Chapter 2

Happy Birthday: 25 Years of DEAD-Box Proteins

Patrick Linder and Frances Fuller-Pace

Abstract

RNA helicases of the DEAD-box family are found in all eukaryotes, most bacteria and many archaea. They play important roles in rearranging RNA–RNA and RNA–protein interactions. DEAD-box proteins are ATP-dependent RNA binding proteins and RNA-dependent ATPases. The first helicases of this large family of proteins were described in the 1980s. Since then our perception of these proteins has dramatically changed. From *bona fide* helicases, they became RNA binding proteins that separate duplex RNAs, in a local manner, by binding and bending the target RNA. In the present review we describe some of the experiments that were important milestones in the life of DEAD-box proteins since their birth 25 years ago.

Key words RNA helicases, ATP-dependent RNA binding, RNA-dependent ATPases

1 Introduction

DEAD-box proteins constitute the largest protein family of RNA helicases [1, 2]. They are present in all eukaryotes, most bacteria, many archaea (www.rnahelicase.org/), and even in viruses (e.g., [3, 4]). The proteins are easily identified based on the presence of twelve conserved sequence motifs that are involved in ATP or RNA binding, or in intramolecular interactions [1]. In eukaryotes many of the DEAD-box proteins are essential, whereas in bacteria they are dispensable under laboratory growth conditions, but may be required for growth in other, suboptimal environments. These proteins are involved in a variety of processes, ranging from transcription to RNA decay, including pre-mRNA splicing, ribosome biogenesis, RNA transport, translation initiation and termination, and organelle gene expression. Accordingly, the number of DEAD-box proteins in eukaryotes ranges from 25 in yeast (26 genes, since two identical eIF4A proteins are encoded by genes *TIF1* and *TIF2*) to over 50 in plants. In a few instances it has been suggested that a particular DEAD-box protein can be involved at several steps in one process (e.g., Prp5, [5]) or participate in multiple processes (e.g., Ded1/Ddx3, [6]; Dbp5. [7]). Interestingly many eukaryotic

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DEAD-box proteins are involved in ribosome biogenesis (14 in yeast) or pre-mRNA splicing (3 in yeast). This is particularly intriguing, since both, ribosome biogenesis and pre-mRNA splicing, require a large number of guide RNAs to allow RNA modifications and correct positioning of protein complexes to occur. In addition to modification and processing by guide RNAs, the compartmentalization of gene expression in eukaryotes complicates the life of an RNA. Thus, eukaryotic DEAD-box proteins participate in directional transport of the mRNA to the cytoplasm, transcription-uncoupled translation initiation involving a ribosome scanning mechanism, translation termination, or the marking in the nucleus of exon–exon junctions for nonsense-mediated decay (NMD) in the cytoplasm [2]. As expected for proteins involved in basic processes in a cell, most DEAD-box proteins, if mutated or absent, cause a general arrest of cell growth (18 out of 25 in yeast) but, in some instances, the mutations may lead to particular phenotypes such as developmental defects as in the case of *vasa* [8].

Our perception of DEAD-box proteins and their role in RNA metabolism has dramatically evolved over the last 25 years. Whereas at the beginning these proteins were found to change the structure of RNA, they were then shown to depend often on single strand extensions for dsRNA unwinding, as expected for *bona fide* helicases. Later, however, it became clear that DEAD-box proteins are ATP-dependent RNA binding proteins and RNA-dependent ATPases, leading to local unwinding of duplexes without translocation, but with ATP hydrolysis-dependent recycling of the protein. In the following paragraphs, we shall describe this evolution and refer to some experiments that led to our current understanding of the functions of DEAD-box RNA helicases.

2 The Discovery of ATP-Dependent RNA Modulation

The godfather of the DEAD-box proteins [9] is, without any doubt, the translation initiation factor eIF4A, since it was its RNA binding activity [10], its ATPase activity [11], and its RNA-structure modulating activity [12] that prompted the idea of RNA helicase activity on secondary structures. The mouse eIF4A protein was also the first DEAD-box protein to be sequenced [13] and is one of the shortest DEAD-box proteins, harboring only the two recA-fold domains and a very short C-terminal extension. eIF4A was found to change the sensitivity of RNA to single strand-specific RNase T_2 treatment in an ATP-dependent manner (Fig. 1) [12], suggesting that it acts as an RNA helicase, denaturing secondary structures to allow scanning of the small ribosomal subunit for the AUG, or by preparation of the landing path of the small ribosomal subunit, when part of eIF4F, the cap-binding complex. Substrates with a 15-nucleotide DNA oligonucleotide complementary to the

