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The Protein Family of RNA Helicases

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ASTRACT: RNA helicases represent a large family of proteins that have been detected in almost all biological systems where RNA plays a central role. They are ubiquitously distributed over a wide range of organisms and are involved in nuclear and mitochondrial splicing processes, RNA editing, rRNA processing, translation initiation, nuclear mRNA export, and mRNA degradation. RNA helicases are described as essential factors in cell development and differentiation, and some of them play a role in transcription and replication of viral single-stranded RNA genomes. Comparisons of the conserved sequences reveal a close relationship between them and suggest that these proteins might be derived from a common ancestor. Biochemical studies have revealed a strong dependence of the unwinding activity on ATP hydrolysis. Although RNA helicase activity has only been demonstrated for a few examples yet, it is generally believed that all members of the largest subgroups, the DEAD and DEAH box proteins, exhibit this activity.

KEY WORDS: RNA helicase, DEAD/DEAH box protein, ATP hydrolysis, unwinding of RNA, ribosomal biogenesis, translation initiation, nuclear pre-mRNA splicing, mitochondrial RNA splicing, mRNA export, RNA degradation.

I. INTRODUCTION

The hydrolysis of nucleoside 5′-triphosphates (NTPs, primarily ATP and GTP) by specific enzymes plays a central role in energy coupling and in the control of biochemical processes. Many of the enzymes that are able to hydrolyze ATP were shown to unwind duplex DNA in the presence of this NTP. Unwinding of double-stranded DNA by these so-called DNA helicases is a prerequisite for DNA replication and repair, explaining their ubiquitous distribution. ATPases with a DNA-dependent helicase activity have been described in a wide variety of organisms, including *Escherichia coli*, *Saccharomyces cerevisiae*, and higher eukaryotes like frog, mouse, and calf (reviewed in Matson and Kaiser-Rogers, 1990).

First amino acid sequence comparisons of DNA helicases of different organisms

revealed a certain similarity between the different proteins. All DNA helicases that are involved in various functions such as recombination, DNA repair, and replication contain a conserved domain, the so-called "Walker-motif A" or "ATPase A" motif (G-X-X-X-X-G-K-T). Based on this box of homology, the NTPases are all grouped in a single large family (Gorbalenya et al., 1988b; Walker et al., 1982).

Meanwhile an increasing number of polynucleotide-dependent ATPases could be added to this family. The discovery that some of these proteins are able to unwind double-stranded RNA as well as DNA-RNA hybrids indicates an essential role of helicases also in the metabolism of RNA. In certain processes such as translation or RNA splicing, the RNA molecules form a specific secondary and tertiary structure that is essential for their function. Frequently, the RNA forms an inactive duplex structure that then becomes activated by conformational changes induced by RNA helicases. Therefore, helicases may represent a key element in the regulation of different cellular processes (Fuller-Pace, 1994; Schmid and Linder, 1992).

The sequence comparison of RNA- and DNA-dependent ATPases led to a new classification of the NTPases based on an altered ATPase A motif. DNA helicases are characterized by the classic Walker motif A (G-X-X-X-X-G-K-T) and belong to the superfamily I, whereas RNA helicases show a variation of this domain (A-X-X-G-X-G-K-T) and form the closely related superfamily II (Gorbalenya et al., 1988a; Gorbalenya et al., 1989; Hodgeman, 1988; Koonin, 1991; Schmid and Linder, 1992).

This review describes the RNA helicases that are so far arranged in superfamily II. The sequence characteristics, possible reaction mechanisms, and the biological functions of the members of this family of enzymes is discussed in detail.

II. THE FAMILY OF RNA HELICASES

The proteins belonging to the RNA helicase family originate from a wide range of organisms ranging from prokaryotes, including viruses, to lower and higher eukaryotes. They seem to be implicated in various cellular processes such as transcription, translation, cell development, cell differentiation, RNA processing, and ribosome assembly.

RNA helicases of superfamily II are characterized by a core region of 290 to 360 amino acids that shows a high sequence homology to the translation initiation factor eIF-4A of mouse (Figure 1A; Schmid and Linder, 1992). This factor is known for a long time to interact directly with mRNA and to exhibit an RNA-dependent ATPase activity, suggesting an RNA helicase function (Abramson et al., 1987; Ray et al., 1985; Rozen et al., 1990). However, until today only for a few other proteins of superfamily II an RNA helicase activity could be demonstrated *in vitro*.

