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One small step for Mot1; one giant leap for other Swi2/Snf2 enzymes?

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Abstract

The TATA-binding protein (TBP) is a major target for transcriptional regulation. Mot1, a Swi2/Snf2-related ATPase, dissociates TBP from DNA in an ATP dependent process. The experimental advantages of this relatively simple reaction have been exploited to learn more about how Swi2/Snf2 ATPases function biochemically. However, many unanswered questions remain and fundamental aspects of the Mot1 mechanism are still under debate. Here, we review the available data and integrate the results with structural and biochemical studies of related enzymes to derive a model for Mot1's catalytic action consistent with the broad literature on enzymes in this family. We propose that the Mot1 ATPase domain is tethered to TBP by a flexible, spring-like linker of alpha helical hairpins. The linker juxtaposes the ATPase domain such that it can engage duplex DNA on one side of the TBP-DNA complex. This allows the ATPase to employ short-range, nonprocessive ATP-driven DNA tracking to pull or push TBP off its DNA site. DNA translocation is a conserved property of ATPases in the broader enzyme family. As such, the model explains how a structurally and functionally conserved ATPase domain has been put to use in a very different context than other enzymes in the Swi2/Snf2 family.

Keywords

TATA-binding protein; TBP; Mot1; BTAF1; Swi2; Snf2; ATPase

1. Introduction

Transcription, the complex process of RNA synthesis from a DNA template, is precisely coordinated to influence critical steps in growth, development and differentiation. Therefore it is not surprising that it is subject to many types of regulation at its various steps. Messenger RNA transcription starts with the formation of a pre-initiation complex (PIC) at the promoter, followed by initiation of RNA synthesis, elongation, and termination [1–5]. PIC formation requires the recruitment of multiple general transcription factors and RNA polymerase II, leading to a complex array of proteins that interact in an orchestrated manner to affect productive synthesis of RNA [2, 6–12]. Although the transcription process is regulated at multiple stages, the rate-limiting step for PIC assembly at many promoters is the

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interaction of the TATA-binding protein (TBP) with the promoter [13–19]. Thus, the TBP-promoter interaction is a major target for regulating transcription.

Mot1 (Modifier of Transcription 1, known as BTAF1 in humans) is one such factor that influences the binding of TBP to promoters and is an essential protein in *Saccharomyces cerevisiae* [20–27]. Mot1 was originally identified as a factor that represses transcription from weak promoters [20, 28–31]. Contemporaneously, a biochemical approach uncovered Mot1 as a TBP-associated factor that removes TBP from DNA in an ATP-dependent reaction [26, 32, 33]. Studies using human cell extracts identified BTAF1 as the defining constituent of a TBP complex, B-TFIID, with transcriptional properties that are distinct from TFIID [26, 27]. Moreover, both the ATPase activity of B-TFIID and its unstable association with DNA were consistent with the biochemical properties of yeast Mot1-TBP complexes. Interestingly, these early efforts focusing on ATPase activity were prompted in part by prior studies in the rat system that uncovered an ATP hydrolysis requirement for accurate transcription initiation [34, 35].

In yeast, the ATP-dependent TBP-DNA dissociation activity of Mot1 fits well with genetic evidence that Mot1 represses transcription [32, 36]. However, it became clear subsequently that Mot1 has complex effects on transcription in vivo, activating perhaps as many genes as it represses [31, 37–41]. In fact, as a consequence of Mot1/BTAF1 action, TBP binds to chromatin in a highly dynamic manner in vivo [42–44], bolstering the relevance of the TBP-DNA dissociation reaction for understanding Mot1 function in vivo. A number of models have been proposed to explain how Mot1-mediated TBP-DNA dissociation might activate rather than repress transcription [21, 45–47]. However, the goal of this review is to focus on the mechanistic question of how Mot1 uses ATP to displace TBP. In vitro, Mot1 can function as a single polypeptide (Figure 1), targeting a relatively simple substrate, the TBP-DNA complex, for ATP-dependent dissociation [36, 48]. In our view, this relatively simple biochemical system has many experimental advantages, and the ongoing elucidation of the Mot1 mechanism provides insight into Swi2/Snf2 ATPases in general. A detailed review of the biochemical data regarding Mot1/BTAF1 is perhaps timely. The Mot1/BTAF1 mechanism has been the subject of considerable speculation, with several different models for Mot1 entertained in the literature [21, 49, 50]. Here we describe the possibilities in the context of available data and argue that the aggregate biochemical and structural evidence place significant constraints on a plausible model for how Mot1 and BTAF1 act on a molecular level, and that ATP-driven DNA translocation is a fundamental feature of the Mot1/BTAF1 catalytic mechanism.