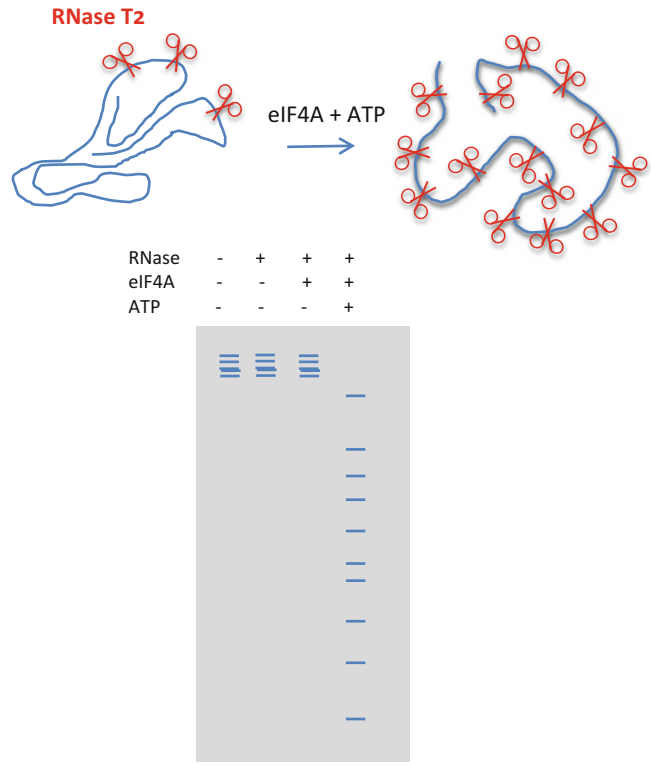

Fig. 1 ATP-dependent unwinding of secondary structures. Before the DEAD-box family was "born," Ray and colleagues [12] had already shown that the susceptibility of RNA to RNase T2 cleavage changed when it was incubated with eIF4A and ATP, but not with only eIF4A or ATP. The figure represents a schematic drawing of a part of one of the figures shown in this report

capped 5′ end of the RNA were unwound by the purified enzymes, albeit much less efficiently with eIF4A than with the cap-binding complex eIF4F (composed of eIF4G, the cap-binding protein eIF4E, and eIF4A).

3 Birth of the DEAD-Box Protein Family

Several proteins harboring sequence motifs similar to eIF4A were described in the late eighties, allowing the description of a new family of proteins (Fig. 2) [14]. Whereas at the time the family was very modest, it is now the largest family of RNA helicases. The early members were the following: (a) the two mammalian eIF4AI and eIF4AII [13], involved in translation initiation, and the yeast eIF4A homologs Tif1 and Tif2 [15] (b) the mouse PL10 protein [16], which is now known as the conserved multifunctional DDX3/Ded1 protein; (c) the translation initiation factor vasa [17],

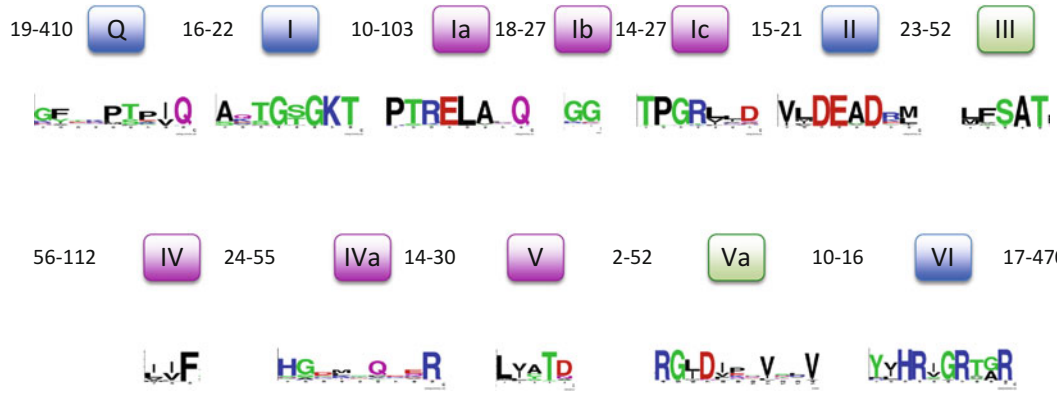

Fig. 2 The DEAD-box proteins from human, the yeast *Saccharomyces cerevisiae*, and the Gram negative and Gram positive model-bacteria *Escherichia coli* and *Bacillus subtilis* were aligned using ClustalW (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The alignment was edited by hand where necessary and the conserved motifs were analyzed by WebLogo (http://weblogo.berkeley.edu/logo.cgi). Distances between the motifs were calculated using the ClustalW alignment. Motifs interacting with ATP and RNA are colored in *blue* and *pink*, respectively. Motifs involved in intramolecular interactions are colored in *green*. *See* Chapter 11 for additional information

