PERSPECTIVE

Regulation of the Dbp5 ATPase cycle in mRNP remodeling at the nuclear pore: a lively new paradigm for DEAD-box proteins

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It is commonly assumed that all DEAD-box ATPases function via a shared mechanism, since this is the case for the few proteins characterized thus far. Hodge and colleagues (pp. 1052–1064) and Noble and colleagues (pp. 1065–1077) now describe a novel model for Dbp5's ATPase cycle in mRNA (messenger RNA)/protein complex (mRNP) remodeling during nuclear export. Notably, unlike other DEAD-box proteins, Dbp5 uses a conformational change distinct from ATP hydrolysis for its activity and requires an ADP release factor to reset its ATPase cycle.

Dbp5 is a canonical DEAD-box protein with a novel mechanism

Dbp5 is one of 25 DEAD-box RNA-dependent ATPases in budding yeast. One or more DEAD-box proteins are associated with every major step of RNA processing (for recent reviews, see Cordin et al. 2006; Jarmoskaite and Russell 2010). This family of proteins is characterized by nine conserved motifs (including the eponymous DEAD motif), which contact ATP and RNA (Fig. 1A). The conserved motifs fold into two RecA-like domains connected by a flexible linker (Fig. 1B). These domains are loosely associated when nucleotide-free, but form a binding pocket for ATP and a binding surface for RNA that are each contacted by conserved residues within the characteristic motifs. Although only a handful of structures exist for DEAD-box proteins, the RecA-like domains in different proteins interact with nucleotides and RNA very similarly (Caruthers et al. 2000; Cheng et al. 2005; Andersen et al. 2006; Sengoku et al. 2006; Del Campo and Lambowitz 2009). DEAD-box proteins accomplish a wide range of cellular tasks, including remodeling or stabilizing structured

RNA, unwinding short helical regions of RNA, stabilizing protein complexes, and directly removing proteins bound to RNA. The functional specificity of individual proteins in the DEAD-box family is due at least in part to their unique N-terminal and C-terminal sequences flanking the two RecA-like domains and their interactions with regulatory protein cofactors.

Several DEAD-box proteins from various organisms have been well characterized, and all function via a similar mechanism (Hilbert et al. 2009). The DEAD-box ATPase cycle begins in the absence of nucleotides and RNA with the two RecA-like domains in an open conformation. Next, ATP binding and RNA binding occur in a cooperative manner. When bound to ATP, DEAD-box proteins have a tight affinity for RNA. ATP binding is sufficient for many DEAD-box proteins to unwind short duplex RNA. Structures of RNA-bound DEAD-box proteins show a sharp kink in the backbone of the ssRNA that is incompatible with A-form helical RNA. ATP hydrolysis is not necessary for the unwinding activity of several DEAD-box proteins, which is still possible in the presence of certain nonhydrolyzable ATP analogs. However, full activity seems to require ATP hydrolysis itself, but not release of the inorganic phosphate (P_i) from the hydrolyzed ATP. Once P_i is released, the ADP-bound DEAD-box protein has a low affinity for RNA and dissociates from it. Finally, ADP dissociates from the DEAD-box protein, which is then ready to begin the ATPase cycle again.

While Dbp5 shares many structural and biochemical features with the other characterized members of the DEAD-box family, Hodge et al. (2011) and Noble et al. (2011) have shown that its mechanism is unique (Fig. 2). The RecA-like domains of Dbp5 are structurally similar to other members of the DEAD-box family in the presence of ATP, ADP, and RNA (Collins et al. 2009; von Moeller et al. 2009; Montpetit et al. 2011). However, Dbp5 has ~90 residues at its N terminus preceding the first RecA-like domain that is important for autoregulating its ATPase activity. Like other DEAD-box proteins, Dbp5 has a tighter affinity for RNA when bound to ATP than to ADP (Tran et al. 2007), and structures show it kinks the backbone of