2. Similarities and differences between Mot1 and other Swi2/Snf2 enzymes

Mot1 is a member of the large and diverse Swi2/Snf2 enzyme family, whose members use ATP hydrolysis to alter protein-DNA interactions in essentially all aspects of chromatin metabolism, including transcription, replication, recombination and repair [51–56]. The characteristic feature of this class of enzymes is that they contain a conserved ~70 kD ATPase domain with the seven classic helicase-related motifs [53, 57, 58]. Swi2/Snf2 ATPases are grouped within the SF2 helicase superfamily, although they have a distinct primary sequence signature defined by the spacing between helicase motifs III and IV, as well as conserved features of their sequences within the domain [57]. This distinguishes these enzymes from genuine helicases within the SF2 group. The Swi2/Snf2 ATPases do not have duplex DNA strand separating activity, as they lack the structural features required to induce or trap DNA strand separation. However, Swi2/Snf2 ATPases retain other features of helicases, including directional DNA translocation [59–63].

2.1. ATPase domain structure and DNA interaction

Structural studies have provided key insights into the mechanism of these enzymes. Many crystal structures of SF1 and SF2 ATPase domains have been reported. These display common features in domain organization and a rich variety of conformational states resulting from nucleotide and/or nucleic acid interaction. Although Swi2/Snf2 enzymes are in the SF2 class, SF1 enzymes are arguably the best studied and the principles of SF1 function have informed models of how other translocases and helicases work [64–67]. The SF1 helicase PcrA has been especially influential in defining general principles of structural organization, as well as how the ATP hydrolysis cycle modulates the conformational state [64]. PcrA possesses four domains designated 1A, 1B, 2A and 2B. 1A and 2A are structurally similar to nucleotide binding domains of the *E. coli* RecA strand exchange enzyme, and thus are referred to as RecA-like domains [66, 68, 69]. In fact, all reported ATPase structures reveal a similar organization in which the 1A and 2A domains are structurally conserved and RecA-like [57, 70, 71]. The nucleotide binding site is defined by the cleft between the 1A and 2A domains [67, 72]. Nucleic acid substrates bind across the cleft, explaining how the ATP hydrolysis cycle converts rigid body reorientations of the RecA domains to movement of the ATPase along DNA or RNA. The 1B and 2B domains are thought to confer functional specificity, for example, by coupling ATP hydrolysis to structural rearrangements of the nucleic acid substrate [67, 73]. Accessory domains that flank the ATPase core may also be involved in enzyme-specific interactions with other subunits or factors [57, 74–79]. In this way, the fundamental biochemical activity of nucleic acid translocation has been exploited to catalyze a great variety of reactions. This general picture of SF1 helicase structure and function is consistent with SF2 helicase structure and function as well. Among the SF2 helicases, NS3 is probably the best understood on a structural level [64, 80, 81]. Again, the picture that emerges is of an enzyme that uses ATP binding and hydrolysis to interconvert conformational states in tandem RecA folds to propel the motor along a nucleic acid substrate [80].

The architecture of Swi2/Snf2 ATPase domains is similar to the structural organization of SF1 and SF2 helicase motor domains. The first Swi2/Snf2 ATPase structures reported were obtained of Rad54 from zebrafish and the archaeal organism *Sulfolobus solfataricus* (Sso) [72, 82]. Interestingly, the SsoRad54 ATPase sequence is among the most closely related sequences known to the yeast Mot1 ATPase sequence [49, 82], arguing that its structure serves as an especially accurate model for the overall fold of the Mot1 ATPase. Both Rad54 ATPases have a similar structural organization [72, 82], and the Rad54-DNA complex revealed residues required for DNA binding [82]. Moreover, the positioning of the RecA domains along duplex DNA supports the idea that Swi2/Snf2 ATPases travel along the minor groove of DNA, principally using interactions with one DNA strand [82]. If the Mot1 ATPase domain interacts with DNA similarly to other SF1 and SF2 enzymes, what would be the predicted DNA requirement for this interaction? The SsoRad54-DNA co-crystal structure shows that the ATPase domain interacts with primarily one strand of duplex B-form DNA extending for 12–15 bp, and the interactions are mediated primarily by domain 1A [82]. Comparison of the SsoRad54 and Mot1 primary sequences shows that a number of the DNA-binding residues in SsoRad54 are conserved in Mot1, and mutational analysis supports the role of Mot1 residues in TBP-DNA interaction [49]. However, Mot1 alone has no detectable DNA binding activity on its own [21, 32, 49, 83–85]. Instead, the interaction of Mot1 with DNA is readily demonstrated by the cooperative interaction between Mot1 and TBP on DNA, yielding a DNase I footprint that extends approximately two turns of DNA upstream from the TATA sequence [49, 84].