identified as required for oogenesis and specification of the posterior–anterior axis of Drosophila embryos; (d) the multifunctional p68/DDX5 protein [18] which plays roles in transcription, pre-mRNA splicing, RNA decay, ribosome biogenesis, and miRNA processing; (e) Mss116 [19], required for mitochondrial gene expression in yeast; and (f) SrmB [20], isolated as a suppressor of a temperature-sensitive mutation in the ribosomal protein gene encoding L24 from *Escherichia coli*. At the time, the results indicated that all these proteins are involved in RNA metabolism and in view of the results with eIF4A, it was suggested that they act as helicases. Today, however, we know that the DEAD-box proteins are non-processive, ATP-dependent, RNA binding proteins, which may locally unwind dsRNA or function simply as RNA binding proteins.

4 RNA-Dependent ATPase Activity

Many different protocols can be used to assay RNA-dependent ATPase activity. In the first description of an ATPase activity by eIF4A [11], the released phosphate from [gamma-32P]-ATP was trapped in an organic phase and separated from the ADP in the aqueous phase. The radioactive phosphate was then measured by scintillation counting. An alternative to this is to separate the released radioactive phosphate by thin layer chromatography (TLC) from the ADP and the non-hydrolyzed ATP, and then measure the released activity by phosphorimaging of the TLC plate [21].

Non-radioactive methods are also available, such as the measurement of released phosphate by malachite green [22, 23] (a detailed protocol is provided in Chapter 11 of this volume). Although this method is easy and straightforward and does not require radioactivity, the "stopped reaction" samples need to be quickly assayed for color changes (yellow to green). An alternative method using non-radioactive ATP is a coupled enzymatic assay, in which pyruvate kinase and lactate dehydrogenase are linked to the hydrolysis of ATP with oxidation of NADH resulting in a decrease in the absorbance at 338 nm [24, 25] (*see* Chapter 10 for details). The K_m for ATP varies in general between 50 and 500 μM and the k_{cat} between 3 and 600 min^{-1} [26]. The ATP level in a cell has been estimated to be 3.5 mM in an exponentially growing *Escherichia coli* [27] and varies from 1 to 10 mM in eukaryotic cells (e.g., [28]). It is important to note that, as in the case of the early descriptions of eIF4A [11], the ATPase activity can be stimulated by partner proteins (e.g., eIF4B for eIF4A [11], Gle1 for Dbp5 [29]) probably by increasing the local concentration of the RNA or by inducing conformational changes of the ATPase. This is expected, since the DEAD-box proteins need to be regulated to keep them from gratuitously hydrolyzing ATP.

Finally, it should be noted that the bacterial DEAD-box protein DbpA shows only a very low ATPase activity, except if is provided with 23S rRNA as a substrate [21]. It was later shown that DbpA has a C-terminal extension with an RNA binding motif that binds specifically to hairpin h92 of the 23S rRNA allowing a high local RNA concentration resulting thereby in a large stimulation of the ATPase activity [30, 31]. The exclusive stimulation of DbpA by a hairpin of 23S rRNA has long served as a paradigm for the specificity of the DEAD-box proteins.

DEAD-box proteins are ATPases that are strictly RNA dependent [11]. If testing a new DEAD-box protein, we routinely include mutant proteins that are deficient for ATP hydrolysis either by changes in the Walker A motif or the Walker B motif to test the purity of our preparations. In some cases RNA copurifies with the protein and it is difficult to show RNA-dependent ATPase activity. If necessary, we therefore isolate our proteins in the presence of RNase A, which is then removed during purification.