Among those are the human nuclear protein p68 (Ford et al., 1988; Hirling et al., 1989), the yeast proteins Dbp5 (Tseng et al., 1998), Brr2 (Raghunathan and Guthrie, 1998), Prp16 and Prp22 (Wang et al., 1998; Wagner et al., 1998) the mammalian Brr2 homolog U5-200kD (Laggerbauer et al., 1998), as well as RNA helicase I and II from HeLa cells (Claude et al., 1991; Flores-Rozas and Hurwitz, 1993). The Dbp5 protein was recently shown to be implicated in nuclear mRNA export (Tseng et al., 1998), and Brr2, Prp16, Prp22, and U5-200kD play an important role in the spliceosome cycle (for recent reviews see Staley and Guthrie, 1998; Hamm and Lamond, 1998), while the biological role of protein p68 and RNA helicase I and II is yet not clear. Interestingly, for the human RNAse A (Lee and Hurwitz, 1992) as well as for the protein *vasa* from *Drosophila melanogaster* an

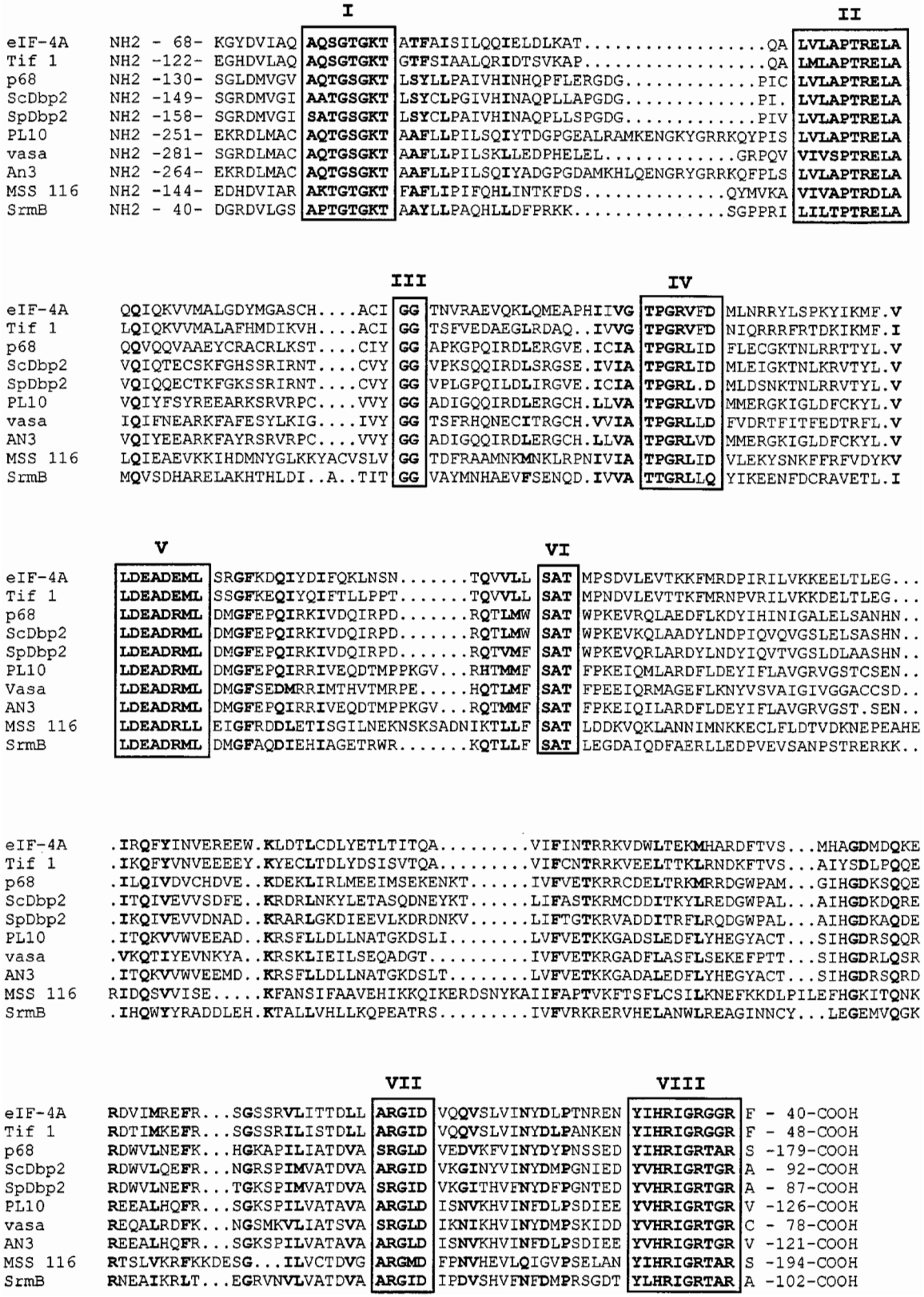

FIGURE 1(A)

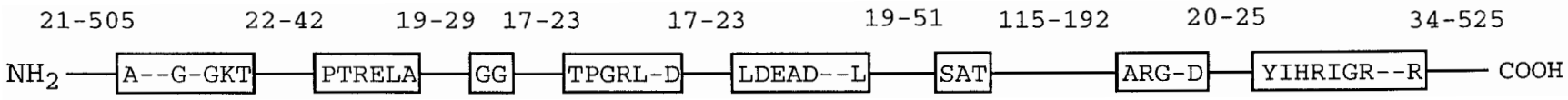

FIGURE 1(B)

FIGURE 1. The core-region of RNA helicases belonging to the DEAD box family (Linder et al., 1989). (A) Alignment of the core-region of some DEAD box proteins of different biological functions. The highly conserved domains are marked by boxes (I–VIII) and boldfaced letters. Single conserved amino acids residues are also marked by boldfaced letters. The individual distances of the core region from the N-terminal and C-terminal end of each protein are indicated at the beginning and the end of the sequences shown. The conserved domains are separated by stretches with low sequence but high length conservation (see also B). The mouse translation initiation factor eIF-4A is the first DEAD box protein for which an RNA helicase activity could be demonstrated (Ray et al., 1985; Abramson et al., 1987). The other RNA helicases represent well-known examples of different cellular functions: The yeast protein Tif1 is a homologue of eIF-4A (Linder and Slonimski, 1988; 