Dbp5 — From nuclear export to translation

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The DEAD-box RNA helicase Dbp5 is an essential and conserved mRNA export factor which functions in the ATP dependent remodeling of RNA/protein complexes. As such it displaces mRNA bound proteins at the cytoplasmic site of the nuclear pore complex. For the regulation of its RNA-dependent ATPase activity during late steps of nuclear transport, Dbp5 requires the nucleoparin Nup159 and its cofactors Gle1 and IP6. In addition to its role in mRNA export, a second important function of Dbp5 was identified in translation termination, where it acts together with eRF1 once the translation machinery has reached the stop codon. Similar to mRNA export, this function also requires Gle1–IP6, however, the counterpart of Nup159 is still missing. Potential other functions of the nucleo-cytoplasmic protein Dbp5 are discussed as well as its substrate specificity and details in its regulatory cycle that are based on recent biochemical and structural characterization. This article is part of a Special Issue entitled: The Biology of RNA helicases — Modulation for life.

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1. Introduction

The DEAD-box protein 5 (Dbp5), also known as Ribonucleic acid–traFFicking protein 8 (Rat8) and DDX19 in human, is an example for a DEAD-box RNA helicase with an ATP dependent RNA/protein complex remodeling activity which functions in the rearrangement/displacement of RNA bound proteins. Dbp5 is an essential protein, which is conserved in eukaryotes [1–4]. In all organisms studied, Dbp5 and its homologues function in the mRNA export from the nucleus to the cytoplasm [1–4]. Additionally, a second important function for this DEAD-box RNA helicase was identified in termination of translation in Saccharomyces cerevisiae [5].

Originally, parts of the DBP5 gene were discovered in the 1990s in a screen searching for DEAD-box protein-encoding genes in S. cerevisiae leaving its function unexplained [6]. In a second screen for temperature-sensitive mutants with mRNA export defects, the yeast gene RAT8 was identified that later turned out to encode the DEAD-box protein 5 [1,7], Snay-Hodge et al. [1] and Tseng et al. [2] independently showed that Dbp5 has an essential function in the nuclear export of mRNAs in S. cerevisiae. Dbp5 had been localized to the nuclear envelope and the cytoplasm. However, a cytoplasmic function for Dbp5 remained unresolved until 2007, when a role of Dbp5 in translation termination was discovered [5]. It is possible that Dbp5 has additional other functions, as a synthetic genetic array revealed several genetic interactions of Dbp5 with P-body (processing body) components [8]. Furthermore, Zhao et al. [4] detected an early recruitment of Dbp5 to the mRNA during transcription and Estruch and Cole [9] showed that Dbp5 genetically and physically interacts with transcription factors, thus suggesting a possible function of Dbp5 in transcription initiation.

In this review we will give an overview of the multifunctional nature of Dbp5 and summarize known details about its ATPase cycle. Finally, we will discuss unresolved questions regarding this nucleo-cytoplasmic DEAD-box RNA helicase.

2. Structural domains in Dbp5

The DEAD-box RNA helicase Dbp5 belongs to the helicase superfamily 2 (SF2). It contains 13 characteristic sequence motifs (Fig. 1) and exhibits the characteristic eponymous sequence Asp-Glu-Ala-Asp (DEAD) in motif II [10]. Dbp5 is an ATP-dependent RNA-binding protein and a RNA-dependent ATPase, which is typical for DEAD-box RNA helicases. ATP-Dbp5 has a high affinity to single-stranded RNA and binding of RNA in turn stimulates the ATP hydrolysis activity leading to the remodeling of bound RNA/protein complexes [2,3,11–13]. The catalytic helicase core of Dbp5 is highly conserved within the SF2 helicases and consists of two structurally similar domains called
RecA-like domains as they exhibit the same fold as the recombination enzyme RecA of *Escherichia coli* [10,14]. Both domains are flexibly connected by a linker region and contribute with conserved residues to the binding of RNA and ATP (Fig. 1). The cleft between the RecA-like domains must be closed in order to provide the binding site for ATP and the catalytic center for its hydrolysis. The unique N-terminal extension flanking the helicase core provides the specificity of the enzyme and ensures autoregulation of its activity [15].