5 ATP-Dependent RNA Unwinding Activity

Following the structural assay of reovirus RNA [12], the first unwinding activities for double-stranded RNAs were reported for p68 and eIF4A by Hirling et al. and Rozen et al., respectively [32, 33]. In the publication by Hirling, the authors used two substrates, one of which had a 162 bp duplex. Although unwinding was only partial, it is not clear how p68 could unwind such a long

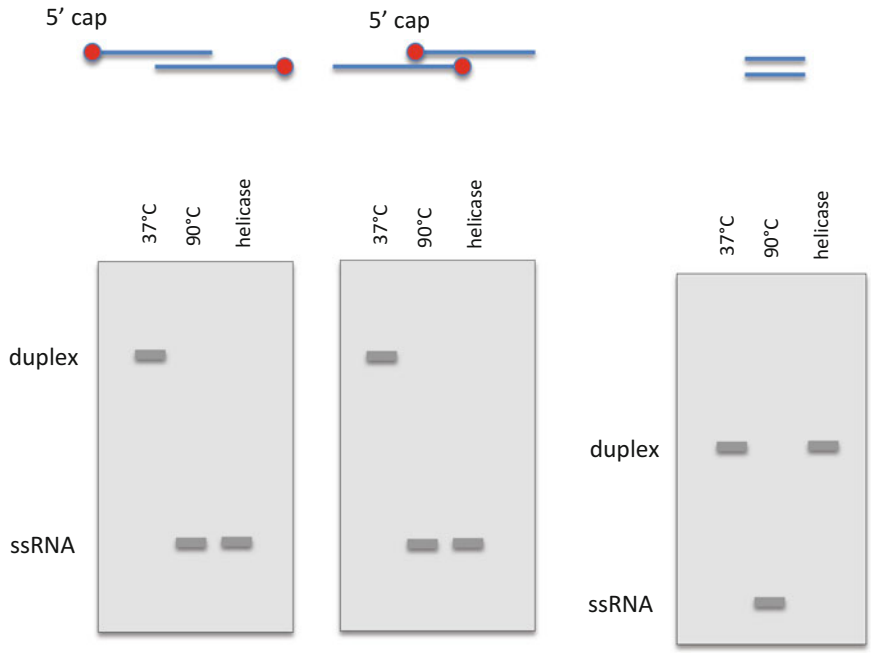

Fig. 3 ATP dependent unwinding of an artifical substrate. The separation of two annealed RNA strands showed for the first time the possibility of unwinding [32, 33]. The report by the Sonenberg laboratory clearly showed bidirectional unwinding that was stimulated by using the cap-binding complex and capped RNA. The drawing is a schematic representation of part of the results shown in the report by Rozen and collaborators

duplex, except with a large excess of protein. In the report by Rozen and coworkers, three substrates were used with either 5′ or 3′ single-stranded overhangs, or without overhangs (Fig. 3). As expected for a helicase, the blunt-ended substrate was not unwound; however, intriguingly, the helicase was able to unwind substrates with 5′-3′ and 3′-5′ polarity. These assays, as in most of the following reports, used duplexes where one strand was radioactively labeled and the reaction products were separated by gel electrophoresis (*see* Chapters 11 and 12 for experimental protocols). It rapidly became clear that DEAD-box proteins unwind only relatively short duplexes and without processivity. In the case of eIF4A, the unwinding can be stimulated by cofactors, such as eIF4B, or become directional, as in the case of eIF4A in the cap-binding complex and capped substrates [33]. In addition to the use of radiolabeled RNA, fluorescence resonance energy transfer (FRET) was used in some cases to measure RNA separation [34, 35] (*see* Chapter 13 for details).

The substrates for in vitro unwinding by DEAD-box proteins can be dsRNA or RNA–DNA hybrids. The fact that dsDNA is in general not a substrate for DEAD-proteins can be used for the in vitro unwinding by DEAD-box proteins. Indeed, by using an

RNA–DNA as substrate for the unwinding analysis, a liberated DNA oligonucleotide can be efficiently trapped by a second, complementary, DNA oligonucleotide, which will form a dsDNA. This new double-stranded nucleic acid is not a substrate for DEAD-box proteins and therefore this setup can easily be used in unwinding assays.

Some DEAD-box proteins bind RNA efficiently and have been shown to accelerate the annealing of two complementary substrates [36, 37]. This reaction is ATP independent and could be an intrinsic in vivo activity for dynamic changes of RNA–protein (RNP) complexes. So far the requirements of DEAD-box proteins to carry out annealing and unwinding have not been defined. As in the case of RNA binding protein Hfq [38], two binding sites should be required, which can easily be achieved in the case of dimerizing DEAD-box proteins.