1989). The helicases Dbp2 of *S. cerevisiae* and *Schizosaccharomyces pombe* (ScDbp2 and SpDpb2) are both related to protein p68, a human nuclear-encoded protein (Ford et al., 1988; Iggo et al., 1991). PL10 of mouse, the vasa protein of *Drosophila melanogaster*, and An3 of *Xenopus laevis* have been shown to be expressed in a developmentally regulated manner (Gururajan et al., 1991; Lasko and Ashburner, 1988; Leroy et al., 1989). The RNA helicase Mss116 has been isolated as a nuclear-encoded mitochondrial splicing factor from *S. cerevisiae* (Séraphin et al., 1987). The SrmB protein, a gene product *of Escherichia coli*, is probably involved in ribosome assembly (Nishi et al., 1988). (B) Schematic representation of the primary structure and the conserved domains common to all DEAD box proteins (Schmid and Linder, 1992). The eight highly conserved amino acid stretches are boxed. Less-conserved residues of the domains are indicated by dashes. The minimal and maximal distances of the conserved domains and the distances of the two flanking domains to the N- and C-terminal end of the different proteins are indicated by numbers.

NTP-independent helicase activity has been demonstrated (Gururajan et al., 1991; Hay et al., 1988). In contrast, the RNA helicase An3 from *Xenopus laevis* and the Mle protein of *Drosophila melanogaster* were shown to exhibit an NTP-dependent helicase activity (Gururajan and Weeks, 1997; Lee et al., 1997). *vasa*, Mle and An3 are involved in cell growth and differentiation (Gururajan et al., 1994; Liang et al., 1994). The first prokaryotic gene product with an RNA helicase activity was the deaD protein isolated from *Escherichia coli* (Jones et al., 1996) and also a few viral proteins such as NPH-II from vaccinia virus and NS3 from hepatitis C virus have been shown to exert RNA helicase activity *in vitro* (Shuman, 1993; Bayliss and Condit, 1996; Kim et al., 1995; Lin and Peterson, 1995).

