from the TOR1 locus of the genomic DNA of HQY1693, and by demonstrating rapamycin-resistant growth of HQY1693 compared to the parental strain BY4741 on YPD medium supplemented with 1 μg/ml rapamycin. Second, the fpr1Δ::kanMX4 allele was introduced into HQY1693 by gene replacement with a fpr1Δ::kanMX4 PCR fragment amplified from genomic DNA of fpr1Δ::kanMX4 strain 2941 to produce HQY1694, which was verified by PCR analysis of the FPR1 locus in HQY1694. Finally, RPL13A in HQY1694 was C-terminally tagged with the coding sequences for two tandem copies of FKBP12 (2xFKBP12) by transformation with a DNA fragment that was PCR-amplified from plasmid pFA6a-2xFKBP12-HIS3MX6 (obtained from EUROscarf), to produce HQY1695, which was verified by PCR analysis of the RPL13A locus in HQY1695. INO80 in HQY1695 was C-terminally tagged with FRB-GFP, producing HQY1697, using a PCR-based method for tagging chromosomal genes by yeast transformation (39) and pHQ2134 (described below) as template. The htz1Δ::natMX4 fragment was introduced into HQY1695 and HQY1697 by gene replacement with an htz1Δ::natMX4 DNA fragment from pHQ2140 (described below) to produce HQY1702 and HQY1701, respectively, and the presence of htz1Δ::natMX4 in these strains was verified by PCR analysis of the HTZ1 locus in the genomic DNA of these strains.

Plasmid constructions

To construct INO80 plasmid pHQ2096, a DNA fragment containing 717 bp of 5′ non-coding DNA, the 4470 bp ORF and 278 bp of 3′ non-coding DNA of INO80 was PCR-amplified from genomic DNA of strain BY4741 with the addition of an EcoRI site at the 5′ end and a HindIII site at the 3′ end and inserted between the corresponding sites of vector YCplac33 (40). To construct integrative tor1–1 plasmid pHQ2135, a PCR fragment containing nucleotides +5503 to +7431 from the 3′ end of the ORF of the tor1–1 allele was PCR-amplified from genomic DNA of tor1–1 strain HHY221 with the addition of a BamHI site at the 5′ end and a SacI site at the 3′ end and inserted between the corresponding sites of Ylpac211 (40). DNA sequence analysis of pHQ2135 revealed the presence of the mutations encoding substitutions S1972I and K2414R mutations. pHQ2134 (pFA6a-FRB-GFP-hphMX4) was constructed by replacing BglIII/SacI-cut HisMX6 fragment of pFA6a-FRB-GFP-HisMX6 (41) with BglII/SacI-cut hphMX4 fragment from pFA6-hphMX4 (42). To construct pHQ2140, 393 bp 5′ promoter of HTZ1 with HindIII/EcoRV sites at 5′ end and EcoRV/HpaI sites at 3′ end was first introduced at HindIII/SacI site at 5′ end as well as SacI–HpaI site. htz1Δ::natMX4 DNA fragment can be cut out by EcoRV and used for gene replacement to make htz1Δ::natMX4.

ChIP-seq analysis of H3, H2B, Rpb3, TBP and Ino80-myc genome-wide occupancies

WT and mutant strains were cultured in synthetic complete medium lacking isoleucine and valine (SC-Iv) to A600 of 0.6–0.8 and sulfometuron methyl (SM) was added at 1 μg/ml for 25 min to induce Gcn4 synthesis prior to treating cells with formaldehyde as previously described (3). ChIP-seq was conducted (43) with modifications described in (3) using polyclonal antibodies against H3 (Abcam, ab1791), H2B (Abcam, ab1790) and TBP (a generous gift from Joe Reese), and monoclonal antibodies against Rpb3 (Neoclon, W0012) and myc epitope (Sigma, 11667203001). DNA libraries for Illumina paired-end sequencing were prepared using DNA Library Prep Kit for Illumina from New England Biolabs (E7370L).

For anchor away strains, yeast cells were cultured in synthetic complete medium lacking isoleucine and valine (SC-Iv) to A600 of 0.3–0.4. rapamycin was added to 1 μg/ml and incubation continued for 1.5 h, after which SM was added to 1 μg/ml for 25 minutes to induce Gcn4 synthesis prior to treating cells with formaldehyde. Western blot analysis revealed nearly WT levels of Gcn4 following SM treatment of all four anchor away strains under study, with the possible exception of the INO80::FRB single mutant (Supplementary Figure S1D).

Paired-end sequencing (50 nt from each end) was conducted by the DNA Sequencing and Genomics core facility of the NHLBI, NIH. Sequence data were aligned to the SacCer3 version of the genome sequence using Bowtie2 (44) with parameters -X 1000 -very-sensitive, to map sequences up to 1 kb with maximum accuracy. PCR duplicates from ChIP-seq data were removed using the samtools rmdup package. Numbers of aligned paired reads from each ChIP-seq experiment and correlation coefficients for genome-wide occupancy profiles of the different replicates are summarized in Table 1. Raw genome-wide occupancy profiles for H3, H2B, TBP, Rpb3 and Ino80-myc were computed using the coverage function in R. To allow the com-
| Strain (genotype/condition) | Sample Name         | All PE reads | PE rmdup reads | Pearson correlation between replicates | Source         |
|-----------------------------|---------------------|--------------|----------------|----------------------------------------|----------------|
| BY4741 (WT, uninduced)      | AGH0220-1 (repl. 1) | 12,305,230   | 11,468,229     | 0.9654 0.9669 0.8495 0.9069 0.9107 | Qiu et al., 2016 |
|                            | AGH0220-2 (repl. 2) | 11,725,253   | 10,818,529     | 0.9640 0.8502 0.9036 0.9044           | Qiu et al., 2016 |
|                            | AGH0220-3 (repl. 3) | 12,511,668   | 11,629,718     | 0.8675 0.9157 0.9145                   | Qiu et al., 2016 |
|                            | AGH58-02 (repl. 4)  | 29,625,671   | 8,011,769      | 0.9229 0.9084                         | Rawal et al., 2018 |
|                            | AGH62-01 (repl. 5)  | 14,993,257   | 8,597,216      | 0.9661                                 | Rawal et al., 2018 |
|                            | AGH62-02 (repl. 6)  | 19,032,734   | 9,965,205      |                                        | Rawal et al., 2018 |
| BY4741 (WT, induced)        | AGH0220-4 (repl. 1) | 9,947,964    | 7,013,803      | 0.9466 0.9387 0.9030 0.9370 0.9150 | Qiu et al., 2016 |
|                            | AGH0220-5 (repl. 2) | 10,810,351   | 10,096,814     | 0.9678 0.8715 0.8715 0.9927 0.9014   | Qiu et al., 2016 |
|                            | AGH58-04 (repl. 4)  | 20,620,578   | 5,466,934      | 0.9364 0.9325                         | Rawal et al., 2018 |
|                            | AGH62-02 (repl. 6)  | 19,798,491   | 5,044,590      |                                        | Rawal et al., 2018 |
| YR092 (ino80Δ, uninduced)  | AGH61-1 (repl. 1)   | 15,593,488   | 7,748,141      | 0.9254 0.9187                         | This study      |
|                            | AGH61-2 (repl. 2)   | 17,669,783   | 7,611,094      | 0.9165                                 | This study      |
|                            | AGH61-3 (repl. 3)   | 15,860,473   | 7,595,531      |                                        | This study      |
| 1586 (snf2Δ, uninduced)    | AGH57-4 (repl. 1)   | 23,934,378   | 15,075,565     | 0.9725 0.9751                         | Rawal et al., 2018 |
|                            | AGH57-5 (repl. 2)   | 24,825,347   | 15,914,213     | 0.9740                                 | Rawal et al., 2018 |
|                            | AGH57-6 (repl. 3)   | 26,587,486   | 16,214,184     |                                        | Rawal et al., 2018 |
| 1586 (snf2Δ, induced)      | AGH0406-1 (repl. 1) | 15,597,956   | 11,985,536     | 0.9633 0.8814 0.9054 0.8974 0.8700   | Qiu et al., 2016 |
|                            | AGH0406-2 (repl. 2) | 17,071,372   | 13,099,928     | 0.8714 0.9158 0.9069 0.8826           | Qiu et al., 2016 |
|                            | AGH25-1 (repl. 3)   | 24,839,200   | 11,548,218     | 0.7962 0.7990 0.7200                   | Qiu et al., 2016 |
|                            | AGH59-01 (repl. 4)  | 14,174,694   | 7,052,399      | 0.9507 0.9473                         | Rawal et al., 2018 |
|                            | AGH59-02 (repl. 5)  | 14,573,693   | 6,456,125      | 0.9447                                 | Rawal et al., 2018 |
|                            | AGH62-05 (repl. 6)  | 20,877,009   | 10,613,818     |                                        | Rawal et al., 2018 |
| 1703 (htz1Δ, induced)      | AGH90-10 (repl. 1)  | 36,624,977   | 28,417,237     | 0.9877 0.9900                         | This study      |
|                            | AGH90-11 (repl. 2)  | 31,409,513   | 25,127,842     | 0.9861                                 | This study      |
|                            | AGH90-12 (repl. 3)  | 30,781,599   | 24,403,241     |                                        | This study      |
### Table 1. Continued

