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Dbp5, Gle1-IP_6 and Nup159

A working model for mRNP export

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Gene expression is a stepwise process involving distinct cellular processes including transcription, mRNA (mRNA) processing, mRNA export, and translation. As mRNAs are being synthesized, proteins associate with the RNA to form messenger ribonucleoprotein particles (mRNPs). Previous studies have demonstrated that the RNA-binding protein composition of these mRNPs is dynamic, changing as the mRNP moves through the different steps of gene expression, and playing a critical role in these events. An important step during this maturation process occurs at the cytoplasmic face of the nuclear pore complex (NPC) where the export protein Gle1 bound to inositol hexakisphosphate (IP_6) spatially activates the ATP-hydrolysis and mRNP-remodeling activity of the DEAD-box protein Dbp5. Recent work from our laboratory and others has provided important insights into the function and regulation of Dbp5. These include a more detailed explanation of the mechanism of Dbp5 RNP remodeling, the role of Gle1-IP_6 in stimulating Dbp5 ATPase activity, and the identification of a novel paradigm for regulation of Dbp5 by Nup159. Based on in vitro biochemical assays, X-ray crystallography, and corresponding in vivo phenotypes, we propose here an updated model of the Dbp5 cycle during mRNP export through the NPC. This takes into account all available data and provides a platform for future studies.

Introduction

Regulation of the life cycle of mRNA-protein (mRNP) complexes is essential for proper gene expression. As mRNA is synthesized, proteins associate co-transcriptionally to mediate each downstream step from export to translation and degradation.[1-3] One family of enzymes, the DEAD-box proteins (DBPs), is intricately involved in these mechanisms and acts in nucleotide-dependent processes such as RNA duplex unwinding and mRNP remodeling (altering the protein composition of an mRNP).[4-7] DBPs are found in all classes of organisms from bacteria through higher plants and animals.[4,8]

By definition, DBPs contain nine canonical amino acid sequence motifs (I-VI, Ia, Ib, Q) that presumably confer similar enzymatic activities to all of the family members (**Fig. 1A**),[6,10] although only a subset have been studied at the enzymological level. The high-resolution protein structures of several DBPs are known, and while they share many structural features, there are also important emerging differences.[8,10,11] Common biochemical characteristics shared by DBPs include ATP binding and hydrolysis activity,[5,6,10] whereby ATP is converted to ADP and inorganic phosphate in an RNA-dependent manner. Structurally, DBPs have two RecA-like domains (subdomain 1: N-terminal RecA domain, and subdomain 2: C-terminal RecA domain)

that are joined by a flexible linker (**Fig. 1**).[8] Most of the conserved motifs line the central cleft between the two domains and provide for conserved ATP binding and hydrolysis activities (**Figs. 1A** and **1B**).[10] The RNA substrate specificity and unique binding sites for potential co-factors and protein-protein interactions occur through less conserved regions, including N- and C-terminal extensions.[11] Interestingly, the unique regions are likely key to the different functions among the family members. However, for DBPs that perform the same function in different organisms, the regions outside the canonical motifs show considerable conservation.[8] Discovering the roles of such non-canonical regions provides an opportunity to understand how DBP ATPase cycles are regulated and substrate selectivity determined.

As an ATPase, a given DBP could cycle through states when it is bound to ATP, ADP·Pi, Pi, ADP, or not bound to any nucleotide (apo).[5] Importantly, studies of other unrelated nucleotide hydrolytic enzymes for both ATP and GTP shed potential insight into how such DBP cycles could be controlled. Protein-protein interactions are important to the mechanisms of modulating the hydrolysis reactions for both GTPases (Ras-like GTPases are stimulated by GTPase activating proteins, or GAPs)[12] and ATPases (Hsp40/J-proteins stimulate Hsp70 chaperone proteins).[13] In addition, different nucleotide-bound or nucleotide free states can be stabilized by interaction partners.[14] For example, the Ran guanine nucleotide exchange factor, RCC1, binds to and alters the Ran nucleotide binding pocket to induce the nucleotide-free state by promoting release of GDP.[15] Factors that regulate DBP nucleotide cycles are speculated to exist, and a few have been reported including ones for two eukaryotic initiation factors (eIF4A for eIF4AIII).[16-21]

One DBP family member, Dbp5 (also called DDX19 in humans), has conserved ATPase activity and is required for mRNP export out of the nucleus.[22-24] Our laboratory and others have shown that the Dbp5 ATPase activity is enhanced by a specific protein binding partner, Gle1, bound to the small molecule inositol hexakisphosphate (IP_6).[25,26] Defining the mechanistic details of this stimulation is an exciting area of active research. As highlighted in recent X-ray crystallographic and biochemical studies, interaction between Dbp5 and Gle1 is stabilized by IP_6.[27,28] Of note, IP_6 alone has no reported effect on Dbp5 activity. Both Gle1 and IP_6 binding to Dbp5 appear to require several residues in Dbp5 outside of the aforementioned conserved DBP regions, thus permitting specific stimulation of Dbp5 by Gle1 bound to IP_6.[28,29] Two recently proposed

