

# DNA in HD: The 3D Folding of Chromatin Targets a Remodeling Enzyme

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It is well known that DNA is wrapped around histone octamers to form the chromatin that exists in the nucleus of a cell. Chromatin inhibits DNA processes including replication and transcription, and it must be modified to allow access to regulatory proteins. Moreover, chromatin fibers are folded into higher order assemblies that influence gene expression. DNA looping is one example of the three-dimensional character of chromatin, where distal DNA regions are brought together and the intervening chromatin is looped out. Although DNA looping has been shown to affect transcriptional regulation, the molecular mechanisms for this are unknown. The Tsukiyama Lab (Basic Sciences Division) has discovered a crucial role for DNA looping in transcriptional repression.

The Tsukiyama Lab studies ATP-dependent chromatin remodeling proteins including Isw2 that use energy from ATP hydrolysis to displace nucleosomes, regulating gene transcription. Isw2 is a highly abundant protein, yet regulates transcription from only a few specific genomic loci. How Isw2 is targeted to *distinct* genomic regions is not clear, so Yadon *et. al.* decided to map Isw2 binding sites throughout the budding yeast genome. One well-studied transcription factor (TF) that recruits Isw2 to specific loci is Ume6. The authors hypothesized that other TFs might recruit Isw2 to additional genomic loci. To test this idea, they investigated TF binding sites at known Isw2 targets and found that 15 TFs were highly enriched at Isw2 genomic loci. Four TFs were then pursued for their potential role in targeting Isw2 to distinct loci.

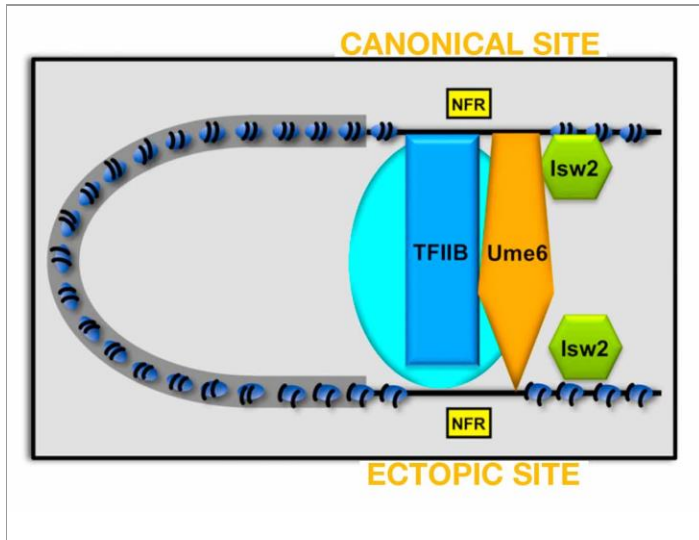
Changes in Isw2 targeting in the four TF mutants was analyzed by chromatin immunoprecipitation of Isw2 on genome-wide tiling microarrays. The authors confirmed that Ume6 is an important regulator of Isw2 localization by showing that Isw2 targeting is strongly reduced at 49% of annotated Ume6 binding sites in an *ume6* mutant. Proper Isw2 targeting was also diminished in the three other transcription factor mutants, but to a lesser extent. Surprisingly, only ~10% of the genomic loci that exhibited Ume6-dependent targeting of Isw2 actually had a Ume6 binding site. Therefore, the chromatin landscape to which Isw2 binds is strongly yet only partly dependent on TF-dependent recruitment.

How does Ume6 target Isw2 to genomic loci that do not have Ume6 binding sites? Yadon and colleagues recognized a pattern in their genome-wide Isw2 microarrays: enrichment of Isw2 at the 5' and 3' ends of the same gene. As gene looping has been shown to generate a similar genetic configuration, the authors wondered whether Isw2 targeting was influenced by it. The general TF TFIIB had been shown to be required for DNA looping, and Yadon *et. al.* found that Isw2 targeting was disrupted at many genomic loci in a TFIIB mutant. These loci were not limited to ones that had Ume6 binding sites, suggesting that DNA looping facilitated by TFIIB plays a role in targeting of Isw2 throughout the genome. Therefore, Ume6 recruits Isw2 both to 'canonical' targets that have an Ume6 binding site as well as 'ectopic' loci that do not. DNA looping promotes targeting of Isw2 to the ectopic sites.

The researchers then investigated whether DNA looping occurs at certain loci to which Isw2 is targeted. Their results revealed the TFIIB-dependent presence of DNA looping between the canonical and ectopic Isw2 targets. Furthermore, Isw2 targeting concomitantly requiring Ume6 and TFIIB prompted Tsukiyama's group to explore a potential role for Ume6 in DNA looping. They found that DNA looping at several tested loci was lost in the absence of Ume6. This reveals a novel role for a transcriptional repressor in DNA loop formation. Lastly, several pieces of evidence indicate a functional role for Isw2 at the ectopic loci: (1) Isw2-dependent chromatin remodeling occurred at the ectopic sites; (2) gene ontology searches revealed that ectopic Isw2 targets were involved in biological process completely distinct from canonical Isw2 targets; and (3) transcript array analysis revealed the upregulation of ectopic Isw2 targets in *ume6* cells.

In summary, the elucidation of Ume6-dependent DNA looping and its role in targeting Isw2 to many genomic loci by Yadon *et. al.* marks a significant advancement in understanding how higher order chromatin conformations affect transcriptional output. DNA looping mediated by Ume6 likely involves additional proteins, and the tools utilized by the authors will be helpful in discovering other factors involved in this phenomenon.

[Yadon AN, Singh BN, Hampsey M, Tsukiyama T.](#) 2013. DNA looping facilitates targeting of a chromatin remodeling enzyme. *Mol Cell.* 50(1):93-103.



*Image adapted from the manuscript*

DNA looping mediates Isw2 targeting throughout the genome. Ume6, a transcription factor that binds to specific DNA sequences, recruits the Isw2 chromatin modifier to DNA elements bound by Ume6 (canonical sites). The authors also found an unanticipated role for Ume6 in DNA looping (also mediated by TFIIB), which localizes Isw2 to ectopic sites.