| Strain (genotype/condition) | Sample Name | All PE reads | PE rmdup reads | Pearson correlation between replicates | Source |
|----------------------------|-------------|--------------|---------------|----------------------------------------|--------|
|                            |             | repl. 2      | repl. 3       |                                        |        |
| HQY1695 (WT<sup>AA</sup>, uninduced) | AGH100-1 (repl. 1) | 19,370,175 | 15,944,625 | 0.9753 | 0.9688 | This study |
|                            | AGH100-2 (repl. 2) | 22,514,696 | 17,298,472 | 0.9695 | | This study |
|                            | AGH100-3 (repl. 3) | 20,992,271 | 17,245,898 | | | This study |
|                            | AGH100-5 (repl. 5) | 22,553,174 | 17,991,047 | | | This study |
|                            | AGH98-4 (repl. 1) | 27,584,074 | 20,131,579 | 0.9814 | 0.9830 | 0.9742 | 0.9735 | This study |
|                            | AGH98-5 (repl. 2) | 28,102,784 | 20,662,142 | 0.9833 | 0.9767 | 0.9743 | | This study |
|                            | AGH98-6 (repl. 3) | 30,309,789 | 21,836,731 | 0.9760 | 0.9740 | | | This study |
|                            | AGH100-4 (repl. 4) | 26,084,944 | 20,789,259 | 0.9767 | | | | This study |
|                            | AGH100-9 (repl. 6) | 21,703,344 | 17,451,155 | | | | | This study |
|                            | AGH80-4 (repl. 1) | 17,452,986 | 12,530,495 | 0.9651 | 0.9638 | 0.9233 | 0.9214 | 0.9201 | This study |
|                            | AGH80-5 (repl. 2) | 13,248,782 | 9,984,908 | 0.9627 | 0.9144 | 0.9105 | 0.9093 | | This study |
|                            | AGH80-6 (repl. 3) | 14,501,763 | 10,091,234 | 0.9256 | 0.9217 | 0.9230 | | | This study |
|                            | AGH100-9 (repl. 4) | 22,391,615 | 18,234,968 | 0.9780 | 0.9751 | | | This study |
|                            | AGH100-10 (repl. 5) | 32,054,634 | 24,850,638 | 0.9778 | | | | This study |
|                            | AGH100-11 (repl. 6) | 27,128,333 | 21,572,319 | | | | | This study |
|                            | AGH98-10 (repl. 1) | 22,514,696 | 17,298,472 | 0.9695 | | | | This study |
|                            | AGH98-11 (repl. 2) | 28,605,692 | 20,789,259 | 0.9767 | | | | | This study |
|                            | AGH98-12 (repl. 3) | 25,309,789 | 21,836,731 | 0.9760 | 0.9740 | | | This study |
|                            | AGH100-4 (repl. 4) | 26,084,944 | 20,789,259 | 0.9767 | | | | | This study |
|                            | AGH100-9 (repl. 6) | 21,703,344 | 17,451,155 | | | | | This study |
|                            | AGH82-4 (repl. 1) | 22,218,508 | 15,268,906 | 0.9644 | 0.9656 | 0.8698 | 0.8707 | 0.8648 | This study |
|                            | AGH82-5 (repl. 2) | 22,684,745 | 14,817,919 | 0.9649 | 0.8689 | 0.8690 | 0.8633 | | This study |
|                            | AGH82-6 (repl. 3) | 25,116,981 | 16,711,191 | 0.8700 | 0.8719 | 0.8626 | | | This study |
|                            | AGH100-12 (repl. 4) | 28,535,817 | 22,639,530 | 0.9709 | 0.9666 | | | | This study |
|                            | AGH100-13 (repl. 5) | 27,284,181 | 22,079,923 | 0.9620 | | | | | This study |
|                            | AGH100-14 (repl. 6) | 30,465,588 | 23,904,663 | | | | | | This study |

<sup>1</sup>PE: reads from paired-end sequencing  
<sup>2</sup>PE rmdup: reads from paired-end sequencing after removing duplicate reads

Comparison between different samples, each profile was normalized to an average of 1 for each chromosome. Heat maps showing alignments of multiple loci were generated in R using custom scripts (https://github.com/rchereji/bamR). To visualize specific loci, BigWig files of samples were loaded in the Integrative Genomics Viewer (IGV) (45). Transcript end coordinates (TSS and TTS) were obtained from Pelechano et al. (2013). 

MACS2 (http://liulab.dfci.harvard.edu/MACS/) was employed to identify TBP binding sites from the TBP ChIP-seq data using two replicate WT<sub>I</sub> cultures and three replicate WT<sub>I</sub>, ino80<sup>ΔI</sup> and ino80<sup>ΔI</sup> cultures (Table 1, TBP ChIP-seq), using a threshold for the q-value of 10<sup>-3</sup>. The TBP occupancy peaks called by MACS2 for 204 SM-induced genes were assessed manually in the Integrative Genomics Viewer (IGV, Broad Institute), which identified TBP peaks in the promoters of 53 genes and 63 genes in the groups of 70 and 134 SM-induced genes, respectively. TBP occupancies in Figure 7B and Supplementary Figure S4B for these peaks were calculated for 161 bp intervals surrounding the summit of each peak by using the custom R script 'plot_binding_at_given_sites.R', modified for a 161 bp window, available at github.com/rchereji/bamR. 

Spearman rank correlations of H3, TBP, Rpb3 and Ino80-myc occupancy changes between WT<sub>I</sub> versus WT<sub>U</sub> or WT<sub>I</sub> versus mutant ino80Δ<sub>I</sub> (tabulated in File S4) were analyzed using Prism8 statistics software.

**RESULTS**

Ino80C enhances eviction of promoter nucleosomes and transcriptional activation of SM-induced genes

Depriving yeast cells of an amino acid, including starvation for Ile/Val achieved with the inhibitor sulfometuron methyl (SM), leads to a substantial reprogramming of transcription, mainly dependent on the transcriptional activator Gcn4 (46–48). By ChIP-seq analysis of Pol II subunit Rpb3,
we previously identified 204 genes showing ≥2-fold induction of Rpb3 occupancies on SM treatment; and ChIP-seq analysis of histone H3 revealed that 70 of these SM-induced genes exhibit a marked reduction in nucleosome occupancy in the interval spanning the consensus positions of the −1_Nuc and +1_Nuc and intervening NDRs at those genes (3). Repeating these experiments with snf2Δ cells lacking the catalytic subunit of SWI/SNF, or in cells depleted of the essential catalytic subunit of RSC (Sth1), we had observed diminished nucleosome eviction for the group of 70 highly-remodeled genes on SM induction; and relatively stronger defects in the double mutant lacking Snf2 and Sth1, suggesting functional cooperation between SWI/SNF and RSC in promoter nucleosome eviction (6). In view of predictions that Ino80C acts to edit the +1_Nucs containing H2A.Z, and that the partially disassembled nucleosomes might be highly susceptible to eviction, we asked whether Ino80C is also required for efficient nucleosome eviction at Gcn4 target genes.

To measure nucleosome eviction, we used ChIP-Seq analysis of sonicated chromatin employing antibodies against H3 or H2B, and quantified histone occupancies in the promoter intervals spanning the −1_Nuc, NDR, and +1_Nuc, dubbed the [−1,NDR,+1] region, which had been defined previously for all genes by sequencing nucleosomes released from chromatin by micrococcal nuclease digestion (MNase-Seq) (3). Three or more biological replicates of each condition were analyzed, with high reproducibility of results among the replicates (Table 1), and the replicate datasets were combined for downstream analyses.

ChIP-Seq analyses of WT cells grown in synthetic complete (SC) medium with or without SM treatment revealed similar, marked eviction of both H3 and H2B from the promoter regions of four canonical Gcn4 target genes, ARG1,
### Table 1. Continued

| Strain (genotype/condition) | Sample ID | All PE reads | PE rmdup reads | Pearson correlation between replicates | Source |
|----------------------------|-----------|--------------|----------------|----------------------------------------|--------|
|                            |           | repl. 2      | repl. 3        | repl. 4                                | repl. 5|
| **BY4741 (WT, uninduced)** | AGH03-1 (repl. 1) | 12,103,981 | 7,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH03-2 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH03-3 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **BY4741 (WT induced)**    | AGH03-4 (repl. 1) | 10,978,154 | 7,859,118 | 0.9963 | 0.9957 | Qiu et al., 2016 |
|                            | AGH03-5 (repl. 2) | 16,862,702 | 11,526,037 | 0.9945 | 0.9936 | Qiu et al., 2016 |
|                            | AGH03-6 (repl. 3) | 14,660,604 | 10,042,576 | 0.9938 | 0.9928 | Qiu et al., 2016 |
| **YR092 (ino80Δ induced)** | AGH64-10 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH64-11 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH64-12 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **1586 (snf2Δ induced)**  | AGH12-7 (repl. 1) | 12,103,981 | 7,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH12-8 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH12-9 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **1703 (htz1Δ induced)**  | AGH92-10 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH92-11 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH92-12 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **HQY1695 (AA-WT, uninduced)** | AGH106-1 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-2 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-3 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **HQY1695 (AA-WT, induced)** | AGH106-4 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-5 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-6 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **HQY1695 (AA-htz1Δ, induced)** | AGH106-7 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-8 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-9 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **HQY1697 (INO80-FRB, induced)** | AGH106-10 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-11 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-12 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
| **HQY1701 (AA-htz1Δ INO80-FRB, induced)** | AGH106-13 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-14 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-15 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |
|                            | AGH106-16 (repl. 4) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-17 (repl. 5) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
| **HQY1702 (AA-htz1Δ, induced)** | AGH106-18 (repl. 1) | 17,103,981 | 11,624,104 | 0.9974 | 0.9966 | Qiu et al., 2016 |
|                            | AGH106-19 (repl. 2) | 15,621,065 | 10,271,685 | 0.9938 | 0.9928 | Qiu et al., 2016 |
|                            | AGH106-20 (repl. 3) | 14,624,526 | 8,895,936 | 0.9963 | 0.9957 | Qiu et al., 2016 |