### 3. The functions of Dbp5

#### 3.1. Does Dbp5 have a function during transcription?

The main cellular localization of Dbp5 is the nuclear rim and the cytoplasm. However, the protein can also be found in the nucleus [4,16]. Zhao et al. [4] showed by immunostaining experiments with the polytene chromosome of the larval salivary gland of *Chironomus tentans* that Dbp5 is associated with transcriptionally active gene loci e.g. Balbani rings or puffs. Immuno-electron microscopic studies revealed an early recruitment of Dbp5 to the pre-mRNA as Dbp5 is already bound to the 5′ end of the growing mRNA during transcription [4]. The authors interpreted that Dbp5 remained associated with the mRNP during nucleoplasmic transport, translocation through the nuclear pore complexes (NPCs) and even during translation at the ribosomes. However, live cell images showing the association of Dbp5 with the mRNP during the whole nucleoplasmic transport and nuclear export are still missing. It is equally well possible that several pools of Dbp5 exist that might have specific functions at certain cellular locations.

Estruch and Cole [9] suggested that Dbp5 is not only recruited to the mRNP during transcription but that it might also have a function in transcription initiation. They showed that Dbp5 genetically and physically interacts with components of the transcription factor complex TFIIH in *S. cerevisiae*. This complex consists of several proteins and has a function in transcription initiation as well as in DNA repair. Especially, mutations in genes of factors that activate transcription initiation partially suppress the growth and mRNA export defects of the temperature-sensitive *rat8-2* strain. In contrast, no effects of strains encoding defective genes that lead to reduced transcription rates, such as mutated elongation factors, were detectable [9]. Moreover, mutations in genes encoding factors repressing transcription initiation, which lead to increased transcription rates as well, were synthetically lethal with *rat8-2*. These results might indicate a role of Dbp5 during transcription initiation. However, it can’t be excluded that these effects might be secondary effects and thus caused by an overall alteration of nuclear mRNA levels, which might lead to an altered requirement of Dbp5 for its function in nuclear transport. Furthermore, no interaction of Dbp5 with chromatin was detectable. It remains to be shown if there is indeed a nuclear function of Dbp5 and further experiments will be needed to answer this question.

#### 3.2. Dbp5 is an mRNA export factor

During transcription and pre-mRNA processing, the composition of the messenger ribonucleoparticle (mRNP) changes constantly. Only capped, fully processed and poly(A)-tail containing mRNPs are competent for export and need to be actively transported across the nuclear envelope through NPCs into the cytoplasm where translation finally occurs (for review of mRNP export see [17,18] or for general nuclear transport see [19]). NPCs are large eight-fold symmetric protein complexes which are composed of nucleoporins that form a channel within the nuclear envelope, a nuclear basket and cytoplasmic fibrils. Small molecules are able to pass the NPC by passive diffusion, but molecules exceeding approximately 40–60 kDa are actively transported by several transport factors [17,18]. In *S. cerevisiae*, export of mRNPs is mediated by the heterodimer Mex67-Mtr2 (TAP-p15 in metazoans) that interacts with the FG-repeat containing nucleoporins of the NPC. Upon translocation, the composition of the mRNP changes again: Several mRNA export factors such as the export receptor Mex67-Mtr2 and the poly(A)-binding protein Nup214 dissociate and shutle back into the nucleus [17]. This remodeling process might ensure directionality of the transport and might prevent the mRNP from diffusing back into the nucleus. Other proteins like the Mex67-adapter and mRNA-binding protein Npl3 remain bound to the mRNP upon transport and might have further functions in the cytoplasm [20].

Already in 1998, the DEAD-box RNA helicase Dbp5 was proposed to have a function in the late steps of mRNA export at the cytoplasmic face of the NPC [1,2]. Temperature-sensitive mutants of Dbp5 showed a rapid accumulation of poly(A)-tail containing mRNAs in the nucleus upon shifts to their non-permissive temperatures [1,2] and were synthetic lethal with mutants of different nucleoporins or known mRNA transport factors such as Gle1 [1]. Fluorescent microscopy experiments revealed a cytoplasmic distribution of Dbp5 with a predominant localization at the nuclear rim [1,2]. The interaction of Dbp5 with the cytoplasmic fibrils of the NPC is mediated by an association with the N-terminal domain (NDT) of the nucleoporin Nup159/Rat7 [1,3,16,21] (in humans CAN/NUP214 [3,22,23]). Yeast strains lacking the NTD of Nup159 or Dbp5 mutants that are unable to bind Nup159 lead to defects in the NPC association and show an exclusively cytoplasmic Dbp5 localization [3,21,24]. Similarly, human Dbp5 with a single amino acid substitution (R259A) results in a protein that is unable to bind Nup214 and has lost the contact to the nuclear envelope [23].

