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REVIEW

Emerging molecular functions and novel roles for the DEAD-box protein Dbp5/DDX19 in gene expression

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Abstract

The DEAD-box protein (DBP) Dbp5, a member of the superfamily II (SFII) helicases, has multiple reported roles in gene expression. First identified as an essential regulator of mRNA export in *Saccharomyces cerevisiae*, the enzyme now has reported functions in non-coding RNA export, translation, transcription, and DNA metabolism. Localization of the protein to various cellular compartments (nucleoplasm, nuclear envelope, and cytoplasm) highlights the ability of Dbp5 to modulate different stages of the RNA lifecycle. While Dbp5 has been well studied for > 20 years, several critical questions remain regarding the mechanistic principles that govern Dbp5 localization, substrate selection, and functions in gene expression. This review aims to take a holistic view of the proposed functions of Dbp5 and evaluate models that accommodate current published data.

Keywords DBP5 · DDX19 · GLE1 · NUP159 · Nuclear pore complex · mRNA export · Gene expression · mRNP · RNPase

Introduction

DEAD-box proteins (DBPs) function ubiquitously throughout the process of gene expression [1, 2]. With 25 of these enzymes identified in yeast and > 35 in humans, they represent the largest group amongst SFII helicases [1, 3]. Generally, DBPs are composed of two RecA-like domains, containing several critical helicase motifs (Q and motif I–VI), which are connected by a flexible linker region (Fig. 1a). This includes the namesake Asp-Glu-Ala-Asp (D-E-A-D) motif contained within motif II. Together these motifs allow DBPs to recognize and hydrolyze ATP, bind nucleic acids, and invoke structural rearrangements on nucleic acid substrates. The highly conserved architecture of DEAD-box proteins has aided scientists in understanding the mechanistic properties governing these ATPases and the diverse processes they engage through common modes of action (refer to Fig. 1b). This includes facilitating formation of ribonucleoprotein (RNP) complexes by acting as a stably bound component (mode 1), using an "RNPase activity" to remodel the structure of RNPs by displacing bound proteins (mode 2), or RNA duplex unwinding (mode 3).

Two DBPs, Sub2 and Dbp5, have been linked specifically to the essential process of messenger RNA (mRNA) export from the nucleus [4–6]. As a component of the TRanscription-EXport (TREX) complex, Sub2 (UAP56 in humans) serves an important role in assembly of the nuclear export competent mRNP [3, 4]. For example, during mRNA transcription, nuclear "export receptors" (e.g., Mex67 in yeast; NXF1 in humans) are directed to newly synthesized transcripts by the THO/TREX complex [3, 7]. These export adapters facilitate docking and passage of mRNPs through the nuclear pore complex (NPC). Upon reaching the cytoplasmic fibrils of the NPC, Dbp5 (DDX19 in humans) is proposed to function in remodeling these complexes to drive the terminal stages of export [5, 6, 8–11]. The remodeling RNPase function of Dbp5 is thought to remove export receptors (e.g., Mex67 and Nab2) and prevent re-association of the transiting mRNP with the NPC after export, thus providing directionality to the process [9, 12].

While Sub2 functions appear limited to the nuclear compartment [3, 4, 13], Dbp5 has been linked to diverse roles in the nucleoplasm and cytoplasm (e.g., transcription and translation), in addition to its essential role in mRNP export

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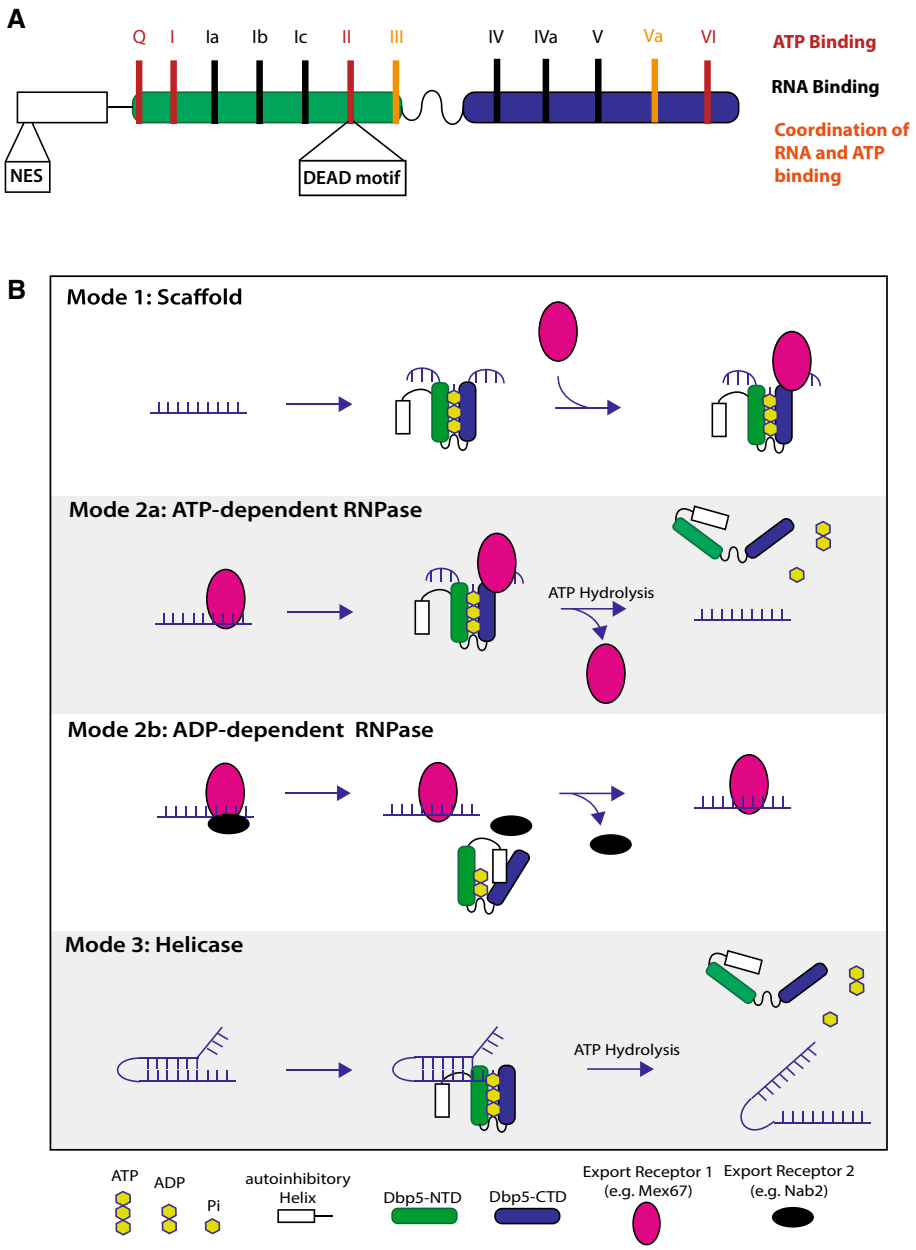