Remarkably, the DNA interaction length observed in the SsoRad54-DNA co-crystal structure is very similar to the length requirement for Mot1 binding to the TBP-DNA complex established from biochemical experiments [49, 84, 86]. DNA segments with

insufficient length for Mot1 interaction are deficient in catalysis of TBP-DNA dissociation as well [84, 86]. Extensive analyses of the DNA requirement have not shown any significant dependence on the DNA sequence per se, again consistent with structural data showing that the RecA domains contact primarily the sugar phosphate backbone rather than bases. Although one study reported that Mot1 does not require a DNA extension flanking the TATA box [85], a large body of evidence employing different types of assays and methodologies, Mot1 mutational analysis, as well as different DNA templates, provides strong and self-consistent support for the DNA interaction and length requirement summarized here [32, 49, 84, 86]. In hindsight, the Mot1 DNA length requirement is relatively unsurprising given the structural evidence that shows a conserved mode of DNA or RNA interaction by SF1 and SF2 enzymes [57, 64]. Mot1/BTAF1 can stabilize binding of TBP to nonconsensus TATA sequences [85, 87]. In our view, this “relaxed” DNA binding specificity is the result of simple cooperativity in the interaction between Mot1, TBP and DNA. However, whether TBP interacts with DNA differently in the Mot1/BTAF1 ternary complex compared to TBP alone awaits structural analysis to define the details of the DNA interface.

2.2. Targeting of Mot1 to TBP

While all members of the Swi2/Snf2 family contain a well-conserved ATPase domain, their particular tasks in the cell are specified by additional domains that mediate interaction with substrate protein-DNA complexes, or other factors. The interaction with TBP occurs through the Mot1 N-terminal domain [22, 83, 88, 89]. The Mot1 N-terminus is composed of a series of 13 computationally identified HEAT repeats (Huntingtin, elongation factor 3, protein phosphatase 2A, target of rapamycin 1) that extend throughout the ~80 to ~150 kD region conserved among Mot1 homologues from different species [90–92]. HEAT repeats form alpha helical hairpins, and in proteins that possess multiple HEAT sequences, the hairpins typically stack on one another to form extended C- or sickle-shaped structures [93]. The predicted curved and extended shape for Mot1/BTAF1 is consistent with hydrodynamic measurements [48] and image reconstruction derived from electron microscopy of negatively stained single particles [94]. The elongated shapes of HEAT repeat proteins, exemplified by importin-beta [95] and the PR65 subunit of PP2A [96], provide extended surfaces for protein-protein interaction [93, 97, 98]. This explains why a relatively long segment of Mot1/BTAF1 N-terminal amino acid sequence is required for interaction with TBP [22, 84, 88]. HEAT repeat (and related repeat) proteins are also interesting structurally because their extended shapes and absence of distal contacts endow them with elastic properties [93, 99–101]. As discussed below, we propose that the inherent ‘springiness’ of the Mot1 N-terminus is an integral component of its catalytic mechanism.

Stable Mot1-TBP complexes can be isolated from cell extracts, and the Mot1-TBP interaction has an affinity in the nanomolar range [26, 27, 33, 85, 102, 103]. On the other hand, fluorescence recovery after photobleaching (FRAP) measurements reveal distinct recovery curves for TBP and Mot1, suggesting that little if any Mot1 and TBP are stably associated with one another in vivo [44]. A clear understanding of the extent and stability of the Mot1-TBP complex in vivo awaits additional experimental work.

3. TBP as a substrate – what kind of obstacle does it pose?

TBP is a saddle-shaped molecule that makes a high affinity interaction with TATA DNA [104–106]. Crystal structures [107–109] show the interaction of the 8 bp TATA Box sequence with the entire length of the concave surface of TBP [12]. TATA DNA arches under the TBP saddle, severely bending towards the major groove and exposing a wide and shallow minor groove to the TBP interaction surface (Figure 2b) [107–109]. The interactions are primarily hydrophobic, involving DNA base edges in the splayed minor groove and