Placed at the cytoplasmic filaments of the NPC, Dbp5 with its ATP-dependent RNA helicase activity is perfectly positioned to trigger the remodeling of the emerging cytoplasmic mRNPs and to initiate the replacement of export factors and in this way to control the directionality of the export process. Lund and Guthrie [25] showed in an in vivo assay that increasing amounts of Mex67 remain bound to the mRNA in the cold sensitive *rat8-7* mutant strain as compared to wildtype, indicating a key role for Dbp5 in dissociation of this export receptor. Moreover, Tran et al. [13] performed an in vitro RNP remodeling assay in which they could show that recombinant purified Nup214 was...
displaced from RNA by Dbp5 and this was even more efficient in the presence of its cofactors Gle1 and IP<sub>6</sub>. Interestingly, ATP hydrolysis was not required for this process, but rather the ADP bound form of Dbp5 itself [13,24]. To be more precise, the conformational change of ATP-Dbp5 to ADP-Dbp5 induced by ATP hydrolysis turned out to be the critical step for mRNP remodeling [24]. Until now, the exact mechanism by which mRNP bound proteins are displaced via Dbp5 at final steps of nuclear transport is not fully understood. Interestingly, Dbp5 does not seem to compete with the RNA for Nab2 binding as no direct interaction between both proteins could be detected [13]. In fact, although ADP-Dbp5 was able to decrease the affinity of Nab2 for RNA the ADP-bound form has a low RNA binding affinity [12,13]. Further experiments will certainly be needed to explain the precise mechanism of how RNP remodeling by Dbp5 is mediated and how Dbp5 can distinguish between proteins that need to be displaced upon transport and proteins that need to remain bound to the mRNA.

3.3. Dbp5 and its cofactors Gle1 and IP<sub>6</sub>

Much biochemical and structural knowledge about Dbp5 was obtained in the last years in order to explain its functionality and the regulation of its activity during mRNA export [2,3,11,12,15,21–24,26–29]. On the one hand, Dbp5 contains several structural and functional similarities in comparison to other DEAD-box RNA helicases, indicating a high degree of conservation within this protein family. However, on the other hand a couple of unique features were discovered for Dbp5, e.g. structural features such as the N-terminal domain that regulates the intrinsic ATPase activity [15], or biochemical features, such as the need for an ADP release factor [24]. Recently, Montpetit et al. [30] provided an interesting collection of methods that allow comparative analyses of the biochemical properties of DEAD-box proteins.

Dbp5 binds RNA and ATP in a conserved manner comparable to other DEAD-box RNA helicases such as Vasa and elf4AIII: Both RecA-like domains tightly clamp ATP and bind the RNA at the opening of the cleft causing a sharp bend of the phosphate backbone of the RNA [15,22]. The RNA associates with its 3' end to the N-terminal and with its 5' end to the C-terminal Dbp5 domain [22]. ATP bound Dbp5 shows the highest affinity for single-stranded RNA. In fact, the presence of a non-hydrolysable ATP analog in an in vitro RNA binding assay resulted in Dbp5 that tightlybound RNA whereas the ability for RNA association was nearly completely abolished without nucleotides or after addition of ADP or ATP [12,13]. Therefore, it seems to be obvious that Dbp5 only transiently binds to RNA and dissociates upon ATP hydrolysis. Furthermore, it is likely that ATP binding occurs before RNA binding [28].

Purified recombinant yeast and human Dbp5 have a low intrinsic ATPase activity, which can be stimulated by the addition of single-stranded RNA [2,3]. Thus, under physiological conditions cofactors are needed to achieve full activation of Dbp5. Actually, in different in vitro assays Weirich et al. [12] and Alcázar-Román et al. [11] independently showed that Gle1 and the small signal molecule inositol 1,2,3,4,5,6-hexakisphosphate (IP<sub>6</sub>) form a complex with Dbp5 and strongly enhance its ATPase activity. Originally, York et al. [31] discovered that the production of IP<sub>6</sub> is required for efficient mRNA export in S. cerevisiae. Enzymes that are responsible for the IP<sub>6</sub> synthesis genetically interact with several transport factors including GLE1, NUP42, NUP159 and DBP5 [31,32]. It is intriguing to speculate that an involvement of small molecules such as IP<sub>6</sub> in the mRNA transport might allow different layers of regulation of this process under various environmental conditions (e.g. cellular stress).