Fig. 1 General organization and potential modes of action for Dbp5. **a** Diagram representation of Dbp5, including the location of the nuclear export signal (NES), RecA-like N-terminal domain (NTD), RecA-like C-terminal domains (CTD), and characteristic DEAD-box proteins motifs. Label color indicates major function associated with each motif; see figure for details. **b** Possible "modes" of action through which Dbp5 and other DBPs may engage RNA substrates. Mode 1 depicts a DBP stably binding RNA to form a scaffold on which other proteins can assemble. Mode 2 indicates an RNPase mechanism in which RNA-binding proteins (RBPs) are displaced by the DBP. Within this mode, most commonly the DBP would act in an ATP-dependent mechanism to displace RBPs through binding the RNA backbone (mode 2a). For Dbp5, data suggest that RNPase activity can result from a unique ADP-dependent mechanism that is independent of RNA-binding (mode 2b). Mode 3 depicts a "helicase" mechanism by which duplexed RNA can be disrupted through the RNA binding and ATP hydrolysis cycle of a DBP

at NPCs. As such, Dbp5 localization is dynamic, and it rapidly shuttles between the nucleoplasm, NPCs, and cytoplasm [8, 14]. Recently, a nuclear export sequence (NES) found in the N-terminus of Dbp5 (denoted in Fig. 1a) has been shown to contribute to movement out of the nucleus in an Xpo1-mediated pathway [15]. In contrast, mechanisms that import Dbp5 into the nucleus are still unclear; however, mutations in residues involved in nucleotide binding (motif VI), hydrolysis (DEAD motif), or co-regulator (Gle1/$InsP_6$) stimulation alter Dbp5 nuclear shuttling [8, 11, 15]. This may suggest catalytic activity and/or RNA binding modulate nuclear localization. One possibility is that nuclear shuttling is facilitated by stable binding to RNAs that undergo retrograde transport from the cytoplasm (e.g., tRNA, TLC1, etc.) [16, 17]. An alternative hypothesis is that Dbp5 has a (yet to be identified) nuclear localization sequence (NLS) within the arginine rich motif VI. Regardless of mechanism, nucleocytoplasmic shuttling allows Dbp5 to participate in the range of roles reported for the enzyme. Detailed reviews of the functions of Dbp5 within mRNA export and translation have been recently published and we direct readers to these articles for more information as well [1, 2, 7, 18–21].

The broad cellular distribution and diversity of reported functions for Dbp5 raises questions regarding how the protein is directed to each task, and how each of these functions are mechanistically connected to Dbp5 enzymology. Throughout this review article, we will evaluate how the different DEAD-box protein modes of action may facilitate

the functions of Dbp5 in different cellular compartments. Extensive research spanning several decades studying highly conserved components of the NPC, and associated RNA export machinery, has been conducted in budding yeast [22]. As such, this article will focus on core findings from *Saccharomyces cerevisiae* with reference to several key studies in other models. Below we discuss biochemical properties of the Dbp5 ATPase cycle, how regulation of the ATPase cycle may promote functions of Dbp5 in different cellular contexts, and what "work" the RNA-binding and/or catalytic cycle might accomplish in each case. This will include a discussion of how co-regulators could modulate enzymatic properties of the protein, and models that accommodate the breadth of research observations related to Dbp5 over the last three decades.

Features of the Dbp5 ATPase cycle with RNA

Characteristic of DBP family members, Dbp5 contains defined motifs that govern nucleotide and RNA binding (Q, I, II, IV, V, VI), ATP selectivity (Q), and hydrolysis/catalytic activity (II) that have been extensively reviewed in other publications (Fig. 1a) [2, 21]. The ATP binding and hydrolysis cycle promoted by these motifs facilitate a series of conformational changes that in turn regulate RNA binding. For example, coordination of ATP by motifs Q, I, II and VI promote formation of a platform for RNA binding by bringing together RNA interaction interfaces on the two RecA-like domains (motif I, IV and V). RNA binding further promotes a "closed state" for the enzyme that stimulates ATPase activity [8, 11, 23]. Importantly, conformational changes influenced by the nucleotide state of Dbp5 (e.g., ATP vs. ADP) impact the affinity of Dbp5 for RNA. In the presence of non-hydrolysable ATP analogs (e.g., AMP-PNP) Dbp5 is reported to have a binding affinity of ~ 40 nM for RNA while RNA binding is not detectable with ADP [9, 10]. This relationship highlights the high level of coordination between nucleotide state, RNA binding, and ATPase stimulation that must be regulated in vivo to direct functional outcomes. In vitro studies have further shown that inorganic phosphate (Pi) release following ATP hydrolysis acts as a slow step within the ATPase cycle of Dbp5 in the presence and absence of RNA [24]. Within the RNA-stimulated ATPase cycle, RNA binding itself is also slow [24]. These represent a potential rate-limiting step within the Dbp5 ATPase cycle that may be modulated by co-regulators in vivo (discussed below). Similar to $Dbp5^{ATP}$, the post-hydrolysis $Dbp5^{ADP\text{-}Pi}$ state is expected to be a high-affinity RNA-binding state, which for other DBPs is key to the cellular function of the protein [25, 26]. By stabilizing this transition state and slowing Pi release, it is feasible for DBPs to form stable assemblies on RNA (e.g., eIF4AIII as part of the exon junction complex) that can direct downstream events or binding of other protein factors (Fig. 1b; mode 1). It is not currently known if a post-hydrolysis state is functionally important for Dbp5.