Although RNA helicase activity has not been demonstrated directly for other members of the superfamily II, circumstantial evidence points toward this function. It is interesting to note that a large number of putative RNA helicases is involved in the nuclear splicing machinery either directly or indirectly (for reviews see Wassarman and Steitz, 1991; Staley and Guthrie, 1998; Hamm and Lamond, 1998). In addition, several putative helicases are also known to be involved in ribosome biogenesis, indicating an important role of those proteins in the manipulation of the RNA structure (reviewed by Fuller-Pace, 1994).

III. THE CORE-REGION OF RNA HELICASES

The alignment of the amino acid sequence of different members of the superfamily II shows a common core-region that is characterized by eight highly conserved domains (Figure 1A). Based on the strong sequence homology in the fifth of the eight structural elements, the so-called DEAD box motif (D-E-A-D: Asp-Glu-Ala-Asp), RNA helicases belonging to superfamily II are also called DEAD box proteins (Linder et al., 1989).

In the core region the DEAD box proteins reveal a 35 to 40% identity of amino acids, and taking evolutionary related amino acids into account the homology is as high as 45 to 60%. Moreover, as can be seen from Figure 1B, there is also a striking conservation in spacing between the conserved protein domains (Linder et al., 1989; Schmid and Linder, 1992).

In contrast, the amino- and carboxy-terminal parts of the RNA helicases are characterized by a high degree of sequence and length variability (Figure 1A and B). It has been suggested that the divergent regions are responsible for individual protein functions, whereas the highly conserved domains are involved in the RNA helicase activity (like ATP-binding and -hydrolysis or binding and unwinding of double-stranded RNA).

IV. THE FUNCTION OF THE CONSERVED DOMAINS

To date, the biochemical function of four of the eight conserved elements (domains I, V, VI, and VIII, see Figure 1A) has been elucidated.

Domain I (A/G-X-X-G-X-G-K-T: Ala/Gly-X-X-Gly-X-Gly-Lys-Thr) has been described as the A motif of ATPases (Walker et al., 1982). The comparison of DEAD box proteins with other ATP- and GTP-binding proteins reveals a difference in this motif. A-X-X-G-X-G-K-T is the consensus sequence for RNA helicases, while ATPases and GTPases have in most cases a glycine instead of alanine at the first position (Gorbalenya et al., 1988b). For the transla-

tion factor Tif1 from *Saccharomyces cerevisiae* it could be shown that replacing the alanine by glycine has no effect on the growth rate. This is not surprising, as both amino acids have uncharged and unbranched side-chains. However, the replacement of the glycine residues with the negatively charged aspartate leads to reduced cell growth and a substitution of alanine by valine is lethal for the cells, suggesting that the isopropyl group near the amino-terminal end of the enzyme causes a conformational change that dramatically inhibits the function of the RNA helicase (Schmid and Linder, 1991). *In vitro* studies of RNA helicase eIF-4A yielded similar results. An exchange of alanine by glycine increases ATP binding, whereas a mutation to valine causes a dramatic decrease of ATP binding (Pause and Sonenberg, 1992). Furthermore, using site-directed mutagenesis, the role of the lysine residue in domain 1 could be elucidated. Based on X-ray crystallography and NMR-analysis, it is thought that the positively charged lysine residue binds to the β- and γ-phosphates of the ATP molecule (Fry et al., 1986). In fact, replacement of lysine with the uncharged amino acid asparagine abolishes binding of ATP (Pause and Sonenberg, 1992; Rozen et al., 1989).