The other cofactor Gle1, also termed Rss1 was identified as an essential mRNA export factor in S. cerevisiae that interacts with the nucleoporin Nup42/Rip1 at the cytoplasmic fibrils next to Nup159 [33–35]. Therefore, a localized activation of Dbp5 at the place of operation can be achieved. Gle1 and Dbp5 interact predominantly via their C-terminal domains [12,27,29]. Especially yeast Dbp5 residues E323, K382 and E473 are necessary for the binding to Gle1 [27]. Additionally, Montpetit et al. [29] showed that the N-terminal RecA-like domain of Dbp5 contributes to the Gle1 interaction, however, to a lesser extent. Interestingly, this kind of complex formation between the DEAD-box RNA helicase and its activator seems to be conserved within this protein family and similar examples are known such as the complex formation between the DEAD-box RNA-helicase elf4A (euarkotic translation initiation factor 4) and its activator elf4F4 and between the NMD factor Upf1 and its activator Upf2 [29]. Both of them show structural similarities despite weak sequence similarity. Unique for Dbp5 is that this interaction involves the signal molecule IP<sub>6</sub> which binds in a positively charged pocket between Gle1 and the C-terminal RecA-like domain of Dbp5 where it might be responsible for stabilizing the binding of both proteins with each other [29,36].

Gle1–IP<sub>6</sub> seems to bind preferentially to ATP-Dbp5 without RNA as in an in vitro binding assay the highest affinity to Dbp5 was detected when it was bound to a non-hydrolysable ATP analog and in the absence of RNA [36]. Addition of RNA might immediately lead to rapid ATP hydrolysis and the subsequent dissociation. Importantly, Gle1–IP<sub>6</sub> does not directly stimulate the ATPase activity of Dbp5 but rather seem to activate Dbp5 by stabilizing a distinct conformation. This particular conformation promotes the dissociation of the RNA and allows the recycling of the enzyme — a step that is rate-limiting [29,36]. Binding of Gle1–IP<sub>6</sub> causes a conformational change in ADP-Dbp5 in which the two RecA-like domains are more open as compared to the RNA bound structure and in which the RNA binding site is altered prohibiting the association of RNA very similar to elf4A and elf4F4 [29].

3.4. Dbp5 traverses multiple regulatory cycles necessary for mRNA export

Dbp5 has another unique feature in comparison to other known DEAD-box helicases: Its intrinsic ATPase activity is regulated by the N-terminal extension of Dbp5 [15]. The amino acids 55–68 of the human Dbp5 form an α-helix in the RNA-free and ADP bound protein that localizes within the cleft between the two RecA-like domains and separates them. In this conformation the ATP binding site, which is normally formed by both domains, is disrupted (Fig. 2). Association of RNA removes the N-terminal extension, induces a closure of the cleft and the building of the ATP binding site leading to the formation of the catalytic center for ATP hydrolysis. A truncated protein lacking the complete N-terminal extension up to Lys92 resulted in the highest ATPase activity that was independent of the presence of RNA. These findings indicate that the helicase core of Dbp5 displays an active ATPase. Its activity and its RNA dependency are regulated by the N-terminal extension of Dbp5. Dbp5 lacking this auto-regulatory domain is still able to bind RNA [22,29], however, it loses the ability to regulate the ATPase activity upon RNA association. This might explain why the first 79 amino acids are non-essential for the function of Dbp5 in yeast cells [1].

