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SURVEY AND SUMMARY

Dead-box proteins: a family affair—active and passive players in RNP-remodeling

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ABSTRACT

DEAD-box proteins are characterized by nine conserved motifs. According to these criteria, several hundreds of these proteins can be identified in databases. Many different DEAD-box proteins can be found in eukaryotes, whereas prokaryotes have small numbers of different DEAD-box proteins. DEAD-box proteins play important roles in RNA metabolism, and they are very specific and cannot mutually be replaced. *In vitro*, many DEAD-box proteins have been shown to have RNA-dependent ATPase and ATP-dependent RNA helicase activities. From the genetic and biochemical data obtained mainly in yeast, it has become clear that these proteins play important roles in remodeling RNP complexes in a temporally controlled fashion. Here, I shall give a general overview of the DEAD-box protein family.

INTRODUCTION

Nucleic acids can be present in single-stranded, double-stranded or even multiple-stranded forms. The advantages of a double-stranded molecule with strands of opposite polarity have been known since the discovery of the double-stranded DNA molecule (1). However, the possibility of finding a matching partner can be of importance not only for DNA but also for RNA. This can be true for extended double-stranded RNA molecules as found in viruses, for local secondary structures as in ribosomes and for short RNA–RNA interactions, as in pre-mRNA splicing or RNA-mediated silencing. The caveat of double-stranded nucleic acids is that at some point they may need to be unwound if the sequence information of the nucleic acid needs to be deciphered or to be used for an alternative sequence-specific binding event. Therefore, an obligatory complement of double-stranded nucleic acids is the presence of enzymes that can unwind these helical molecules, i.e. helicases. Since the two strands are held together by base pairing, helicases require energy for unwinding. Text books discuss in detail helicases required for initiation and elongation of DNA replication, but only rarely helicases that are involved in the separation of RNA strands. Nevertheless, genes encoding helicases make up a considerable portion of the coding information of a eukaryotic genome (2) and many of these helicases have a preference or even an exclusive requirement of RNA molecules. Several reviews on different aspects of DEAD-box proteins have been published in recent years (3–12). Here, I shall give a general overview of the DEAD-box field as it stands today.

WHAT IS THE DEAD-BOX FAMILY?

One of the earliest descriptions of an RNA helicase was the report that incubation of globin mRNA with the translation initiation factor eIF4A and ATP changed the susceptibility of the mRNA to nucleases (13). Thus, eIF4A altered the structure of the mRNA in such a way, that the RNase digestion pattern changed. This change was dependent on a source of energy in the form of ATP. The translation initiation factor eIF4A could therefore be considered as a helicase that melts (local) secondary structures and makes the RNA accessible to nucleases. Since then, many RNA helicases involved in a variety of cellular processes have been described.

In 1988, Gorbalenya *et al*. (14) defined a group of NTPases and showed that they had several common sequence elements. This analysis, together with the description of a number of proteins involved in RNA metabolism (p68, SrmB, MSS116, vasa, PL10, mammalian eIF4A, yeast eIF4A) resulted, based on the sequence of eIF4A, in the birth of the DEAD-box protein family (15). Today, the alignment of all annotated sequences in SwissProt from all species reveal nine conserved sequence motifs with very little variation (15,16) (Figure 1). The simultaneous presence of these motifs is a criterion for inclusion of a protein within the family, although an enzymatic activity has been demonstrated only for a limited number. Motif II (or Walker B motif) has the amino acids D-E-A-D, which gave the name to the family. This motif, together with motif I (or Walker A motif), the Q-motif and motif VI, is required for ATP binding and hydrolysis (16–19). Motifs Ia and Ib, III, IV and V have been characterized less well but may be involved in interaction with

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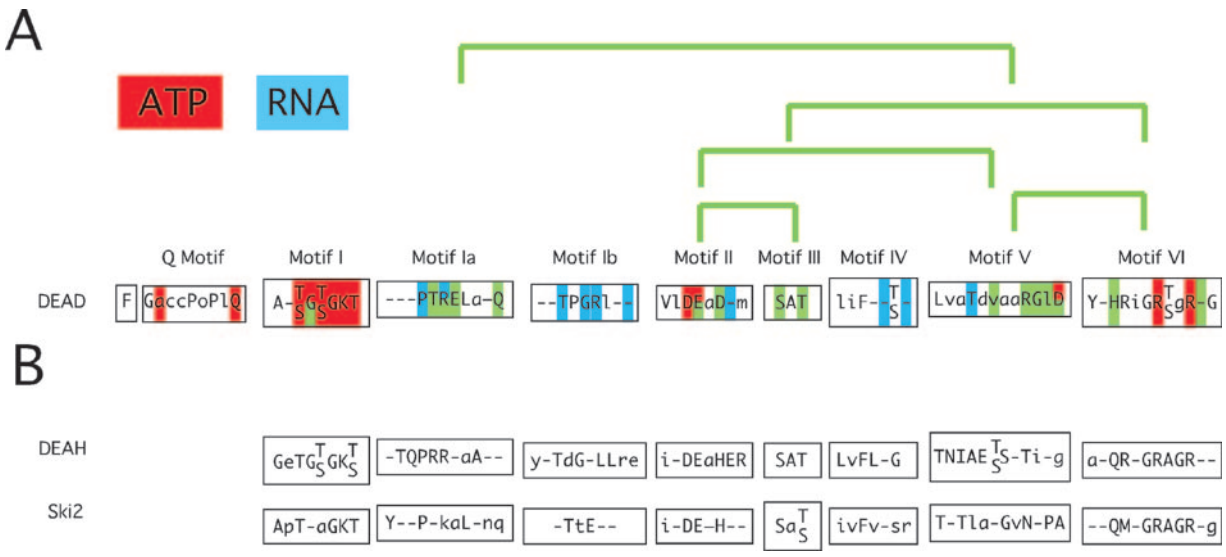