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Figure 1. Dbp5 structural model. (A) Schematic representation of yeast Dbp5. The N-terminal region of Dbp5 does not contain any known motifs and its structure is unknown. The two domains of the conserved helicase core are shown in blue and purple. The nine motifs conserved among DEAD-box proteins are shown in yellow boxes within the two RecA-like domains. The Q motif contacts the adenine base of ATP; motifs I, II, and VI contact the phosphate groups of ATP; and motifs Ia, Ib, IV, and V contact the RNA backbone. Motif II contains the conserved D-E-A-D residues for which the family of proteins is named. (B) Cartoon of domain arrangement of Dbp5 bound to ATP and RNA. The structure of the gray N-terminal region of Dbp5 is unknown. A flexible linker connects the two RecA-like domains. (C) Cartoon showing which faces of Dbp5 bind Gle1-IP₆ and Nup159. Both have been shown in vitro to simultaneously bind Dbp5 in the presence of ADP and absence of RNA.

its bound RNA (Collins et al. 2009; von Moeller et al. 2009; Montpetit et al. 2011). Importantly, the remainder of Dbp5's ATPase cycle is novel for the DEAD-box family. Dbp5 seems to require both ATP hydrolysis and P_i release for its mRNA (messenger RNA)/protein complex (mRNP) remodeling activity, which is achieved by the conformational change it undergoes from its ATP-bound state to its ADP-bound state (Tran et al. 2007; Noble et al. 2011). Dbp5 has a tight affinity for ADP and requires an additional protein cofactor, Nup159, to help it release ADP for

further rounds of catalytic activity (Noble et al. 2011). Nup159 is the first known ADP release factor for a DEAD-box protein.

Dbp5 confers directionality to mRNP nuclear export

Dbp5 is involved in several cellular processes relating to mRNA metabolism. It is diffusely localized across the cell, but is enriched at the cytoplasmic side of the nuclear envelope at steady state (Schmitt et al. 1999). Dbp5 has no known specific RNA targets, suggesting that it is spatially controlled by interactions with cofactors. Dbp5 associates with nascent mRNA transcripts in the nucleus (Zhao et al. 2002; Estruch and Cole 2003), and, when Dbp5 is impaired, nuclear RNAs are hyperadenylated (Hillgren and Parker 2001). This suggests that Dbp5 has a role in mRNA quality control. Dbp5 is also involved in recognizing termination codons and recruits the protein synthesis release factor eRF3 (Gross et al. 2007; Bolger et al. 2008). Interestingly, Dbp5 has been genetically linked to P-body components (Scarcelli et al. 2008), and may therefore have undiscovered roles in mRNA metabolism. However, the best-characterized role for Dbp5 thus far is its remodeling of mRNPs exiting the nuclear pore complex (NPC).

Dbp5 acts in an ATP-dependent manner to remodel mRNPs exiting the NPC to confer directionality to mRNP export (for recent review, see Stewart 2010). The hydrophilic mRNA is able to pass through the NPC aided by the Mex67–Mtr2 heterodimer, which passively interacts with the hydrophobic proteins that line the pore. However, Mex67–Mtr2 do not contribute to the directional flow of the mRNP. Dbp5 establishes directional mRNP transport by removing Mex67–Mtr2 from the mRNP at the cytoplasmic face of the NPC. Mex67–Mtr2 then return to the nucleus, while the mRNA remains in the cytoplasm to be translated by ribosomes.

Recent work by Hodge et al. (2011) and Noble et al. (2011) now elucidates how Dbp5's specificity for remodeling newly exported mRNPs is achieved. Dbp5's ATPase

Figure 2. Simplified model of Dbp5 ATPase cycle. (1) The cycle begins with a nucleotide-free Dbp5, which is ATPase-repressed by its NTD. (2) Next, Gle1-IP₆ binding to Dbp5 stimulates it to bind ATP. (3) Then, an mRNP binds the Dbp5-Gle1-IP₆-ATP complex. After ATP hydrolysis, Dbp5 changes its conformation and releases the remodeled mRNA. (4) Nup159 binds to Dbp5 and stimulates it to release ADP.

cycle is regulated by two proteins found at the cytoplasmic face of the NPC. Gle1, which binds the nucleoporin Nup42 (Strahm et al. 1999), stimulates Dbp5's ATP binding and hydrolysis. Nup159 acts to release ADP from Dbp5. Here, we review the new model for Dbp5's ATPase cycle, and how it is novel for the DEAD-box family of proteins.