Furthermore, the nucleoporin Nup159 (Nup214 in human) is directly involved in the regulation of the ATPase cycle of Dbp5 in addition to its function to localize Dbp5 to the cytoplasmic fibrils. The N-terminal β-propeller core of Nup159 interacts with the N-terminal domain of Dbp5 and this interaction is primarily mediated via the surfaces of the proteins [21–23]. Especially the amino acids Asp221 (human DDX19: Asp223), Arg256 (259) and Arg259 (262) of yeast Dbp5 contribute to the binding to Nup159 as mutation of these residues completely abolishes the association with the nucleoporin [22,23]. This Nup159 binding site partially overlaps with the binding site for RNA [22,23,29]. Therefore binding of Nup159 and RNA must be mutually exclusive which was verified in a competition assay [22]. Furthermore, the binding affinity between human Nup214 and DDX19 analyzed by surface plasmon resonance was reduced in the presence of ADP or AMP-PNP, a non-hydrolysable ATP analog [22]. These findings indicate that two distinct complexes exist during the ATPase cycle: Dbp5 bound to ATP and RNA as well as nucleotide free Dbp5 bound to Nup159, which might be important for substrate release. Binding of Nup159 to Gle1–IP<sub>6</sub> and ADP...
bound Dbp5 induces an additional conformational change in Dbp5 forming a more open cleft between the two RecA-like domains which facilitates ADP displacement [29]. In an in vitro ADP binding assay purified recombinant Dbp5 tightly bound ADP and only the addition of recombinant Nup159 lacking the C-terminus stimulated ADP release whereas the presence of Gle1–IP6 or RNA did not affect the dissociation of ADP [24]. Direct binding of Nup159 to Dbp5 seems to be necessary for the complete ADP release as nucleoporin mutants without the ability to bind Dbp5 failed to stimulate the ADP dissociation. Therefore, Dbp5 is the first known DEAD-box RNA helicase with an ADP release factor. The question still remains, if this is a unique feature for Dbp5 or if other DEAD-box RNA helicases might require similar factors. Thus, it will be interesting to search for possible release factors for the other DEAD-box proteins.

Taken together, the data about the regulation and the ATPase cycle of Dbp5 led to the current model [14,24,28,29,37] (Fig. 2). Dbp5 without nucleotides or binding partners has an open conformation with only loosely associated RecA-like domains. When exactly ATP binding occurs is not clear yet. However, since ATP bound Dbp5 displays the highest affinity for RNA and Gle1–IP6, it is likely that ATP-binding occurs first. ATP bound Dbp5 still possesses a relatively open structure with the N-terminal extension folded to an α-helix in the cleft between both helicase core domains holding them apart and interfering with the catalytic center. The cooperative association of mRNA and Gle1–IP6, then causes the displacement of the N-terminal extension, tight closure of the cleft between both RecA-like domains and full formation of the ATP binding site and the catalytic center leading to ATP hydrolysis. The corresponding conformational change from ATP-Dbp5 to ADP-Dbp5 seems to be critical for the displacement of mRNA associated proteins by a currently unknown mechanism. Gle1–IP6 might stimulate the activity of Dbp5 by stabilizing the ADP bound form and in this way promote the consequent displacement of the RNA, which is rate limiting for enzyme recycling and leaves the enzyme in a partially open structure with an altered RNA binding site. Finally, Nup159 interacts with the partially overlapping RNA-binding site, resulting in a complete opening of the RecA-like domains and the release of ADP. The fully recycled enzyme is now ready for a new ATPase cycle.

This Dbp5 ATPase cycle involving Nup159 and Gle1–IP6 tethered to Nup42, is restricted to the cytoplasmic filaments of the NPC as both nucleoporins are localized to the cytoplasmic fibrils and thus determine the place (the cytoplasmic site of the NPC) and the time of action (when the mRNP begins to show up in the cytoplasm). Again, the question remains how Dbp5 might get there. Does it shuttle with the mRNA or does it simply wait at the cytoplasmic side of the NPC? Moreover, it is possible that a single Dbp5 molecule performs multiple ATPase cycles and mRNP remodeling events at the NPC [24,28,38]. This would not necessitate the export of Dbp5 along with the mRNA. Yeast Dbp5 mutants that are unable to bind RNA

Fig. 2. Model for the ATPase cycle of Dbp5 during mRNA export. Dbp5 (illustrated in yellow) with its open conformation can bind ATP (I). This leads probably to a cooperative binding of its cofactors Gle1–IP6 (in gray) and the exported mRNP (II). Binding causes a conformational change, which results in the removal of the N-terminal extension from the protein center and to a closer conformation of both RecA-like domains leading to ATP hydrolysis (III). The transition to the ADP bound form leads to the specific displacement of mRNP bound proteins and to the dissociation of the mRNA (IV). This in turn allows the binding of Nup159. The interaction induces a further conformational change leading to the release of ADP (V). The recycled enzyme can now start a new cycle for mRNP remodeling.
were still localized to the NPC indicating that mRNA binding is not necessary for NPC association [28]. Furthermore, Siebrasse et al. [38] analyzed the transport of single mRNA molecules through the NPC in C. tentans and showed that Dbp5 arrives mostly at the NPC from the cytoplasm. In this study it was shown that Dbp5 dissociated after approximately 55 ms into the cytoplasm indicating that several Dbp5 molecules might act repeatedly on one mRNP at the same time since transport of large mRNPs might require a longer time. Studies in yeast have shown that Dbp5 accumulates in the nuclei of mex67-5 mutants [16], in which poly(A)-tail containing mRNAs are retained in the nuclei as well [39]. This could be interpreted in a way that Dbp5 shuttles with the mRNA to the cytoplasm. However, it is also possible that an mRNA encoding a Dbp5 export factor causes a secondary defect. Interestingly, a rat7/mup159 mutant in which mRNA is not exported to the cytoplasm and accumulates in the nucleus, Dbp5 does not co-accumulate in the nucleus [16]. Additionally, yeast Dbp5 accumulates in the nuclei of xpo1-1 mutants, encoding a defective Ran-dependent exportin, indicating at least alternative export routes [16]. It is conceivable that two parallel ways might exist for Dbp5 to leave the nucleus: bound to mRNA and bound to Xpo1. The Xpo1 route might be faster and in this way a cell might be able to react rapidly and supply Dbp5 at all places where it is needed.