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ARG4, HIS4 and CPA2, on SM-induction (Figure 1A & Supplementary Figure S1A(i)–(iv), light versus dark colors, data within dashed lines). As expected, highly similar results were observed among the three biological replicates for each condition (Supplementary Figure S1B). Averaging results for all 70 SM-induced genes indicated similar reductions in the mean H2B and H3 occupancies in response to SM treatment (WTI versus WTU) in the regions corresponding to the consensus positions of the –1, Nuc, NDR and +1, Nuc at these genes (Figure 1B, WTI versus WTU traces). Using the known sequence coordinates of the [−1,NDR,+1] intervals for each gene (3), and calculating the average H3 or H2B occupancy per nucleotide in these intervals, we identified reductions in median occupancies of H3 and H2B across the promoters of ~47% and ~40%, respectively, of the group of 70 genes on SM-treatment of WT cells (Fig-
## Table 1. Continued

**TBP ChIP-seq**

| Strain (genotype/condition) | Sample ID | All PE reads | PE rmdup reads | Pearson correlation between replicates | Source |
|-----------------------------|-----------|--------------|----------------|----------------------------------------|--------|
|                             |           | repl. 2      | repl. 3        |                                        |        |
| BY4741 (WT, uninduced)      | AGH121-1  | 16,885,453   | 10,236,588     | 0.9992                                 | This study |
|                             | AGH121-2  | 27,562,033   | 12,530,926     | 0.9993                                 | This study |
|                             | AGH121-3  | 22,937,418   | 11,812,400     |                                        | This study |
| BY4741 (WT induced)         | AGH121-4  | 24,785,155   | 14,448,990     | 0.9962                                 | This study |
|                             | AGH121-5  | 4,584,274    | 3,832,277      |                                        | This study |
| YR092 (ino80Δ uninduced)    | AGH121-7  | 26,930,283   | 18,269,275     | 0.9983                                 | This study |
|                             | AGH121-8  | 15,095,156   | 10,094,991     | 0.9994                                 | This study |
| YR092 (ino80Δ induced)      | AGH121-9  | 22,489,806   | 12,887,915     |                                        | This study |

1PE: reads from paired-end sequencing  
2PErmdup: reads from paired-end sequencing after removing duplicate reads

**Ino80-myc ChIP-seq**

| Strain (genotype/condition) | Sample ID | All PE reads | PE rmdup reads | Pearson correlation between replicates | Source |
|-----------------------------|-----------|--------------|----------------|----------------------------------------|--------|
|                             |           | repl. 2      | repl. 3        |                                        |        |
| BY4741 (WT, induced)        | AGH101-17 | 14,884,109   | 12,055,647     | 0.9913                                 | This study |
|                             | AGH101-18 | 11,931,449   | 9,790,387      |                                        | This study |
| HQY1687 (INO80-myc, uninduced) | SMC-4   | 12,766,025   | 10,672,236     | 0.9789                                 | This study |
|                             | SMC-5    | 12,576,299   | 10,692,034     | 0.9829                                 | This study |
|                             | SMC-6    | 14,347,932   | 12,259,057     |                                        | This study |
| HQY1687 (INO80-myc, induced) | SMC-1   | 11,035,014   | 6,454,383      | 0.9878                                 | This study |
|                             | SMC-2    | 11,562,224   | 6,699,211      | 0.9872                                 | This study |
|                             | SMC-3    | 14,314,644   | 10,113,486     |                                        | This study |

1PE: reads from paired-end sequencing  
2PErmdup: reads from paired-end sequencing after removing duplicate reads

## Table 2. Yeast strains used in this study

| Name            | Parent       | Relevant genotype                                                                 | Reference         |
|-----------------|--------------|-----------------------------------------------------------------------------------|-------------------|
| BY4741          | BY4741       | MATαhis3Δ1 leu2Δ met15Δ ura3Δ                                                   | Research Genetics |
| YR092           | BY4741       | MATαhis3Δ1 leu2Δ met15Δ ura3Δ ino80Δ::kanMX4                                    | This work         |
| Y24517          | BY4741       | MATαhis3Δ1 leu2Δ met15Δ ura3Δ his3Δ1/Δ his3Δ1/Δ leu2Δ met15Δ/MET15               | EUROscarf         |
| HQY1687         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ his3Δ1/Δ his3Δ1/Δ met15Δ/Δ MET15                  | Research Genetics |
|                 | SMC-2        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ his3Δ1/Δ his3Δ1/Δ met15Δ/Δ MET15                  | Research Genetics |
| HQY1693         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
|                 | SMC-2        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
| HQY1695         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
| HQY1697         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
| HQY1699         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
| HQY1701         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
| HQY1702         | SMC-1        | MATαhis3Δ1 leu2Δ met15Δ ura3Δ tor1–1                                          | This work         |
Figure 1. Ino80C contributes to promoter nucleosome disassembly, together with SWI/SNF and RSC, at SM-induced genes. (A) IGV profiles of histone H3 and H2B occupancies from ChIP-seq with sonicated chromatin for canonical Gcn4 target gene HIS4, before (darker colors) and after (lighter colors) SM treatment in WT strain (rows 1–2); and SM-treated ino80Δ mutant (darker colors) and WT (lighter colors) (rows 3–4). Dotted lines encompass the relevant [−1, NDR, +1] interval. (B) Plots of H3 and H2B occupancies at each base pair, normalized to the average occupancy on the respective chromosome for each gene, calculated from combined ChIP-Seq data of sonicated chromatin from 3 or more biological replicates, averaged over the 70 SM-induced genes and aligned to the TSS. (C) Notched box plots of H3 and H2B occupancies per nucleotide in the [−1, NDR, +1] region calculated from combined ChIP-seq data for each strain for the 70 SM-induced genes. Each box depicts the interquartile range containing 50% of the data, intersected by the median; the notch indicates a 95% confidence interval (CI) around the median. If the notches of two plots do not overlap, there is 95% confidence that the true medians of the two distributions differ. Asterisks signify significant differences between the mean values connected by brackets according to the Mann–Whitney U test: *** P < 0.001; ** P < 0.01, * P < 0.05. Median values are given in parenthesis below the column labels. (D) Venn diagram depicting involvement of Ino80, Snf2, or Sth1 in promoter nucleosome eviction at the 70 exemplar genes based on effects of the indicated mutations on the mean H3 occupancies per bp in the [−1, NDR, +1] regions determined by H3 ChIP-Seq analysis of three biological replicates each of WT or mutant strains under inducing conditions. Genes included in each set showed significant differences between WT and mutant mean values in the Student’s t-test (P < 0.05). All results were obtained from isogenic WT strain BY4741 and ino80Δ mutant YR092. ChIP-Seq data for isogenic snf2Δ and P_{TET}-STH1 strains were reported previously (Table 1) and presented here for comparison.

ure 1C, columns 1–2 and 5–6), all in good agreement with our previous results (6). (In these and all other notch box plots, non-overlapping notches in two plots indicates that the two medians are significantly different with ≥95% confidence. P-values from Mann–Whitney U tests are also generally indicated with asterisks.) As expected, similar but less pronounced eviction of H3 and H2B was observed for the remaining, less remodeled subset of 134 genes from among the complete group of 204 SM-induced genes (Supplementary Figure S2A, columns 1–2 and 5–6). The H3 and H2B occupancies at these latter 134 genes are somewhat higher in uninduced cells, and also exhibit smaller reductions on SM treatment, compared to the group of 70 genes (Supplementary Figure S2A versus Figure 1C). The similar occupancy reductions observed for H3 and H2B at both sets of genes is consistent with complete disassembly of +1 and −1 nucleosomes during SM-induced transcription.

We next examined the effect of deleting Ino80, catalytic subunit of Ino80C, on promoter nucleosome eviction at the SM-induced genes. Western blot analysis revealed nearly WT levels of Gcn4 protein on SM treatment of an ino80Δ deletion mutant compared to the isogenic WT strain (Supplementary Figure S1D, lanes 1–6). Accordingly, we proceeded with H3/H2B ChIP-seq analyses of this ino80Δ
strain. As illustrated for HIS4 in Figure 1A, both H2B and H3 occupancies were substantially elevated in the promoter region of induced ino80Δ versus WTI cells (rows 3–4, dark versus light colors between dashed lines). The mean occupancies of both H3 and H2B for the group of 70 genes are higher in the SM-treated mutant compared to WT1 cells (Figure 1B, ino80Δ1 versus WT1 traces); and quantification of histone occupancies for each gene revealed increased median occupancies of ~1.4-fold between ino80Δ1 and WT1 cells (Figure 1C, columns 2–3 and 6–7). Box-plot analyses of data from individual replicates (Supplementary Figure S1C) led to the same conclusion reached from Figure 1C, that ino80Δ increases the median H3 occupancies in the promoter regions of the 70 SM-induced genes. H3 and H2B eviction defects were also observed in ino80Δ cells for the group of 134 induced genes (Supplementary Figure S2A, columns 2–3 and 6–7). These findings are similar to those obtained previously for an isogenic snf2Δ mutant (6) included here for comparison (Figure 1C, cf. snf2Δ1 and ino80Δ1 results); except that ino80Δ conferred a significantly greater increase in H3/H2B occupancies compared to snf2Δ for the set of 134 genes (Supplementary Figure S2A, cf. snf2Δ1 and ino80Δ1 results). Thus, it appears that Ino80C functions on par with SWI/SNF in evicting promoter nucleosomes at these two groups of SM-induced genes.