Figure 1. A schematic presentation of the conserved motifs of the DEAD-box family. (**A**) Consensus sequence of the DEAD-box family. Residues identified in the structure of the Vasa protein (70) to interact with ATP (red), RNA (blue) or involved in intra-protein interactions (green) are highlighted. (**B**) Consensus sequences of the DEAH-box and Ski2 family. The consensus sequences (capital letters represent amino acids conserved at least 80%, lower case letters represent amino acids that are conserved 50–79%) are taken from Tanner and Linder (10).

RNA (20) and in intramolecular rearrangements necessary for remodeling activity (Figure 1).

Proteins related to eIF4A in sequence can be found in all eukaryotic cells and in most eubacteria and archaebacteria. The genome of the yeast *Saccharomyces cerevisiae* encodes 25 DEAD-box proteins (21,22). Interestingly, it has two genes (*TIF1* and *TIF2*) encoding exactly the same eIF4A protein, and it encodes two related proteins Ded1 and Dbp1. The deletion of *DED1* is lethal, whereas the deletion of *DBP1* is not lethal under normal laboratory conditions. However, overexpression of Dbp1 can suppress the lethal deletion of *DED1* (23), indicating (but not proving) a functional redundancy. A comparison with another fungal species, *Ashbya gosypii*, which is considered to be the free living eukaryote with the smallest genome (24), reveals all the DEAD-box proteins found in *S.cerevisiae*, with the exception of Dbp1 and Prp28 (involved in pre-mRNA splicing, see below), and with only one eIF4A copy. Thus, the DEAD-box proteins of *A.gosypii* could represent the minimal number of such proteins required for a free-living eukaryote.

In multicellular eukaryotes, several additional DEAD-box proteins can be found. A search in the human genome revealed 38 DEAD-box proteins (Table 1), which can tentatively be classified into 32 subfamilies. These subfamilies have been defined by iterative blast searches against the SwissProt/trEMBL databases, using all human DEAD-box proteins. Approximately 250 best scoring sequences from each blast search were then used for a ClustalW analysis to identify related sequences. In some cases, where two human or two yeast proteins clustered together, the members of the putative subfamily from other model organisms were analyzed further to determine whether there were one or two proteins within this subfamily. If other model organisms had only one protein, the subfamily was defined as such. However, if most model organisms had also two representatives within the putative subfamily, the subfamily was divided into two. An example is the separation of the Ddx3/Ded1 and Vasa subfamilies. Drosophila and other multi-cellular eukaryotes have two or more DEAD-box protein related to Ded1 or Vasa. However, with the exception of the yeast *S.cerevisiae*, unicellular eukaryotes have only one of these proteins (25) and therefore these proteins have been divided in two subfamilies. Another example would be the subfamily of proteins homologous to the yeast Dbp5 protein. In the human genome three proteins, Ddx19A, Ddx19B and Ddx25, are very similar to Dbp5 and are therefore being included in the same subfamily. It is clear, that this definition of subfamilies is somehow arbitrary and should be regarded as a working tool to compare proteins and predict functions. In some cases cross species complementation could be demonstrated (26,27) but in any case, experiments are needed to characterize these subfamilies further. The Ddx7 (28) protein has no homologs in other mammals and a tblast against the human genome does not report any significant similarity. It is therefore excluded from the list presented here. According to the criteria defined above, 11 human DEAD-box proteins have no direct or obvious counterpart in yeast (Ddx1, Ddx4/vasa, Ddx20/DP103, Ddx21/RNA helicase Gu-alpha, Ddx28, Ddx50/RNA helicase Gu-beta, Ddx41/abstrakt, Ddx42, Ddx43, Ddx53, Ddx59). Although it may be expected that the human genome contains more DEAD-box proteins than the simple budding yeast, it may seem surprising that three DEAD-box proteins present in yeast (Dbp3, Mss116, Mrh4) have no obvious counterpart in humans. The DEAD-box proteins Mss116 and Mrh4 have been shown to be required for gene expression in yeast mitochondria (29,30). It is tempting to speculate that these proteins are simply not required in human mitochondria, because the structural organization of human mitochondrial genes is different from that of yeast mitochondrial genes, which harbor many introns. In contrast, Ddx28, may be involved in mitochondrial gene expression in human