3.5. Dbp5 functions in translation termination

The role of Dbp5 in mRNA export is known for many years and certainly best understood. Surprisingly, the question of what might be the function of the protein in the cytoplasm was not addressed until 2007, when Dbp5 was detected on cytoplasmic polyribosome containing mRNAs in S. cerevisiae and a new function for Dbp5 was identified in translation termination [5].

Eukaryotic translation termination begins when the translating ribosome reaches a stop codon and involves the eukaryotic release factors eRF1 and eRF3 (encoded in S. cerevisiae by SUP45 and SUP35, respectively) [40]. In the ribosomal A-site all three kinds of stop codons are recognized and bound by eRF1 which subsequently promotes the hydrolysis of the ester bond of the peptidyl-tRNA in the peptidyl transferase center and finally the release of the polypeptide chain. The GTPase eRF3 is necessary for efficient stop codon recognition and peptidyl-tRNA hydrolysis. GTP hydrolysis by eRF3 leads to a conformational change and proper positioning of eRF1 in the peptidyl transferase center. For a long time only these two factors were known to mediate the termination process, until Dbp5, than Gle1 and the iron–sulfur containing and ATP-binding cassette protein Rti1 (RNase L inhibitor, ABCE1 in human) were found to be additional essential factors in S. cerevisiae that participate in the process of translation termination [5,41,42].

For Dbp5 it was shown that the protein was detectable in the polyosome-containing fraction of sucrose density fractionation experiments indicating that Dbp5 is part of actively translated mRNAs [5]. Further, mutants of DBP5 were hypersensitive to translation inhibitors, in contrast to mutants of NUP159. A first hint for an involvement of Dbp5 in the process of termination was obtained by genetic analyses in which high copy DBP5 suppressed growth defects of temperature-sensitive mutants of eRF1, eRF3 and the eRF3 interacting and poly(A)-tail binding protein Pab1. Furthermore, different temperature-sensitive mutants of DBP5 were synthetically lethal with the eRF1-, eRF3- and Pab1-mutants [5]. It is of interest to note that a physical RNA-independent interaction between Dbp5 and eRF1 was detected, but not with eRF3 or Pab1. Moreover, the temperature-sensitive mutant rat8-2 showed after shift to non-permissive temperature an increased readthrough activity comparable with the level of the eRF3 mutant sup35-21 in a luciferase dual reporter assay. Overexpression of DBP5 was able to suppress the enhanced readthrough activity of the termination factor mutants sup45-2 and sup35-21. This led to the conclusion that Dbp5 might be involved in the recognition of stop codons. Dbp5 might be part of the early termination complex since a simultaneous presence of Dbp5 and eRF3 was not detectable. Furthermore, in the mutant rat8-2 the interaction between eRF1 and eRF3 was disrupted and eRF3 was no longer part of the polyosome-containing fraction in sucrose density fractionation experiments [5].