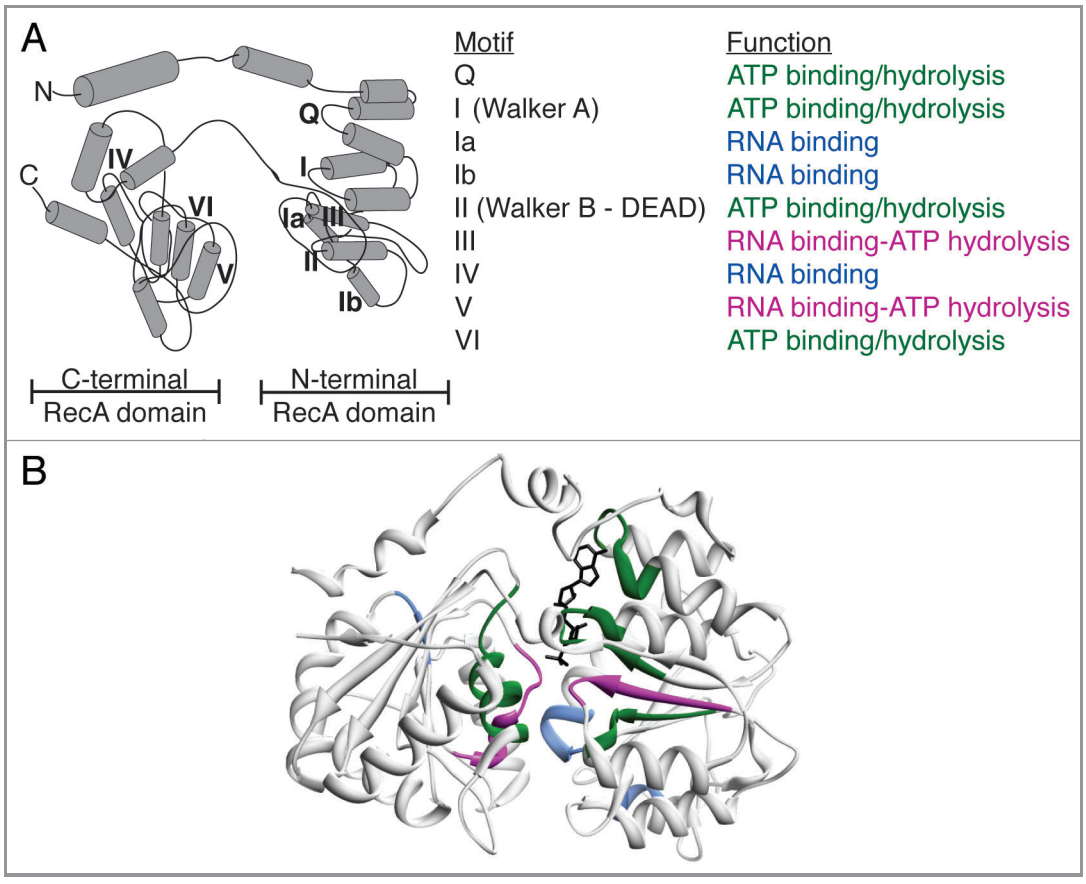

Figure 1. Structural organization of a DEAD box protein. (A) Topology model of the RecA-like helicase domains of a generic DEAD-box protein. The positions of the conserved sequence motifs within the labeled RecA helicase domains are indicated with Roman numerals. (B) Structure of human Dbp5 bound with ATP analog (black) [PDB 3GOH].[9] Highlighted conserved sequence motifs include those involved in nucleotide binding and hydrolysis (Green); RNA binding and ATP hydrolysis (Pink); RNA binding (Blue).

models have outlined potential mechanisms by which Gle1-IP_6 stimulates Dbp5.[28,30-32] Here we discuss how the data impact each model, and propose a role for IP_6-bound Gle1 in promoting loading of ATP onto Dbp5 as a key step in the mechanism, with RNA binding to Dbp5 linked to Gle1-IP_6 release.

The requirement for Dbp5 and Gle1 in mRNA export is coordinated at the NPC through their independent interactions with the nuclear pore complex (NPC). Specifically, Dbp5 binds to the NPC protein, Nup159.[24,33] Prior studies have established that Dbp5 does not require binding to Nup159 for stimulation by Gle-IP_6, although loss of this interaction leads to temperature sensitive defects in mRNA export.[33,34] Others have suggested that the overlapping binding sites for Nup159 and RNA on Dbp5 reflect roles pre- and/or post-ATP hydrolysis for Nup159.[35] Our recent work reveals a novel role for Nup159 in triggering the release of ADP from Dbp5.[32] Here we evaluate extensive complementary data from our recent work[27,30,32,36] and that of others[9,28,29,35,37,38] and present an updated model of the export process at the NPC. In addition, we illustrate how work on Dbp5 provides an important paradigm for the DBP family and a context for future studies.