Table 1. A tentative assignment of yeast and human DEAD-box protein subfamilies

Human	SwissProt	Alias	Function	Reference	Yeast	SwissProt	Function	Reference
DDX1	Q92499	DEAD-box protein-retinoblastoma	Amplified in retinoblastoma, cellular co-factor of HIV-1 Rev, nucleolar	(105,143,144)	—	—		
DDX2A	P60842	eIF4A I	Translation initiation	(9)	Tif1 Tif2	P10081	Translation initiation	(145)
DDX2B	Q14240	eIF4A II						
DDX3Y	O15523	DBY		(146,147)	Ded1 Dbp1	P06634 P24784	Translation initiation, re-mRNA splicing, mRNA export	(90,125, 126,148)
DDX3X	O00571	DDX3, mDEAD3	Similar to mouse PL10, *Xenopus* An3, and *Drosophila* Bel; required for Rev-dependent export of intron-containing HIV-1 RNA, nucleolar	(105,149,150)				
DDX4	Q9NQI0	vasa	Translation initiation, imilar to *Drosophila* vasa that interacts with eIF5B	(151,152)	—	—	—	—
DDX5	P17844	p68, HLR1	transcription, pre-mRNA splicing, mRNA stability and ribosome biogenesis, nucleolar	(105,153,154)	Dbp2	P24783	ribosome biogenesis, interacts with Upf1 and is involved in NMD	(155)
DDX17	Q92841	p72	nucleolar	(105,156)				
DDX6	P26196	p54, RCK	Oncogene RCK, translation initiation of c-myc mRNA, nuclear assembly of stored mRNP particles, mRNA masking in analogy to clam homolog	(137,138,157–159)	Dhh1	P39517	Assists decapping, Required for mRNA storage,	(135,160)
DDX10	Q13206		nucleolar	(105,161)	Dbp4	P20448	Ribosome biogenesis	(162)
DDX17	See subfamily DDX5/DDX17							
DDX18	Q9NVP1	MrDb,	Nucleolar, Myc-regulated	(105,163)	Has1	Q03532	Ribosome biogenesis	(108)
DDX19A	Q9NUU7	mRNA export	DEAD box protein	(53)	Dbp5	P20449	mRNA export	(52,111)
DDX19B	Q9UMR2							
DDX25	Q9UHL0	GRTH	Gonadotropin-regulated testicular RNA helicase	(164)				
DDX20	Q9UHI6	DP103, Gemin3, survival of motor neurons (SMN)-interacting protein	Spliceosomal snRNP biogenesis	(165,166)	—	—	—	—
DDX21	Q9NR30	Nucleolar RNA helicase II, Nucleolar RNA helicase Gu Gu-alpha	Ribosomal RNA production, co-factor for c-Jun-activated transcription	(105,167–169)	—	—	—	—
DDX50	Q9BQ39	RNA helicase Gu-beta DDX21B according to Abdelhaleem *et al*.	Localizes to nuclear speckles containing splicing factor SC35 Co-factor for c-Jun-activated transcription, nucleolar	(61,105,170,171)	—	—	—	—
DDX23	Q9BUQ8		Pre-mRNA splicing	(172)	Prp28	P23394	pre-mRNA splicing	(72,173,174)
DDX24	Q9GZR7		nucleolar	(105,175)	Mak5	P38112	Ribosome biogenesis	(176)
DDX25	See subfamily DDX19A/DDX19B/DDX25							
DDX27	Q96GQ7		Nucleolar	(105)	Drs1	P32892	Ribosome biogenesis	(177)
DDX28	Tr_Q9NUL7	MDDX28	Mitochondrial and nuclear localization	(31)	—	—	—	—
DDX31	Q9H8H2		Nucleolar	(105)	Dbp7	P36120	Ribosome biogenesis	(178)
DDX39	O00148	URH49	Pre-mRNA splicing and export	(118)				
BAT1	Q13838	UAP56		(179–181)	Sub2	Q07478	Pre-mRNA splicing and export	(85,115, 117,182)
DDX41	Q9UJV9		DEAD-box protein abstrakt homolog	(183,184)	—	—	—	—