conserved hydrophobic side chains on the DNA binding saddle. Critical for the interaction are four phenylalanine residues that partially intercalate between base steps at either end of the TATA sequence and induce kinks in the duplex [107–109]. These wedge residues explain in part how the bound DNA becomes so deformed. Coupled with the bending, there is severe local unwinding of TATA DNA [107–109]. Although the bound DNA is highly deformed, the TBP-DNA complex can be nonetheless exceptionally long-lived. In vitro studies show that TBP-DNA complexes have a half-life of ~15–60 minutes [105, 110]. Such stability can explain why an enzyme is required to dissociate TBP from DNA in vivo. It was recently proposed [50] that the distorted conformation of TATA DNA is a key reason why Mot1 acts more efficiently to remove TBP from TATA-containing promoters than TATA-less promoters in vivo [111]. The mechanistic proposal is that the bent and unwound TATA DNA acts as a spring to facilitate Mot1-mediated dissociation by snapping back to the unconstrained state during TBP release [50]. With ATP hydrolysis as an energy source, this is thermodynamically plausible, particularly if differences in DNA trajectory (for example) are also exploited by Mot1 for differential recognition of TATA-containing and TATA-less TBP complexes. Thus, while the stability of TBP-DNA complexes makes them potentially formidable substrates for dissociation, Mot1 may exploit the constrained state of the DNA as part of the catalytic mechanism. At the moment, there is no direct experimental evidence in support of this provocative model. Other factors may also explain Mot1's differential effect on TATA-containing and TATA-less promoters, including the presence of other TBP-associated factors that mask or bias TBP recognition [46, 47, 112].

4. Possible mechanisms for ATP hydrolysis-coupled TBP displacement

The central question for this review is how does Mot1 couple ATP hydrolysis to disrupt the TBP-DNA interaction? Models for Mot1 action fall mainly into two classes. In the first set of models, Mot1 functions as an ATP-dependent DNA translocating motor (Figure 2d-f). Although direct evidence for DNA translocation is lacking for Mot1, such a mechanism is consistent with some experimental data as well as the broad literature on enzymes in Swi2/Snf2 family. Alternatively, Mot1 has been speculated to use the ATPase domain to do something else- a notable hypothesis involves using ATP hydrolysis to induce a conformational change in TBP (Figure 2g). We strongly favor the idea that TBP-DNA dissociation occurs via a mechanism involving DNA translocation activity, and here review the various mechanistic results that led to this view. Also highlighted are gaps in our understanding and questions worthy of future effort.

4.1 “DNA-based” Mechanisms for Mot1 Action

The ATPase activity suggested early on that Mot1 might possess helicase activity or some related activity that drives a change in DNA conformation [36]. DNA strand separation activity was ruled out not only because Mot1 displays no helicase activity in standard strand displacement assays in vitro [36], but because interstrand DNA crosslinks do not abrogate Mot1's TBP-DNA dissociation activity (Figure 2d) [48, 84]. An alternative is that ATP hydrolysis induces DNA twist or bulge propagation through the TATA box. Such a mechanism would provide a parallel with chromatin remodeling enzyme function [113–116]. To test this idea, single-stranded gaps were introduced in the DNA between the TBP-TATA complex and upstream DNA required for Mot1 binding and catalysis [84]. Here again, Mot1's TBP-DNA dissociation activity was not affected, implying that while binding to flanking DNA is important, ATP hydrolysis does not induce torque on DNA in order to release TBP from the TATA box (Figure 2f) [84].

It was reported that Mot1 can readily remove TBP from a conformationally constrained mini-circle DNA template, a result which was interpreted to mean that Mot1 does not utilize DNA bending as an obligate part of the catalytic mechanism [84]. However, a 156 bp

constrained mini-circle does not actually approximate the severity of the bend angle in the TBP-DNA complex. For this reason, whether the highly bent DNA in the TBP-DNA complex contributes to the Mot1 catalytic mechanism remains an open question. As discussed above, Mot1 was recently proposed to somehow exploit the 'spring-loaded' nature of the TBP-DNA complex to efficiently target TATA-containing TBP complexes for dissociation [50].

The final class of 'DNA-based' models posits that the Mot1 ATPase is a DNA tracking motor. In this view, the ATPase domain engages with duplex DNA and uses ATP hydrolysis to track either towards or away from the TBP-DNA complex (Figure 2e). Translocation towards TBP has been hypothesized to displace it from the TATA box by a snowplow mechanism [117]. Alternatively, translocation away from TBP might remove TBP by pulling it off its binding site on DNA. As discussed in section 2.1, ATP-driven DNA translocation is consistent with a wealth of structural and biochemical results for other ATPases in the enzyme family. While the available evidence strongly implicates translocation as part of the catalytic mechanism, this is not to imply that the highly bent TATA DNA, or unique properties of the Mot1-TBP-DNA complex, do not contribute to the TBP-DNA dissociation reaction.

4.2. Roles for TBP allostery or conformational change?

A very different possibility is that Mot1 uses ATP hydrolysis to induce a conformational change in TBP (Figure 2g). We disfavor this idea because, as discussed above, the HEAT repeats that form the TBP interaction surface and tether the ATPase domain to TBP are inherently elastic. Presumably, conformational changes in TBP relevant for Mot1 catalysis would need to be comparably dynamic in order to be put to use in the catalytic cycle. However, TBP is comprised of a single, small domain and a nonessential, flexible N-terminus. The numerous structures of TBP that have been reported bear a striking resemblance to one another with only very modest deviations in the relative orientations of secondary structural elements [107–109, 118, 119]. It is difficult to envisage how the conformationally pliable Mot1 enzyme might induce the kind of wholesale structural rearrangement in TBP structure that would likely be required to disrupt DNA binding.