Examining the effect of ino80Δ in cells untreated with SM revealed no significant increases in promoter occupancies of H3 or H2B for the group of 70 SM-induced genes, but significant increases for the less remodeled group of 134 induced genes (Supplementary Figure S2B, C, columns 1–2 and 4–5). This suggests that Ino80C functions constitutively to remove nucleosomes from the promoter regions at many of the 134 induced genes. In contrast, its contribution is substantially greater under inducing versus noninducing conditions for most of the 70 more highly induced genes. The snf2Δ mutation had no effect on H3/H2B occupancies for either group of genes in uninduced cells (Supplementary Figure S2B, C, columns 1, 3 and 4, 6), indicating that its role in nucleosome eviction is limited primarily to inducing conditions, as previously concluded (6).

Using H3 ChIP-seq data from the biological replicates of ino80Δ1 and WT1 cultures, and our previous results on SM-induced snf2Δ cells or P_TET-STH1 cells deplet ed of the catalytic subunit of RSC (6), we determined the subsets of the 70 SM-induced genes showing significantly higher H3 occupancies in the [-1, NDR, +1] intervals in each mutant versus WT cells (P < 0.05). As shown in Figure 1D, there is ≈70% overlap between the groups of 41 and 42 genes showing a significant increase in H3 occupancies on elimination of Ino80 or Snf2, respectively, indicating that Ino80C and SWI/SNF both contribute to promoter nucleosome eviction at the 29 genes found at the intersection of the two gene sets. About 25–30% of the genes requiring one of these CRs did not appear to require the other, at least when each CR was eliminated from otherwise WT cells (Figure 1D, the 12–13 genes in the non-overlapping portions of pink and tan sets). Similar overlaps were seen for the groups of genes showing significantly impaired H3 eviction on depletion of Sth1 versus elimination of Snf2 or Ino80 (Figure 1D), indicating extensive cooperation among the three CRs in promoter nucleosome eviction at SM-induced genes. For the 10 genes that did not overlap with either the Ino80C-, SWI/SNF- or RSC-dependent genes (Figure 1D, genes in white set), it was possible that Ino80C, SWI/SNF and RSC exert largely redundant functions at these genes, such that inactivating each CR alone has little impact on nucleosome eviction in strains containing the other two CRs intact. Indeed, examining our previous H3 ChIP-seq data (6) revealed that 9 of these 10 genes exhibit a significant defect in H3 eviction in the snf2Δ P_TET-STH1 double mutant (6).

Differential effects of eliminating Ino80C versus SWI/SNF function for different members of the group of 70 induced genes is also revealed by a heat-map depiction of H3 occupancy changes for each gene. The map in Figure 2A(ii) depicts the magnitude of H3 eviction in SM-treated versus untreated WT cells (as blue hues) across the region surrounding the +1_Nuc for each gene in the two groups of 70 or 134 SM-induced genes. Each group of genes is ordered on the previously determined WT induced levels of Pol II subunit Rpb3 across the CDSs (3), a measure of transcription, as indicated by the heat-map in Figure 2A(i). As expected, the amount of H3 eviction is considerably lower for the less-remodeled group of 134 genes (lower sector) versus the 70 induced genes (upper sector). The difference heat-maps in Figure 2A(iii) and (iv) displays the increases in H3 occupancies across the same regions confounded by ino80Δ (iii) or snf2Δ (iv) under inducing conditions, for the same ranking of genes. Consistent with previous results (6), the snf2Δ mutation confers increased occupancies, frequently in the vicinity of the -1_Nucs, for most of the group of 70 induced genes (Figure 2A(iv), upper, yellow & red hues); but has much smaller effects on H3 eviction at the 134 induced genes (Figure 2A(iv), lower). In contrast, ino80Δ tends to increase H3 occupancies over larger distances both upstream and downstream of the +1_Nuc for both groups of induced genes, but again confers much larger eviction defects for the 70 versus 134 SM-induced genes (Figure 2A(ii), upper versus lower). Interestingly, the results in Figure 2A(iii) and (iv) indicate a spatial segregation of H3 occupancy increases in response to ino80Δ vs. snf2Δ, suggesting that Ino80C and SWI/SNF remodeling activities are focused on distinct regions of the promoter.

The defect in nucleosome eviction conferred by ino80Δ is associated with a marked reduction in transcriptional activation of SM-induced genes, as judged by Rpb3 ChIP-seq analysis. In the heat-maps shown in Figure 2B, genes were again ordered according to their Rpb3 occupancies in induced WT cells for both groups of 70 and 134 induced genes (panel (i) upper & lower sectors, respectively). The Rpb3 difference map in panel (ii) shows that, in ino80Δ cells, the Rpb3 occupancies under inducing conditions are reduced for most of the 70 induced genes, with the greatest reductions (darkest blue hues) seen for the genes most highly transcribed in WT1 cells, located near the top of the map. Similar findings were observed for the 134 SM-induced genes, except for the relatively smaller reductions in Rpb3 levels for the genes near the top of the map and the minimal reductions seen for most of the remaining, less highly transcribed genes (Figure 2B(ii), lower vs. upper). The snf2Δ mutation also reduces the Rpb3 occupancies for a large proportion
of genes in both sets of SM-induced genes (Figure 2B(iii), upper & lower), but has smaller effects compared to ino80Δ for the most highly transcribed members of the group of 70 genes (top of upper sector), while reducing transcription of a somewhat larger proportion of the group of 134 genes (lower sector). These differences between the snf2Δ and ino80Δ mutations are in accordance with the differential requirements for Ino80C and SWI/SNF in evicting promoter nucleosomes at different SM-induced genes, described above in Figure 1D.

Quantifying the Rpb3 occupancies for each of the individual genes revealed that ino80Δ significantly decreases the median induced Rpb3 levels for both groups of induced genes (Figure 2C, columns 2–3 and 6–7), similar in magnitude to the effects of snf2Δ on the same two sets of genes (Figure 2C, columns 3–4 and 7–8). When considered as a proportion of the increase in median Rpb3 occupancy in induced versus uninduced WT cells (column 2 versus column 1), ino80Δ confers an ≈40% reduction in the induced median Rpb3 occupancy for the 70 genes, and a ≈18% reduction for the 134 SM-induced genes. Noting that the median induced Rpb3 level in WT cells is ≈2-fold higher for the group of 70 versus 134 genes (Figure 2C, cf. columns 2 & 6), these findings suggest a relatively greater requirement...
for Ino80C for robust transcription of the more highly expressed subset of SM-induced genes, as we concluded previously for SWI/SNF (3,6).

To examine the relationship between defects in H3 eviction and transcription conferred by ino80Δ, we sorted the genes according to the difference in average H3 occupancy between ino80Δ1 and WT1 cells across the [-1,NDR,+1] intervals, as shown in panel (i) of Figure 2D, and constructed corresponding Rpb3 difference maps for this same gene order. Interestingly, panels (ii) and (iii) show that the genes at the top of both maps tend to show the greatest decreases (ii) and largest fold-reductions (iii) in Rpb3 occupancy in ino80Δ1 versus WT1 cells (blue shades), consistent with the notion that nucleosome eviction by Ino80C generally enhances transcriptional activation on SM-induction. This inference is supported by a significant negative Spearman (ranked) correlation between the changes in H3 occupancies in the [-1,NDR,+1] intervals and the changes in Rpb3 occupancies in the CDS conferred by ino80Δ under inducing conditions for the group of 204 SM-induced genes (ρ = -0.55, P<0.0001). The snf2Δ mutation also reduces the induced levels of Rpb3 for a proportion of genes in both groups of SM-induced genes (Figure 2B(iv), upper & lower, blue hues), but the fold-changes are not clearly associated with the H3 eviction defects seen in ino80Δ1 versus WT1 cells shown in panel (i). Rather, they are better correlated with the H3 eviction defects seen in snf2Δ1 versus WT1 cells, as revealed by imposing a different ranking of genes according to their H3 eviction defects in snf2Δ cells. In this instance, the decreases and fold-reductions in Rpb3 levels in snf2Δ1 versus WT1 cells (Supplementary Figure S2D, (ii) & (iii)) are now more clearly associated with the corresponding increases in H3 occupancies (panel (i)). These results support our previous conclusion that nucleosome eviction by SWI/SNF enhances transcriptional activation of SM-induced genes (6).

