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A Role for Sfn2-Related Nucleosome-Spacing Enzymes in Genome-Wide Nucleosome Organization

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The positioning of nucleosomes within the coding regions of eukaryotic genes is aligned with respect to transcriptional start sites. This organization is likely to influence many genetic processes, requiring access to the underlying DNA. Here, we show that the combined action of Isw1 and Chd1 nucleosome-spacing enzymes is required to maintain this organization. In the absence of these enzymes, regular positioning of the majority of nucleosomes is lost. Exceptions include the region upstream of the promoter, the +1 nucleosome, and a subset of locations distributed throughout coding regions where other factors are likely to be involved. These observations indicate that adenosine triphosphate–dependent remodeling enzymes are responsible for directing the positioning of the majority of nucleosomes within the *Saccharomyces cerevisiae* genome.

Chromatin has the potential to influence all genetic processes that act on the underlying DNA. The application of genomic technologies to study chromatin organization has revealed a striking alignment with respect to transcribed regions, consisting of a nucleosome-depleted region upstream of the transcriptional start site (TSS) followed typically by an array of nucleosomes whose positioning decays with progression into the coding region (1–3). This organization appears to be a conserved feature of the organization of eukaryotic genomes, and an assortment of factors have been proposed to contribute to its establishment (2, 3).

Prime candidates are remodeling enzymes related to the yeast Snf2 protein that have been shown to be capable of repositioning nucleosomes (4). Of these enzymes, Isw1- and Chd1-containing remodeling enzymes have been shown to be particularly effective in repositioning nucleosomes in vitro (5–7). These enzymes share structural motifs that may adapt them for the purpose of nucleosome spacing (8), exhibit sensitivity to an epitope in the N-terminal tail of histone H4 (9, 10), and have been shown to alter chromatin at specific loci in vivo (11–15). This prompted us to investigate the extent to which deletion of any one of these proteins contributes to the overall organization of nucleosomes in vivo. To do this, we took advantage of recently published data for *ISW1* (14) and *ISW2* (15) and our own data for a strain in which the CHD1 gene had been deleted. Numerous alterations to chromatin structure are apparent in each strain. However, when the average chromatin structure with respect to TSSs is aligned for all yeast genes, the individual deletions were observed to have relatively minor effects (Fig. 1, A to C).

The phenotypes associated with deleting individual *ISW1, ISW2*, or *CHD1* genes are relatively minor, whereas deletion of all three genes results in synthetic phenotypes (6). This led us to investigate chromatin organization in strains deleted for all combinations of these enzymes. Micrococcal nuclease digestion of chromatin isolated from these strains indicated the presence of spaced nucleosomes, except in the case of the *isw1Δ, chd1Δ* and *isw1Δ, isw2Δ, chd1Δ* strains (fig. S1). To characterize chromatin organization in these strains in more detail, nucleosomal DNA fragments were isolated and subject to paired-end sequencing.

The locations of nucleosome dyads were estimated as the midpoint of each paired-end read. A plot illustrating how the dyads map to a representative chromosomal locus (chromosome I coordinates 100,000 to 120,000) is illustrated in fig. S2. In the wild-type strain, a clear periodic enrichment of nucleosomal dyads is observed with a mean spacing of ~15 base pairs (bp). In the *isw1Δ, chd1Δ* and *isw1Δ, isw2Δ, chd1Δ* strains, many nucleosomes were observed to be less organized than in the wild-type strain. However, it is also notable that while many nucleosomes lose positioning relative to the TSS in the triple mutant, a subset of nucleosomes are retained. Alignment of nucleosomal dyads with the TSS reveals that nucleosome organization is grossly perturbed in these strains (Fig. 1, D and E). Especially prominent is a loss of nucleosome positioning through the coding regions while depletion of nucleosomes within the vicinity of the -1 nucleosome is unaffected. The
loss of chromatin organization is observed in genes expressed at high and low levels (Fig. 2, A and B), but it is notable that nucleosome read depth increases within coding regions of highly transcribed genes after deletion of ISW1, ISW2, and CHD1 (Fig. 2A). It is possible that this reflects a role for spacing enzymes in the previously observed retrograde movement of nucleosomes counter to the direction of RNA polymerase (16).

In contrast to the dramatic effects on the organization of chromatin in the coding region, deletion of ISW1, ISW2, and CHD1 had relatively minor effects on chromatin organization in the vicinity of the 3′ ends of genes (Fig. 2C). In concert, these observations suggest that spacing enzymes play a major role in the organization of nucleosomes, especially within coding regions.