Interpreting these data, the following model for translation termination was suggested [5,43] (Fig. 3). Dbp5 and its known RNA/protein complex remodeling activity might be involved in the accurate positioning of eRF1 at the termination codon in the A-site of the ribosome and therefore might ensure proper stop codon recognition. Dbp5 might then need to dissociate from the termination complex to allow the entry of eRF3 that supports efficient translation termination including polypeptide release. How Dbp5 enters the ribosome is currently unknown. It might either enter the complex on its own, join in bound to eRF1 or accompany the ribosome during the whole process of translation. Furthermore, until now it has not been shown whether eRF1 and eRF3-GTP bind as a complex to the terminating ribosome. Bolger et al. [41] and Alcázar-Román et al. [36] showed that the known Dbp5 cofactors Gle1 and Ip6 are also involved in the process of translation termination. Gle1 mutants were also hypersensitive to translational inhibitors [41] and displayed an increased readthrough activity to a similar extent as shown for Dbp5 mutants [36]. Furthermore, Gle1 co-migrates with translating ribosomes, genetically and physi- cally interacts like Dbp5 with eRF1 and the temperature-sensitive mutant gle1-4 failed to recruit eRF3 to the polyosomes after shift to non-permissive temperature [41]. Interestingly, Dbp5 and eRF1 were still part of the polyosome-associated fraction in the gle1-4 mutant indicating that the missing stimulation of the ATPase activity of Dbp5 does not affect the recruitment of Dbp5 and eRF1 to the terminating ribosome but permits the association of eRF3. Therefore, potential remodeling of the termination complex in the ribosome allowing eRF3 to enter seems to require the same Gle1 dependent ATPase cycle of Dbp5 as shown for mRNA export. These findings are supported by the fact that Ip6 is required for translation termination as well: Yeast strains lacking the Ip6 producing kinase Ipk1 are synthetic lethal with the eRF1 mutant sup45-2 and had defects in stop codon recognition [41]. Additionally, Gle1 mutants with single amino acid exchanges that disrupt Ip6 binding display enhanced readthrough activities similar to the level of the ipk1A strain [36]. These results indicate that Ip6 together with Gle1 supports the function of Dbp5 not only in mRNA export, but also in translation termination. The same ATPase cycle as described for Dbp5 in mRNA export might be necessary for the remodeling activity of Dbp5 during translation termination. If so, the most pressing question is which factor is responsible for the ADP release at the ribosome? The counterpart for mRNA export, Nup159, is tethered to the nuclear pore.

It is of interest to note that, while the function of Dbp5 in translation seems to be restricted to the process of translation termination, Gle1 seems to have an additional function in translation initiation, which is also independent of Ip6 [41,44]. Despite the experiments that nicely show an involvement of Dbp5 in efficient stop codon recognition, the exact function of Dbp5 and its cofactors during translation termination are still unknown and the mechanism of their activity remains unclear and needs further experimental data.

3.6. The role of Dbp5 during cellular stress

Stress conditions like heat shock to 42 °C or addition of up to 10% ethanol lead to a rapid adaption of gene expression in S. cerevisiae (for review see [45]). General transcription and splicing are reduced, bulk poly(A)-tail containing mRNAs accumulate in the nucleus and non-translated mRNAs from the cytoplasm are stored in P-bodies or stress-granules. In contrast, the transcription of genes induced by stress such as the heat shock protein-encoding genes (HSP) is

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increased and their mRNAs are specifically transported out of the nucleus and translated in the cytoplasm. This mechanism offers a specific and rapid expression of proteins needed to cope with the stress situation. However, how the selective mRNA transport under stress conditions is mediated is currently unclear.

Dbp5 seems to be involved in the transport of mRNAs under normal and under stress conditions as mutants prevent the export of both regular mRNAs and the stress induced SSA4-mRNA that encodes the heat shock protein Hsp70 [46]. Yeast Dbp5 has a unique insertion of six amino acids at positions 412-417 (NGQADP) which is conserved in Dbp5 homologues in all species in contrast to other DEAD-box RNA helicases [46]. Yeast strains expressing Dbp5 lacking this insertion showed no defects at 16 to 37 °C but revealed a nuclear accumulation of the SSA4-mRNA upon shifting it to 42 °C, indicating that this insertion is necessary for the functionality of Dbp5 during heat stress [46].

Different stress conditions lead to different cellular reactions. Interestingly, in contrast to heat stress in which Dbp5 showed the normal localisation predominantly at the nuclear envelope, Dbp5 was mislocalized to the nucleus upon addition of 10% ethanol which caused a nuclear accumulation of the SSA4-mRNA upon shifting it to 42 °C, indicating that this insertion is necessary for the functionality of Dbp5 during heat stress [46].

Different stress conditions lead to different cellular reactions. Interestingly, in contrast to heat stress in which Dbp5 showed the normal localisation predominantly at the nuclear envelope, Dbp5 was mislocalized to the nucleus upon addition of 10% ethanol which caused a nuclear accumulation of the SSA4-mRNA upon shifting it to 42 °C, indicating that this insertion is necessary for the functionality of Dbp5 during heat stress [46].