Domain V, the DEAD box (L-D-E-A-D-X-X-L: Leu-Asp-Glu-Ala-Asp-X-X-Leu), represents a specific form of the ATPase B motif (Walker et al., 1982). The highly conserved sequence stretch D-E has not only been found in RNA helicases but also in many proteins that are implicated in DNA and RNA replication (Gorbalenya and Koonin, 1989; Hodgeman, 1988; Koonin, 1991). The analysis of crystallographic data and NMR spectroscopy indicates that the negatively charged aspartate residue may interact with the Mg of the ATP (Fry et al., 1986). Consistent with these results are *in vitro* studies using eIF-4A that have demonstrated that the alteration of D-E-A-D to N-E-A-D or D-Q-A-D inhibits ATP hydrolysis. However, binding of ATP was not impaired, indicating that the different functions can be assigned to different motifs. The ATPase A motif seems to be responsible for the binding and the B motif for the hydrolysis of ATP (Pause and Sonenberg, 1992).

Domain VI, the SAT-motif (Ser-Ala-Thr), is localized proximal to the DEAD box and has been described exclusively for RNA helicases. The replacement of S-A-T with A-A-A leads to an altered functional behavior of the translation initiation factor eIF-4A *in vitro*. The modified protein is capable of ATP binding and hydrolysis as well as RNA binding, but no RNA helicase activity could be detected (Pause et al., 1993; Pause and Sonenberg, 1992). Although there is no change in net charge, it is possible that the defect in RNA helicase activity is due to conformational changes caused by the shorter side chains of the alanine residues. Those results confirm the separation and assignment of distinct functions to different conserved regions.

Domain VIII is characterized by the YIHRIGRXXR box (Tyr-Ile-His-Arg-Ile-Gly-Arg-X-X-Arg) that represents a motif that is, like SAT, unique to RNA helicases. For translation initiation factor eIF-4A biochemical data indicate that this domain is critical for the binding of RNA *in vitro*. Changing the basic residues histidine or arginine to the uncharged glutamine diminish or abolish RNA binding and simultaneously reduce ATP hydrolysis. It appears that RNA binding depends on ATPase activity. Indeed, a decreased ATP binding results in reduced ATP hydrolysis and helicase activity, as observed after substitution of alanine for valine in the ATPase motif A. Similarly, mutations in the DEAD box cause an impaired ATP hydrolysis and RNA binding, while the binding of ATP remains unchanged. In addition, mutations in the SAT box affecting neither ATP binding nor hydrolysis have no effect on RNA binding but destroy heli-

case activity. Several lines of evidence have revealed that ATP binding can occur in the absence of RNA, whereas ATP hydrolysis is necessary for RNA binding (Pause et al., 1993).

From these data the following model for the ATP hydrolysis-dependent RNA interaction of eIF-4A has been proposed: Initially, the ATP binds weakly to the translation initiation factor inducing a conformational change. This allows the subsequent binding of RNA, which results in ATP hydrolysis. The hydrolysis of ATP is presumed to increase the affinity of eIF-4A for RNA binding and, due to the tighter RNA/protein interaction, unwinding of double-stranded RNA may occur (Pause et al., 1993).

V. THE SUBGROUPS OF RNA HELICASES

With the growing number of identified putative RNA helicases and other related proteins it became clear that the members of superfamily II fall into different subgroups. Three subgroups have yet been identified. The first subgroup is formed by the classic DEAD box proteins, the other two are named DEAH and DEXH based on their deviating ATPase B motif (see Figure 2). The latter two subgroups are more heterogeneous than the DEAD box proteins with respect to their sequence and biochemical function. Some of the proteins that share the DEAH motif are involved in the splicing of yeast nuclear introns and exhibit an RNA-dependent ATPase activity (reviewed in Wassarman and Steitz, 1991; Staley and Guthrie, 1998; see the *Nuclear Pre-mRNA Splicing* section for details). While these proteins show a significant homology to the DEAD box family, other proteins of the DEAH box group show a greater variability of the core region. It appears that domains I and V, which are functionally involved in ATP binding and hydrolysis, are conserved, while the boxes, which are most likely specific for RNA binding and unwinding (domains VI and VIII), are highly variable. Some proteins, such as the Rad3 from *Saccharomyces cerevisiae* and recB and recC of *Escherichia coli*, have been demonstrated to play a role in the modification of DNA. These proteins form their own subgroup called DEAH* (Figure 2; Gorbalenya and Koonin, 1991; Matson and Kaiser-Rogers, 1990). RNA helicases carrying the DEXH motif include the *maleless* protein (Mle) of *Drosophila*; the homologous human gene product, RNAse A; and the *plum pox potyvirus* CI protein (Kuroda et al., 1991; Lain et al., 1990; Lee and Hurwitz, 1993). For the last two proteins, an RNA helicase activity has been demonstrated (Lain et al., 1990; Lee and Hurwitz, 1993), and it was shown that the DEXH subgroup is more closely related to the DEAD box proteins than proteins belonging to the DEAH* subgroup (Fuller-Pace, 1994).