Gle1-inositol hexakisphosphate (IP₆) stimulates ATP binding to Dbp5

Like many DEAD-box proteins, Dbp5 binds a partner protein, Gle1, which stimulates its ATPase activity. One surface of Gle1 binds Nup42, part of the cytoplasmic face of the NPC (Strahm et al. 1999), while another surface binds Dbp5 across both of its RecA-like domains (Fig. 1C; Montpetit et al. 2011). The interaction with Dbp5 is stabilized by the small molecule IP₆, associated with Gle1 (Montpetit et al. 2011). Gle1-IP₆ helps stabilize the closed conformation of the two RecA-like domains of Dbp5, which is necessary for ATP binding. Hodge et al. (2011) and Noble et al. (2011) show that interaction with Gle1-IP₆ increases Dbp5's ability to bind ATP by using a mutation in the DEAD motif of Dbp5 that prevents ATP hydrolysis: Dbp5(E240Q) (also noted as Dbp5^{EQ}). In vitro, addition of Gle1-IP₆ increases the amount of bound ATP by ~2.5-fold (Noble et al. 2011). Although Dbp5(E240Q) still binds Gle1-IP₆, ATP, RNA, and some Nup159 in vitro, it is lethal to cells when present as the only form of Dbp5, confirming the necessity of ATP hydrolysis for Dbp5 activity and not merely ATP binding (Hodge et al. 2011).

Gle1-IP₆ binding is necessary for Dbp5's mRNP remodeling activity. Hodge et al. (2011) created a mutant, Dbp5(R369G), that competes with wild-type Dbp5 for Gle1-IP₆ ATPase stimulation in vitro. Dbp5(R369G) cannot bind RNA, and therefore cannot remodel mRNPs, making it lethal to cells in the absence of wild-type Dbp5. In vivo, the competition for Gle1-IP₆ results in a dominant-negative mRNP export phenotype. This phenotype is diminished when Gle1 is overexpressed or the Dbp5 residues that interact with Gle1 are mutated. Dbp5(R369G) still properly localizes to the NPC. These data show Gle1-IP₆ binding is necessary for Dbp5's mRNP remodeling activity and its NPC localization.

It is important to keep in mind that Dbp5 is associated with many cellular processes, and in vivo phenotypes may be due to effects on any of them. For example, Gle1-IP₆ binding and ATP hydrolysis are also necessary for Dbp5's role in termination codon recognition (Bolger et al. 2008), and Dbp5(E240Q) has been shown to inhibit proper translation termination as well as mRNP export (Gross et al. 2007). Dbp5's requirement for ATP binding and hydrolysis in its other cellular roles remains unknown.

Interestingly, the contacts formed between Dbp5 and Gle1 are similar to those formed between the DEAD-box ATPase eIF4A and its interacting partner, eIF4G, which are necessary for cap-dependent mRNA translation initiation. Crystal structures have been solved for both the Dbp5-Gle1-IP₆ complex and eIF4A-eIF4G (Oberer et al. 2005; Schutz et al. 2008; Dossani et al. 2009; Montpetit

et al. 2011). Both eIF4G and Gle1 are composed of HEAT repeats—anti-parallel α -helical hairpins that fold into a curved structure. eIF4G binds across both of eIF4A's RecA-like domains, as Gle1 does with Dbp5, with additional protein contacts making up for the lack of a small molecule like IP₆ (Oberer et al. 2005). By helping to close the two RecA-like domains, Gle1 and eIF4G each act like a “soft clamp” to hold the domains in a conformation productive for ATP binding (Oberer et al. 2005; Schutz et al. 2008; Dossani et al. 2009; Montpetit et al. 2011). The structural similarities and similar binding sites of these ATPase stimulators suggest that they may represent a common paradigm for contacting and regulating DEAD-box proteins.