Although it is difficult to prove a direct role for Chd1 and Isw1 in establishing nucleosome positioning, the following evidence supports such a role. First, these enzymes are especially proficient in nucleosome spacing in vitro (5–7, 12) and may contribute to the adenosine triphosphate (ATP)–dependent organization of chromatin recently observed in vitro (17). Second, Isw1 and Chd1 are found associated with the coding regions of highly transcribed genes consistent with functions related to elongation (fig. S3). Third, although there are alterations to the transcription of some genes after deletion of CHD1 and ISW1 (Fig. 2D), these are relatively minor and alterations to chromatin structure do not correlate with them (fig. S4).

To study how chromatin at individual genes is affected by deletion of spacing enzymes, we performed hierarchical clustering analysis to identify groups of genes with similar nucleosome organization. Unexpectedly, when clustering was performed based on the distributions of nucleosome dyads in both the wild-type and the triple-deletion mutant, a subset of nucleosomes that retain organization could be identified (Fig. 3, A to C). Interestingly, the location of these nucleosomes varies with respect to the transcriptional start within different groups of genes. These locations are also favored locations for in vitro nucleosome assembly (Fig. 3D) and show enrichment for the predicted affinity for nucleosomes (Fig. 3E). This suggests that DNA sequence acts as a more important, although not necessarily sole, determinant of nucleosome read depth at this subset of locations.

An attractive explanation for our observations is that the nucleosome-free region at the –1 region acts as a barrier for the establishment of the +1 nucleosome. It is notable that the depletion of nucleosomes in the –1 region is the feature of chromatin organization least affected by deletion of ISW1 and CHD1. DNA sequence features and/or the assortment of other factors known to act in this region may combine to maintain the depletion of nucleosomes in this region (18–21). The +1 nucleosome is the most prominent nucleosome remaining in the absence of spacing enzymes (Fig. 3A), and it is attractive to speculate that its location may be set adjacent to the –1

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**Fig. 1.** Nucleosome organization is disrupted upon deletion of *ISW1* and *CHD1*. Fragments of nucleosomal DNA protected from digestion by micrococcal nuclease were subject to paired-end sequencing from wild-type (green line) and mutant (red line) yeast strains. Nucleosomal dyads were assigned as the center of each fragment and aligned to the TSS. The read depth (A and C to E) or hybridization signal (B) normalized per base pair is plotted against the distance from the TSS.

**Fig. 2.** Chromatin changes in the absence of remodeling enzymes do not directly correlate with transcription. Nucleosomal reads were aligned to the TSS of the 10% highest transcribed genes (A) or 10% lowest transcribed genes (B). In (C), reads were aligned to the 3′ ends of genes. Genome-wide changes to transcription upon deletion of genes encoding remodeling enzymes are relatively modest (D).
region. Exclusion from the 147-bp territory of the +1 nucleosome could, to a limited extent, direct positioning of the +2 nucleosome through mechanisms that have been discussed previously (22). However, in the absence of spacing enzymes, the +2 nucleosome is far less prominent and subsequent nucleosomes barely discernible (Fig. 1, D and E). The majority of nucleosomes within the coding regions of genes are positioned in register with the +1 nucleosome as a result of the action of spacing enzymes that position successive nucleosomes within ~15 bp of their neighbors.

Nucleosome positioning has been observed in vitro in the absence of ongoing transcription (17) and occurs in vivo at genes transcribed at low levels (Fig. 2D). Nonetheless, an assortment of evidence connects the functions of Chd1 and Isw1 to transcription (II, 23–26).

Indeed, previous studies have shown that increased intragenic transcription and histone H3 acetylation is observed after deletion of CHD1 and ISWI (25), which may occur as a consequence of the presence of disorganized chromatin. Perturbation to chromatin structure by even a few base pairs can profoundly influence an assortment of genetic processes, including transcription and DNA replication. (15, 27). Given the substantial defects to chromatin after deletion of ISWI and CHD1, it is perhaps surprising that this strain survives reasonably well (6). Our data indicate that substantial transcription is possible in the absence of correct nucleosome spacing within coding regions (Fig. 2D). Chromatin organization within open reading frames may have a more important role in tuning the sensitivity and kinetics of transcriptional responses (28) rather than as an obligate requirement.

**Fig. 3.** A subset of nucleosomes retain organization in the absence of Isw1, Isw2, and Chd1. Clustering of the nucleosome distribution surrounding the TSS of each gene in wild-type and isw1Δ, isw2Δ, chd1Δ strains reveals the presence of a subset of nucleosomes that retain organization (A and B). The same gene order was used to plot the nucleosome read depth in an isw1Δ, chd1Δ mutant (C), chromatin assembled by salt dialysis (29) (D), the predicted affinity for nucleosomes (30) (E), and sonicated DNA (29) (F). Examples illustrating the variation of these parameters in individual clusters and their statistical significance are shown in fig. S5.

**References and Notes**