Dbp5 Nucleotide Cycle

Understanding how DBPs cycle through stages of nucleotide binding, hydrolysis, and release of products will be fundamental to revealing their molecular mechanisms of action.[5] Our recent work has focused on investigating the nucleotide cycle of Dbp5. Earlier this year, we proposed a model whereby the nucleotide cycle of Dbp5 has distinct stages.[32] This is consistent with the proposed mechanism of several other nucleotide-dependent hydrolytic enzymes that are RNA or DNA helicases,[39,40] and is strongly supported by evidence for both *S.cerevisiae* and human Dbp5 indicating distinct nucleotide-dependent protein conformations.[9,32,36,37] X-ray crystallographic structures of both yeast and human Dbp5 show clear differences in the relationship of the two RecA-like domains that form the nucleotide binding pocket when Dbp5 is bound to different nucleotides.[9,28,37] In support of these results, in 2007, using limited trypsin digestion of recombinant purified yeast Dbp5 in the presence of various nucleotides, we found clear differences in the proteolytic products for Dbp5 bound to ADP vs. bound to either ATP or no nucleotide.[36] More recently, we independently showed using circular dichroism that the in-solution conformation of Dbp5 bound to ADP is quite different from its conformation when bound either to no nucleotide or to ATP analogs.[32] Moreover, the conformations of Dbp5 with no nucleotide and ATP-bound are very similar.

Based on the X-ray crystallographic structures, when bound to ADP, an N-terminal helix of human Dbp5 (residues 55–68) is localized between the two RecA-like domains with the enzyme in a relatively open conformation.[9] In the presence of the ATP analog AMP-PNP, this N-terminal helix appears displaced from between the RecA-like domains, the overall structure is more closed, and Dbp5 has the ability to bind RNA.[9] Others have reported an increase in ATPase activity in vitro when Dbp5's N-terminal helix is absent, and based on the in vitro and structural data, proposed an auto-inhibitory mechanism of action for this helix.[9,28] However, deletion of a portion of the yeast Dbp5 N-terminal domain (*dbp5Δ9–79*, including the N-terminal helix) does not perturb mRNA export or the overall growth of *S. cerevisiae* cells.[33] From this, while it apparently contributes to the nucleotide binding pocket, the dampening effect of the N-terminal helix is not a rate-limiting, regulatory step in the Dbp5 nucleotide cycle in vivo. The positioning of the N-terminal helix also cannot be solely responsible for the Dbp5 conformational change in vivo. We propose that regulation of the Dbp5 ATPase activity by protein binding partners compensates for loss of the N-terminal helix in vivo in the dbp5Δ9–79 protein.

There is now substantial evidence that Dbp5 protein interaction partners play direct roles in modulating its nucleotide cycle.[25-28,30,32] Gle1 bound to IP_6 enhances ATP binding by wild-type Dbp5 approximately 2–4 fold,[30,32] which could account for some of the reported 5–6 fold stimulation of ATPase activity by Gle1-IP_6.[25,26] In support of this role, a dominant negative (DN) Dbp5 protein (Dbp5-R369G) with severely diminished RNA binding inhibits yeast cell growth and mRNA export by sequestering Gle1.[30] The Dbp5-R369G protein shows a 16.7 fold increase in ATP binding in the presence of Gle1-IP_6.[30] Additionally, Dbp5 causes a further elevation in IP_6 binding with Gle1 when the ATP analog, AMP-PMP, is present.[27] Together, this implies that while Gle1-IP_6 stabilizes ATP binding, at the same time ATP stabilizes the Dbp5-Gle1-IP_6 interaction, resulting in an overall priming of Dbp5 for ATP hydrolysis. Cooperative binding enhancement for RNA and ATP has also been shown for other DBPs.[41-43] This leads us to propose a mechanism for Gle1-IP_6 stimulation of Dbp5 ATPase activity whereby Gle1-IP_6 binds to Dbp5 to enhance ATP binding, which then facilitates RNA binding to Dbp5. Overall, Gle1-IP_6 promotes the likelihood of ATP hydrolysis. It is possible that Gle1-IP_6 also functions to inhibit ATP release. Further kinetic analysis is required to determine if the Gle1-IP_6 stimulatory role can be solely attributed to enhanced ATP binding by Dbp5.

Our recent work has shown that following ATP hydrolysis, the bound ADP is not efficiently released from full length yeast Dbp5.[32] The release of ADP is critical to allow re-cycling of Dbp5. We discovered that the N-terminal domain (NTD, residues 2–387) of Nup159 acts to promote Dbp5 release of ADP in vitro through direct protein-protein interaction.[32] Strikingly, our in vitro biochemical results are directly supported by predictions based on recent X-ray crystallographic structures of complexes assembled from combinations of $\Delta 90dbp5^{L327V}$ protein, $\Delta 243$ $gle1^{H337R}$ protein, IP_6, ADP, and nup159NTD.[28] The $\Delta 90dbp5^{L327V}$ and $\Delta 243$ $gle1^{H337R}$ proteins lack the designated N-terminal amino acid spans (Δ90 and Δ243) and also have changes based on their respective gain-of-function point mutations. When bound to nup159NTD, the relative position changes for the Dbp5 N-terminal RecA-like domain and in specific residues the nucleotide binding pocket.[28] This provides a potential

