# Mot1 Says TATA to TBP

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Eukaryotic transcription is a complex, highly regulated process involving hundreds of molecular components or factors. One such factor is TATA-binding protein (TBP), so named for its ability to bind the DNA sequence TATA. TBP is essential for transcriptional initiation by all three RNA polymerases and is regulated by many other proteins. A TBP-regulating factor is modifier of transcription 1 (Mot1), which uses the energy of ATP hydrolysis to remove TBP from the DNA template (Wollman *et al*., 2011). The finding that Mot1 removes TBP from DNA led to the suggestion that it may act as a global transcriptional repressor. However, numerous studies have shown that loss of Mot1 both increases and decreases transcription, and how Mot1 upregulates transcription has been unclear. A study performed by Dr. Gabriel Zentner, a postdoc in Dr. Steven Henikoff's lab (Basic Sciences Division) now demonstrates that the primary site of action for Mot1 is at promoters that contain robust TATA boxes and are expected to have high-affinity TBP binding sites (Zentner and Henikoff, 2013). This in turn ensures that there is sufficient soluble TBP available to bind TATA-less promoters, which are likely to be have lower-affinity TBP binding sites. "Mot1 was first characterized in [Dr.] Steve Hahn's lab here at the Fred Hutchinson Cancer Research Center. While its TBP-displacing activity has been very well studied in vitro, how it can positively regulate transcription in vivo has been quite a puzzle. Our results provide a satisfying explanation for the ability of Mot1 to both positively and negatively regulate transcription in vivo," says Dr. Zentner.

To determine the genome-wide relationship between Mot1 and TBP, the authors mapped the distribution of both proteins on uncrosslinked chromatin in budding yeast and found that Mot1 and TBP bound essentially the same sites throughout the genome. To examine the effects of TBP loss on the association of Mot1 with chromatin, the authors used a genetic technique called Anchor-Away (Haruki *et al*., 2008), which allows rapid, conditional depletion of specific nuclear proteins. Depletion of TBP from the nucleus led to a nearly complete loss of Mot1 from chromatin, indicating that TBP is required for Mot1 recruitment to chromatin.

In vitro, Mot1 must bind to DNA upstream of TBP in order to remove TBP from DNA, but it has been unclear if this feature of Mot1's in vitro action is relevant in vivo. Using information about the length and position of Mot1-bound DNA fragments, the authors showed that, at several hundred sites of

TBP binding, the majority of Mot1 is indeed bound to DNA upstream of TBP, in line with its in vitro mode of action.

To examine the effects of compromising Mot1 function on TBP binding to the genome, the authors used a temperature-sensitive Mot1 allele that was defective in its interaction with TBP. While the authors observed both increases and decreases in TBP binding upon Mot1 inactivation, many more promoters were associated with decreases than increases. Analysis of the underlying DNA sequences of sites of TBP gain and loss revealed that sites of gain contained robust TATA boxes, which are expected to be high-affinity TBP binding sites. In contrast, sites that lost TBP did not show strong TATA sequences, and are expected to be lower-affinity binding sites for TBP.

From these data, the authors concluded that the major function of Mot1 in vivo is to clear TBP from its intrinsically preferred binding sites (those containing strong TATA motifs) to ensure that sufficient soluble TBP is available to bind its intrinsically disfavored sites (those without TATA boxes). "We're now working on the connections between Mot1 and other proteins that regulate the association of TBP with the genome in order to get a better understanding of basic transcriptional regulatory mechanisms," says Dr. Zentner.

Zentner GE, Henikoff S. Mot1 Redistributes TBP from TATA-Containing to TATA-Less Promoters. *Mol Cell Biol* 33(24):4996-5004.

See also: Wollmann P, Cui S, Viswanathan R, Berninghausen O, Wells MN, Moldt M, Witte G, Butryn A, Wendler P, Beckmann R, Auble DT, Hopfner KP. 2011. Structure and mechanism of the Swi2/Snf2 remodeller Mot1 in complex with its substrate TBP.*Nature* 475(7356):403-407.

Haruki H, Nishikawa J, Laemmli UK. 2008. The Anchor-Away Technique: Rapid, Conditional Establishment of Yeast Mutant Phenotypes. *Mol Cell* 31(6):925-932.

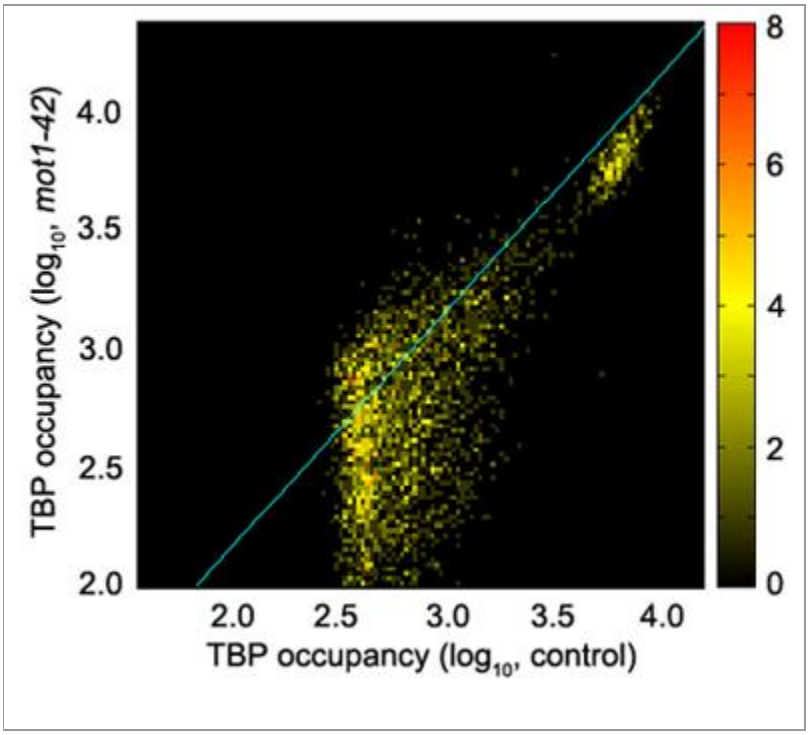


*Image provided by Dr. Gabriel Zentner*

Density plot comparing TBP binding at ~3,000 sites in the Mot1 wild-type (control) and mutant (mot1-42) strains. A larger number of points fall below than above the diagonal, indicating that more sites lose than gain TBP in mot1-42.