Recently, evidence has emerged from analyses of other enzyme systems that allostery can occur in small single domain proteins without accompanying structural rearrangements [120, 121]. Although such effects challenge the conventional understanding of how allosteric effects occur, they have been explained in thermodynamic terms [121, 122]. These effects are reminiscent of the observation that a solvent-exposed surface of TBP can mediate rather dramatic effects on DNA binding, even though the surface is distal to the DNA binding surface [123]. Remarkably, this regulatory surface of TBP overlaps with surface residues implicated in Mot1 binding [89]. The combined observations lead to the intriguing but speculative idea that Mot1 binding per se to the 'top' surface of TBP can modulate TBP's DNA binding behavior, perhaps predisposing it to ATPase mediated dissociation that occurs subsequent to Mot1-TBP-DNA ternary complex formation.

A conceptually related idea is that Mot1 activity is regulated in vivo by another factor. The TBP-interacting NC2 heterodimer is a good candidate [45, 102, 124]. A close correspondence has been observed between Mot1 and NC2 localization in vivo, and moreover, Mot1 and NC2 were co-associated with TBP in cell extracts [102]. These results extend prior work [39, 89, 125] demonstrating cooperation between Mot1 and NC2, and suggest that NC2 may demarcate or facilitate TBP-DNA complex dissociation by Mot1 in vivo [50, 102]. Mot1 can stabilize TBP binding to nonconsensus DNA sites, a result that was interpreted to mean that the Mot1-TBP complex has "relaxed" DNA binding specificity [85]. This could be due to the cooperative interaction between Mot1 and TBP, both of which

interact with DNA, and/or it could be due to the ability of Mot1 to alter the molecular details of the TBP-DNA interaction. Distinguishing among these possibilities will probably require a high-resolution structure of the Mot1-TBP-DNA ternary complex.

5. An Integrated Model for Mot1 Catalytic Activity

It is increasingly well established that Swi2/Snf2 enzymes use ATP to translocate along one DNA strand [59, 61, 62, 126]. On a structural level, Swi2/Snf2 enzymes resemble helicases, but without the accessory domains that lead to DNA strand separation [57, 64]. Chromatin remodelers, like SWI/SNF and RSC, have been suggested to disrupt histone-DNA contacts through DNA translocation [63]. They bind to an internal site on the nucleosome, draw in DNA from the entry/exit site and pump it towards the dyad, thus sliding the DNA into and around the nucleosome (Figure 3A), [60, 62, 127]. Single molecule techniques using optical and magnetic tweezers, as well as AFM imaging, have provided information about the translocation properties of chromatin remodelers on nucleosomes and DNA [128–130]. Direct visualization of the translocation motion of molecules like Rad54 and Rdh54 on DNA has also been observed [131, 132]. A mechanism for Mot1 based on DNA translocation activity (Figure 3B) explains most of the available biochemical data and is consistent with the general mechanistic framework for enzymes in the Swi2/Snf2 family. In the following sections we explain how Mot1 fits within this general mechanistic framework.

5.1. Evidence for conserved structural organization and function of the Mot1 ATPase

Based on the structure of a Swi2/Snf2 enzyme Rad54, mutations in homologous residues in Mot1 were made that were predicted to affect its DNA binding and ATPase activities. As expected, the mutants were defective in coupling ATP hydrolysis to TBP displacement and those residues that were predicted to be important for ATP binding showed reduced TBP-stimulated ATPase activity [49]. These experiments support the idea that not only does the Mot1 ATPase have a similar structure as the SsoRad54 ATPase domain, but that Mot1 utilizes a conserved mechanism for ATP hydrolysis-coupled TBP-DNA disruption.

