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DNA looping-dependent targeting of a chromatin remodeling factor

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Eukaryotic genomes are highly complex structures that must be efficiently packaged into relatively small nuclei in order to accommodate multiple DNA-dependent processes, from transcription and DNA replication to DNA repair and recombination. The compaction of genomes is hierarchically achieved at two distinct levels: (1) the compaction of DNA into nucleosome arrays and (2) the three-dimensional (3D) folding of nucleosome arrays within the nucleus. The compaction of genomes is required for the proper regulation of DNA-dependent processes, and disruption of either is associated with complex human diseases.[1,2]

The 3D folding of nucleosome arrays within the nucleus is highly dynamic, with discrete chromosomes occupying distinct non-random "territories".[3] Within each chromosome territory, specific DNA "loops" are formed that uniquely juxtapose distally located DNA loci, bringing them into close proximity.[3] DNA loops have been implicated in transcriptional regulation and transcriptional memory, although the molecular mechanisms for these phenomena remain to be determined.

The compaction of DNA into nucleosome arrays is accomplished by wrapping DNA around an octamer of histone proteins. Eukaryotic organisms regulate DNA-dependent process through nucleosome arrays using highly conserved ATP-dependent chromatin remodeling enzymes that utilizing the energy released from ATP hydrolysis to slide, evict or replace histones within nucleosomes.[4] ATP-dependent chromatin remodeling enzymes are highly abundant, yet function only at very specific loci. How such abundant enzymes are targeted to specific loci genome-wide remains a very important unanswered question.

In our recent article,[5] we established that the primary mechanism for the targeting of Isw2, a highly conserved ATP-dependent chromatin remodeling enzyme in *S. cerevisiae*, is through sequence-specific transcription factor (TF)-dependent recruitment. Using chromatin immunoprecipitation on whole genome tiled microarrays (ChIP-chip), we showed that the TFs Ume6, Cin5, Sok2 and Nrg1 target Isw2 to their binding sites genome-wide. These "canonical" Isw2 targets represent the classical model of protein targeting. Unexpectedly, we found that more than half of the TF-dependent Isw2 targets do not have the corresponding TF binding site. This suggested that TFs target Isw2 to specific loci via a previously unknown mechanism(s). A hint for this mechanism(s) came from the observation that Isw2 is targeted to both the 5'- and 3'-ends of the same gene at a highly statistically significant frequency. Because the 5'- and 3'-ends of yeast genes have been shown to form DNA loops,[6] we hypothesized that DNA looping may mediate Isw2 targeting to loci that do not have TF binding sites (**Fig. 1**). Using Ume6-dependent Isw2 targets as a model, we demonstrated by chromosome conformation capture (3C) that DNA looping does indeed take place between an Isw2 target with a Ume6 binding site (canonical targets) and one lacking a Ume6 binding site (ectopic targets). We further discovered that DNA looping-dependent ectopic Isw2 targets require both the general TF TFIIB and the sequence-specific DNA binding repressor Ume6. Finally, we provided evidence suggesting that Ume6-dependent DNA looping is associated with both chromatin remodeling and transcriptional repression.

Therefore, our results reveal two distinct mechanisms for TF-dependent targeting of a chromatin remodeling factor (**Fig. 1**): (1) targeting directly to its binding sites (canonical targets) and (2) via DNA looping (ectopic targets). Significantly, our finding that DNA looping-dependent Isw2 targeting likely takes place very widely across the budding yeast genome, suggests a model where the 3D folding of nucleosome arrays within the nucleus is intimately linked to both the regulation of chromatin structure and DNA-dependent processes. In addition, our results identified a molecular mechanism by which DNA looping affects DNA-dependent processes and a novel biological function of DNA looping.

Our results have raised several interesting questions. For example, are there different biological consequences associated with recruitment of Isw2 to canonical vs. ectopic targets? Bioinformatics analysis does in fact suggest this might be the case: canonical targets are associated with genes involved in meiosis and DNA recombination, while ectopic targets are associated with housekeeping genes involved in translation and glucose metabolism. Isw2 is also known to repress non-coding RNA (ncRNA),[7,8] but the role for DNA looping-dependent targeting of Isw2 in the repression of ncRNA remains unknown. It is possible that canonical and ectopic targets have different specificities for coding

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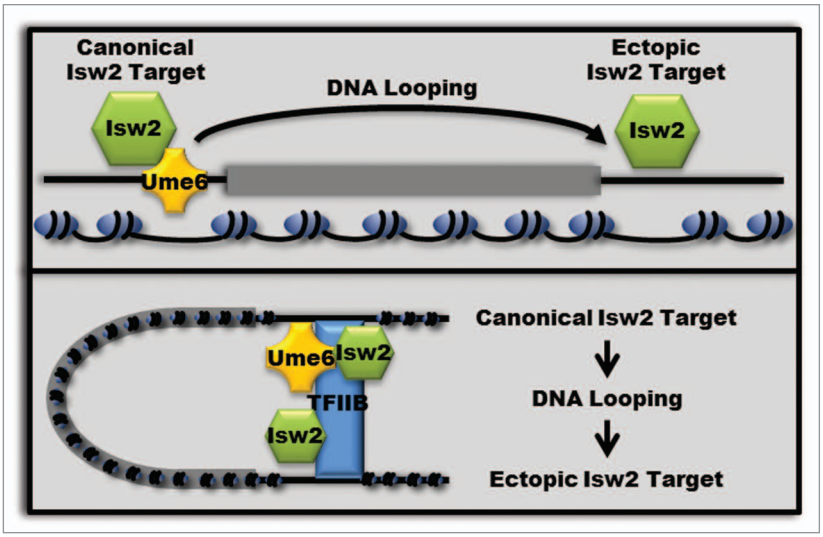

Figure 1. Two distinct mechanisms of TF-dependent Isw2 targeting. Transcription factor Ume6 can target Isw2 to the vicinity of its binding sites via physical interactions (canonical targets) or by TFIIB- and Ume6-dependent DNA looping (ectopic targets).

and ncRNA. Finally, it is unknown how dynamic DNA looping-dependent Isw2 targeting is. It is likely that DNA looping is a far more dynamic and transient process than TF binding to its recognition sites. If this were the case, DNA looping-dependent Isw2 targeting may lead to more variable chromatin remodeling at ectopic targets within a cell population, which would lead to variable transcriptional repression. Investigating biological functions of DNA looping-dependent Isw2 targeting will likely reveal novel aspects of chromatin regulation.

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