mechanism for promoting release of ADP from the nucleotide binding site in the Dbp5 interdomain cleft.[28] However, the Δ90dbp5^{L327V} protein is missing the N-terminal helix and this results in altered ATP hydrolysis in vitro and possibly altered nucleotide binding.[9,28] This could explain why the Δ90dbp5^{L327V}-Δ243 gle1^{H337R}-IP$_6$-nup159NTD complex has ADP bound. Further tests are necessary to determine the relative in vitro ADP affinity and release rates of the Δ90dbp5^{L327V} protein as compared with wild-type full length Dbp5.

We recently discovered that an altered dbp5 protein, dbp5-R259D R256D (dbp5^{RR}) with changes in critical residues of the Nup159 binding interface, has intrinsically enhanced ADP release.[32] Interestingly, even at high temperatures, the *dbp5^{RR}* mutant alone does not result in reduced growth of yeast cells.[32] This indicates that as long as ADP release can occur efficiently, binding to Nup159 is not essential for Dbp5 function. Additionally, while deletion of the Nup159NTD causes growth and mRNP export defects,[33,34] expression of the mutated *dbp5^{RR}* allele rescues the *nup159ΔN* growth defect.[32] In vivo, compensation in the absence of Nup159NTD can also be accomplished by overexpressing wild type Dbp5.[33] This indicates that the primary function of the Nup159NTD is to aid in Dbp5-ADP release for enzyme recycling. Of note, the human homolog of Nup159, Nup214, interacts with both yeast and human Dbp5.[24] Therefore, we think it is highly likely that Nup214 will play a similar role in triggering ADP release from human Dbp5. We also predict that other DBPs might utilize unique ADP release factors that are yet to be identified.

RNP Remodeling

DEAD-box proteins catalyze rearrangements of both RNA-RNA and RNA-protein complexes through helicase and remodeling activities, respectively.[7] Both of these enzymatic activities harness distinct structural changes that occur during the nucleotide cycle.[10] It is unclear, however, if these activities share similar or distinct molecular mechanisms. Until recently, it was thought that all DEAD-box proteins require the ATP hydrolysis event for both duplex unwinding and RNP remodeling. For several DBPs, this mechanism is not true: DEAD-box proteins eIF4A, Msn16, and Ded1 require ATP binding but not hydrolysis to promote RNA duplex unwinding.[44,45] Surprisingly, it was demonstrated that ATP hydrolysis occurs primarily to recycle the enzyme for additional rounds of helicase activity.[44,45] Previous studies reported that both yeast and human Dbp5 have ATP-dependent helicase activity;[23,24,26] however, additional studies are needed to investigate this further and to test whether Dbp5 utilizes ATP binding or ATP hydrolysis to facilitate duplex unwinding.[24,26]

Several studies have analyzed the in vitro ability of DBPs to remove proteins from RNA.[36,46-48] Prior to our recent studies,[32,36] the DBPs tested reportedly required ATP hydrolysis to remove proteins from RNA.[47] We find that the conformational change that occurs in the transition from the Dbp5-ATP form to the Dbp5-ADP form is required for the remodeling of the mRNP.[32,36] Importantly, for Dbp5, this conformational change can be functionally driven in vitro by conversion from the nucleotide-free form of Dbp5 to the ADP-bound form.[32,36] Analysis of the X-ray crystal structures of hDbp5 shows that the RNA binding interface is dramatically changed by the transition from the ATP to the ADP state (Fig. 2).[9,28] Such a change in the RNA binding site during the transition from binding ATP to binding ADP might represent a shared mechanism utilized by many DBPs to remodel RNPs. Further studies are needed to test this possibility. Taken together, our new data combined with previous studies demonstrate that DBPs clearly utilize nucleotide dependent conformations in distinct manners for mRNP remodeling vs. RNA duplex unwinding.

An important remaining question involves understanding the mechanism by which specific proteins are recognized for remodeling by DBPs. This question has been difficult to address due to the fact that remodeling assays conducted in vitro have not used physiological substrates and have not shown in vitro specificity for their remodeling target. Furthermore, very few studies have identified remodeling targets in vivo. We have shown that the RNA binding protein Nab2 is released by Dbp5 from the mRNP both in vitro and in vivo,[36] and others have evidence that the mRNA export receptor Mex67 is an in vivo Dbp5 remodeling target.[49] Two studies have suggested that Kap104, the karyopherin that mediates nuclear import of Nab2, might also function to facilitate Nab2 removal from mRNA during export.[50,51] To directly test this possibility, we conducted in vitro remodeling assays and found that recombinant Kap104 does not mediate release of Nab2 from a Nab2-mRNP.[36] It is certainly possible that Kap104 performs an indirect role in the in vivo mRNP remodeling process by reducing the cytoplasmic availability of Nab2 following its release by Dbp5 at the NPC cytoplasmic face (**Fig. 3**). Overall, identifying in vivo remodeling targets of DBPs will be critical allow a greater understanding of gene expression regulation.