An additional layer of ATPase regulation is conferred by an auto-inhibitory N-terminal alpha-helical extension in Dbp5, which can be positioned between the two catalytic domains responsible for coordinating nucleotide binding. This serves to prevent formation of the ATP-bound closed state and lowers the basal Dbp5 ATPase activity [23, 27, 28]. Dbp5 also has a higher affinity for ADP (~ 0.4 mM) than ATP (~ 4 mM) [11, 29]. Given these facts it is not surprising that in the absence of co-regulators and RNA, Dbp5 exhibits a relatively low ATP hydrolysis rate of ~ 0.04–0.14 s^{-1} [10, 24, 30]. Upon RNA-binding, it is envisioned that auto-inhibition is relieved and the closed state promoted, which leads to a maximal increase in ATPase activity of ~ 6- to 20-fold [5, 6, 10, 24, 30]. It has also been observed that the extent of this stimulation may vary with different RNA substrates, with poly(A), poly(U) or poly(C) showing robust stimulation compared to poly (G) and tRNA as weaker stimulators [5, 6]. Further work is required to establish whether these differences can be explained by altered binding conformations between Dbp5 and these RNAs, which may be of biological relevance.

Unlike helicases outside of the SFII enzymes, most DEAD-box proteins (including Dbp5) are inherently non-processive and are often only able to unwind short duplexes of RNA [2, 5, 6, 10, 31, 32]. Dbp5 exhibits the ability to unwind short RNA duplexes with low melting temperatures (Tm) in an ATP-dependent manner [5, 6, 10]. It has been reported that Dbp5 is able to perform such functions in the absence of co-regulators when provided in twofold excess of short duplexes containing 5′ single-stranded overhangs [10]. Earlier reports also showed Dbp5 has the capacity to unwind duplexes containing 3′ overhangs, but only in the presence of co-regulators [5, 6]. Importantly, it has yet to be shown if this "helicase" activity (Fig. 1b; mode 3) has functional significance for Dbp5 cellular roles. Due to lack of evidenced processivity, the core cellular functions of Dbp5 are not predicted to be dependent on unwinding duplexes. Instead, the proteins' RNA binding and ATPase cycle have been proposed to drive RNPase activity (Fig. 1b; mode 2) to facilitate functions such as mRNP export [1, 2, 7, 18–21].

Function and regulation of Dbp5 at NPCs

Regulation of the Dbp5 ATPase cycle at the NPC

Published data suggest Dbp5 promotes directional mRNA export using ATP hydrolysis to displace mRNA export receptors at NPCs [6, 8, 9, 11, 12]. The observable

steady-state enrichment of Dbp5 at NPCs is facilitated by an interaction with the cytoplasmic nucleoporin Nup159 (NUP214 in humans) [6, 33, 34]. Nup159 binds the NTD of Dbp5 in a manner that occludes RNA binding and would be incompatible with a closed conformation of the enzyme [6, 23, 33, 34]. This is consistent with in vitro observations that nucleotide and RNA (which promote the closed state) weaken Dbp5–Nup159 binding and that Nup159 inhibits RNA-stimulated ATPase activity [6, 23, 33, 34]. Notably, disruption of the Dbp5–Nup159 interaction in vivo is not lethal, but cells lacking this interaction can exhibit poor growth, temperature sensitivity, and mRNA export defects, suggesting the interaction is functionally important [11, 35]. A mutant that is an exception to these phenotypes is Dbp5^{RR} (R256D and R259D), which disrupts a critical salt bridge between Dbp5 and Nup159, causing Dbp5 enrichment at NPCs to be lost without negatively impacting growth or bulk mRNA export status [11]. This is reported to be the result of the Dbp5^{RR} mutations causing accelerated ADP release from Dbp5, which bypasses the necessity for co-regulators at NPCs to promote nucleotide exchange. Specifically, it has been suggested that Nup159 may act as a nucleotide exchange factor (NEF) in this process [11]. This is in line with structural analyses of Dbp5^{ADP}–Gle1/InsP$_6$–Nup159 showing that, within the ternary complex, Dbp5 RecA-like domains are positioned in an open conformation that could allow for nucleotide release [23]. In contrast, recent results reporting nucleotide turnover at the millisecond timescales (kinetics relevant to mRNA export), suggest that Nup159 does not act as an ADP release factor [29]. Together, these data indicate that further studies are needed to understand if ADP release is a regulated event in vivo. Furthermore, it highlights that while the structural details of the Dbp5–Nup159 interaction are well defined, the in vivo function of this interaction requires investigation.

A second cytoplasmic nucleoporin, Gle1, participates in an essential interaction with Dbp5 at NPCs and is known to activate Dbp5 ATPase activity in vitro [10, 23, 27, 30, 35–40]. This suggests that in vivo Gle1 could function to accelerate a slow step of the Dbp5 RNA-stimulated ATPase cycle (i.e., P_i release and/or RNA binding). The interaction between Dbp5^{ADP} and Gle1 has been elucidated by X-ray crystallography and shows that Gle1 binds both RecA-like domains of Dbp5^{ADP}, which is aided by the endogenous small molecule inositol hexakisphosphate (InsP$_6$) that bridges the interaction between Gle1 and the CTD of Dbp5 [10, 23, 27, 36, 37]. The binding of Gle1 positions the two RecA-like domains of Dbp5 in an open conformation that would exclude RNA binding and is incompatible with the auto-inhibited conformation of Dbp5 [23]. Moreover, Gle1 accelerates RNA release from preformed Dbp5-RNA complexes in vitro, even when ATP hydrolysis is inhibited by mutation of the DEAD motif (Dbp5^{E240Q}) [23]. This suggests Gle1 could aid the release of RNA from either an ATP or post-hydrolysis ADP-Pi state. In addition, by limiting conformational flexibility of the two RecA-like domains and blocking access of the N-terminal auto-inhibitory domain, Gle1 may also promote RNA binding upon exchange of ADP for ATP. Indeed, Gle1 has been reported to promote formation of an ATP bound Dbp5 state [10, 11]. These observations raise the possibility that Gle1/InsP$_6$ binding could promote multiple rounds of Dbp5 ATP hydrolysis by enhancing aspects of both RNA binding and release. Such a paradigm might parallel conformational regulation observed for eIF4A that transitions between open, semi-open, and closed conformations promoted by eIF4G binding in different nucleotide, RNA, and co-regulator (eIF4B) contexts [41–43]. It is expected that future structural analyses and in vitro biochemistry of Dbp5 with Gle1 and RNA are key to testing such possibilities.