5.2. Evidence for two conformations of the Mot1 ATPase

Structures of SF1 and SF2 ATPases show the RecA domains in either a “closed” conformation, in which the ATP-binding catalytic cleft is properly formed, or an “open” conformation in which one RecA domain has undergone a large rigid body rotation with respect to the other and as a consequence, the ATP binding site does not exist [57, 82, 133]. In the crystal of the SsoRad54-DNA co-complex, the ATPase is in the open conformation, suggesting that the open and closed states represent snapshots of ATPase conformations along the normal catalytic path [82]. In support of this idea, the existence of open and closed conformational forms has been observed directly in solution [134]. This suggests that ATP binding and hydrolysis regulate the interconversion between the open and closed states (Figure 4a, b, c, e). Since DNA binds across the ATP catalytic cleft, RecA domain reorientation in this way would push on the minor groove, explaining how ATP hydrolysis is coupled to DNA translocation [57, 82]. In fact, biochemical evidence supports this general model as being applicable to Mot1. In solution, Mot1-TBP-DNA ternary complexes are of two types: ~60% are more stable than TBP-DNA alone (Figure 4b), whereas the remaining complexes are *less* stable than TBP-DNA alone (Figure 4d) [49]. Importantly, the ternary complexes with reduced stability were observed in the absence of ATP, implying that Mot1 can catalyze an ATP-independent TBP-DNA destabilization reaction [49]. A mutation in the hinge connecting the two RecA domains abolished the biphasic behavior, giving rise only to complexes with greater stability than TBP-DNA [49]. FRET experiments also revealed evidence for an ATPase conformational change that occurs in SsoRad54 after ATP hydrolysis but before ADP release [134]. This fits nicely with the observation that binding

of ATP is insufficient for Mot1 to catalyze TBP-DNA dissociation; hydrolysis of ATP is critical for completion of the ATP-dependent reaction (Figure 4b, c, e) [89]. Taken together, the available results strongly support an ATP-driven conformational cycle for Mot1 that is mechanistically similar to cycles inferred for related enzymes.

5.3. Mot1 ATP hydrolysis rate and translocation

The ATP-independent destabilization of TBP-DNA by Mot1 was completely unexpected [49]. We suggest that when Mot1 binds to TBP-DNA, the ATPase can dock onto the upstream DNA (see section 2.1) in either the closed or open conformation. If the complex assembles in the closed conformation, ATP is not required for the dissociation reaction to proceed. In this case, we suggest that TBP-DNA dissociation occurs because an induced-fit of Mot1 to TBP-DNA destabilizes the TBP-DNA interface. If the complex assembles in the open conformation, ATP binding is required to drive the conformational transitions that result in TBP-DNA dissociation.

In the absence of ATP, there is no energy source to promote multiple rounds of the catalytic cycle, so if the ATP-independent reaction involves the same conformational changes as the ATP-dependent reaction, an implication is that TBP-DNA dissociation by stably bound Mot1 can probably be accomplished by just one round of ATP hydrolysis. This is consistent with observations of 10 bp displacements of the nucleosome by RSC under single ATP turnover conditions [130]. Comparison of the ATP hydrolysis rate with TBP-DNA dissociation rate indicated that as many as ~13 ATPs are hydrolyzed by Mot1 for each TBP-DNA complex dissociated [49]. The stoichiometry is undoubtedly inflated by ATP hydrolysis by free Mot1, but it is also possible that Mot1-mediated catalysis is relatively inefficient or that more than one ATP is required per dissociation event. An additional caveat is that the ATP-independent dissociation reaction is not observed using all TATA-containing DNA templates [86], so the predominant Mot1 reaction mechanism appears to be DNA sequence dependent on some level.

ATPases that interact with DNA move in one direction by tracking along one of the two DNA strands. Since DNA has an intrinsic symmetry, the tracking directionality is dictated by other interacting factors [64]. A wealth of information has emerged on how this occurs on a molecular level. Conceptually, the mechanism is akin to the inchworm model proposed by Yarranton and Gefter more than 30 years ago [135]. Presumably, the asymmetric binding of Mot1 to the TBP-DNA complex [84] is responsible for ATP-driven tracking on upstream DNA either toward or away from TBP bound to the TATA Box. Despite the large body of evidence that SF1 and SF2 enzymes are nucleic acid translocating enzymes, and that chromatin-remodeling enzymes in particular are DNA tracking enzymes, it's been clear for some time that Mot1 does not track processively on DNA [117]. Short-range translocation, using triplex helix displacement assays for example, have also failed to reveal translocation activity (W-S. Park and D.T.A., unpublished observations). However, translocation of one or a few base pairs would not be detectable by these assays. This distinction in tracking ability can be understood in terms of the targeted substrate. Rather than reorganizing an extensive protein-DNA interface as required by a chromatin-remodeling enzyme, Mot1's task is comparatively simpler. Displacement of TBP from DNA need not require a processive translocase because the TBP-DNA interface is much smaller; disruption of one or a few TBP-DNA interactions would be sufficient to induce dissociation of the TBP-DNA complex. Additionally, BTAF1 has been shown to regulate the DNA binding surface of TBP through its N-terminal HEAT repeats [22]. A pre-formed yeast Mot1-TBP complex does not bind DNA well, consistent with a role for Mot1 in regulating the TBP-DNA binding surface accessibility, and in this case the effect is regulated by ATP hydrolysis [49, 84]. Together, the results suggest that domains on Mot1 may regulate TBP-DNA binding or ensure irreversibility of TBP-DNA dissociation via interactions with TBP's DNA binding surface.