**Ino80C functions broadly throughout the genome in promoter nucleosome eviction**

Previously, we uncovered a prominent role for SWI/SNF in promoter nucleosome eviction for the most highly transcribed subset of genes that are expressed constitutively in untreated and SM-treated WT cells (3,6). This limited role of SWI/SNF is evident in the H3 occupancy difference maps of Figure 3A for a group of 3619 genes chosen on the basis of exhibiting ≤1.2-fold increases in Rpb3 occupancy on SM-treatment of WT cells, and sorted by their Rpb3 occupancies in WT1 cells. As expected from their largely constitutive expression, this group of genes shows little change in H3 occupancies on SM-treatment of WT cells (Figure 3A(i)); and the overwhelming majority exhibit only a small increase in H3 promoter occupancy in SM-treated snf2Δ versus WT1 cells, as indicated by the yellow strip positioned just upstream of the +1 Nuc in Figure 3A(iii). Accordingly, there is little or no difference in median H3 or H2B occupancies in the [-1,NDR,+1] intervals for this group of 3619 genes in snf2Δ1 versus WT1 cells (Figure 3B, column 4 versus 2 & col. 8 versus 6). In ino80Δ1 cells, by contrast, a considerably larger H3 eviction defect was observed across the group of 3619 genes, which was more pronounced for the most highly transcribed genes positioned at the top of the difference map (Figure 3A(ii)). As noted above for the 70 SM-induced genes, the H3 occupancy increases for the 3619 constitutive genes appear to extend across a larger region surrounding the +1 Nuc in ino80Δ1 compared to snf2Δ1 cells (Figure 3A(ii) versus (iii)); although except for the most highly transcribed genes at the top, the occupancy increases conferred by ino80Δ are generally confined to the region immediately upstream of the +1 Nuc (Figure 3A(ii)). The most highly transcribed subset at the top of the map are exceptional in showing occupancy increases both upstream and downstream of the +1 Nuc in ino80Δ1 cells (Figure 3A(ii)), as was observed for the 70 SM-induced genes analyzed above in Figure 2A(iii).

Consistent with the H3 difference maps in Figure 3A(ii), the median H3 and H2B occupancies in the [-1, NDR, +1] intervals are significantly elevated, each by ≈10%, in ino80Δ1 versus WT1 cells (Figure 3B, column 3 versus 2 & column 7 versus 6), in contrast to the much smaller increases conferred by snf2Δ (Figure 3B, column 4 versus 3 & column 8 versus 7). As would be expected for constitutively transcribed genes, ino80Δ also confers significant increases in median H3/H2B occupancies in cells that were not treated with SM (Supplementary Figure S3A, column 2 versus 1 & column 5 versus 4), whereas snf2Δ again has a relatively smaller impact on promoter histone occupancies for the constitutively expressed genes in uninduced cells (column 3 versus 1 & column 6 versus 4).

Interestingly, the group of 3619 constitutive genes exhibit a moderate ≈15% increase, rather than a decrease, in median Rpb3 occupancies in ino80Δ1 versus WT1 cells, which was also observed for snf2Δ1 cells (Figure 3C). Previously, we found that defective histone eviction is associated with reduced transcription in snf2Δ cells only for the most highly transcribed subset of constitutively expressed genes (3). To explore if this also holds true for ino80Δ, we divided the 3619 genes into ten equal deciles based on Rpb3 occupancies. Genes in all deciles show increased H3 occupancies in the [-1, NDR, +1] interval in ino80Δ1 versus WT1 cells, but with relatively larger increases for Decile 1 containing the most highly transcribed genes, whose changes are on par with the increased H3 occupancies seen for the 70 SM-induced genes (Figure 4A, cf. green versus orange boxes, columns 1–4 versus columns 5–22). Interestingly, examining Rpb3 occupancy changes for the same deciles shows that ino80Δ confers reduced Rpb3 levels only for Deciles 1 and 2, as it does for the set of 70 SM-induced genes, whereas Deciles 4 to 10 exhibit increased Rpb3 levels in ino80Δ1 versus WT1 cells (Figure 4B; note different y-axis scales for deciles 2–10 versus decile 1 and the 70 SM-induced genes). These findings suggest that ino80Δ selectively reduces the relative transcription levels of only the most highly transcribed groups of constitutive genes. Previously, we interpreted similar findings for snf2Δ and gcn5Δ mutants by proposing that nucleosome eviction is a rate-limiting step for transcriptional activation only for the strongest promoters, and that impairing these promoters by eliminating Snf2 or Gcn5 increases the pool of transcription factors, co-factors, or Pol II available to the weaker promoters, for which recruitment of such factors would be a more rate-limiting step (3). (Note that because Rpb3 occupan-
A

Figure 3. Ino80C acts broadly in promoter nucleosome disassembly at the 3619 constitutive genes. (A) Heat maps of H3 occupancy differences on SM induction of WT cells (i), or between mutant and WT cells under inducing conditions (ii)-(iii), for the 3619 constitutive genes, sorted by their WT Rpb3I occupancies and color-coded as in the scale to the right of panel (iii). (B) Box plots of H3 (left) or H2B (right) occupancies in the [−1,NDR,+1] intervals for the indicated strains for the 3619 constitutive genes, as in Figure 1C. (C) Box plots of Rpb3 occupancies for the indicated strains for the 3619 constitutive genes, as in Figure 1C. All results were obtained from isogenic WT strain BY4741 and ino80Δ mutant YR092. ChIP-Seq data for an isogenic snf2Δ mutant were reported previously (Table 1) and presented here for comparison.

cies are expressed relative to the average occupancy in each strain, the apparent increases in median Rpb3 occupancies for deciles 3–10 in the mutant might signify only smaller than average declines rather than absolute increases in transcription.) We cannot however eliminate the alternative explanation that the increased Rpb3 occupancies conferred by ino80Δ for many lowly expressed genes reflects a slower rate of Pol II elongation in the absence of Ino80C.

Identification of genes highly dependent on Ino80C for promoter nucleosome eviction

In an effort to characterize the subset of yeast genes that are most highly dependent on Ino80C for promoter nucleosome remodeling, we identified a group of 399 genes showing the largest observed increases in H3 occupancies in ino80Δ versus WT1 cells. Figure 5(i)–(iv) presents genome browser views from two replicate datasets for four such ‘Ino80-hyperdependent’ genes (henceforth Ino80-hyp genes) revealing greatly increased promoter H3 occupancies in ino80Δ versus WT1 cells (cf. rows 5–6 versus 3–4, data between dotted lines), comparable or exceeding the occupancies in uninduced WT cells (cf. rows 5–6 versus 1–2). As would be expected, the reductions in promoter H3 occupancies in ino80Δ cells were essentially eliminated by introducing WT INO80 on a plasmid (Figure 5(i)–(iv), cf. rows 7–8 versus 5–6). A subset of 154 of the entire group of 399 genes exhibit >1.2-fold induction of Rpb3 occupancies on SM induction of WT cells and was dubbed the ‘Induced’ subset of Ino80-hyp genes. (Most of the 154 genes in the induced subset of Ino80-hyp genes exhibit induction ratios between 1.2- and 2.0-fold, and thus only 37 belong to the group of 204
Figure 4. Ino80C enhances relative transcription only at the most highly expressed constitutive genes. (A, B) H3 or Rpb3 occupancies in WT1 or ino80Δ1 cells plotted for the 70 SM-induced genes (columns 1–2) or for deciles of the 3619 constitutive genes sorted in descending order of Rpb3 occupancies in WT1 cells. Note different y-axis scales in (B) for columns 1–4 and columns 5–22. All results were obtained from WT strain BY4741 and ino80Δ1 mutant YR092.

SM-induced genes discussed above showing >2-fold Rpb3 induction.) The remaining 245 Ino80-hyp genes belong to the group of 3619 constitutively expressed genes (the ‘Constitutive’ subset). As shown in the H3 difference heat-maps of Figure 6A(ii), both the Induced and Constitutive subsets of Ino80-hyp genes exhibit strong increases in H3 occupancy, generally even greater than observed for the group of 70 SM-induced genes in Figure 2A(iii) (upper sector); although, for the Ino80-hyp genes the increases are relatively more confined to the +1 Nuc and regions upstream (cf. Figure 6A(ii) versus Figure 2A(iii)). By comparison, snf2Δ confers much smaller increases in H3 occupancies for the Ino80-hyp genes, producing marked increases only for the most highly transcribed Induced genes shown at the top of the upper sector of the map in Figure 6A(iii). Consistent with the H3 difference maps, the median H3 and H2B occupancies in the [−1,NDR,+1] intervals of the Ino80-hyp genes are substantially higher in ino80Δ1 versus WT1 cells, whereas snf2Δ confers much smaller increases in median H3 and H2B occupancies (Figure 6B, columns 3–4 versus 2, and columns 7–8 versus 6). The ino80Δ mutation also confers marked increases in promoter histone occupancies at the Ino80-hyp genes in cells untreated with SM, while snf2Δ confers much smaller defects in histone eviction under these conditions as well (Supplementary Figure S3B). Thus, as a group, the Ino80-hyp genes are much more dependent on Ino80C than SWI/SNF under both inducing and non-inducing conditions.