6 Protein Displacement

Consistent with a remodeling activity of RNP complexes, the laboratories of Anna Pyle and Eckhard Jankowsky have shown that DEAD-box proteins can also displace proteins from RNA. Firstly the viral DEAH-box protein NPH-II was found to displace the RNA binding protein U1A in active manner from its substrate [39]. In a subsequent report, it was shown that the processive NPH-II was capable of displacing even the tightly bound TRAP complex (Tryptophan RNA-binding Attenuation Protein) in an ATP-dependent reaction, whereas the non-processive DEAD-box protein Ded1 was unable to displace TRAP [40]. However, in the case of the exon-junction complex, EJC, displacement by Ded1 was possible. These findings add a level of complexity for the function of DEAD-box proteins in the dynamic rearrangements of RNA–protein complexes. Such an RNPase activity could apply to many processes, such as ribosome biogenesis, pre-mRNA splicing, mRNA export, and others.

7 Genetic Analysis of DEAD-Box Proteins

Some of the DEAD-box proteins were identified by virtue of the high conservation of several of the motifs, either by PCR [41] or simply by genome sequencing. Model systems such as the yeast *Saccharomyces cerevisiae* and the prokaryotic *Escherichia coli* are particularly suitable for genetic analysis and many DEAD-box proteins were identified—sometimes by serendipity—through genetic screens (Table 1).

Genetic screens also permitted the identification of the targets of DEAD-box proteins. In one example, a cold-sensitive splicing

Table 1
First identification of DEAD-box proteins from *S. cerevisiae* and *E. coli*

Gene name	protein	Deletion phenotype	Primary function	First description
Saccharomyces cerevisiae				
YJL138c	Tif2p	Dead[a]	Translation initiation	Suppressor of a mitochondrial non-sense mutation [15]
YKR059w	Tif1p	Dead[a]	Translation initiation	Suppressor of a mitochondrial non-sense mutation [15]
YDR021w	Fal1p	Dead	Ribosome biogenesis	Identified from genome sequencing based on conserved motifs [56]
YDL160c	Dhh1p	Viable	Transcription, mRNA storage	Multicopy suppressor of the *caf1* mutation in the Ccr4-Not complex [57]
YOR046c	Dbp5p	Dead	mRNA export	PCR amplification using motif I and motif II-specific primers [41] and by gene disruption [58, 59]
YDL084w	Sub2p	Dead, N	Pre-mRNA splicing, mRNA export	Isolated as a high-copy suppressor of the splicing mutant brr1 [43]
YNL112w	Dbp2p	Viable	Nonsense-mediated decay (?)	Identification by yeast 2-hybrid screen [60], cloning by low stringency hybridization using the p68 gene [61]
YGL078c	Dbp3p	Viable	Ribosome biogenesis (60S)	PCR amplification using motif I and motif II-specific primers [41] and by gene disruption [62]
YOR204w	Ded1p	Dead	Translation initiation	Suppressor of a splicing mutation in *prp8* [63]
YPL119c	Dbp1p	Viable	Translation initiation (?)	Suppressor of a *ded1* mutation [64]
YHR065c	Rrp3p	Dead	Ribosome biogenesis (40S)	PCR amplification using motif I and motif II specific primers [65]
YHR169w	Dbp8p	Dead	Ribosome biogenesis (40S)	Identified from genome sequencing based on conserved motifs [66]
YGL171w	Rok1p	Dead	Ribosome biogenesis (40S)	Multicopy suppressor of the *kem1* mutant, deficient in nuclear fusion [67]
YLL008w	Drs1p	Dead	Ribosome biogenesis (60S)	Complementation of the cold-sensitive ribosome biogenesis mutant *drs1* [68]
YDL031w	Dbp10p	Dead	Ribosome biogenesis (60S)	Identified from genome sequencing based on conserved motifs [69]
YBR237w	Prp5p	Dead	Pre-mRNA splicing	Complementation of the splicing mutant *prp5* [70]
YLR276c	Dbp9p	Dead	Ribosome biogenesis (60S)	Identified from genome sequencing based on conserved motifs [71]

(continued)

Table 1
(continued)