5.4. Macromolecular springs and how translocation may be put to use

While models in which Mot1 dissociates TBP-DNA complexes by inducing DNA twisting or strand separation have been largely ruled out, it remains possible that DNA unbending facilitates the dissociation reaction [50] (see section 3). The release of the bent DNA in the TBP-DNA complex and its relaxation to a straightened form has been proposed to drive dissociation like the release of tension in a spring [50]. An alternative, and perhaps complementary idea, is that spring-like HEAT repeats that tether the Mot1 ATPase to TBP fulfill a similar function by storing elastic tension generated as the ATPase domain walks along DNA. As discussed in section 2.2, HEAT repeat proteins are inherently springy, and biochemical and computational data support the notion that the Mot1 N-terminus has such properties as well. We propose that the HEAT linker in Mot1 is not simply a passive connector of the ATPase to TBP, but that its stretchable and compressible character would allow the ATPase to translocate along DNA, and then displace TBP when a critical threshold of force is achieved. Remodelers can generate forces of up to ~12 pN [129]. Although an estimate is highly speculative, based on measurements of the spring-like behavior of ankyrin-B repeats [101], and assuming that the spring constants of HEAT and ankyrin repeats are similar, the HEAT repeats of Mot1 could stretch by ~0.7 nm in response to ATP-driven DNA translocation. This corresponds to a stepping distance of ~2 bp along DNA. Additionally, if the Mot1 ATPase behaves similarly to chromatin remodeling ATPases [49, 53, 60, 61, 89], the prediction is that ATP hydrolysis induces it to walk away from the TBP-DNA complex. This prediction is consistent with photocrosslinking results that show the Mot1 ATPase domain interacts primarily with the “top” strand of DNA [49] and a typical 3' to 5' translocation direction for SF2 ATPases [59–62, 64]. It is unknown if DNA translocation alone would be sufficient for TBP-DNA dissociation. However, even among enzymes that induce structural changes in their substrates as a result of ATP hydrolysis, nucleic acid translocation is fundamentally the mechanistic basis for the observed catalytic activity [136, 137].

In summary, based on the conserved structural organization and function of the ATPase domain and the biochemical evidence, the catalytic mechanism of Mot1 resembles the mechanism of other Swi2/Snf2 enzymes. As DNA translocation is a conserved feature of the ATPases of the Swi2/Snf2 family, we propose that the catalytic mechanism by which Mot1 displaces the TBP-DNA complex involves short range DNA tracking, which serves to pull or push TBP off its binding site on DNA. Additionally, the elastic nature of the Mot1 N-terminus that tethers the ATPase domain to TBP-DNA might contribute to the displacement reaction by storing energy until a dissociation threshold is reached.

6. Open questions and future directions

The model for Mot1 action proposed here is consistent with the available evidence but several of its features lack direct support. To define steps in the catalytic cycle in molecular detail, we require structural data, combined with biochemical and biophysical analyses of single complexes. X-ray crystallography is showing promise in elucidating the structure of the Mot1-TBP complex (P. Wollmann, S. Cui, R. V., O. Berninghausen, M.N. Wells, M. Moldt, G. Witte, A. Butryn, P. Wendler, R. Beckman, D.T.A., and K.-P. Hopfner, unpublished observations). High-resolution structures will be critical for designing experiments to rigorously test specific facets of the model. Measurements of force necessary to displace TBP along DNA would provide constraints for the motor requirement. In addition, measurements of springiness of the Mot1 N-terminus in the context of the required force for TBP-DNA displacement would help test whether elasticity has the potential to play a catalytic role. Single molecule approaches such as FRET [138] will also be useful for determining if and how the TBP-DNA complex changes when stably bound by Mot1 in the absence of ATP. Such approaches that register DNA trajectory could be implemented to test

the model that release of bent DNA drives the TBP-DNA dissociation reaction[50]. There is evidence that the Mot1-TBP-DNA ternary complex can be stabilized by nonhydrolyzable or transition state analogs of ATP [88, 89]; these will be interesting to characterize using single molecule approaches as well. Obtaining direct evidence for short-range DNA tracking may continue to prove difficult. From a thermodynamic standpoint, it is important to bear in mind that Mot1-mediated TBP-DNA dissociation may not proceed through a single pathway even if the sole function of the ATPase is to step along DNA [139].