Potential stepwise interactions of Dbp5 at the NPC

While questions remain as to the functions of both Nup159 and Gle1 in regulating Dbp5 ATPase activity, published data can be used to propose a series of interactions between these nucleoporins and Dbp5 to promote RNP export. In Fig. 2, multiple pathways leading to RNP binding and release from Dbp5 are presented in a series of "states". This includes potential interactions that may be occurring to support these transitions. It is not currently known if each of these states are occupied in vivo, what fraction of Dbp5 follows each path, and if the states occupied by Dbp5 vary with the RNP substrate. Each of these points are important issues that must be addressed in the future to further refine models of Dbp5-mediated RNP export.

To start, it has been reported that Dbp5-ADP binding affinity is ~ tenfold higher than Dbp5-ATP [11, 29], as a result, under physiological conditions, it is calculated that a significant fraction of Dbp5 in the cell may be ADP-bound (state 1a*) [24]. Nup159 is required for enrichment of Dbp5 at NPCs, with the Dbp5–Nup159 interaction precluding RNA binding [23, 33]. When bound to nucleotide, Dbp5 binding to Nup159 is also weakened from ~ 20 to ~ 0.6 nM (Dbp5^{ADP}) and ~ 1 µM (Dbp5^{ATP}) in vitro [6, 33, 34]. These biochemical and physiological observations motivate us to propose Nup159 bound pools of Dbp5 may be ADP-bound (state 1a), but likely lack nucleotide (favoring state 1a → 1b or state 1b* → 1b transitions). Dbp5 molecules positioned at the cytoplasmic fibrils may subsequently bind ATP (state 1 → 2), which promotes a closed state that is primed to engage RNA. As a result, we envision a rapidly cycling pool of Dbp5 at NPCs composed of states 1a/1a*, 1b/1b*, and 2, which fits well with measurements of the dwell time of Dbp5 at NPCs that is estimated to be ~ 0.05–0.8 s [8, 14].