Table 1. *Continued*

Human	SwissProt	Alias	Function	Reference	Yeast	SwissProt	Function	Reference
DDX42	Tr_Q86XP3	SF3b125 DEAD-box protein	Pre-mRNA splicing, splicing	(185)	—	—	—	—
DDX43	Tr_Q9NXZ2		Displays tumor-specific expression	(186)	—	—	—	—
DDX53	Tr_Q6NVV4	CAGE	CAGE is expressed in a variety of cancers but not in normal tissues except testis,	(187)	—	—	—	—
DDX46	Tr_Q7L014		Pre-mRNA splicing	(185)	Prp5	P21372	Pre-mRNA splicing	(46,47,188)
DDX47	Q9H0S4		Co-transfection of GABARAP and DDX47 cDNA into a tumor cell line induces apoptosis, nucleolar localization	(105,106)	Rrp3	P38712	Ribosome biogenesis	(189)
DDX48	P38919	NMP265/NUK34, eIF4A III	DDX48 is a component of the EJC; has also been found in proteomic studies of the nucleolus	(98,105,190)	Fal1	Q12099	Ribosome biogenesis	(100)
DDX49	tr_Q9Y6V7		nucleolar	(105)	Dbp8		Ribosome biogenesis	(191)
DDX50	See subfamily DDX21/DDX50							
DDX51	Tr_Q8IXK5		Nucleolar	(105)	Dbp6	P53734	Ribosome biogenesis	(192)
DDX52	Q9Y2R4		nucleolar	(105,106)	Rok1	P45818	Ribosome biogenesis	(193)
DDX53	See subfamily DDX43/DDX53							
DDX54	Q8TDD1	DP97	nucleolar	(105,106,194)	Dbp10	Q12389	Ribosome biogenesis	(195)
DDX55	Tr_Q8NHQ9		Nucleolar	(105)	Spb4	P25808	Ribosome biogenesis	(196)
DDX56	Q9NY93	noH61, DDX21	associates with nucleoplasmic 65S preribosomal particles,nucleolar	(105,197)	Dbp9	Q06218	Ribosome biogenesis	(198)
DDX59	tr_Q8IVW3				—	—	—	—
—					Dbp3	P20447	Ribosome biogenesis	(33)
—					MSS116	P15424	Mitochondrial gene expression	(30,199)
—					Mrh4	P53166	Mitochondrial function	(29)

The yeast DEAD-box proteins have been described previously (21). The human subfamilies have been determined with the help of Abdelhaleem *et al*. (2003), a search for DDX genes in SwissProt, a search in the human gene nomenclature search site (www.gene.ucl.ac.uk/nomenclature/), and by running a blast search using yeast eIF4A against the *initio proteins* of the human genome (http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html). Representative samples (∼250 sequences) from the blast searches using every individual human DEAD-box protein defined above was used for a second round of blast analysis for confirmation and for ClustalW analysis at EBI and a tentative tree has been established by using the TreeView (Rod page, http://taxonomy.zoology.gla.ac.uk/rod/treeview.html) program. Proteins related to DDX2A and DDX2B, DDX3Y and DDX3X, DDX5 and DDX17, DDX19A and DDX19B and DDX25, DDX21 and DDX50, DDX39 and BAT1, DDX43 and DDX53, form each one subfamily, respectively. Based on this analysis and the absence of any significant match in a blast with the human genome, the DDX7 entry (28) has been removed from the list. References are given for information but are by far not exhaustive. More information on RNA helicases can be found on http://www.helicase.net and http://www.medecine.unige.ch/∼linder/RNA_helicases.html.

mitochondria, insofar as it shows nuclear and mitochondrial localization (31,32). The yeast Dbp3 protein is involved in ribosome biogenesis and it is one of the rare DEAD-box proteins that are not essential for growth under normal laboratory conditions (33). In contrast to eukaryotes, bacterial genomes encode far fewer DEAD-box proteins and some bacterial species seem not to encode DEAD-box proteins at all (5,8). Today, searches in SwissProt reveal ∼205 annotated sequences and >700 different entries in SwissProt and trEMBL. Based on the activity of eIF4A and on the sequence alignments, it is thought that the members of the DEAD-box family have similar biochemical activities.

THE DEAD-BOX FAMILY IS DISTINCT, BUT NOT ALONE

Bioinformatic searches have revealed related proteins that share some motifs with the DEAD-box family, but have other distinguishing motifs that are conserved within their own family (34). The related proteins belong to the DEAH and Ski2 families, which together with the DEAD-box family are often referred to as the DExD/H families. However, based on their sequences, the families are clearly distinct, despite the similarities they share (Figure 1B). In other words, no protein has been found so far that could belong to two families, as judged from the conserved motifs. This could simply be explained by a co-evolution of the different motifs within one family. However, another perspective is that the different families serve different purposes in RNA metabolism in a cell. In this respect, it is interesting to note that biochemical and structural analyses have revealed certain similarities amongst members of the various families, but also differences. For example both DEAD-box and DEAH-box proteins are stimulated by RNA in their NTPase activity, but DEAD-box proteins use only ATP, whereas DEAH box proteins are more promiscuous in their NTP usage (16,35).

WHAT DO WE KNOW ABOUT THE BIOCHEMICAL ACTIVITIES OF DEAD-BOX PROTEINS?

