A role for Snf2-related nucleosome-spacing enzymes in genome-wide nucleosome organization

Published in final edited form as:
Science. 2011 September 23; 333(6050): 1758–1760. doi:10.1126/science.1206097.

A role for Snf2 related nucleosome spacing enzymes in genome-wide nucleosome organization

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Abstract

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 indicated that ATP-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 genes consisting of a nucleosome depleted region upstream of the transcriptional start site (TSS) followed typically by an array of nucleosomes who's 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, ISWI 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

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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 TSS's is aligned for all yeast genes, the individual deletions were observed to have relatively minor effects (Fig 1A-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. MNase 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 100,000-120,000) is illustrated in Figure S2. In the wild type strain a clear periodic enrichment of nucleosomal dyads is observed with a mean spacing of approximately 15 bp. In the *isw1Δ*, *chd1Δ* and *isw1Δ*, *isw2Δ*, *chd1Δ* strains, many nucleosomes were observed to be less organized than in the wt 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. 1DE). 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. 2AB), but it is notable that nucleosome read depth increases within coding regions of highly transcribed genes following deletion of *ISW1, ISW2* and *CHD1* (Fig 2A). It's 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 this. Firstly, these enzymes are especially proficient in nucleosome spacing in vitro (5-7, 12) and may contribute to the ATP dependent organization of chromatin recently observed in vitro (17). Secondly Isw1 and Chd1 are found associated with the coding regions of highly transcribed genes consistent with functions related to elongation (Fig S3). Thirdly, although there are alterations to the transcription of many genes following deletion of *CHD1* and *ISW1* (Fig 2D), alterations to chromatin structure do not correlate with these changes to transcription (Fig S4).

In order to study how chromatin at individual genes is affected by deletion of spacing enzymes, hierarchical clustering analysis performed to identify groups of genes with similar nucleosome organization. Surprisingly, when clustering was performed based upon the distributions of nucleosome dyads in both the wild type and triple deletion mutant, a subset of nucleosomes that retain organization could be identified (Fig. 3A-C). Interestingly, the location of these nucleosomes varies with respect to the transcriptional start site coinciding with the +1, +2, +3 and +4 positions within different groups of genes. These locations are also favored locations for in vitro nucleosome assembly (Fig. 2D) and show enrichment for the predicted affinity for nucleosomes (Fig. 2E). This suggests that DNA sequence acts as a

more important, though 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 region. Exclusion from the 147bp territory of the +1 nucleosome could to a limited extent direct positioning of the +2 nucleosome via 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 discernable (Fig.1D,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 approximately 15 bp of their neighbor.

It is attractive to speculate that the action of Chd1 and Isw1 could be linked to transcription as the periodic nucleosomal pattern decays with increasing distance from the +1 nucleosome. There are also existing links between both Isw1 and Chd1 and transcription (11, 23-26). Following inactivation of RNA polymerase a shift in nucleosome positioning towards thermodynamically favored locations has been observed (16). This loss of organization is not as severe as that observed following deletion of spacing enzymes, possibly because pol II was inactivated for up to 120 minutes and a substantial proportion of nucleosome positioning may be stable over this time frame.

Perturbation to chromatin structure even by a few base pairs can profoundly influence an assortment of genetic processes including transcription, and DNA replication. (15, 27). This makes it important to understand the principles underlying nucleosome positioning. Here we provide evidence that chromatin remodeling enzymes participate in this process. Previous studies have shown that increased intragenic transcription and histone H3 acetylation is observed following deletion of *CHD1* and *ISW1* (25). This may occur as a consequence of the presence of disorganized chromatin. Given the defects to chromatin and it is perhaps surprising that an Isw1, Chd1 delete strain survives reasonably well (6). Our data indicate that substantial transcription is possible in the absence of correct nucleosome spacing (Fig. 2D). Chromatin organization may have a more important role in tuning the sensitivity and kinetics of transcriptional responses (28) rather than as an obligate requirement.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Sequence data was generated at the Genepool Facility at the University of Edinburgh and Genotypic Technology Ltd, Bangalore. TG, VS and TOH were supported by Wellcome Trust Senior Fellowship 064414. MS and JLW were supported by the Stowers Institute. We thank Anitha Nair for useful advice and sending strains, Jim Allan and members of the TO-H lab and Wellcome Trust Centre for Gene Regulation and Expression for useful discussions.

