orientations. Although RBX1 is critical for efficient NEDDylation[14], a more recent study indicates that Hrt1 (RBX1 in mammals) and Dcn1 work synergistically as a dual NEDD8 E3 ligase[15]. It is suggested that Dcn1 places the UBC12-associated Hrt1 adjacent to the cullin NEDD8 acceptor lysine, thereby enhancing NEDDylation[16]. Therefore, this dual E3 mechanism might function to limit orientations that would allow UBC12's catalytic site to encounter the cullin's acceptor lysine[15]. On the other hand, ligation of NEDD8 to cullin might also assist SCF to adopt conformations that allow recruitment of distinct E2 conjugation enzymes to a particular substrate. It is likely that once NEDDylation occurs, the RING domain is unable to reach this conformation, so the Cdc34-bound complex uniquely selects conformations that favor substrate ubiquitination rather than cullin NEDDylation.

Although it is well established that spatiotemporal organization of the E2 and E3 enzymes is implicated in the control of specific substrate modifications[17], how the dynamic changes of E2 and E3 are regulated *in vivo* remains unclear. The study by Calabrese *et al.*[3] reveals the critical role of conformational flexibility in the RBX1 RING ligase for controlling NEDDylation of cullins as a signal to switch on cullin–RING ubiquitin ligase activity. Clearly, there are more challenges ahead for those who seek a better understanding of the regulatory mechanisms within the CRL-assembled complexes, and future studies will certainly soon reveal new surprises.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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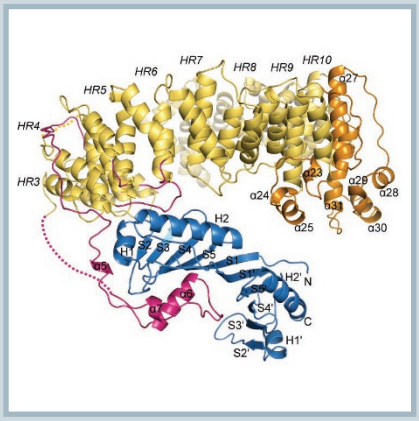
A bottle opener for TBP



The ATP-dependent remodelers are implicated in many DNA transactions. Although considerable mechanistic insight has been gained through biochemical, biophysical and *in vivo* analyses, structural information that shows how these complexes work to move proteins relative to DNA has been elusive. Auble, Hopfner and colleagues (*Nature* doi:10.1038/nature10215, advance online publication 6 July 2011) have now examined the structure of Mot1, an ATPase in the Swi2/Snf2 family that acts as a single polypeptide. The fact that Mot1 can act alone makes it a good candidate for structural analysis, given that a number of ATP-dependent chromatin remodelers in this family act as members of large complexes. Mot1 is highly conserved in the eukaryotes and is a regulator of TATA box–binding protein (TBP). Because it quite specifically displaces TBP from DNA, it has a simple substrate facilitating analysis, and it has an interesting role in transcriptional regulation. The authors have solved the structure of the N-terminal domain (NTD) of *Encephalitozoon cuniculi* Mot1 in complex with EcTBP to 3.1-Å resolution. This region has previously been implicated in TBP binding and lacks the ATPase that is located in the Mot1 C-terminal region. The authors found that the NTD of Mot1 consists of 16 HEAT repeats that are organized in a horseshoe shape (yellow and orange in image). The Mot1 repeats wrap around a single TBP molecule (blue in image), of interest because TBP can also form dimers. Mot1 interacts across the convex side of TBP, a region known to interact with other proteins. However, Mot1 also unexpectedly wraps around and interacts with the DNA-binding surface of TBP, immediately suggesting a mechanism by which it might prevent TBP interaction with DNA. The authors called this region of Mot1 the 'latch' (magenta in image). The interactions observed in the structure overlap with some seen in the TBP–TBP dimer and the TBP–DNA structure. Indeed, TBP adopts a very similar conformation in all of these structures.

Mutational analysis in the literature, both *in vivo* and *in vitro*, as well as further analysis presented by the authors support the concept of Mot1 interactions across the convex back of TBP. Indeed, a broad set of interactions in the N-terminal domain of BTAF1 (the human ortholog of Mot1) and *Saccharomyces cerevisiae* Mot1 has previously been implicated in TBP interactions, consistent with the present structure. In order to test the role of the latch region in TBP interactions, the authors generated deletion mutants of the full-length and Mot1 (NTD) proteins that lack latch residues. As expected, these deletion mutants did in fact interact with TBP, given that they contain the HEAT repeats required for interaction across the convex side of TBP; however, in this case, the NTD of Mot1 without the latch interacted with a TBP dimer. This supports the idea that the latch may be involved in disrupting the TBP–TBP dimer. The latch mutant Mot1 (NTD) also inhibited DNA binding *in vitro* when incubated with TBP prior to DNA exposure. It was not, however, absolutely required for inhibiting the TBP-DNA interaction, based on experiments with the Mot1 latch mutant containing the C-terminal ATPase domain. This suggests a model in which the ATPase region can displace TBP from DNA, and the latch facilitates or makes this more efficient, perhaps by interacting with the hydrophobic face of TBP and preventing DNA rebinding.

In order to assess the overall structure of the complex, the authors generated a three-dimensional reconstruction of EcMot1–TBP that includes its Swi2/Snf2 DNA-binding domain. The complex is C-shaped, with a globular protrusion, and the authors used deletion analysis to map the identities of different regions of this structure and then to help dock in the above crystal structure. These analyses suggest that Mot1 could feasibly interact with other TBP complexes and thus provide a number of avenues for further studies of how a relatively simple remodeling system can work to displace a protein from DNA.

Sabbi Lall