In comparison to the enormous number of DEAD-box proteins present in protein databases, only few RNA helicases from the DEAD-box family have been characterized biochemically (36). As expected from the presence of the Walker A and Walker B motifs typical for NTPases, DEAD-box proteins show ATPase activity. Normally, this activity is dependent on RNA, although in some instances an RNA-independent activity has been reported (36). Further experiments are needed to determine whether these differences are intrinsic to the analyzed proteins themselves, or dependent on the purification of the proteins. In general, stimulation of the ATPase activity is not dependent on a particular RNA species. Indeed, in many cases such as in the scanning process of the 40S ribosomal subunit in translation initiation or in mRNA export from the nucleus, sequence specificity for the substrate would be in contradiction to its function. This implies that their specificity relies on the interaction with other RNP components. In the case of eIF4A it has been shown, for a long time, that its RNA-dependent ATPase activity is stimulated by eIF4B, although the molecular details of this stimulation are still not known (37). More recently the stimulation of the activity of eIF4A by eIF4H and eIF4G has also been described previously (38,39). In the case of eIF4G, it has been suggested that eIF4G forms a 'soft clamp' that stabilizes eIF4A in a closed active conformation (39). Interestingly eIF4A can also be stimulated by pateamine A, a natural marine product that inhibits translation initiation and decreases the interaction of eIF4G and eIF4A (40,41). In contrast to these examples of stimulation by other proteins, in the case of proteins from the bacterial DbpA subfamily, a large stimulation by a hairpin structure of the 23S rRNA can be observed (42–44). This stimulation is dependent on a C-terminal domain that contains an RNA recognition fold motif (45). To a lesser extend, the yeast Prp5 protein, involved in pre-mRNA splicing, is stimulated in its activity by the snRNA U2 (46,47). It is noteworthy that Prp5 interacts with components of the U2 RNP (48). It is likely that, for other DEAD-box proteins, other stimulating or regulatory conditions/environments will be found in the near future [e.g. eIF4AIII, below, and Dbp8 (49)].

DEAD-box proteins are often referred to as RNA helicases. This implies that the proteins unwind, in an energy-dependent manner, double-stranded RNA molecules. Such an activity has indeed been demonstrated for several DEAD-box proteins (50–69). In most cases, however, unwinding activity is limited to short duplexes, indicating that it is not processive. Two simple explanations can be offered. First, recombinant proteins out of their biological context may not be efficient or processive. This is also true for proteins that are considered to have highly processive activities, such as the DNA polymerase that requires a clamping factor to become processive in its activity. The second explanation would be that indeed the DEAD-box proteins are not processive even *in vivo*, since they do not need to unwind lengthy double-stranded structures. In this scenario, which is at present the most likely one, their requirement would be a local action to unwind a limited double-stranded RNA or dissociate a protein from the RNA (see below), to allow further steps in a process to occur. The recently published data on the structure of the Drosophila Vasa protein with non-hydrolyzable ATP and an RNA substrate are clearly consistent with this view (70). In this structure, the Vasa protein bends the bound RNA in such a way that a double-stranded nucleic acid would be partially unwound (71). Clearly, the destabilization of the double-stranded RNA by virtue of the binding of the helicase to the double-stranded substrate, would suggest a non-processive and local dissociation activity (71).

Following the idea of a local dissociation activity, it has been shown recently, by genetic and biochemical experiments, that DEAD-box proteins are able to dissociate proteins from RNA molecules. Genetic experiments demonstrated that mutations in the genes encoding DEAD box proteins Prp28 and Sub2 can be suppressed by mutations in genes encoding proteins that are part of RNPs (72,73). In the case of Prp28, it has been shown that mutations in the U1 snRNA or the U1-C protein bypass the requirement of Prp28 (72). Similarly, deletion of Mud2 bypasses the requirement of Sub2 (73) and it has been shown recently that a mutation in the export factor Mex67 can suppress a mutation in *DBP5* (74). These results suggest that either these DEAD-box proteins can directly dissociate RNA–protein complexes or modify RNA structures that stabilize RNA–protein interactions. How this applies to the structure of Vasa, remains to be determined.

Thus, DEAD-box proteins are modulators of RNP complexes [see also (6)]. This modulating function is dependent on the presence of RNA, since the ATPase activity of most, if not all, DEAD-box proteins is dependent or largely stimulated by the presence of RNA. In order to limit the activity in time and space, RNA helicases may only transiently associate with an RNP complex. However, they also may be part of a complex for a certain period as found in proteomic studies of successive intermediates in pre-ribosomal particles (75–83). In this case it is likely that a conformational change, induced by the binding or dissociation of another subunit of the complex, brings the RNA substrate in such a position as to activate the ATPase activity of the DEAD-box protein. The DEAD-box protein would then induce a further conformational change in the RNP structure. This change will in turn modify the structure in such a way that it might no longer be a substrate for this particular RNA helicase. This would be an easy and elegant way to limit the activity of DEAD-box proteins and to provide a force for a unidirectional development of an RNP complex.