Gene name	protein	Deletion phenotype	Primary function	First description
YJL033w	Dbp4p	Dead	Ribosome biogenesis (40S)	PCR amplification using motif I and motif II-specific primers [41] and as multicopy suppressor of U14 snoRNA [72]
YFL002c	Spb4p	Dead	Ribosome biogenesis (60S)	Suppressor of poly(A)-binding protein gene deletion [73]
YDR243c	Prp28p	Dead	Pre-mRNA splicing	Cold-sensitive mutation in pre-mRNA splicing [74]
YMR290c	Has1p	Dead	Ribosome biogenesis	Identified from genome sequencing based on conserved motifs [75]
YBR142w	Mak5p	Dead	Ribosome biogenesis (60S)	Identified as Maintenance of Killer plasmid [76] and by genome sequence analysis [77]
YDR194c	Mss116p	Viable	Mitochondrial RNA splicing	Complementation of nuclear mutation affecting mitochondrial splicing [19]
YNR038w	Dbp6p	Dead	Ribosome biogenesis (60S)	Identified from genome sequencing based on conserved motifs [78]
YKR024c	Dbp7p	Viable	Ribosome biogenesis (60S)	Identified from genome sequencing based on conserved motifs [79]
YGL064c	Mrh4	Viable	Mitochondrial gene expression	Low-copy suppressor of mitochondrial splicing mutant [80]
YDR332w	Irc3	Viable	ND	Identified from genome sequencing based on conserved motifs [81]
Escherichia coli				
	SrmB	Cold sensitive	Ribosome biogenesis	Multicopy suppressor of a temperature-sensitive L24 protein [20]
	CsdA	Cold sensitive	Ribosome biogenesis, RNA turnover	Multicopy suppressor of a temperature-sensitive S2 protein [82]
	RhlB	Viable	RNA turnover, degradosome	Identified from genome sequencing based on conserved motifs [83]
	DbpA	Viable	Ribosome biogenesis	Low stringency hybridization using mammalian p68 [84]
	RhlE	Viable		Identified by serendipity in analyzing a neighbor gene [85], targeted interaction search with degradosome components [86]

[a]The eIF4A protein encoded by *TIF1* or *TIF2* is essential and a double mutant is not viable, whereas single mutants are N, a *sub2* deletion is viable depending on the strain, (?) suggested functions

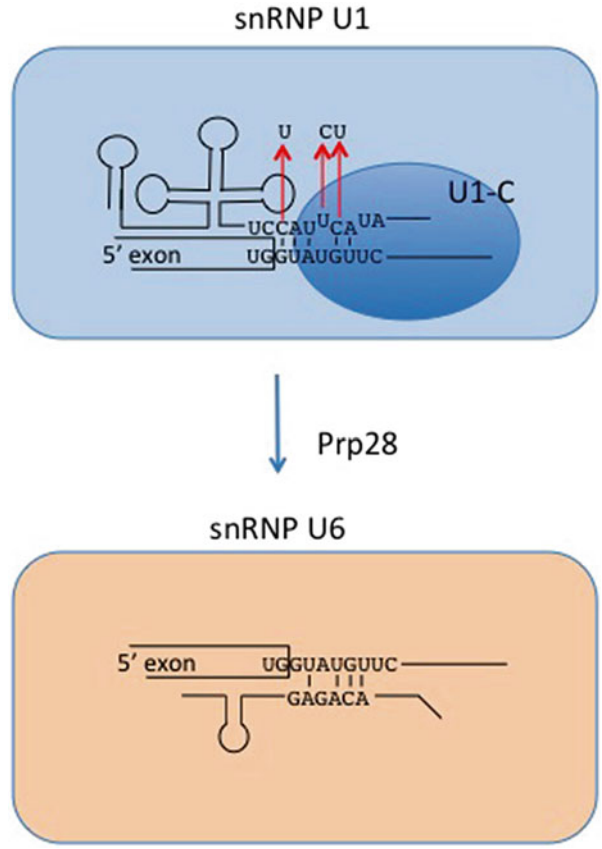

Fig. 4 In vivo genetic data that are consistent with an RNP modeling activity. Work in the laboratory of T.H. Chang showed that mutations in the U1-C protein or the U1 snRNA allow bypassing the requirement of Prp28 [42]. The replacement of the U1 snRNP by the U6 snRNP is schematically represented in the figure. The *red arrows* indicate residues that, if mutated, allow bypassing the Prp28 requirement

mutant of the otherwise essential *prp28* gene in *S. cerevisiae* allowed the isolation of mutations in the U1 snRNP complex [42]. The U1 snRNP is aligned to the exon–intron junction using a guide snRNA that needs to be removed in order to allow binding of the U6 snRNP. The isolated suppressor mutation was in the gene encoding U1-C. In addition to the mutation in the protein gene, the authors also ectopically expressed a mutant version of the U1 snRNA and observed suppression of Prp28 essentiality when they changed bases that form Watson-Crick base-pairs between the pre-mRNA and the snRNA (Fig. 4).

The interpretation of this result is that weakening the interactions between U1 snRNP and the pre-mRNA bypasses the requirement for Prp28. Together with a study showing that the essentiality of the Sub2 splicing factor is partially suppressed in the absence of

the branch-point binding protein Mud2 [43], this report was very rewarding since it was the first genetic demonstration that a DEAD-box protein was required for unwinding of a duplex or destabilizing an RNP complex. Nevertheless, a deletion of *SUB2*, which encodes a DECD protein, will not restore full growth since Sub2 has other functions in the cell. In this context, it would be interesting to create an intron-free yeast that would allow us to determine whether some splicing factors have other functions in the cell and if certain introns, such as that of Dbp2 [44], affect growth of the cell if deleted.