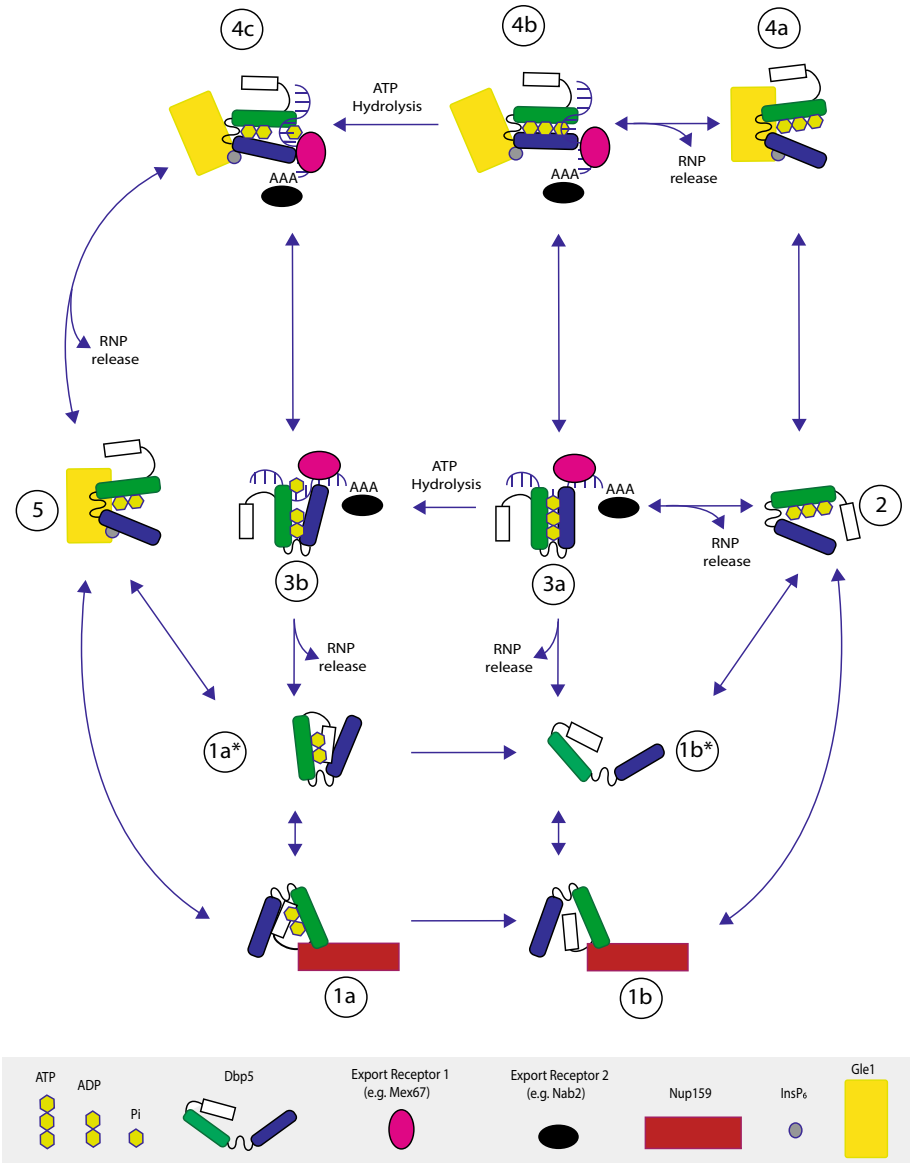

Fig. 2 Potential binding states of Dbp5 at NPCs supporting RNP export. A stepwise cycle of possible interactions involving Dbp5, ATP, ADP, inorganic phosphate (P_i), Gle1/$InsP_6$, Nup159, RNA, and RBPs at the cytoplasmic face of an NPC is depicted. Arrow heads indicate directionality with many events having the potential to proceed in either direction. Note that not all possible states are shown due to space limitations and the position of the Dbp5 NTD in each state is postulated as current X-ray crystallography data lack information on the position of the Dbp5 NTD domain. The cycle is presented starting with Dbp5 or $Dbp5^{ADP}$ undergoing cycles of binding (states 1a/b) and release (states 1a*/b*) from Nup159, which enriches Dbp5 at the cytoplasmic fibrils of the NPC. Nucleotide exchange allows Dbp5 to enter an ATP (state 2), which weakens interaction with Nup159. $Dbp5^{ATP}$ can either proceed to bind RNA directly (state 3a) or bind Gle1/$InsP_6$ (state 4a) prior to binding RNA (state 4b). RNA binding promotes a closed conformation and displacement of the auto-inhibitory NTD of Dbp5 (states 3a or 4b) with ATP hydrolysis leading to formation of a $Dbp5^{ADP\text{-}Pi}$ complex (state 3b or 4c). Gle1/$InsP_6$ binding to both RecA-like domains of Dbp5 (state 4a or 5) relieves auto-inhibition of Dbp5 and promotes separation of the NTD and CTD domains to potentially promote the release of RNA from either the ATP or ADP-Pi bound forms of Dbp5 (states 4c→5 or 4b→4a). In the $Dbp5^{ATP}$–Gle1/$InsP_6$ complex (state 4a), which could be formed through the exchange of ADP for ATP from state 5, the domains of Dbp5 may be well positioned for RNA binding (state 4a→4b), which could promote cycles of RNA-binding and release without ATP hydrolysis (4a←→4b). Finally, a Nup159–Dbp5 interaction may promote release of Gle1/$InsP_6$ from Dbp5 (state 5→1a) resulting in regeneration of state 1, thus completing the cycle. Within this scheme, RNP remodeling to promote export (i.e., displacement of export factors from the RNP) may occur as a result of Dbp5 binding to the RNA (state 3a or 4b), hydrolysis (state 3a→3b or 4b→4c), or the subsequent release of Dbp5 from the RNA (e.g., state 4c→5). Additionally, the $Dbp5^{ADP}$–Gle1/$InsP_6$ complex (state 5) would be a state capable of RNA binding-independent remodeling (Fig. 1b; mode 2b)

This locally concentrated pool of Dbp5 would be available to participate in mRNP export due to its proximal positioning to Gle1 and the exit site of mRNAs from the nucleus. Estimates suggest that an NPC transports an mRNP every ~ 2–6 s, with the transport event itself lasting ~ 0.2 s [44–46]. The millisecond dynamics of Dbp5 cycling at NPCs in relation to the seconds frequency of export suggests that the vast majority of Dbp5 is binding and releasing NPCs (e.g., cycling on and off NPCs between state 1 and 2), without participating in an mRNA export event. This idea is supported by phenotypic characterization of the Dbp5 NES ($Dbp5^{L12A}$) and $Dbp5^{RR}$ mutants that lack an obvious steady-state localization of Dbp5 at NPCs, yet do not impact bulk mRNA export [11, 15]. Such observations further suggest a model where Dbp5 at NPCs could be engaged in non-mRNP export activities (e.g., nuclear import or ncRNA transport, see further discussion below). We raise these observed differences between kinetic measurements of mRNP export and other observations involving Dbp5 (e.g., localization and essential role in mRNP export) to highlight important questions to be addressed.

In a scenario where Dbp5 alone engages an RNP, $Dbp5^{ATP}$ would bind the RNA (state 2 → 3a), possibly hydrolyze ATP, and if so, transition to the ADP-Pi post hydrolysis state (state 3b) [8, 11]. From the RNA-bound state 3a or 3b, Dbp5 may subsequently release the RNA without the aid of other factors, resulting in a return to state 1. However, given the essential nature of Dbp5–Gle1 interactions [36, 47], the ability of this interaction to accelerate Dbp5 ATPase activity in vitro [10, 30], and Gle1/$InsP_6$ mediated RNA release from Dbp5 [23], it is likely that Gle1 facilitates one or more of these events during RNA export. In doing so, Gle1 could engage $Dbp5^{ATP}$ (state 2 → 4a), the $Dbp5^{ATP}$-RNA complex (state 3a → 4b) or post-hydrolysis complex (state 3b → 4c). Notably, Gle1 bound Dbp5 (state 4a or 5) might be well positioned (i.e., auto-inhibitory domain displaced and RecA-like domains organized in an open conformation) to promote multiple rounds of RNA binding on the same or different RNA without fully releasing Dbp5 (e.g., cycling between states 4a → 4b or undergoing a transition from state 5 → 4a upon exchange of ADP for ATP). Through these changes in state, the binding and release of Dbp5 from an RNP would accomplish the work needed to support RNP export.

Finally, from state 5, data suggest that Nup159 binding to Dbp5 weakens the interaction between Dbp5–Gle1 [29], which may aid in the release of Gle1 from Dbp5 (state 5 → 1a). Yet, the Dbp5–Nup159 interaction is not essential [34], suggesting that other transitions occur in vivo (state 5 → 1a* or 5 → 4a). A return to state 1 completes the cycle described here with Dbp5 being released from the NPC or remaining associated with Nup159.

Possible RNPase activities that promote Dbp5 functions at the NPC

As a result of the cycle of RNA binding and release by Dbp5, displacement of export factors would occur from the RNP. A major question is how the enzymatic cycle of Dbp5, and/or the energy derived from this activity, is ultimately used to promote export. It has been proposed that Dbp5 activity at the pore facilitates RNPase remodeling events (Fig. 1b; mode 2) which displace mRNA export adapters (e.g. Mex67 and Nab2) from mRNAs exiting the nucleus [9, 12]. Indeed, catalytic mutants of Dbp5 have been shown to increase cellular Mex67 levels on mRNAs in yeast, and Dbp5 has been reported to displace Nab2 from RNA in vitro [9, 12]. For several DEAD-box proteins, including Dbp5, structural analysis reveals that nucleotide dependent RNA binding induces a local kink in the phosphate backbone of the RNA substrate [23, 43, 48–50]. This structural rearrangement may facilitate hydrolysis-independent unwinding of duplexes or displacement of proteins (Fig. 2; state 3a), with hydrolysis ensuring constant recycling and availability of the enzyme (i.e., Dbp5 does not remain locked on RNAs in the cytoplasm/nucleus) [21]. In other cases, hydrolysis is known to cause DBPs to transition to a higher affinity binding state that are functionally relevant [25, 49–51], which may occur here in the context of mRNP export to alter RNP structure (state 3b). It is known that an ATPase deficient mutant of $Dbp5^{E240Q}$ is lethal [8], but this does not differentiate these two possible modes of action for Dbp5 on an mRNP. As such, it is unclear if mRNP remodeling as envisioned here (i.e., displacement of export factors from the mRNP) occurs as a result of Dbp5 binding to the RNA (state 3a or 4b), hydrolysis (state 3a → 3b or 4b → 4c), or the subsequent release of Dbp5 from the RNA (e.g., state 4c → 5).