Dbp5 remodels mRNPs via a conformational change distinct from ATP hydrolysis

Many DEAD-box proteins tested to date seem to use the energy from ATP hydrolysis not for RNA remodeling per se, but for enzyme recycling to reset the ATPase for multiple rounds of activity. This has been demonstrated using the well-characterized DEAD-box proteins Ded1, eIF4A, and Mss116. All three proteins are able to unwind a short duplex RNA in the presence of a nonhydrolyzable ATP analog, ADP-beryllium fluoride (Liu et al. 2008). However, after a single round of unwinding, the protein remains bound to one of the RNA strands instead of releasing it, and therefore cannot unwind any more duplex RNA. When ATP is present, many rounds of duplex RNA unwinding are accomplished. This shows that ATP hydrolysis is necessary for RNA to be released and the enzyme to recycle, while ATP binding alone is sufficient for unwinding activity. However, maximal duplex unwinding may require ATP hydrolysis, but not P_i release (Henn et al. 2008; Cao et al. 2011). Interestingly, another nonhydrolyzable ATP analog, ADPNP, does not result in activity for any proteins tested, including Dbp5 (Tran et al. 2007; Liu et al. 2008). Although the RecA-like domain conformations have been shown to be similar in the presence of ADP-beryllium fluoride and ADPNP (Del Campo and Lambowitz 2009), seemingly small differences in the analogs used for these mechanistic investigations can clearly have a large impact on the enzymatic activity.

Importantly, the mechanism by which Dbp5 couples ATP hydrolysis and mRNP remodeling activity is distinct from that of other DEAD-box ATPases, in which ADP alone is unable to stimulate enzymatic activity (Liu et al. 2008; Del Campo et al. 2009). However, Dbp5 has robust in vitro activity in the presence of ADP (Tran et al. 2007; Noble et al. 2011). This remodeling activity is interpreted as being the result of a conformational change in Dbp5 between its starting nucleotide-free state, which is structurally similar to the ATP-bound state, and its final ADP-bound state (Tran et al. 2007; Noble et al. 2011). Although such a transition is unlikely to take place in vivo due to the low concentration of ADP, this experiment nicely distinguishes ATP hydrolysis itself from a structural transition that results from it, likely when P_i is released. Although the NS3 helicase, a member of the related DExD/H-box

family of RNA-dependent ATPases, has been suggested to perform its mechanical function upon P_i release (Wang et al. 2010). Dbp5 is the first example of a canonical DEAD-box protein suggested to function this way.

The mechanism by which Dbp5 removes specific proteins from mRNPs is still unknown. Dbp5 is known to remove the poly(A)-binding protein Nab2 from mRNPs as well as Mex67/Mtr2. It has been shown in vitro that Dbp5 does not interact directly with or displace Nab2 from poly(A) RNA (Tran et al. 2007). Instead, it inhibits the reassociation of Nab2 after a noncatalyzed dissociation event, probably by restructuring the free RNA such that it is incompatible with Nab2 binding. It is still unknown whether Dbp5 interacts directly with Mex67/Mtr2 or actively displaces it. Also, it is unknown how Dbp5 avoids removing proteins from the exported mRNP that are necessary for mRNA translation.

Interestingly, Dbp5 autoregulates its own ATPase activity, which is stimulated by Gle1-IP₆. Gle1-IP₆ increases the affinity of Dbp5 for RNA and stimulates Dbp5's ATP hydrolysis. Full-length Dbp5 exhibits very low in vitro ATPase activity in the absence of RNA and Gle1-IP₆. However, deleting the N-terminal 91 amino acids of Dbp5 results in a high ATPase level that is Gle1-IP₆-independent and is not further stimulated by RNA (Collins et al. 2009; Montpetit et al. 2011). Also, although full-length Dbp5 binds only a small percent of RNA in the presence of ATP, the truncated Dbp5 is able to saturate RNA binding in the presence of ATP (Montpetit et al. 2011). Therefore, the N-terminal region of Dbp5 represses its ATP hydrolysis and RNA-binding activities. While this N-terminal region is often deleted in order to crystallize Dbp5 for structural analysis, one structure of ADP-bound Dbp5 with a portion of the N-terminal region shows that residues 55–68 form an α helix that inserts between the two RecA-like domains (Collins et al. 2009). This suggests that the N-terminal region acts as a regulator of Dbp5 ATPase activity by interacting directly with the ATP-binding cleft. Truncating the N terminus of Dbp5 increases its affinity for RNA to the same extent as Gle1-IP₆ binding to wild-type Dbp5 (Montpetit et al. 2011), suggesting that the N-terminal sequence in Dbp5 also represses its binding to RNA in the absence of Gle1-IP₆. Gle1-IP₆ may stimulate Dbp5's ATPase and RNA-binding activities by shifting Dbp5's catalytically repressive N-terminal region upon binding. Although Dbp5 is the only known example of a DEAD-box protein that has an ATPase-repressing domain, it is possible that any of the many members of the DEAD-box family that have not been thoroughly characterized biochemically could also have similar autoregulatory domains.