Model for the Mechanism of mRNP Export

Targeting of the mRNP to the NPC nuclear face and through the NPC central channel is dependent upon the mRNA export factors Mex67 and Mtr2. By interacting with both the mRNP and Nups, Mex67 directly facilitates the translocation[52,53] (**Fig. 3**, Step 1). These steps in the export mechanism have been recently reviewed and remain an active area of investigation.[2] For the terminal export step (**Fig. 3**, Step 2), the essential sequence of events that occur at the NPC cytoplasmic face are linked to precise, localized activation of Dbp5 ATPase activity by Gle1-IP$_6$.[25,26] This is needed to catalyze Dbp5-triggered release of RNA-binding proteins and ensure directional and irreversible transport of mRNPs out of the nucleus.[30,54] Remodeling is also required for recycling of transport factors and nuclear mRNA binding proteins[36,49] (**Fig. 3**, Step 8).

At the critical location where mRNP remodeling occurs as the final step of the export process (**Fig. 3**, Steps 3–7), Dbp5 interacts with ATP, ADP·Pi, ADP, Gle1-IP$_6$, Nup159, and RNA, although these

interactions do not occur simultaneously. The very recent studies by our group[30,32] and Montpetit et al.[18] each propose similar yet clearly distinct models for the ordering of these events. Both models are in agreement that it is critical for Dbp5 to interact with Gle1-IP_6 at the NPC. Moreover, they both propose that Gle1-IP_6 activates the Dbp5 ATPase activity which is RNA dependent with the ATP-bound Dbp5 having the greatest affinity for RNA (and the ADP-bound form the lowest). Finally, the evidence is clear for independent binding to RNA and Nup159, with their overlapping binding sites, and both models incorporate this. The major differences between the two models as published concern the respective function(s) for Gle1-IP_6 and RNA during the export cycle. We find that Gle1-IP_6 promotes ATP binding to Dbp5 which is required for binding of RNA by Dbp5. Additionally, we propose that RNA binding is linked to Gle1-IP_6 release from Dbp5.[30,32] In contrast, the model presented by Montpetit et al.[28] proposes that Gle1 and IP_6 binding to Dbp5 serves to release RNA from Dbp5. The Montpetit model was based in part on in vitro experiments showing an increase in the off-rate for the dbp5-E240Q-RNA interaction in the presence of Gle1-IP_6; however, the K_d for dbp5-E240Q-RNA binding in the presence of Gle1-IP_6 is reportedly unchanged.[28] Therefore, both on-rates and off-rates for dbp5-E240Q binding of RNA must be similarly changed in the presence of Gle1-IP_6 and suggests an alternative mechanism for Gle1-IP_6 action. Indeed, our findings indicate instead that RNA binding to Dbp5-ATP is needed for Gle1 release.

The phenotypes of two DN mutants (*DBP5-R369G* and *DBP5-R426G*) that display altered RNA binding,[30] contributed directly to the model detailed in Noble et al.[32] and its updated version in **Figure 3**. First, high levels of Dbp5-R369G and Dbp5-R426G protein act in a dominant negative fashion by competing with wild-type Dbp5 for limited Gle1 binding sites at the NPC.[30] Second, the *DBP5-R369G* DN phenotype is dramatically reduced by introducing into the *DBP5-R369G* gene a second mutation (*E323K*) which reduces the Dbp5 interaction with Gle1 (*dbp5-E323K/R369G*).[30] Given the DN mutants have markedly diminished RNA binding,[25] this indicates that Dbp5 binding to RNA is required for efficient release of Gle1. This conclusion is further supported by our studies showing that increasing RNA concentrations reduce the binding of Gle1 to Dbp5 (as measured by decreased cooperative binding of IP_6).[27] The fact that Gle1-IP_6 facilitates the loading of ATP onto Dbp5 is based on in vitro binding studies[30,32] and is further supported by in vivo data showing that introduction of an ATP binding mutant (*K144Q*) dramatically reduces the *DBP5-R369G* DN phenotype (*dbp5-K144Q/R369G*).[30] This suggests that the formation of the ATP/Dbp5/Gle1 complex is critical for the DN phenotype to be manifested. Taken together, these data are consistent with the idea that ATP/Dbp5/Gle1 and ATP/Dbp5/RNA complexes are present at distinct steps in the cycle, whereby Dbp5 binding to RNA serves to promote both efficient release from Gle1 and mRNP remodeling. This further suggests that RNA is the major activator of Dbp5-ATP hydrolysis. To further test this model, structural analysis of a Dbp5-ADP·BeF_3-Gle1-IP_6 complex would be valuable.