7. Conclusions

Swi2/Snf2 enzymes appear to share many fundamental structural and biochemical properties, and yet are tailored to perform numerous unique functions in vivo. Most function in multi-subunit complexes and perform complex biochemical reactions such as chromatin remodeling. Given this complexity, we argue that analysis of the Mot1 mechanism provides some unique perspective on Swi2/Snf2 enzyme function. In particular, Mot1 appears to drive TBP-DNA dissociation by a mechanism comparable to that employed by chromatin remodeling enzymes to initiate protein-DNA rearrangements that typically require many more ATP hydrolytic events. As such, analysis of Mot1 provides insight into conserved aspects of Swi2/Snf2 motor function.

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Research Highlights

1. Different models for the Mot1 mechanism are discussed.
2. A key aspect of the Mot1 mechanism is proposed to be DNA translocation, driven by an ATP-mediated conformational cycle.
3. Rather than functioning as a simple tether, the spring-like nature of the Mot1 N-terminus is proposed to contribute to the catalytic mechanism.
4. The Mot1 catalytic mechanism is proposed to be analogous to the initial ATP-driven steps in chromatin remodeling, arguing that mechanistic insights derived from analysis of Mot1 are applicable to other Swi2/Snf2 ATPases.

Figure 1. Comparison of Mot1 to native chromatin remodeling complexes

In comparison to the multi-subunit SWI/SNF remodelers (containing 8–15 subunits) and ISWI remodelers (containing 2–4 subunits), Mot1 (and BTAF1) can be isolated from cell extracts as a single polypeptide and can function biochemically as a single polypeptide, thereby offering a simple system to gain insight into the mechanisms of members of this broad classes of enzymes [36, 48, 77]. Mot1 possesses a conserved ~70 kDa C-terminal ATPase domain and an extended N-terminal HEAT repeat region that mediates interaction with TBP [22, 89]. HEAT repeat proteins generally assume C-shaped structures [93]; such a shape is supported in the case of Mot1 and BTAF1 by biochemical and EM data [48, 94]. For simplicity, the flanking domains of the ATPase subunits of the chromatin remodelers are not shown.

Figure 2. Possible models for the catalytic mechanism of Mot1

TBP binds and bends the DNA (a, b). Mot1 binds to the DNA adjacent to the TATA box and recognizes the 'top' surface of TBP through its N terminal HEAT repeats (c). DNA based mechanisms for the displacement of TBP are shown in d-f. Evidence suggests that the catalytic mechanism does not involve DNA strand separation or twist/bulge propagation (d, f). Instead, Mot1 might translocate on the DNA upstream from the TATA box, thereby displacing TBP (e). A possible allosteric mechanism for Mot1 is shown in (g). In this scenario, Mot1 might induce a conformational change in TBP that alters the binding interaction of TBP with DNA. In all of these mechanisms, the 'spring-loaded' nature of TBP-TATA complex resulting from the highly distorted TATA DNA might facilitate TBP-DNA dissociation [50]. In all of these scenarios, the reaction is depicted to generate free TBP and Mot1, consistent with previous proposals [85]. However, the stability of the Mot1-TBP complex in vivo is currently unresolved.

Figure 3. Comparison of chromatin remodeling enzyme DNA translocation with proposed short-range DNA tracking by Mot1

(A) A chromatin remodeler binds to the nucleosome at a fixed site and uses ATP hydrolysis to translocate along DNA, thereby pumping the DNA in the direction shown by the arrow. The yellow star represents a particular DNA site shown as a reference for DNA movement [63, 140]. (B) Model showing the proposed translocation of the Mot1 ATPase domain on DNA upstream and away from TBP. This results in upstream DNA being pumped toward the TBP-DNA complex (arrow). This translocation direction is predicted by analogy with other Swi2/Snf2 family members, as well as the crosslinking pattern of Mot1 to primarily one particular strand of upstream DNA [49, 63, 64].

Figure 4. Conformational changes in the ATPase domain drive TBP-DNA displacement

SF2 ATPases exist in open or closed conformational forms [57, 82]. There is biochemical evidence that the Mot1 ATPase exists in two separate conformational forms as well (a, d) [49]. Heterogeneity in the kinetic behavior of Mot1-TBP-DNA ternary complexes suggests that Mot1 can bind to TBP-DNA in either conformation. Ternary complex formation in the closed state is highly unstable, even in the absence of ATP (d). In contrast, ATP binding to the catalytic cleft induces transition of the open to the closed state (b). ATP hydrolysis (c) leads to TBP-DNA dissociation and product release (e). Importantly, ATP binding is not sufficient for TBP-DNA dissociation in the stable ternary complex population; rather, ATP hydrolysis and product release appear to be key, consistent with published data on SsoRad54 [134]. TBP is shown in blue, Mot1 in red, ATP and the products of its hydrolysis (ADP and Pi) in green.