BIOLOGICAL FUNCTIONS OF DEAD-BOX PROTEINS

DEAD-box proteins have been described to be necessary for, or involved in, many different processes of RNA metabolism. In eukaryotic cells, in particular, these range from the transcription to the degradation of RNA, and include pre-mRNA splicing, mRNA export, ribosome biogenesis, translation initiation and gene expression in organelles (Figure 2).

Transcription

Recently several RNA helicases of the DEAD-box family have been described to be involved in transcription [see the contribution by Fuller-Pace (4)].

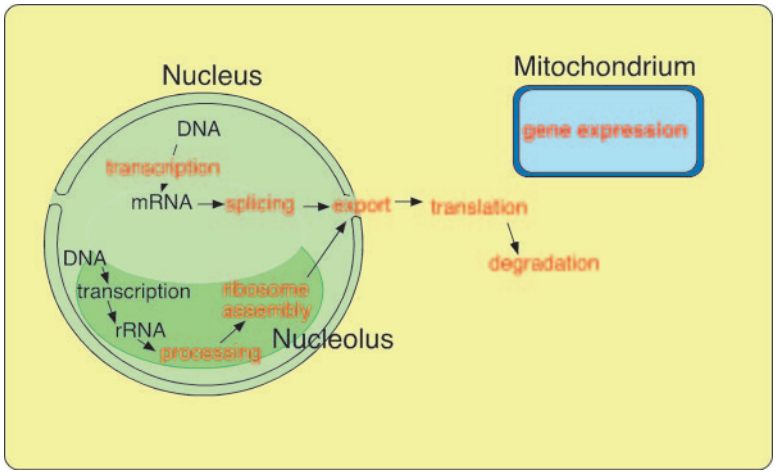

Figure 2. Schematic presentation of cellular processes that require DEAD-box proteins in eukaryotic cells.

Pre-mRNA splicing

Splicing of pre-mRNAs has become a paradigm for the analysis of the function of DEAD/DExH proteins. Although the removal of an intron by two transesterification reactions is energetically neutral, the splicing reaction requires ATP. This could be explained by temporal modification reactions, such as phosphorylation, or by active remodeling of the spliceosome. Indeed the formation of the spliceosome, the rearrangements within the spliceosome during the splicing reactions and the final release of the product, as well as the recycling of the components, require the rearrangement of five large RNP complexes (snRNPs U1, U2, U4, U5 and U6). Proteomic approaches of the spliceosome suggest >200 proteins involved in this process (84). Part of these rearrangements need to occur rapidly and in a controlled fashion, and therefore most likely require an energy input, which, at least partially, may be attributed to the function of DEAD-box proteins. In yeast, three DEAD-box proteins have been shown to be required for *in vivo* splicing [Sub2, Prp28 and Prp5 (85)]. In higher eukaryotes, p68 was shown to be involved in constitutive and alternative mRNA splicing (86,87), and its homolog p72 has been implicated in alternative splicing (88). Other proteins, such Ded1p (see below), may also be involved in splicing (89,90), although their role in this process has not been established definitely. In addition to the known DEAD-box proteins, other DExD/H proteins are required for splicing to occur, namely the DEAH proteins Prp2, Prp16, Prp22, Prp43 and the Ski2-like protein Brr2 (85,91–97). Interestingly, DEAD-box proteins are required for establishment of a functional spliceosome, whereas DEAH-box proteins are (indirectly) required for the transesterification reactions, the release of the mRNA, and the recycling of the spliceosome components.

In addition to these 'classical' splicing DEAD/H-box proteins, the proteomic approaches of spliceosomes from higher eukaryotes also revealed the presence of other DEAD-box proteins such as homolog of the Drosophila abstrakt, eIF4AIII, Ddx35 and Ddx9 (84). The eIF4AIII protein has been shown to be an important component of the exon-junction complex (EJC) (98). In the case of eIF4AIII it has been reported recently that its ATPase activity is inhibited by the presence of another component of the EJC (99). Interestingly, a homologous protein, Fal1, from yeast is involved in ribosome biogenesis (100).

Ribosome biogenesis

Pre-ribosomal complexes with well over 100 transacting factors, including small nucleolar RNAs (snoRNAs) and many proteins of different activities (101,102), represent another example of a complex and highly dynamic RNP. Many NTPases have been shown to be involved in ribosome biogenesis in prokaryotes and eukaryotes. Beside many DEAD-box proteins, DEAH-box proteins, a Ski2-like RNA helicase (Dob1/Mtr4), and AAA proteins, are required for ribosome biogenesis. Whereas the number varies in prokaryotes and is relatively small [i.e. 0 in *Borrelia burgdorferi*; 3 in *Escherichia coli*, (5)], 14 DEAD-box proteins have been shown by genetic experiments to be required for ribosome biogenesis in *S.cerevisiae* (21,103,104). Most of these DEAD-proteins from *S.cerevisiae* have counterparts in higher eukaryotes, indicating that their requirement is conserved. This is further supported by the fact that most of the human DEAD-box proteins homologous to those required in ribosome biogenesis in yeast can be detected in proteomic approaches of human nucleoli (105,106). One of the rare exceptions is Dbp3, required for the MRP RNase assisted cleavage at A3 (33). This protein is highly conserved amongst fungi, but has no obvious counterpart in higher eukaryotes, as judged from blast searches and ClustalW analyses (Table 1). Interestingly, Dbp3, together with Dbp7, is not absolutely essential for ribosome biogenesis in yeast. However, in contrast to their bacterial DEAD-box counterparts (5), the other proteins involved in ribosome biogenesis in yeast are essential for cell viability. Moreover, they are highly specific and cannot be replaced by each other, even when overexpressed.