Beyond the role of hydrolysis, it also remains unclear if or how Dbp5 is directed to sites where relevant export adapters are bound to mRNA. One possibility is that this is mediated through direct protein–protein interactions with export factors. For Mex67, RNA-independent interactions with Dbp5 have been reported in vitro; but for Nab2, direct binding to Dbp5 has not yet been detected by pull down assays [9, 52]. It is also possible that the binding of Mex67 to the FG-repeats within Nup159 ideally position the export factor next to Dbp5, but this has not yet been demonstrated. Interestingly, recent work has shown that fusing Mex67 to a NPC component allows for ongoing mRNA export, suggesting that any essential interactions occurring between Dbp5 and Mex67 happen at NPCs [53]. Moreover, it is thought that there are multiple export factors per mRNP, raising the possibility that multiple remodeling events must occur either by the same enzyme or multiple Dbp5 molecules acting in concert on a single mRNP [54]. Given the eightfold symmetry of the NPC [55], multiple molecules of Dbp5 could

act simultaneously, but this raises further questions relating to how such events would be coordinated.

Strikingly, the in vitro displacement of Nab2 from mRNA is reported to occur specifically with ADP bound Dbp5, by $Dbp5^{ADP}$ appearing to decrease affinity between Nab2 and RNA (Fig. 1b; mode 2b) [9]. As noted, $Dbp5^{ADP}$ does not bind RNA [9], suggesting Nab2 remodeling would be independent of Dbp5-RNA binding, ATP hydrolysis, and the energy derived of this cycle. As such, Dbp5 may instead function by decreasing Nab2 affinity for nucleic acid substrates through a direct protein–protein interaction [9, 10]. Indeed, RNA-independent interactions between Dbp5 and Mex67 are reported to be strengthened by the presence of nucleotide (ATP) in vitro [52]. Such changes in the bound state, e.g., Dbp5 vs. $Dbp5^{ATP}$ vs. $Dbp5^{ADP}$, might therefore facilitate nucleotide dependent interactions between Dbp5 and Nab2. Alternatively, co-factors such as Gfd1, that are present at the pore and shown to physically and genetically interact with both proteins may also regulate this function [56]. However, as noted previously, no such direct interaction between Dbp5 and Nab2 has been reported and the mechanism underlying this unique remodeling remains unclear. Furthermore, it is not known how Dbp5 specificity for Nab2 is conferred without causing rearrangements that displace non-export receptor components of the mRNP (e.g., Pab1 is also remodeled in vitro by $Dbp5^{ADP}$, but is not thought to be removed during export and is present on cytoplasmic mRNPs [9]).

While the models discussed above have centered on knowledge gained by the studies of mRNA export, individual states in this cycle (Fig. 2) may further serve roles in export of ncRNA substrates given that Dbp5 has been implicated in the export of ribosomal RNA (rRNA), the telomerase RNA TLC1, and transfer RNA (tRNA) [15, 52, 57]. It is unclear how Dbp5 supports each of these pathways and whether it utilizes the RNPase mechanisms proposed for mRNP export. For example, Gle1 stimulation of Dbp5 ATPase activity is reported to be dispensable for rRNA export, as Gle1 and Dbp5 ATPase mutants do not exhibit the dominant-negative export defects observed for bulk mRNA with these same mutants [52]. Furthermore, Neumann et al. propose that Nup159 mediated positioning of Dbp5, rather than catalytic activity, supports rRNA export. Because Mex67 is present on translating ribosomes, it is also postulated that Dbp5 does not displace Mex67 from rRNA during export as is reported for mRNA. Rather a nucleotide-dependent and RNA-independent physical interaction between $Dbp5^{ATP}$ (state 2) and Mex67 is speculated to prevent "back-sliding" of the ribosomal subunit particle into the nucleus [52]. It is unknown if such a mechanism would also be possible for mRNAs or tRNAs, being that rRNAs, tRNAs, and mRNAs all share the export receptor Mex67 [12, 44, 58–64]. Recent work has also indicated that a nuclear pool of Dbp5 is involved in tRNA export, raising the possibility that Dbp5 could engage tRNAs in the nucleus and transit through NPCs bound to tRNA. If so, these Dbp5 molecules transiting the pore with RNA could occupy "state 3a/3b" acting as a stable scaffold (Fig. 1b; mode 1) [15].

Overall, while much is known about the role of Nup159 and Gle1/$InsP_6$ in regulating Dbp5 nucleotide state, RNA binding, and critical conformational changes, the precise Dbp5-dependent mechanism(s) of mRNA and ncRNA export has yet to be fully uncovered. Specifically, a lack of knowledge about the protein composition of an exporting mRNP and stoichiometry of the individual components, as well as what "work" Dbp5 performs in vivo during mRNP export, leaves many open questions to be addressed. Currently, reconstitution of this process is an extremely technical challenge given the size, complexity, and membrane association of NPCs. A related issue is the speed of export in the context of the models presented. In vitro, maximal Dbp5 ATPase activity with RNA and Gle1/$InsP_6$ is ~ 1 ATP/s, which is slow as compared to the estimated ~ 80 ms mRNPs spend at the cytoplasmic face of an NPC during export [10, 27, 30, 44–46]. While subsecond residence times of Dbp5 at the NPC would agree with the speed of the terminal steps of export, the comparatively slow ATP hydrolysis cycle does not [8, 14]. This discrepancy is compounded by any need for multiple remodeling events. Given the rapid dynamics of Dbp5 at each NPC, one possibility is that Dbp5 does not need to complete a full ATPase cycle to promote mRNP export. Hence, we pose the following questions for consideration. Are there unknown regulators of Dbp5 that accelerate ATPase activity to a level that matches the kinetics of mRNP export? Alternatively, is it possible that not all events of the proposed mRNP export cycle occur at NPCs? Could Dbp5 leave the NPC with an mRNP for subsequent ATP hydrolysis and mRNP remodeling in the cytoplasm? This would account for the slower hydrolysis cycle of Dbp5, as well as the dynamics of Dbp5 molecules at NPCs. Does Dbp5 target and displace specific export factors from an mRNP or does it bind RNAs indiscriminately to bias directional release into the cytoplasm? Is a general RNA binding activity how Dbp5 supports the export of both mRNA and ncRNAs? If so, is mRNP remodeling ultimately the result of competition for the mRNA by abundant cytoplasmic RNA-binding proteins and the act of translation? We expect that such questions are central to understanding NPC-associated Dbp5 functions in gene expression and can be addressed in the future using live-cell imaging approaches, in vitro reconstitution strategies, and the powerful genetics and cell biology of the *Saccharomyces cerevisiae* system.