Our model further contends that the critical change in the Dbp5 RNA binding site is accomplished through conversion from the ATP-bound to the ADP-bound state.[32,36] In the study by Montpetit et al., the authors propose that binding of Gle1-IP_6 to Dbp5 markedly alters the RNA binding site.[28] This conclusion is based on comparing the crystal structure of the ADP-bound Δ90dbp5^{L327V}-Δ243 gle1^{H337R}-IP_6 complex to that for ADP·BeF_3-bound Δ90dbp5^{L327V}, wherein the RNA binding site was more open and less positively charged in the ADP-bound Δ90dbp5^{L327V}-Δ243 gle1^{H337R}-IP_6 structure[28] However, it is unclear if the addition of Gle1-IP_6 or ADP contributed to the structural change observed in the RNA binding site. As described above, previous studies have clearly demonstrated that both human and yeast Dbp5 have dramatically different conformations in the ATP-bound form vs. the ADP-bound form.[9,36] Thus, to examine this in greater detail, we compared the relative surface charge of the RNA binding pockets of the previously published structures of human Δ53dbp5 bound to AMP-PNP and RNA or to ADP with the yeast Dbp5 structures published in the Montpetit study. Specifically, the solvent-accessible electrostatic potential for the four Dbp5 structures were mapped (**Fig. 2**). As published in the Montpetit study, we confirmed the RNA binding pocket is altered in the ADP-bound Δ90dbp5^{L327V}-Δ243 gle1^{H337R}-IP_6 structure as compared the ADP·BeF_3 bound Δ90dbp5^{L327V} structure (**Fig. 2C and D**). However, strikingly, we also found that the RNA binding pocket displayed a loss of positive charge when human Δ53dbp5 was bound to ADP alone as compared with when it was bound to ANPNP (**Fig. 2A and B**). Thus, the change from ATP to ADP alone is sufficient to mediate the change in electrostatic potential, with Gle1-IP_6 not contributing directly to the environment of the RNA binding pocket or mRNP remodeling. Moreover, this result is consistent with reported nucleotide dependent affinities of Dbp5 for RNA, where ADP-bound Dbp5 has the lowest affinity for RNA.[26,28,36] Importantly, we propose that the change in Dbp5 conformation when ATP is hydrolyzed is responsible for the release of RNA. This model is directly supported by our studies showing that in vitro mRNP remodeling is only dependent on the Dbp5 conformational change from no nucleotide to ADP states, and is efficient in the absence of Gle1-IP_6.[32,36]

Finally, the in vitro and in vivo data together highlight a mechanism for efficient cycling of both Gle1 and Dbp5 at NPCs (**Fig. 3**, Steps 1–8). During export, when the mRNP exits the NPC (Steps 1–2), it encounters both Gle1 and Dbp5 at the cytoplasmic fibrils (Step 2). Gle1 binds to Dbp5 and mediates ATP loading (Step 3). The ATP/Dbp5/Gle1 complex then binds to RNA (Steps 4–5). Binding to RNA stimulates the ATP hydrolysis event, release of Gle1, and displacement of a protein from the mRNP (Steps 5–6). It is unclear if these events happen sequentially or simultaneously. The hydrolysis event triggers a conformational change within Dbp5 (as it goes from ATP-bound to ADP-bound) and this drives remodeling of the mRNP via changes in the RNA