VI. THE BIOLOGICAL FUNCTIONS OF RNA HELICASE

The ability to bind RNA and to modulate its secondary and tertiary structure makes the proteins of superfamily II well suited to participate in various biological processes. It has been shown that RNA helicases are involved in translation initiation, ribosome biogenesis, nuclear mRNA export, RNA degradation, and nuclear as well as mitochondrial RNA processing. Other proteins of the family have been implicated in cell growth, division, and differentiation. Some viral RNA helicases have been suggested to play a role in the replication of RNA.

A. Translation Initiation

The best characterized member of the RNA helicase family, the translation initia-

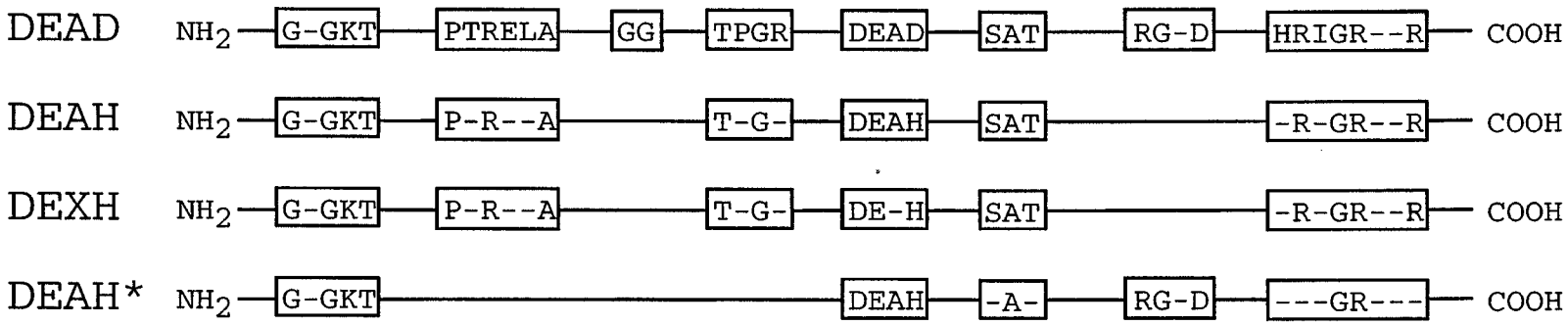

FIGURE 2. Variations in the conserved domains of the proteins arranged in the different subgroups of superfamily II (Fuller-Pace, 1994). The DEAD, DEAH, and the DEXH box proteins belong to the family of RNA helicases. The proteins of the DEAH* subgroup show the strongest variations in the conserved regions and are characterized by a DNA helicase activity (see text for detailed description).

tion factor eIF-4A from mouse, plays a key role in the first step of translation together with the two translation initiation factors eIF-4B and eIF-4F. In eukaryotic cells, eIF-4A exists both in the free form and as a part of the cap-binding complex eIF-4F, which is composed of three different subunits: the 46-kDA protein eIF-4A, the 24-kDA translation initiation factor eIF-4E, and the 220-kDA polypeptide eIF4G (p220) (see Figure 3; Grifo et al., 1984). It is thought that the eIF-4F complex melts the secondary structures of mRNA molecules in the presence of ATP, free eIF-4A, and translation factor eIF-4B (Rozen et al., 1990). This allows subsequent binding of the 43S preinitiation complex that consists of the 40S ribosomal subunit, the initiator Met-tRNA, eIF-2, eIF3, eIF1A, and GTP to the mRNA (reviewed by Sonenberg, 1988; Hershey, 1991; Rhoads, 1991). This results in the formation of the 48S preinitiation complex, which moves along the mRNA in 5′→3′ direction until it encounters the first initiation codon, a process called scanning (Kozak, 1989). Once the 48S preinitiation complex has reached the start codon, the initiation factors are released. The subsequent binding of the ribosomal 60S subunit leads to the formation of the 80S initiation complex (Figure 3). This starts the process of elongation during which the open reading frame is translated into protein (reviewed by Linder and Prat, 1990; Voorma et al., 1994).