Fig. 3. Model for the function of Dbp5 during early steps of translation termination in S. cerevisiae. When and how Dbp5 (in yellow) and its cofactors Gle1–IP6 (in gray) enter the ribosome is currently unclear. The stop codon in the ribosomal A-site might be recognized by eRF1 (in green) bound to Dbp5 (Ia). Entry of Gle1–IP6 in turn might stimulate ATP-hydrolysis. In contrast, eRF1 and Dbp5 might enter the terminating ribosome together with the cofactors of Dbp5 (Ib) or Dbp5 might accompany the ribosome already during earlier phases of translation upon arrival at the termination codon (Ic). After stop codon recognition, Gle1–IP6 stimulates the ATP hydrolysis of Dbp5 and thus might induce the remodeling of the RNA/protein complex leading to an accurate positioning of eRF1 on the stop codon. Dissociation of Dbp5 allows the recruitment of eRF3 (in blue, II). This in turn might facilitate the subsequent hydrolysis of the peptidyl-tRNA and the polypeptide chain release (not shown).

4. Concluding remarks and open questions

Dbp5 was initially discovered as a nuclear mRNA export factor. However, in the meantime the multifunctional nature of the protein is exposed. Dbp5 has been identified to be involved in several cellular processes of RNA metabolism, ranging from transcription, mRNA-export to translation, which occurs at different cellular locations (nucleus, nuclear rim and cytoplasm). Therefore, it is of great interest to find out whether different pools of Dbp5 exist or whether one single Dbp5 molecule does all jobs and accompanies the mRNA through its whole life cycle starting from transcription in the nucleus, translocation into the cytoplasm and ending with translation and mRNA decay. In this light it will be interesting to know whether a similar ATPase cycle of Dbp5 discovered for its deficiency of Dbp5 at the NPC in the presence of ethanol causes the transport block for bulk mRNAs and contributes to the regulation of their export especially under ethanol stress conditions in contrast to heat stress.

Interestingly, in the rat8-2 strain dbp5 is mislocalized in cytoplasmic granules upon a shift to the non-permissive temperature and the protein co-localizes there with other mRNA export factors such as Mex67 and probably with non-translated mRNAs [8]. Therefore, these foci found in mRNA export mutants were called RNA export granules (REGs) which differ from P-bodies but overlap completely after heat shock to 42 °C. The physiological relevance of these phenomena is still unclear.

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role in mRNA export is needed for the other processes as well. In this case, a cytoplasmic and nuclear ADP release factor such as Nup159 at the NPC should exist and awaits its discovery.

Moreover, many details of the exact role and function, as well as additional cofactors of Dbp5 during translation termination are missing and need to be analyzed in further experiments. Further, the regulation of Dbp5 under different environmental conditions such as cellular stresses remains elusive and it will be a challenging task to analyze for example if the IP6 level is involved in modulating Dbp5 activity in vivo. Even the intensively analyzed role of Dbp5 in mRNA export still leaves several questions open. Apparently, mRNPs binding of Dbp5 is sequence unspcific and a wide range of different mRNPs could be remodeled. However, detailed in vivo information is missing and thus it is still possible that Dbp5 for instance when bound to certain regulatory proteins might develop sequence specificity. Moreover, it is unclear how Dbp5 discriminates between proteins that have to be removed from the mRNPs and proteins that need to remain bound after translocation through the NPC? The displaced proteins might exhibit special recognition motifs for Dbp5 but there is nothing known yet. Additionally, the exact mechanism for the displacement of mRNA bound proteins is still vague and needs to be enlightened as well.

In spite of several conserved structural and regulating properties of Dbp5 such as RNA and ATP binding or the complex formation with its cofactors, some unique characteristics for Dbp5 as a DEAD-box RNA helicase were found including the autoregulatory N-terminal extension and the use of Nup159 as an ADP release factor. Furthermore, Dbp5 is the only DEAD-box RNA helicase known to use a conformational change upon ATP hydrolysis for the remodeling of RNA-protein complexes. Therefore, new analysis of the other family members regarding these regulatory features will be a challenge.

References


[27] C.A. Hodge, H.V. Color, P. Stafford, C.N. Cole, Rat8p/Dbp5p is a shuttling transport factor that interacts with Rat7p/Nup159p and Gle1p and suppresses the mRNA export defect of xpo1-1 cells, EMBO J. 18 (1999) 5778–5788.