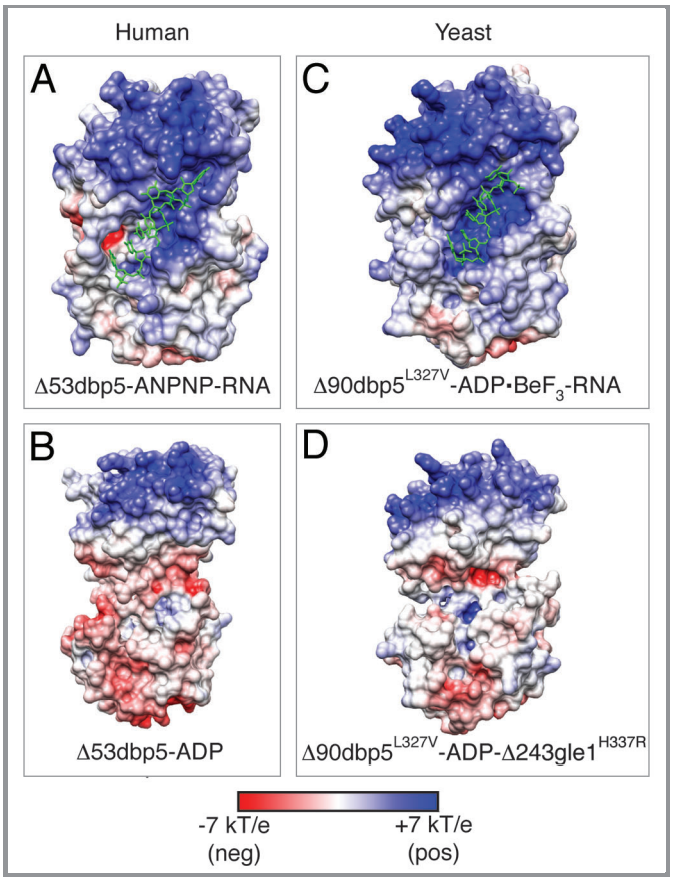

Figure 2. Solvent electrostatics of *S. cerevisiae* and human Dbp5. Solvent-excluded surface views of (A) human Δ53dbp5-ANPNP-RNA protein [PDB 3G0H],[9] (B) human Δ53Dbp5-ADP protein [PDB 3EWS],[9] (C) yeast (*S. cerevisiae*) Δ90dbp5^{L327V}-ADP·BeF_3 protein [PDB 3PEW],[28] and (D) yeast (*S. cerevisiae*) Δ90dbp5^{L327V}-ADP-Δ243 gle1^{H337R}-IP_6 proteins [PDB 3RRN][28] are shown colored by solvent accessible electrostatics contoured at −7 (*red*) to +7 *k*T/e (*blue*). The electrostatic potential was mapped to a molecular surface calculated using UCSF Chimera with default settings[55,56] as indicated by the PDB coordinates in brackets above. RNA is shown in green.

binding pocket (Steps 5–6). Resolving how the change in the RNA binding pocket of Dbp5 causes the release of a protein(s) from the mRNP will require further study. Dbp5-ADP is then recycled by interaction with Nup159 to release ADP (Step 7). With the overlapping RNA and Nup159 binding sites on Dbp5,[35] the NPC plays a critical role in regulating remodeling cycles. Overall, such a mechanism would allow a single Dbp5 molecule to remain at the pore and thereby to perform multiple remodeling events (**Fig. 3**).

Future Directions

Recent work has advanced substantially our mechanistic understanding of how Dbp5 functions to facilitate and regulate mRNP export. Through nucleotide-dependent conformational changes, Dbp5 mediates remodeling of mRNP complexes to allow directional export out of the nucleus. As summarized above, control of the ATP-ADP nucleotide cycle by Gle1-IP_6 and Nup159 provides a means by which Dbp5 is activated and recycled for multiple rounds of remodeling. Despite all that is known about Dbp5 and its interaction partners, a significant number of questions remain whose answers are needed to fully understand the mechanism of mRNP export. It is still unclear whether a single Dbp5 can mediate multiple linked rounds of remodeling for the same mRNP, whether several Dbp5 molecules work on a single mRNP, or both. These distinctions will be important to understanding whether Dbp5 enzyme recycling is required for Dbp5 to function repeatedly and efficiently in mRNP export, or for whether an individual Dbp5 can move from acting in mRNA export to performing downstream roles in the mRNP life cycle. An earlier study showed that Dbp5 works in translation termination to regulate association of termination factors with polysomes.[57] This role requires ATPase activity and possibly even mRNP remodeling, similar to the mechanism of Dbp5 function in mRNP export.[57] We have shown that Gle1 and IP_6 are important for proper translation termination and this is likely through regulation of Dbp5.[58] However, there is no reported role for Nup159 in translation termination.[57,58] Thus, if Dbp5 is to cycle during termination, we propose that an unidentified, translation-specific Dbp5-ADP release factor exists.[32] Further work is required to identify such a translation-specific release factor, and to determine if Dbp5 enzyme cycling for multiple remodeling rounds is necessary during translation termination.

For both mRNP export and translation termination, significant additional work is required to pinpoint the potential targets of Dbp5 action. Both Nab2 and Mex67 proteins are confirmed in vivo remodeling targets during mRNP export in *S. cerevisiae*.[36,49] At this point, neither the structure nor composition of an export competent mRNP is known, and most of the RNA binding proteins that are found in mRNPs passing through NPCs could be Dbp5 targets during export. However, not all RNA binding proteins in the mRNP are remodeled during export (e.g., CBP, the Cap binding complex).[59] It is also unclear whether the sequence/structure of the mRNP itself is directly or indirectly recognized by Dbp5. Likewise, although Dbp5-Gle1-IP_6 function is required for proper eRF3/Sup35 incorporation into the translation termination complex, the mechanism is not defined.[57,58] It is possible that Dbp5 itself is non-selective in terms of mRNA or RNA-binding proteins. As such, the critical determinants in Dbp5 specificity could lie in the factors that control its spatial and temporal activation (e.g., Gle1, Nup159). Additional studies are needed to identify the molecular basis for Dbp5 recognition of in vivo mRNP