Nup159 stimulates ADP release by Dbp5

Excitingly, Noble et al. (2011) have discovered that Dbp5 requires an ADP release factor, a novel finding for a DEAD-box ATPase. Unlike other DEAD-box proteins, Dbp5 has a tight affinity for ADP. This is likely because Dbp5 uses ATP hydrolysis as part of its remodeling activity rather than for enzyme recycling like other DEAD-box

proteins. Dbp5's recycling requires regulated release of its bound ADP to reset its ATPase cycle.

Noble et al. (2011) show that, once Dbp5 is bound to ADP in vitro, it does not release ADP, even in the presence of competing ADP or ATP. Although Gle1-IP₆ stimulates the ATP binding of the nucleotide-free form of Dbp5, it does not affect the ADP release or exchange for ATP over a 24-h period. However, when the N-terminal domain (NTD) of Nup159 (also known as Rat7 or Nup214 in humans) is present, significant amounts of ADP are exchanged within minutes (Noble et al. 2011). A recent crystal structure shows that the NTD of Nup159 binds Dbp5 and opens the RecA-like domains (Montpetit et al. 2011). This weakens the interaction between Dbp5 and ADP, and is likely how Nup159 causes ADP release.

Nup159 probably acts after Dbp5 has released the remodeled mRNP. RNA and Nup159 binding are mutually exclusive due to their similar binding site on Dbp5 (Fig. 1C; Napetschnig et al. 2009; von Moeller et al. 2009; Montpetit et al. 2011). Also, the human homolog of Nup159 has tight affinity for ADP-bound and nucleotide-free Dbp5, but has weak affinity for ATP-bound Dbp5 (von Moeller et al. 2009). Therefore, Nup159 likely binds only to RNA-free, ADP-bound Dbp5. It is currently unknown whether Dbp5 releases mRNA itself, or whether Nup159 also has an active role in displacing the remodeled mRNA.

Interaction with Nup159 is also necessary for NPC localization of Dbp5 in vivo. Cells expressing Nup159 with a truncated NTD do not exhibit normal enrichment of Dbp5 at the nuclear envelope (Noble et al. 2011). Similarly, a Dbp5 double mutant [Dbp5(R256D/R259D)] that does not bind Nup159 in vitro also lacks enriched nuclear envelope localization and is diffuse throughout the cytoplasm. Interestingly, cells with the Nup159 NTD truncation only exhibit a temperature sensitive phenotype, and Dbp5(R256D/R259D) does not have an observable cell growth phenotype (Noble et al. 2011). Since an mRNP export phenotype is not observed in these mutant cells, Dbp5 must either make use of another unknown ADP release factor, potentially associated with one of its other cellular roles, or exchange ADP for ATP through an unknown mechanism in order to remodel mRNPs.

The requirement for an ADP release factor is a novel finding for DEAD-box proteins, but it is unclear whether it is unique. In general, there is only a limited amount of mechanistic data on a small number of DEAD-box proteins. Therefore, it could be the case that ADP release factors are necessary for any of the yet-uncharacterized DEAD-box proteins that may be found to couple activity to the conformational change between the ATP- and ADP-bound states, like Dbp5. Regulated ADP release and enzyme recycling may be necessary for Dbp5's regulation to prevent any off-target activity, since Dbp5 is found in all parts of the cell and is associated with many different cellular processes.