The interactions of translation initiation factors, mRNA, and cofactor ATP have been studied *in vitro*. eIF-4A has been demonstrated to have an RNA-dependent ATPase activity, as well as an ATP-dependent RNA helicase activity (Abramson et al., 1987; Rozen et al., 1990). Furthermore, it was shown that the presence of eIF-4B stimulates RNA helicase activity that can proceed in 5′→3′ as well as in 3′→5′ direction (Abramson et al., 1988; Rozen et al., 1990). Interestingly, eIF-4B exhibits a similar behavior relative to the eIF-4F complex. The complex unwinds mRNA duplex structures in an ATP-driven reaction only when combined with eIF-4B. The conversion of double-stranded RNA (dsRNA) into single-stranded RNA (ssRNA) leads to the dissociation of the complex, which indicates higher affinity of eIF-4F for dsRNA. This assumption is supported by binding studies that show that eIF-4F binds stronger to dsRNA and ss/dsRNA junctions than to ssRNA (Jaramillo et al., 1991).

Sequencing of the genes that encode the human translation initiation factor eIF-4B and the putative homologous gene *TIF3* of *S. cerevisiae* has revealed that both proteins contain an RNA binding motif, a so-called RNP or RRM domain (ribonucleoprotein or RNA recognition motive), as well as a carboxy-terminal RNA binding region (Altmann et al., 1993; Milburn et al., 1990). Apart from the motifs specific to RNA helicases, such a binding motif does not exist in the DEAD box protein eIF-4A. This raises the question about the relation of both initiation factors. One possible explanation is that the proteins bind with different specificity to mRNA. Indeed, eIF-4A shows a strong preference for ssRNA, while eIF-4B has a higher affinity for the ssRNA/dsRNA junction of native mRNA (Abramson et al., 1987). It has been supposed that eIF-4A may be essential for the initial binding to regions of single-stranded RNA. eIF-4B might be required for the recognition of double-stranded RNA structures during the unwinding process, as well as for mediating an efficient binding between eIF-4A and dsRNA (Linder and Prat, 1990; Pause et al., 1993).

Based on these results, the mechanism of translation initiation can be explained by the following model (Figure 3). In the initial step the translation initiation factor eIF-4E recognizes and binds to the 5′ cap structure of the mRNA. This interaction induces the subsequent binding of eIF-4A and the p220