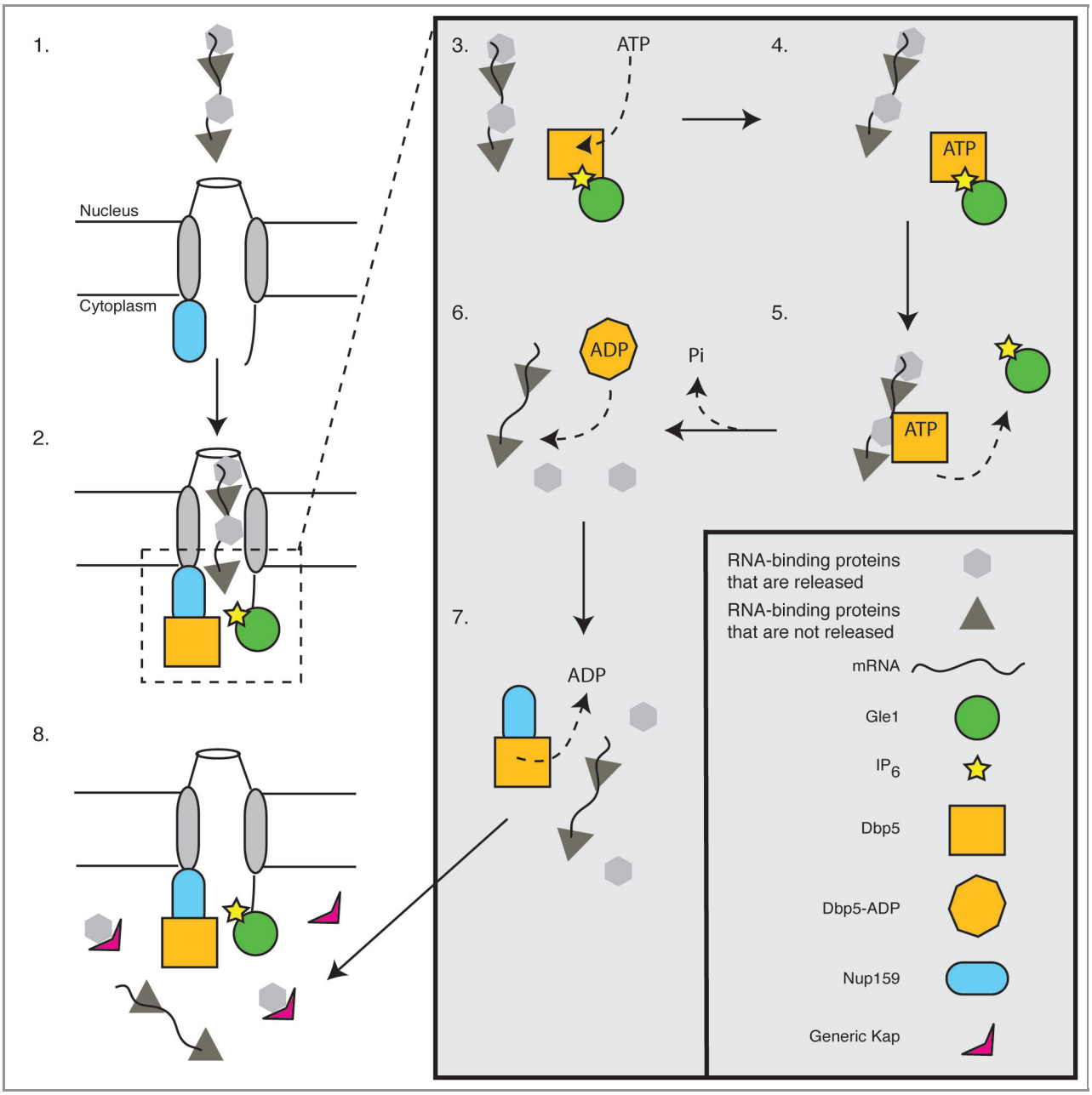

Figure 3. Working model for Dbp5/Gle1-IP_6/Nup159 mRNA export cycle. During export, the mRNP exits the NPC where it encounters both Gle1-IP_6 and Dbp5 at the cytoplasmic fibrils (Steps 1–2). Gle1-IP_6 binds Dbp5 and enhances ATP loading (Steps 3–4). The ATP/Dbp5/Gle1-IP_6 complex then binds to the mRNP, which stimulates both the release of Gle1-IP_6 and the ATP hydrolysis event (Step 5). The change from ATP to ADP triggers a conformational change that drives both the remodeling of the mRNP and release of the mRNA from Dbp5 (Step 6). Dbp5-ADP is then recycled by interaction with Nup159 to release ADP (Step 7), and positioning for binding to Gle1 to begin the cycle again. The released RNA-binding proteins bind to cytoplasmic karyopherins for import back into the nucleus (Step 8).

remodeling substrates. The fact that Dbp5 acts in two distinct steps during gene expression also raises the question as to whether there are separate pools of Dbp5 and Gle1 (one for each process). We recently measured the in vivo Dbp5-NPC association rate and determined that it is highly dynamic (with a half recovery < 1 sec), suggesting the potential for exchange between pools if they exist.[30]

Overall, our knowledge of the Dbp5 cycle of action provides a paradigm for how DBP nucleotide hydrolysis cycles are regulated by specific activators and release factors. The non-canonical structural regions are prime targets for interacting with such regulatory factors. For Dbp5, the common DN phenotypes for the equivalent *S. cerevisiae DBP5-R369G* and human *DBP5-R372G* mutants also sets the precedent for cross-species analysis of other *dbp5* mutants and other DBPs.[30] We predict that future additional insights into the mechanism by which Dbp5-Gle1-IP_6

act will be important for continuing to resolve how many other DBP family members work during gene expression.

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