New model for the Dbp5 catalytic cycle

Taken together, the two new studies by Hodge et al. (2011) and Noble et al. (2011) provide evidence for a comprehensive

model explaining the regulation of mRNP remodeling by Dbp5 during nuclear export at the NPC (Fig. 2). As an mRNP exits the nuclear pore, it passes Nup42, the nucleoporin that interacts with Gle1-IP₆. This interaction may serve to increase the local concentration of Gle1-IP₆, which in turn increases the local concentration of Dbp5. Since Dbp5 does not have any known specific RNA targets, concentrating Dbp5 at the NPC may also serve to target it specifically to newly exported mRNPs. Given that ATP and RNA bind to Dbp5 cooperatively, in principle, either may occur first. However, since Gle1-IP₆ stimulates ATP binding and may help to recruit Dbp5 to the exiting mRNP, ATP binding may occur first. When Dbp5 binds the mRNP, Gle1-IP₆ stimulates Dbp5 to hydrolyze its bound ATP. The Dbp5-ADP-P_i then releases P_i and transitions to its ADP-bound conformation, which has low affinity for RNA. This conformational change presumably does the mechanical work of mRNP remodeling. In an as-yet-undetermined mechanism, Mex67-Mtr2 and Nab2 are released and return to the nucleus, and the mRNA proceeds to a ribosome to be translated. Dbp5-ADP, having weaker affinity for RNA and higher affinity for Nup159 than Dbp5-ATP, then binds the NTD of Nup159, located nearby on the NPC (Alber et al. 2007). Once bound, Nup159 causes Dbp5 to release its bound ADP, and one full Dbp5 cycle is complete.

Open questions

While these studies elucidate how Dbp5 is able to remodel mRNPs exported from the nucleus in a regulated manner, many questions about Dbp5 remain. It is unclear at this point how the cycle is reset and whether Nup159 directly hands the nucleotide-free Dbp5 to Gle1-IP₆. Also, it is unknown whether a single Dbp5 is handed off between Gle1-IP₆, an mRNP, and Nup159 to accomplish multiple cycles of remodeling, or whether different copies of Dbp5 continuously occupy all of these factors such that multiple cycles of remodeling occur simultaneously. Gle1-IP₆ and Nup159 are able to bind Dbp5 simultaneously *in vitro* (Montpetit et al. 2011), but this has not yet been demonstrated *in vivo*. It is still unclear precisely how Dbp5 remodels an mRNP and how its activity is regulated such that only nuclear factors such as Mex67-Mtr2 are removed. Also, while the data support that Dbp5 accomplishes mRNP remodeling via a conformational change, careful experiments need to be performed to show that, after ATP hydrolysis, phosphate release and remodeling are correlated. Similarly, the role of the N-terminal sequence of Dbp5 in regulating its ATP and RNA binding must still be determined. Dbp5 has multiple roles in the cell, and it remains to be discovered whether it has other ADP release factors aside from Nup159. Alternatively, it is possible that Dbp5 acting in one of these other cellular functions must also bind Nup159 at the NPC to reset its nucleotide state. Since Dbp5 also interacts with transcriptional, translational, and P-body components, its functional specificity may depend on its cellular location and other interacting factors.

These new studies have made Dbp5 a rare example of a member of the DEAD-box RNA-dependent ATPase family, where there are strong structural, biochemical, and genetic functional data that describe its mechanism and regulation. Notably, Dbp5 requires a regulatory factor, including its own NTD, to control each step of its ATPase cycle. The dearth of mechanistic detail for other DEAD-box proteins could mean that Dbp5's mechanism and use of cofactors will turn out to be common methods of regulation and function throughout this family of ATPases. In the meantime, Dbp5 is the only known DEAD-box protein that (1) has a putative ATPase autoregulatory domain, (2) functions via a conformational change after ATP hydrolysis, and (3) has an ADP release factor, Nup159. This foundation of a model for the regulation and function of the Dbp5 ATPase cycle allows the remaining open questions for mRNP remodeling during nuclear export to be addressed, and poses important questions for studies on other DEAD-box ATPase studies.

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