Ribosome biogenesis is an ideal playground for DEAD-box proteins. Eukaryotic ribosomes are composed of 4 rRNAs and 78 proteins (102). Three of the mature rRNA species are transcribed as a large pre-rRNA and are, during the assembly reaction, processed to the three mature 18S,

5.8S and 25S rRNAs. In addition to the processing reactions, the 18S and 25S rRNA are modified by pseudouridylation and methylation. These modifications are guided by snoRNAs (107) that are complementary to the rRNA. Thus, DEAD-box proteins could play roles in reorganizing the pre-ribosomal complexes by dissociating snoRNAs from the pre-rRNA, to allow new and mutually exclusive RNA–RNA interactions to occur. Moreover, as in pre-mRNA splicing, DEAD-box proteins may be involved in RNP remodeling by altering RNA–protein interactions [see contribution by (6)]. Whereas all DEAD-box proteins involved in ribosome biogenesis in yeast have been characterized genetically, little is known about these proteins in higher eukaryotes. Nevertheless, most of them have been found in proteomic analyses of nucleoli, the cradle of ribosomes (105,106).

Genetic analyses of ribosome biogenesis and of the DEAD-box proteins required for this process in yeast indicate functions of RNA helicases by dead-end products that can be detected. By this criterion, six DEAD-box proteins are required for early cleavages and affect synthesis of the small ribosomal subunit, whereas eight DEAD-box proteins are required for the synthesis of the large ribosomal subunit. Nevertheless, this analysis of dead-end products in ribosome biogenesis may not reflect appropriately the actual function of DEAD-box proteins. It is likely that the absence of a protein of this family does not induce an immediate defect in ribosome biogenesis or assembly, but only a delayed processing/assembly defect. Moreover, a strong defect may mask a weaker effect in a completely different step. As an example, it is intriguing that Has1 is mainly found associated with pre-60S particles, but the genetic analysis reveals a clear 40S deficit (108). It is therefore important to characterize interacting partners of these enzymes. Genetic screens, such as the search for synthetic lethal interactions, suppressor analyses and complex purification, will certainly help to go in this direction (109,110).

Nuclear export

In eukaryotic cells transcription and translation occur in separate compartments. Therefore the mature mRNA needs to be exported, through nuclear pores, from the nucleus to the cytoplasm. A defect in the yeast Dbp5/Rat8 gene results in accumulation of poly(A) mRNA in the nucleus, clearly indicating a role in mRNA export (52,111). In a beautiful experiment, it has been shown that a mutation in the Nup159 protein of the nuclear pore complex leads to a cytoplasmic localization of Dbp5, rather than at the nuclear rim (53). These data suggest a role of Dbp5 on the cytoplasmic side of the nuclear pore. In addition, recent data show that Dbp5 localizes to Balbiani ring of *Chironomus tentans* (112) and demonstrate genetic and physical interactions of yeast Dbp5 with the transcription machinery (113). This suggests that Dbp5 needs to be loaded on the mRNA early, travels along to the nuclear pore, where it is required for export. A genetic interaction between *mex67* and *dbp5* suggests that Dbp5 is required for the release of Mex67 (74). Another DEAD-box protein, Uap56/Bat1 in higher eukaryotes and Sub2 in yeast (that are in reality DECD proteins), has also been shown to be required for export of mRNA, in addition to its role in pre-mRNA splicing (114–117). Interestingly this splicing factor is also required for export of mRNAs that do not contain introns, arguing against the simple scenario that Sub2 remains bound to the message after splicing. Thus, Uap56/Bat1/Sub2 proteins play two roles in the life of an mRNA. Intriguingly, two highly homologous (89% identity) DECD proteins, Ddx39 and Bat1, are encoded by the human genome (118).