More recently, an elegant suppressor screen of the cold-sensitive *srmB* deletion mutant revealed a chaperone-like function of this DEAD-box protein in *E. coli* ribosome biogenesis [45]. Briefly, an *E. coli* strain deleted for SrmB and carrying only one rDNA copy on a plasmid was used to isolate suppressors that allowed growth at lower temperatures. The analysis of three strains revealed mutations in three repeated sequences. Two mutations changed a repeated sequence in the 23S rRNA, whereas the third affected the 5S rRNA in a sequence complementary to these repeats. It seems therefore that SrmB is required at lower temperatures to prevent or undo erroneous annealings in the rRNA.

8 Non-processive Local Unwinding

The genetic data regarding DEAD-box protein functions, coupled with the observed conformational changes of RNA caused by eIF4A and ATP, the denaturation of short duplexes by DEAD-box proteins in an ATP hydrolysis-dependent fashion, as well as the early structural data that showed similarities amongst helicases of different sorts, clearly hinted towards a classical helicase mechanism. This would suggest binding of the DEAD-box protein to single-stranded extension and translocating along this loading strand to displace a complementary nucleic acid or a bound protein. However, there were several unresolved questions, such as bidirectional unwinding and very low processivity. The latter could eventually be explained by the absence of processivity factors in the in vitro reaction, similar to DNA polymerase in the absence of the sliding clamp [46].

Two major discoveries showed another way. First, the structure of vasa in the presence of a short RNA revealed a kinked substrate [47]. Such a kink is unlikely to be compatible with efficient translocation and it would not allow the binding of duplex substrate. Thus, ATP-dependent binding would allow local denaturation, but without translocation.

Second, a landmark paper that changed our perception of DEAD-box proteins and distinguishes them from other helicases comes from the Jankowsky laboratory [48]. In this work, the

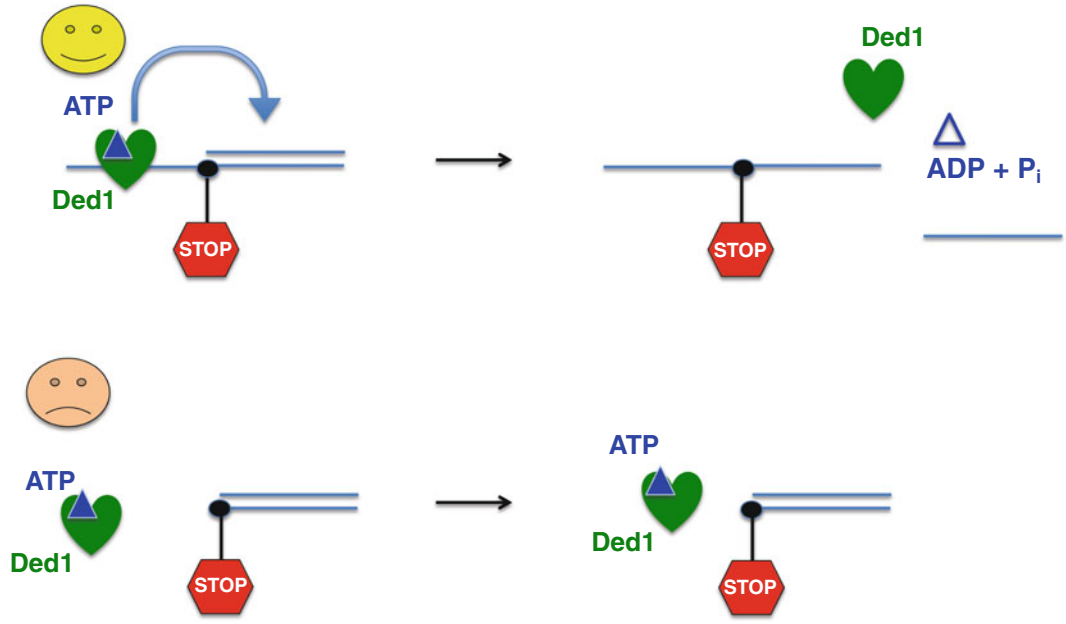

Fig. 5 Work in the Jankowsky laboratory, together with the structure of vasa with an RNA molecule and the structural insights into RNA binding by individual domains of Mss116 [47, 48, 52], changed our perception of DEAD-box proteins and explained the requirement for single-stranded extensions for the non-processive unwinding. The work by Yang and Jankowsky [48] showed that linking the single-stranded loading strand and the duplex by a streptavidin–biotin complex does not prevent unwinding, but that the single-stranded extension remained necessary for unwinding. This indicated that DEAD-box proteins do not translocate like classical helicases