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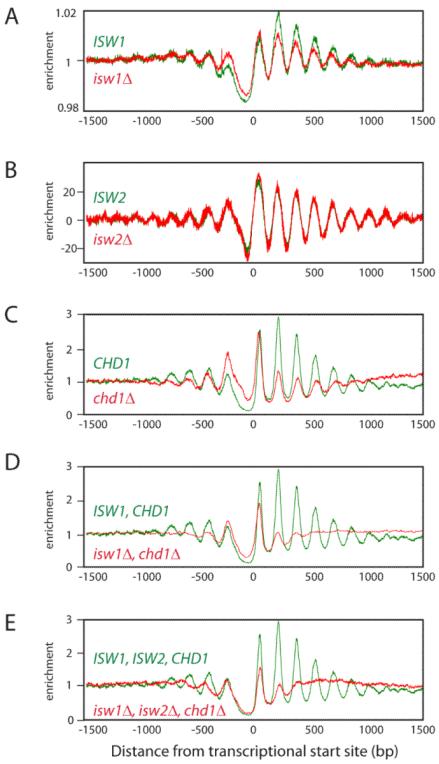

Figure 1. Nucleosome organization is disrupted upon deletion of *ISW1*, and *CHD1*

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, C-E) or hybridization signal (B) normalized per base pair is plotted against the distance from the TSS.

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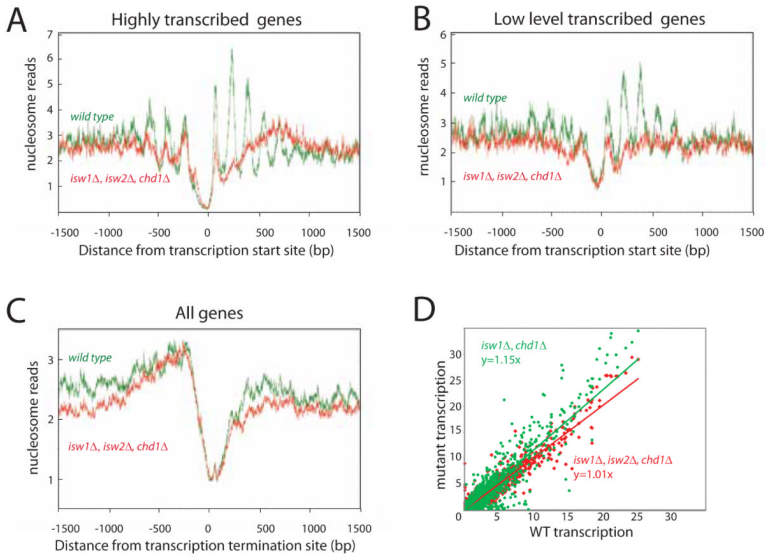

Figure 2. Chromatin changes in the absence of remodeling enzymes do not directly correlate with transcription

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**).

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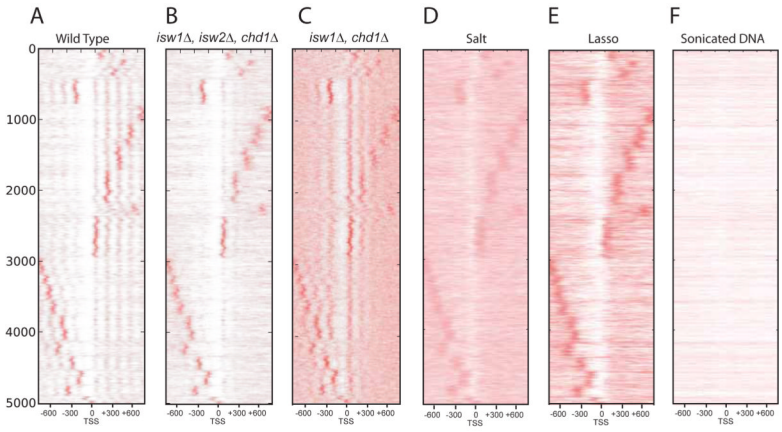

Figure 3. A subset of nucleosomes retain organization in the absence of Isw1, Isw2 and Chd1

Centroid clustering of the nucleosome distribution surrounding the TSS of each gene in wild type and *isw1Δ*, *isw2Δ*, *chd1Δ* strains strains reveals the presence of a subset of nucleosomes that retain organization (**A**,**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 Figure S5.

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