Translation initiation

The translation initiation factor eIF4A was the first DEAD-box protein described to have a RNA-dependent ATPase activity (119). Several reviews have been published about eIF4A (9,25) and I summarize here only its essentials. The translation initiation factor eIF4A is a very abundant protein (120,121). It is part of the cap-binding complex eIF4F but is also present in a free form. Its biochemical activities are greatly stimulated by eIF4B, eIF4H and eIF4G (37–39,122). It has been proposed that eIF4A helps to unwind secondary structures in the 5′-untranslated region (5′-UTR), which are inhibitory for the scanning process of the small ribosomal subunit (123). Experimental evidence supporting this hypothesis has been reported in an *in vitro* translation system with increasing secondary structures in the 5′-UTR (20) and by the analysis of cell cycle defects in *Schizosaccharomyces pombe* (124). Interestingly, however, a mRNA substrate with the initiator AUG positioned 8 nt downstream of the cap structure is still absolutely dependent on eIF4A in a yeast *in vitro* translation system (17).

The laboratory of Tien-Hsien Chang and our laboratory have demonstrated that another DEAD-box protein, Ded1, is also required for translation initiation *in vivo* and *in vitro* (125,126). Although its precise role in translation initiation remains elusive, the laboratory of John McCarthy has shown, by testing mRNAs with 5′-UTR of various lengths, that Ded1 plays a role in the scanning process *in vivo* and *in vitro* (127). In multicellular organisms, yet another DEAD-box protein, Vasa, has been shown to play an important role in translation initiation via its interaction with IF2 (128). Interestingly, Vasa is highly related to Ded1, but is absent in fungi, in accordance with its role in embryonic development.

Degradation

Elegant studies demonstrated the requirement for DEAD-box proteins in RNA degradation in *E.coli* (5,129,130). In eukaryotes, RNA degradation occurs mainly via the multisubunit exosome, assisted by RNA helicases of the Ski2 family (131–134). However, no DEAD-box protein seems to be directly required for the progression of the exosome. The Dhh1 protein plays an essential role in mRNA degradation through its implication in decapping of the mRNA (135,136). Interestingly, proteins from the same subfamily play important functions in masking mRNAs in higher eukaryotes (13) (137,138).

Organelle gene expression

In yeast, two DEAD-box proteins are required for mitochondrial gene expression, Mss116 and Mrh4. The Mss116 protein was shown to be involved in mitochondrial splicing.

However, a strain with no mitochondrial introns still required Mss116 for growth on non-fermentable carbon sources (139). Intriguingly, overexpression of Mss116 does suppress the absence of a completely unrelated helicase, Suv3, which is involved in mitochondrial RNA turnover (140). The Mrh4 protein was isolated as a low-copy suppressor of a point mutation in the mitochondrial aI5γ intron, although a block in the splicing reaction could not be observed in a Δ*mrh4* strain (29). As mentioned above, both these DEAD-box proteins have no direct homolog in humans, which could be related to differences in the mode of mitochondrial gene expression in yeast and humans. A human DEAD-box protein, Ddx28, has been reported to localize to the mitochondria (31). However, its function is not known.

DEAD-box proteins from Trypanosomes have also been shown to be required for editing (141). Interestingly these proteins have homologs only in another kinetoblast organism: Leishmania. It is thus tempting to speculate that these proteins are required for the guide RNA assisted editing of mitochondrial RNA, characteristic for these organisms (142).

Altogether, the emerging picture of DEAD-box proteins depicts a large family of proteins that possess a non-processive dissociation activity. This activity is particularly used in many RNA metabolic processes in eukaryotic cells. It is likely that in these processes, the DEAD-box proteins are important place-holders or check-point proteins, allowing processes to proceed efficiently in one direction and connected with previous or following steps in the RNA metabolism machinery.

DISCUSSION AND OPEN QUESTIONS

Eukaryotic cells have a large number of DEAD-box proteins, most of which are essential, as judged from genetic experiments in yeast. From our actual knowledge of these proteins, we can deduce that they are involved in many if not all steps of RNA metabolism. Although they present a high degree of similarity within the core region, which is responsible for the enzymatic activities of these proteins, they are all highly specific for their function and cannot be replaced by each other.

Despite intensive studies in many laboratories, the exact role of these proteins remains elusive. Although we know that they are required for the dynamics of RNP complexes, such as the establishment of the spliceosome, the biogenesis of ribosomes, export of mRNA through the nuclear pore or the presence of EJCs on spliced mRNAs, their exact function remains unclear. Do they need their enzymatic activity for an active remodeling of RNP structures, or do they play a more passive role and use the enzymatic activity to leave the complex to make place for new interactions within the RNP complex?

Yet another enigma is their regulation. As far as we know, DEAD-box proteins require the presence of RNA for stimulation of their enzymatic activity. Also, it is clear that the Q-motif plays an important role in ATP recognition and thus in regulation. Nevertheless, the activity needs to be tightly controlled in time and space. Presumably, it is regulated by the interaction with specific RNA sequences or other proteins of the RNP complexes.

Finally, some DEAD-box proteins may function as a sort of 'check point' control. In a dynamic RNP assembly, such as the spliceosome or the ribosome, the cell needs to control the correct functionality of these super-machines to avoid erroneous splicing or protein synthesis. In this view, a particular DEAD-box protein can only be activated if the intermediate structure is correct. If the structure is not correct, the synthesis has to wait or be abandoned.

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