double-stranded substrate was linked with a streptavidin–biotin linker to a single-stranded extension that served for loading. In this case, the DEAD-box protein Ded1 is still able to unwind the duplex, although it is unlikely to translocate across the linker region (Fig. 5). Moreover, in the absence of a single-stranded loading strand, Ded1 was unable to unwind the duplex. Thus, the single-stranded extension is required to increase the local concentration of the enzyme. Additional experiments showed that the loading strand could be DNA, but that at least one of the duplex strands must be RNA.

Thus, DEAD-box proteins bind in an ATP-dependent fashion to the substrate, locally denature the duplex and hydrolyze the ATP to dissociate from the nucleic acid for recycling [49, 50]. It should, however, be pointed out that some DEAD-box proteins, such as RhlE from *E. coli*, can unwind blunt-ended duplex substrates as efficiently as partially single-stranded substrates [51].

Importantly, two reports showed that unwinding does not require ATP hydrolysis, but that it is required for recycling the protein. In the report by Liu and coworkers, it was shown that unwinding can occur by using the nonhydrolyzable ATP analog

ADP-beryllium fluoride, which mimics the prehydrolysis state of ATP in ATPase [50]. This is also consistent with the report form the Russel laboratory, in which it was shown that a single ATP is sufficient for unwinding a duplex [49]. Thus, binding of the DEAD-box protein to a dsRNA will locally unwind the substrate whereas the hydrolysis of ATP will allow recycling of the protein. Importantly, ATP analogs are not all equal, but represent different states that will induce different behaviors of the DEAD-box proteins with respect to binding and release [50].

Finally, elegant structural data on Mss116 and its separated two helicase domains provided a scenario of RNA unwinding [52]. Mallam and coworkers showed that the C-terminal part of the core domain acts by binding the duplex RNA. Upon closing of the two domains of the core, one strand is kinked, whereas the other one is excluded, leading to local strand separation. The structure of the C-terminal part of the core domain with the duplex RNA showed also that the accommodated duplex needs to be in the A-form, explaining why a dsDNA cannot be bound by Mss116.

9 To Hydrolyze or Not to Hydrolyze the ATP?

A eukaryotic cell contains several DEAD-box proteins that are able to hydrolyze ATP in the presence of RNA. Therefore, there must be tight control over this activity to avoid futile energy consumption. One way would be to keep the DEAD-box protein away from the RNA to limit hydrolysis, or to use partner proteins that delay ATP hydrolysis or prevent the release of the inorganic phosphate so as to mimic an intact ATP. The latter senario is used at least by eIF4AIII which, together with helper proteins forms the exon-junction complex (EJC) and thereby marks the exon–exon junction [53]. This labeling of the previous presence of an intron, allows the cell to detect premature stop codons and to feed such RNAs into the pathway of nonsense-mediated decay (NMD). The DEAD-box protein eIF4AIII, which is 67 % identical (81 % similar) to the translation initiation factor eIF4AI, forms a complex with the three proteins MAGOH, Y14, and MLN51. The MAGOH-Y14 proteins prevent release of the Pi upon hydrolysis and thereby clamp eIF4AII and thereby the entire complex onto the RNA [54, 55]. This bound complex then serves as a "flag," which is recognized by the NMD machinery.

10 Conclusion

Since the birth of the DEAD-box protein family in 1989, our vision of these important proteins has profoundly changed. From a handful of proteins that unwind dsRNA in an ATP-dependent manner,

the family has grown to be the largest family of RNA helicases that clamp to dsRNA and thereby locally unwind a short stretch of the duplex. This local unwinding is consistent with the presence of short duplexes in several processes of RNA metabolism, such as ribosome biogenesis and pre-mRNA splicing, and the absence of long double-stranded RNA molecules in a normal cell. Research in the past 25 years has also shown that DEAD-box proteins are involved in several processes, such as pre-mRNA splicing, mRNA export, ribosome biogenesis, and RNA degradation, but can also participate in an ATPase-independent manner in basic processes such as transcriptional regulation. Future work will decipher the regulation of these proteins and provide a better picture on the function of these proteins on a molecular and structural level.

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