The Induced subset of Ino80-hyp genes shows a marked reduction in median Rpb3 occupancy in ino80Δ1 versus WT1 cells (Figure 6C, columns 1–2). Examination of the Rpb3 difference maps in Figure 6D(ii)–(iii), with genes
Sorted on WT1 Rpb3 occupancies, indicates that both the absolute decreases and fold-reductions in Rpb3 levels conferred by ino80Δ1 are greatest for the genes most highly expressed in WT1 cells located at the top of the maps (blue hues in upper sectors). The 245 genes in the Constitutive subset of Ino80-hyp genes also show reduced Rpb3 occupancies in ino80Δ1 cells that is confined largely to the most highly transcribed genes of the group (Figure 6D(ii)–(iii), blue hues at top of lower sectors). Interestingly, however, the weakly expressed members of this group tend to exhibit increased rather than decreased Rpb3 occupancies in ino80Δ1 cells versus WT1 cells (Figure 6D(ii)–(iii), yellow or red hues at bottom of lower sectors), despite substantial histone eviction defects in ino80Δ1 cells for the entire group of Constitutive genes (Figure 6A(ii), lower). The significant negative association between expression levels and changes in expression conferred by ino80Δ was clearly evident when the 399 Ino80-Hyp genes were sorted into quartiles according to their Rpb3_I occupancies in WT1 cells and the median changes in Rpb3_I occupancies conferred by ino80Δ compared among the four quartiles (Supplementary Figure S3C). The opposite effects of ino80Δ on transcription for the highly versus weakly expressed members of the Constitutive group effectively cancel out as the median Rpb3 occupancies for the group are very similar in ino80Δ1 versus WT1 cells (Figure 6C, columns 4–5). Together, these findings extend to the Ino80_hyp cohort our previous conclusion that nucleosome eviction by Ino80C is rate-limiting for transcription of only the most highly expressed genes.

Defects in promoter nucleosome eviction in ino80Δ cells are associated with reduced promoter occupancies of TBP

We sought next to determine whether the defects in nucleosome eviction in ino80Δ cells are associated with impaired recruitment of TBP, by conducting ChIP-Seq of TBP. TBP occupancy peaks were identified from two biological replicates of SM-induced WT cells using the program MACS2 (49). TBP peaks were identified for 53 (76%) and 63 (47%) of the groups of 70 and 134 SM-induced genes and, as expected, the mode occupancies were located on average ≈50 bp upstream of the TSS, and were substantially larger in WT1 versus WTU cells (Figure 7A & Supplementary Figure S4A, red versus blue traces). Quantification of TBP occupancies in the 161 bp surrounding the mode occupancies at individual genes revealed an increase in median occupancy on SM-induction of WT cells of ≈3-fold and ≈2-fold for the groups of 70 and 134 induced genes, respectively (Figure 7B and Supplementary Figure S4B). These results are consistent with the idea that TBP recruitment to the promoters of these genes is stimulated by SM. Importantly, the TBP occupancies for both groups of genes were significantly diminished in ino80Δ1 versus WT cells, particularly under inducing conditions (Figure 7A & Supplementary Figure S4A).
Figure 6. Ino80C strongly enhances promoter nucleosome disassembly at all Ino80-hyp genes but stimulates transcription only at the most highly expressed subset. (A) H3 occupancy difference maps for SM induction of WT cells (i), or between mutant and WT cells under inducing conditions (ii)–(iii), for the 399 Ino80-hyp genes, divided between SM-induced (upper) and constitutive (lower) genes, sorted by their WT Rpb3 occupancies and color-coded as in the scale to the right of panel (iii). (B) Box plots of H3 (left) or H2B (right) occupancies in the [–1, NDR, +1] intervals for the indicated strains for all 399 Ino80-hyp genes, as in Figure 1C. (C) Box plots of Rpb3 occupancies for the indicated strains for the induced (left) or constitutive (right) Ino80-hyp genes, as in Figure 1C. (D) Heat-maps of WT1 Rpb3 occupancies (i), or of differences (ii) or ratios (iii) of Rpb3 occupancies between ino80Δ and WT1 cells, color-coded as in the scales on the right of each panel. In all 3 maps, genes are sorted on the Rpb3 occupancies in WT1 cells, as in (i). All results were obtained from isogenic WT strain BY4741 and ino80Δ mutant YR092. ChIP-Seq data for an isogenic snf2Δ mutant were reported previously (Table 1) and presented here for comparison.

S4A, purple versus red & green versus blue; Figure 7B & Supplementary Figure S4B, column 2 versus 4 & 1 versus 3), suggesting that defective promoter nucleosome eviction in ino80Δ cells impairs TBP recruitment.

The difference heat-maps in Figure 7C strongly suggest that the increases in TBP occupancies on SM-induction of WT cells (panel ii) are associated with increased Rpb3 occupancies (panel iii), which is confirmed by a significant positive correlation between the changes in TBP and Rpb3 occupancies in induced versus uninduced WT cells for all 204 SM-induced genes (ρ = 0.54, P < 0.0001). Consistent with this, the TBP occupancy increases on induction (panel ii) scale with the induced Rpb3 levels in WT cells (panel i), for both groups of SM-induced genes (upper & lower panels). Moreover, the most highly expressed members of the two groups of SM-induced genes show a pervasive reduction in TBP occupancies in ino80Δ versus WT cells, which is generally associated with reduced Rpb3 occupancies (Figure 7C (iv)–(v), genes at top of maps). A similar association between decreases in TBP and Rpb3 occupancies is evident for the two groups of Ino80-hyp genes in the heat maps of Supplementary Figure S4C (i) versus (ii), These impressions are supported by significant positive correlations between changes in TBP and Rpb3 occupancies conferred by ino80Δ under inducing conditions for the 116 SM-induced genes with defined promoter TBP peaks (described in Figure 7A and Supplementary Figure S4A) (ρ = 0.77, P < 0.0001); and for the 399 Ino80-Hyp genes (ρ = 0.40, P < 0.0001), consistent with the idea that reductions in TBP recruitment contribute to the decreased transcription of many of these genes in ino80Δ cells. Supplementary Figure S5 shows genome browser results for four exemplar genes in which increased promoter H3 occupan-
Ino80C is recruited and also promotes TBP recruitment at SM-induced genes. (A) Average TBP occupancies relative to the TSS at 53 genes of the 70 genes with TBP peaks called by MACS2 analysis of TBP ChIP-Seq data for the indicated strains. The solid lines show the averages of ≥2 replicates; shaded areas show the ranges of values for individual replicates. (B) Box plots of TBP occupancies for the indicated strains calculated from combined ChIP-Seq data from ≥2 biological replicates for the subset of SM-induced 70 genes from (A) calculated for the 161 bp intervals centered on the mode occupancies for each peak. (C) TBP and Rpb3 occupancy difference maps for the SM-induced genes. (ii) & (iv) TBP difference maps for SM induction of WT cells (ii), or between ino80ΔI and WT1 cells (iv) for the 70 SM-induced (upper) and 134 SM-induced genes (lower), sorted by their WT1 Rpb3 occupancies (shown in panel i) and color-coded as in the scale to the right of each panel. (iii) & (v) Rpb3 difference maps for SM induction of WT cells (iii), or between ino80ΔI and WT1 cells (v). (D) Ino80-myc occupancy difference maps for SM induction of WT cells calculated from combined ChIP-Seq data from three biological replicates for the 70 SM-induced (upper) and 134 SM-induced genes (lower) sorted by their WT1 Rpb3 occupancies and color-coded as in the scale to the right. All results were obtained from WT strain BY4741 and ino80Δ mutant YR092.

Ino80C is recruited and also promotes TBP recruitment at SM-induced genes. (A) Average TBP occupancies relative to the TSS at 53 genes of the 70 genes with TBP peaks called by MACS2 analysis of TBP ChIP-Seq data for the indicated strains. The solid lines show the averages of ≥2 replicates; shaded areas show the ranges of values for individual replicates. (B) Box plots of TBP occupancies for the indicated strains calculated from combined ChIP-Seq data from ≥2 biological replicates for the subset of SM-induced 70 genes from (A) calculated for the 161 bp intervals centered on the mode occupancies for each peak. (C) TBP and Rpb3 occupancy difference maps for the SM-induced genes. (ii) & (iv) TBP difference maps for SM induction of WT cells (ii), or between ino80ΔI and WT1 cells (iv) for the 70 SM-induced (upper) and 134 SM-induced genes (lower), sorted by their WT1 Rpb3 occupancies (shown in panel i) and color-coded as in the scale to the right of each panel. (iii) & (v) Rpb3 difference maps for SM induction of WT cells (iii), or between ino80ΔI and WT1 cells (v). (D) Ino80-myc occupancy difference maps for SM induction of WT cells calculated from combined ChIP-Seq data from three biological replicates for the 70 SM-induced (upper) and 134 SM-induced genes (lower) sorted by their WT1 Rpb3 occupancies and color-coded as in the scale to the right. All results were obtained from WT strain BY4741 and ino80Δ mutant YR092.