Nuclear functions of Dbp5

The processes of nuclear mRNA biogenesis and export are known to be coupled by the functions of various protein complexes, including the THO/TREX complex [65]. Early observations that Dbp5 accesses the nuclear compartment led to a general hypothesis that Dbp5 could link nuclear events to mRNA export [35, 66]. For example, immunological visualization of the very large Balbiani Ring (BR) mRNP in *Chironomus tentans* indicated a potential co-transcriptional recruitment of Dbp5 and role for the protein prior to export [66]. Similarly, genetic and physical interactions were identified between Dbp5 and early transcriptional machinery in yeast [67, 68]. Estruch et al. report multiple transcription factor IIH (TFIIH) components that either suppress or exacerbate defects caused by perturbing Dbp5. Based on genetic interactions identified in their initial study and a later follow-up, the authors proposed a role for Dbp5 shortly after formation of the pre-initiation complex and prior to elongation [67, 68]. A nuclear function for Dbp5 that bridges transcription and mRNA export is an attractive model that evokes ideas such as gene-gating as an elegant mechanism to tightly control early stages of gene expression [69]. While human DDX19 has been shown to fractionate with chromatin following UV irradiation (discussed below), it is important to note that efforts to ChIP Dbp5 in yeast have not yet been successful [67, 70]. Additionally, the Dbp5 NES mutant revealed a nucleolar pool of the protein, raising the possibility of additional interactions within the nucleolus [15].

Given data indicating a potential co-transcriptional Dbp5 recruitment to chromatin [66], it is possible that these interactions could facilitate the aforementioned assembly of a larger export competent RNP (Fig. 1b; mode 1), similar to how eIF4AIII anchors the exon-junction complex (EJC) onto RNA [51]. In contrast to this hypothesis, recent evidence indicates nuclear pools of Dbp5 may not actually be required for bulk mRNA export, rather nuclear Dbp5 may function to support tRNA export [15]. Furthermore, no co-regulators have been identified that can inhibit Dbp5 RNA release in a manner observed for MAGOH and Y14 with eIF4AIII [51]. While this possibility still exists for ncRNAs, especially given the observed weak activation of Dbp5 by tRNA, current evidence that nuclear pools of Dbp5 are dispensable for mRNA export has important implications for current models [5, 6, 15]. This includes the potential that: (1) Dbp5 nuclear loading onto an mRNP is not a requirement for mRNA export, (2) Dbp5 nucleocytoplasmic shuttling is not required to support mRNA export, (3) and the essential functions of Dbp5 in mRNA export are performed at the cytoplasmic face of NPCs.

Another proposed nuclear function of Dbp5 is regarding cellular response to stress. Dbp5 has been reported to accumulate in the nucleus following ethanol stress and UV irradiation [70–72]. While ethanol broadly impacts Xpo1-mediated shuttling of NES-containing proteins, Hodroj et al. report that the human homolog of Dbp5 (DDX19) re-localized to the nucleus upon UV-induced DNA damage via an ATR-dependent mechanism [70, 72]. The ATR target, CHK1 kinase, phosphorylated DDX19 at residue Serine 93 (S93) inducing nuclear re-localization. It is proposed that nuclear pools of DDX19 in this context are critical to DNA metabolism and R-loop resolution. Conservation of this role has not been confirmed in yeast; however, two N-terminal phospho-sites (S69 and S86) as well as Serine-162 have been identified to be phosphorylated in response to DNA damage through large scale yeast proteomic screens [73]. Serine-86 has also been identified as a site for post-translational modification (PTM) in two separate proteomic screens [73, 74], while the S162A mutation was reported to be temperature sensitive in a recent alanine scanning mutagenesis study of Dbp5 [15]. However, none of these PTMs have been further validated and their regulation and functional significance remain uncharacterized. Moreover, a precise role for DDX19 in R-loop metabolism has not been elucidated. Another nuclear DBP, Dbp2 (DDX5 in humans), has also been implicated in R-loop regulation in budding yeast. Specifically, Dbp2 binds RNA at sites of R-loop formation, loss of Dbp2 leads to increased R-loops, and the protein (along with fellow DBPs Ded1 and Mss116) binds and destabilizes G-quadraplex RNA in an ATP-independent manner [75–80]. The implicated role of DDX19 in R-loop metabolism raises questions as to whether the protein may have a similar function to Dbp2 in DNA metabolism or perhaps a more unique stress-specific role on chromatin [78].

Yet, as novel nuclear functions for Dbp5/DDX19 continue to be studied, it is critical to consider the linearity of the gene expression pathway and the possibility that disruptions in RNA export caused by mutation in Dbp5 may indirectly impact other nuclear processes supporting gene expression. Future studies will need to clarify if Dbp5 directly acts on chromatin, if nuclear Dbp5 has preference for transcripts that reflect a direct role in R-loop metabolism, and what role PTMs play in regulating these nuclear functions.