Ino80 occupancies in the promoter regions of induced genes are elevated by SM treatment

We conducted ChIP-seq analysis of myc-tagged Ino80 to determine whether Ino80C is recruited to the promoters of SM-induced genes during their induction. The data were corrected for non-specific immunoprecipitation by Myc antibodies by subtracting occupancies calculated from ChIP-seq data obtained from the isogenic untagged strain. The results revealed marked increases in Ino80-myc occupancies upstream of the TSS in WT1 versus WTU cells, which generally scale with the induced Rpb3 levels at the groups of 70 and 134 SM-induced genes (cf. Supplementary Figure S6A & B, upper & lower panels); as summarized in the difference map of Ino80-myc occupancies shown in Figure 7D. Similar findings were observed for both groups of Ino80-hyp genes (Supplementary Figure S6C, upper & lower panels), as well as for the group of 3619 constitutively expressed genes (Supplementary Figure S6D). Increased Ino80-myc occupancies on SM induction is further illustrated for the four exemplar genes described above in Supplementary Figure S5 (Ino80-myc tracks). Thus, for all of these gene sets, the most highly expressed genes tend to have the highest Ino80C occupancies, consistent with a direct role for Ino80C in promoter nucleosome eviction during transcriptional activation. Supporting this inference, we observed a signif-
Figure 8. Ino80C plays a larger role than H2A.Z in promoter nucleosome eviction genome-wide. (A–C) Box plots of H3 occupancies in the [–1,NDR,+1] intervals for the indicated strains for the 70 SM-induced genes (A), Ino80-hyp genes (B) and Constitutive genes (C), as in Figure 1C. (D) H3 occupancy difference maps for SM induction of WT cells (i), or between mutant and WT cells under inducing conditions (ii)–(iii), for the Ino80-hyp genes, sorted by degree of H3 eviction in WT cells on SM-induction, color-coded as in the scale to the right of panel (iii). (E) Box plots of Rpb3 occupancies for the indicated strains for the 70 SM-induced genes, as in Figure 1C. All results were obtained from WT strain BY4741, ino80Δ mutant YR092, and htz1Δ mutant 1703. The data for WTΔ, WTΔI, and ino80ΔI strains in panels A–C & E are re-plotted from Figures 1C, 6B, 3B & 2C, respectively, for direct comparison with the htz1Δ results.

Figure 8A–C, columns 3–

icant negative correlation between the changes in Ino80-myc occupancies and the changes in H3 occupancies in the [–1,NDR,+1] intervals on induction of WT cells for both the 204 SM-induced genes ($\rho = -0.47$, $P < 0.0001$) and the 399 Ino80-hyp genes ($\rho = -0.34$, $P < 0.0001$). Moreover, we observed significant positive correlations between the induced Ino80-myc occupancies in WTI cells and the changes in H3 promoter occupancies conferred by ino80Δ versus WT under inducing conditions for the SM-induced genes ($\rho = 0.55$, $P < 0.0001$) and Ino80-hyp genes ($\rho = 0.68$, $P < 0.0001$). As shown in Figure 7D and Supplementary Figure S6A–D, Ino80-myc occupancies generally do not extend beyond 500 bp upstream of the TSS. The fact that the H3 occupancy increases in ino80Δ cells observed at some genes can extend between 500 bp and 1000 bp upstream of the TSS (Figure 2A(iii), upper & Figure 6A(ii), upper) might indicate that Ino80C can migrate some distance from its most stably bound positions within NDRs.

Nuclear depletion of Ino80 in cells lacking H2A.Z impairs promoter nucleosome eviction

Finally, we sought to determine whether defects in promoter nucleosome eviction conferred by inactivation of Ino80C occur strictly in the context of replacing H2A.Z-H2B dimers with conventional H2A-H2B dimers—a process that might facilitate eviction of H3-H4 tetramers (30). If this ’H2A.Z-editing’ model is correct, then (i) deletion of HTZ1 or INO80 should confer highly similar defects in promoter H3 occupancies, and (ii) inactivation of Ino80C should produce no additional eviction defect in a strain lacking HTZ1. To test the first prediction of this model, we compared the effects of deleting HTZ1 or INO80 on promoter nucleosome eviction. In contrast to ino80Δ, htz1Δ did not confer a statistically significant increase in median H3 occupancies in the [-1,NDR,+1] intervals of the groups of 70 SM-induced genes and Ino80-hyp genes, and actually led to a decrease rather than increase in occupancies for the 3619 constitutive genes (Figure 8A–C, columns 3–
Figure 9. Ino80C promotes nucleosome eviction independently of H2A.Z. (A) Box plots of H3 occupancies in the [-1, NDR, +1] intervals for the indicated strains for the 70 SM-induced genes (A) and Ino80-hyp genes (B), as in Figure 1C. (C) H3 and Rpb3 occupancy difference maps for the Ino80-hyp genes. Panel (i) shows H3 difference (ΔH3) and Rpb3 difference (ΔRpb3) maps for SM induction of WT cells (i). Panels (ii), (iii) & (iv) show H3 difference (ΔH3) and Rpb3 difference (ΔRpb3) maps between induced mutant and WT1 cells. color-coded as in the ΔH3 and the ΔRpb3 scale to the right of panel (iv). All results were obtained from WT strain HQY1695, htz1Δ strain HQY1702, INO80-FRB strain HQY1697, and htz1Δ INO80-FRB strain HQY1701, all four of which contain FKBP12-tagged RPL13A, fpr1Δ and the tor1Δ allele. All strains were treated with Rap for 1.5h followed by SM treatment for 25 min (for the induced cultures).

4 versus 2), thus suggesting a role for Ino80C in nucleosome eviction that is independent of H2A.Z. This possibility is further supported by extensive gene-to-gene differences in the patterns of increased H3 occupancies conferred by ino80Δ versus htz1Δ for the Ino80-hyp group of genes (Figure 8D), both in magnitude and position within the promoter regions, whereas the H2A.Z-editing model predicts highly similar H3 occupancy changes in the two mutants at all genes. For the group of 70 SM-induced genes, for which ino80Δ reduces the median induced Rpb3 occupancy (Figure 2C), htz1Δ does not confer a significant decrease in transcription (Figure 8E, cf. rows 2 & 4), in accordance with its relatively smaller effect on H3 eviction (Figure 8A).

To test the second prediction of the H2A.Z-editing model, we used ‘anchor away’ (41) to deplete an FRB-tagged version of Ino80 from the nucleus by addition of rapamycin (Rap) to cultures of WT, INO80-FRB, htz1Δ and htz1Δ INO80-FRB strains, all harboring the FKBP12-tagged allele of RPL13A encoding the cytoplasmic ‘anchor’ protein and the fpr1Δ and tor1Δ alleles that confer rapamycin insensitivity of the TORC1 kinase complex (41). Anchor away was shown produce substantial nuclear depletion of the INO80-FRB product, and to confer sensitivity to hydroxyurea (HU) (29). We confirmed that Rap treatment of our INO80-FRB strain confers sensitivity to HU, albeit not to the same degree seen for deletion of INO80 (Supplementary Figure S7), which suggests an incomplete nuclear depletion of the INO80-FRB product. Consistent with the latter interpretation, Rap treatment of the INO80-FRB strain does not confer a significant increase in the median H3 occupancy of the 70 SM-induced genes on SM-induction (Figure 9A), unlike the marked H3 eviction defect we observed for these genes in the ino80Δ mutant and resembling instead the weaker phenotype of the htz1Δ strain (Figure 8A). Nevertheless, anchor-away of Ino80 does confer increased H3 occupancies for the Ino80-hyp genes, even though htz1Δ does not (Figure 9B, columns 3–4); and most importantly, combining INO80-FRB with htz1Δ leads to significantly greater H3 eviction defects compared to htz1Δ alone for both groups of genes (Figure 9A, B, columns 5 & 3). Examination of the H3 difference maps reveals that the additive effect of combining INO80-FRB with htz1Δ
occurring for nearly all genes among the Ino80-hyp groups of genes (Figure 9C, ΔH3, (iv) versus (ii)). These findings are at odds with the model that the role of Ino80C in promoter nucleosome eviction is confined to H2A.Z-containing nucleosomes, and instead indicates a prominent role for Ino80C in evicting canonical nucleosomes containing H2A. Finally, examination of the Rpb3 occupancy changes reveals that most of the reductions in transcription of the Ino80-hyp genes observed in the INO80-FRB htz1Δ double mutant also occur in the INO80-FRB single mutant, with minimal effects in the htz1Δ single mutant (Figure 9C, ΔRpb3, (iv) versus (iii) & (ii)). The lack of a strong effect of htz1Δ on Rpb3 occupancies is consistent with the idea that the role of Ino80C in activating transcription involves evicting promoter nucleosomes irrespective of whether they contain H2A or H2A.Z.

**DISCUSSION**

Recently, we presented evidence that SWI/SNF and RSC function comparably in evicting promoter nucleosomes for the cohort of SM-induced genes, most of which are induced by Gcn4, and also for the subset of constitutively expressed genes with the highest transcription levels under these growth conditions (6). Despite the fact that Ino80C is capable on its own of reconstituting a near-native nucleosomal organization in vitro (19), it had not been implicated in genome-wide NDR formation in vivo. Moreover, only a few reports indicated a role for Ino80C in disassembly of the +1_Nuc, where this activity appeared to be coupled to its proposed nucleosome-editing function in replacing H2A.Z with H2A (21,30,32). Other studies however found no evidence for a broad role of Ino80C in evicting promoter nucleosomes, apparently to prevent prolonged induction of certain genes and block non-coding transcription (20). Ino80 appears to operate coordinate with Mot1 and NC2 to suppress non-coding transcripts (35).

Here, we uncovered a prominent role for Ino80C in the eviction of promoter nucleosomes and transcriptional activation of SM-induced genes. Roughly 60% of the 70 genes exhibiting the greatest promoter nucleosome eviction on SM-induction in WT cells display a significant increase in histone H3 occupancies in the region encompassing the −1_Nuc, NDR, and +1_Nuc in SM-treated ino80Δ cells. Eliminating Ino80 also conferred a smaller, albeit significant, reduction in promoter histone eviction and transcription for the group of 134 SM-induced genes that exhibit less nucleosome eviction and lower Pol II occupancies in WT induced cells compared to the 70 induced genes. For both groups, the degree of promoter H3 eviction defect was correlated with the degree of transcriptional defect, consistent with the idea that overcoming the steric impediment of promoter nucleosomes to PIC assembly and transcription initiation is frequently required for robust transcriptional activation of SM-induced genes. Further support for this conclusion was provided by our finding of reduced promoter occupancy of TBP in ino80Δ cells that is correlated with the reductions in Pol II occupancies observed at the two groups of SM-induced genes.

We also identified a group of 399 genes exhibiting the most pronounced defects in promoter H3 eviction among all genes in ino80Δ1 versus WT1 cells, dubbed Ino80-hyp genes, which included a subset of 37 of the 204 SM-induced genes. Although all members of the Ino80-hyp group showed H3 eviction defects similar in magnitude to the group of 70 SM-induced genes, only a subset showed transcription defects, which generally corresponded to the most highly expressed members of the cohort. A similar finding was made for the larger group of 3619 constitutively expressed genes, for which reductions in median Rpb3 occupancies in ino80Δ1 versus WT1 cells were observed only for the two deciles with the highest transcription levels in WT1 cells. We interpret these findings, as previously (3), to indicate that promoter nucleosome eviction is rate-limiting for the most highly transcribed genes, whereas another step in transcriptional activation, such as recruitment of co-factors, general transcription factors, or Pol II itself, would be more rate-limiting at lowly expressed genes.

For both the Ino80-hyp and constitutive groups of genes we examined, including the Induced subset of the Ino80-hyp genes, we observed increased promoter histone occupancies in ino80Δ cells under both non-inducing and SM-inducing conditions. The same is also true for the group of 134 SM-induced genes (Supplementary Figure S2C), but not for the more highly induced and remodeled group of 70 SM-induced genes (Supplementary Figure S2B). These findings imply a constitutive role for Ino80C in evicting promoter nucleosomes that contributes to the larger reductions in nucleosome occupancy achieved on Gcn4 induction at a subset of the SM-induced genes (Supplementary Figure S8). This situation differs from that inferred previously for SWI/SNF and RSC based on our findings that eliminating or depleting Snf2 or Sth1 conferred considerably larger increases in promoter histone occupancies under inducing versus non-inducing conditions (6) (snf2Δ data in Figure 1C versus Supplementary Figure S2B and S2A versus Supplementary Figure S2C), supporting the idea that SWI/SNF and RSC are actively recruited by Gcn4 to its target genes. Ino80C is also strongly recruited on SM induction to the most highly-expressed members of the group of 70 induced genes (Figure 7D), consistent with the much greater contribution of Ino80C to nucleosome eviction under inducing versus noninducing conditions at these genes.

It was of interest to explore the properties of gene promoters that might render the 399 Ino80-Hyp genes unusually dependent on Ino80C for promoter nucleosome eviction compared to other genes. The Ino80-Hyp genes have significantly greater Pol II occupancies compared to all expressed genes (Supplementary Figure S9A, columns 5–6). Differences in expression among the Ino80-Hyp genes is not a significant determinant of their differing requirement for Ino80C in promoter nucleosome eviction, however, as the WT Rpb3_I occupancies do not vary systematically among quartiles of the 399 genes divided according to their increases in promoter H3 occupancy conferred by ino80Δ (Supplementary Figure S9A, cols. 1–4). In agreement with their higher than average expression level, both the induced and constitutive subsets of the Ino80-Hyp genes exhibit wider than average NDRs (Supplementary Figure
S9B, columns 1–3 versus 4; Supplementary Table S1) and lower than average H3x occupancies within their NDRs (Supplementary Figure S9C, columns 3–4; Supplementary Table S1) in WT cells under inducing conditions. In addition to showing a heightened dependence on Ino80C for eviction of promoter nucleosomes in SM-induced cells, the Ino80-hyp genes also exhibit a greater than average dependence on SWI/SNF and RSC, as the mean increases in H3x occupancies in their promoter regions are greater for the Ino80-hyp group versus all genes in the ino80Δ, snf2Δ and doxycycline-treated P_TET-STH1 mutants versus WT (Supplementary Figure S9D, columns 1 versus 2, 3 versus 4, & 5 versus 6). However, the defect in H3 eviction for these genes is much greater in ino80Δ cells compared to the other two remodeler mutants (Supplementary Figure S9D, cf. columns 1, 3 & 5), even though the ino80Δ and P_TET-STH1 mutations confer comparable increases in promoter H3x occupancies genome-wide (Supplementary Figure S9D, cf. columns 2 & 6). Thus, as a group, the Ino80-hyp genes are highly expressed with a heightened dependence on all three chromatin remodelers, but they are particularly dependent on Ino80C for eviction of promoter nucleosomes under SM-inducing conditions.

Interestingly, the Ino80-Hyp genes are significantly enriched for the 283 genes belonging to the induced environmental stress response (iESR) group (50) (Supplementary Table S1; $P = 3 \times 10^{-17}$, hypergeometric distribution), which was even more pronounced for the 154 SM-induced members of the Ino80-Hyp cohort ($P = 6 \times 10^{-28}$) (Supplementary Figure S9E). Consistent with this, both the entire group ($P = 6 \times 10^{-16}$) and the induced subset ($P = 2 \times 10^{-15}$) of the Ino80-Hyp genes are enriched for direct targets of the stress-regulated transcriptional activator Msn2 (Supplementary Table S1), as well as for Msn2 binding motifs, and for changes in expression in msn2 mutants (Supplementary Table S1). In contrast, the Ino80-Hyp genes are significantly depleted of the group of 585 stress-repressed ESR genes encoding ribosome biogenesis factors and ribosomal protein genes (Supplementary Table S1; $P = 1 \times 10^{-12}$). As might be expected, the induced subset of Ino80-Hyp genes is also enriched for direct targets of Gcn4 (51) (Supplementary Table S1; $P = 3 \times 10^{-9}$), and for binding of various other TFs involved in stress responses, amino acid metabolism or utilization of different carbon or nitrogen sources (Supplementary Table S1); and GO analysis reveals a significant enrichment for gene functions involved in carbohydrate metabolism (Supplementary Table S1). In line with the role of these TFs in regulating responses to stresses or changes in nutrients, the Ino80-Hyp group is enriched for genes recently designated as ‘coactivator redundant’ (Supplementary Table S1, $P = 3 \times 10^{-16}$), shown to be dependent on both TFIID and SAGA for robust transcription and to be enriched for ‘condition-regulated genes’; whereas they are significantly unenriched for the ‘TFIID’-dependent group ($P = 1 \times 10^{-21}$) that require TFIID but are relatively independent of SAGA (52) (Supplementary Table S1). Thus, the Ino80-hyp genes are enriched for genes activated in response to stress or changing nutrients and utilize SAGA to achieve efficient transcription. Similar to our findings for the most highly remodeled SM-induced genes (Figure 7D), members of the Ino80-hyp group induced during other stresses or nutrient limitations may exhibit active recruitment of Ino80C to support their transcriptional activation.

In this study, we also addressed whether Ino80C acts in promoter nucleosome eviction strictly in the context of its proposed role in H2A.Z-editing, wherein Ino80C-elimination of an H2A.Z:H2B dimer would render the partially disassembled promoter nucleosome highly susceptible to eviction from the DNA. Our results do not support this model, as we observed that nuclear depletion of Ino80 conferred considerable defects in promoter H3 eviction even in an htz1Δ mutant lacking H2A.Z. Moreover, complete elimination of Ino80 in the ino80Δ mutant conferred much stronger defects in promoter nucleosome eviction and transcription than did eliminating H2A.Z by htz1Δ. Together these results strongly suggest a prominent role for Ino80C in evicting canonical, H2A-containing promoter nucleosomes rather than being limited to those containing H2A.Z. Consistent with this conclusion, defects in GAL gene induction conferred by the ino80ΔΔ900 mutation were found to be greater than that given by htz1Δ (32); and only a limited overlap (~10%) was observed between sets of genes exhibiting reduced expression in ino80Δ versus htz1Δ cells (27). Nevertheless, we do not exclude the possibility that the presence of H2A.Z may render promoter nucleosomes more susceptible to Ino80C-mediated eviction and thereby contribute to more efficient transcription.

In summary, our results demonstrate an important role for Ino80C in eviction of promoter nucleosomes that appears to enhance recruitment of TBP and stimulate transcription of a sizable number of yeast genes. As this function can be observed in cells lacking H2A.Z, it clearly operates on canonical, H2A-containing nucleosomes and is not restricted to those containing H2A.Z. Thus, we propose that Ino80C functions on par with CRs in the SWI/SNF family, i.e. RSC and SWI/SNF in yeast, in remodeling nucleosomes to increase promoter DNA accessibility in a manner that is rate-limiting for transcription initiation at many highly expressed genes. For many of the less-induced group of 134 SM-induced genes, Ino80C appears to clear nucleosomes from promoters even in noninducing conditions; whereas for many of the more highly activated members of the group of 70 SM-induced genes, Ino80C function is critically required only during inducing conditions, when it is recruited along with SWI/SNF and RSC, to evict nucleosomes, increase promoter DNA accessibility, and achieve elevated transcription levels (Supplementary Figure S8).

DATA AVAILABILITY
All raw and processed sequencing data generated in this study have been submitted to the NCBI Gene Expression Omnibus ( GEO; http://www.ncbi.nlm.nih.gov/geo/) under accession number GSE142273.

SUPPLEMENTARY DATA
Supplementary Data are available at NAR Online.

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