Cytoplasmic functions of Dbp5

Like nucleoplasmic and pore associated pools of Dbp5, cytoplasmic localization of the protein has been proposed to have important functions in regulating gene expression. It has been reported that Gle1/$InsP_6$ mediated Dbp5 activation is important for proper translation termination [38, 81–83]. In addition, Gle1 is reported to function in

translation initiation through a physical contact with eIF3 in a mechanism that is independent of $InsP_6$ and Dbp5 [39, 40]. The role of Dbp5 in translation termination and its relationship to nonsense mediated decay (NMD) has been recently reviewed in detail by the Krebber group [18]. Briefly, during translation termination it is proposed that Dbp5 recruits the polypeptide release factor eRF1 (Sup45 in yeast) to ribosomes already containing termination factors Rli1 and eRF3 (Sup35 in yeast) at the STOP site. Functional eRF1 seems to be important for Dbp5 recruitment to the ribosome, which may also be promoted by an RNA-independent interaction with ribosome-bound Rli1 during termination. Dbp5 prevents premature termination by occupying a mutually exclusive binding site in the CTD of eRF1, which prevents an interaction with eRF3 until Dbp5 is removed. This interaction between eRF1 and eRF3 is critical for progression of termination as it promotes polypeptide and tRNA release via eRF3-mediated GTP hydrolysis. Subsequent removal of eRF3 allows eRF1 to also stimulate ATPase activity of Rli1 which promotes ribosome disassembly [81, 82]. In this way, Dbp5 is proposed to help regulate the sequential progression of the terminal steps of translation prior to ribosome recycling.

Yet, many finer details of how the ATPase cycle of Dbp5 is regulated to support this mechanism are still unclear. The observation that disrupting Nup159–Dbp5 interaction abrogates Dbp5–eRF1 interaction, has led Beißel et al. to propose a mechanism in which Nup159-mediated recycling of $Dbp5^{ADP}$ is critical for translation termination [81]. Similarly, such mutations in Nup159 also result in translation read-through defects like those reported to occur when Dbp5 function is perturbed. The idea that nucleotide state and recycling of Dbp5 is critical for cytoplasmic functions is further supported by the fact that Dbp5–eRF1 binding is stabilized in the presence of non-hydrolysable ATP analogs. As is the case for mRNA export, further investigation of how Nup159 may alter Dbp5 nucleotide release in vivo is required before further conclusions can be drawn.

Nevertheless, proper ATPase function and cycling does appear to be important to proper cytoplasmic homeostasis in other manners as well. It has been observed that in temperature sensitive Dbp5 catalytic mutant *rat8-2*, mRNA export factors (including Mex67 and Pab1) accumulate in RNA export granules (REGs) with Dbp5 that are distinct from P-bodies [84]. However, results from a genetic screen with *rat8-2* revealed synthetic lethality with P-body components, leading to the observation that REGs can coalesce with P-bodies under conditions of heat stress. These observations may indicate that without proper enzymatic cycling of Dbp5, mRNP aggregates can form in the cytoplasm. Formation of phase-separated condensates and stress granules (SGs) have been shown to be regulated by other DEAD-box proteins in vivo and in vitro (e.g., eIF4A and Dhh1) [85, 86]. Consistent with this, recent studies in mammalian systems have shown that DDX19 overexpression can prevent formation of drug-induced SGs similar to eIF4A [86]. These links between Dbp5, REGs and translation control highlight a potential requirement for Dbp5 RNPase activity to regulate RNP function and localization downstream of mRNP export.

Each of these discoveries provides a putative cytoplasmic function of Dbp5 requiring active investigation to understand Dbp5 regulated gene expression. As these avenues of research are pursued, we expect it will be critical to consider how these functions may be related to, or unique from, mRNP processing by Dbp5 at NPCs. For example, further investigation in to how Dbp5/Gle1 are recruited to translating ribosomes, the spatial regulation of these roles (e.g., at NPCs or within the cytoplasmic compartment away from the NPCs), and nature of RNP changes induced by Dbp5 will be critical. As more Dbp5 functions are reported, this will allow important distinctions between what could be multiple distinct functions performed by Dbp5 vs. a universal function for Dbp5 in regulating RNP composition in a spatially regulated manner.

Concluding remarks and future perspectives

With this review, we aim to draw attention to the progress made in understanding the functions of Dbp5 in gene expression and the many important unanswered questions in need of investigation. This includes questions posed within this review involving the spatial regulation of the in vivo Dbp5 ATPase cycle, how Dbp5 mechanistically supports mRNA and ncRNA biology, and what role(s) Dbp5 fulfills during stress. To aid in this, we have used published data to propose a series of interactions between Dbp5, RNA, and regulators that could define Dbp5 regulation in vivo (Fig. 2). We anticipate future experimentation in budding yeast will test and refine this model by adding/removing interaction states using both in vitro (e.g., reaction rates and binding constants) and in vivo (e.g., mutational analysis and imaging) methods. For example, structural data are still missing on position of the NTD of Dbp5 when bound to nucleotide, RNA, and/or coregulators. This is functionally important and goes beyond auto-inhibition of the enzyme, as RNA-stimulation of Dbp5 requires the NTD [7].

The high level of conservation observed between NPC and RNA export machineries amongst yeast and metazoans further motivates continued investigation in both systems [55]; in addition to the properties of orthologs in other organisms. In mammals, two isoforms of the protein exist (DDX19A and DDX19B) that share only 46% similarity in sequence identity to the yeast ortholog [83]. How these differences relate to both the documented functions and regulation of yeast Dbp5 (e.g., spatial regulation, localization,

and functions in ncRNA biology) is just beginning [37, 87]. This is particularly pertinent as recent work has linked functions of DDX19A and DDX19B to human health, especially within the context of viral infection [18, 88–90]. Core to addressing these questions will be a mechanistic understanding of Dbp5 regulation by co-regulators, the work that Dbp5 performs on an RNP, and the spatiotemporal dynamics of these activities. As a DBP, key to this will be understanding the interaction of Dbp5 with nucleotide, which is central to the ability of these enzymes to engage RNA. Similar to the Ran-GTP gradient, which regulate other nucleocytoplasmic transport events [91–93], we expect the sub-cellular distribution of different Dbp5 nucleotide states is critical and likely regulated in vivo by co-regulators and possibly PTMs. This makes future characterization of Dbp5-nucleotide distributions and the identification of spatially restricted nucleotide exchange an important goal. Long term, we expect that a focus on these questions aimed at defining the core regulatory principles of Dbp5 will lead to a coherent model that unifies the diverse functions of Dbp5 in gene expression.

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