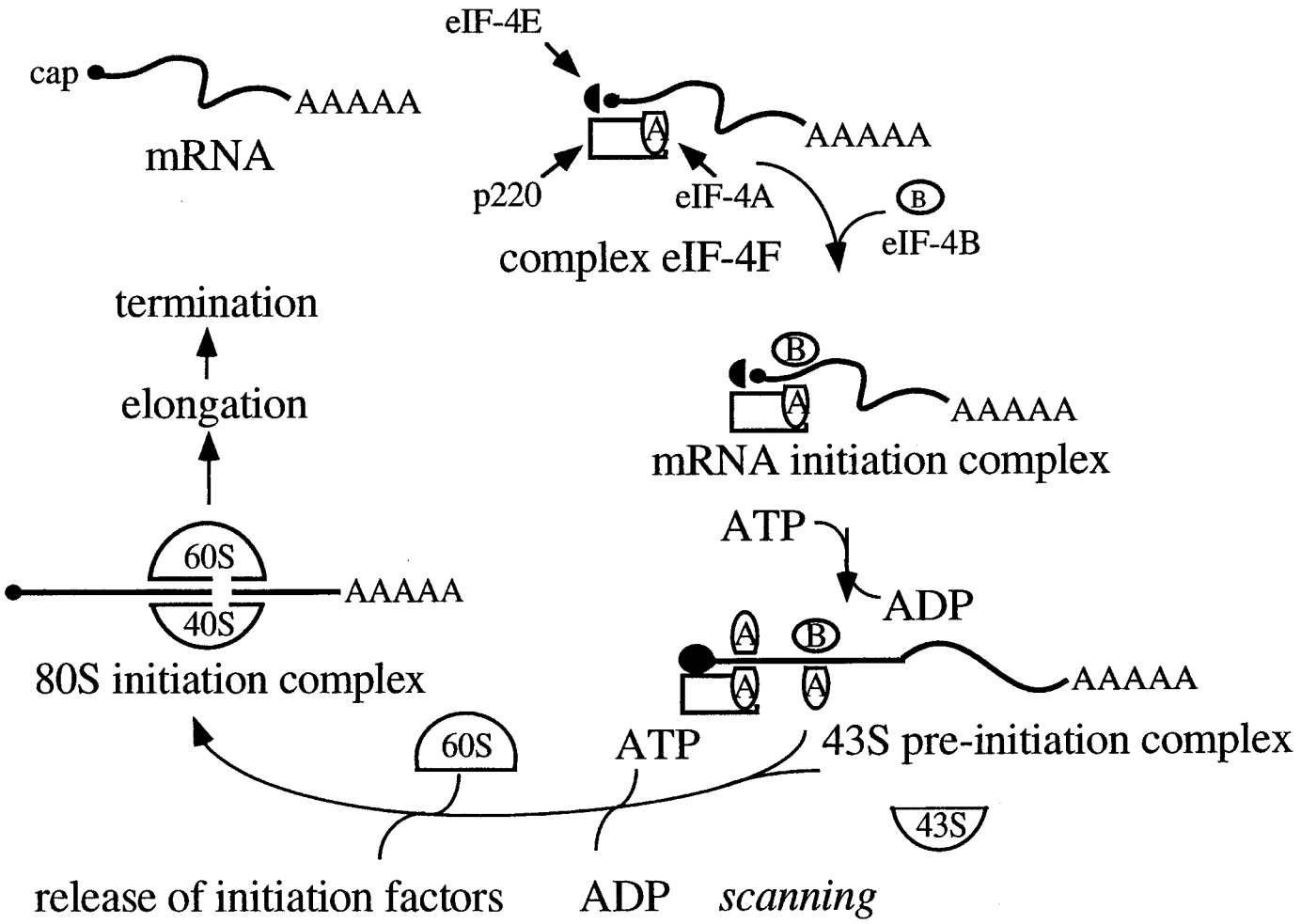

FIGURE 3. Model of the initiation of translation in eukaryotic cells (Linder and Prat, 1990). The translation initiation factor eIF-4A is represented by an A and the protein eIF-4B by a B. The black semicircle symbolizes the translation initiation factor eIF-4E, and the rectangle represents the p220 protein. The 5′ cap structure of mRNA is indicated by a filled black circle (see text for detailed description).

protein leading to the complex eIF-4F. It is likely that the cofactor ATP has an essential function during this first interaction between eIF-4A and the mRNA. ATP binds weakly to eIF-4A in an RNA-independent manner, enabling the first contact with the mRNA. This forces a conformational change of eIF-4A, which improves ATP binding and hydrolysis. The hydrolysis of ATP results in an higher affinity of eIF-4A for the mRNA and the following binding of eIF-4B starts the translocation of the small ribosomal subunit (Pause et al., 1993). The complex moves along the mRNA and unwinds the duplex RNA structures in the untranslated 5′ region in an ATP-dependent reaction. It is likely that this process requires additional free eIF-4A (Jaramillo et al., 1991; Pause et al., 1993; Schmid and Linder, 1991).

Several proteins have been identified that show a high degree of homology to the mouse initiation factor eIF-4A, and two highly conserved forms (eIF-4AI and eIF-4AII) have been identified in mammalian cells (Nielsen and Trachsel, 1988). In rabbit reticulocyte lysate, eIF-4AII is preferentially associated with the eIF-4F cap binding complex, while the factor eIF-4AI exists both in free and in complexed form (Conroy et al., 1990). This different behavior is also reflected by the different levels of expression of the two proteins. It has been found that both forms are required *in vitro* for maximum translation, and hence eIF-4AI and eIF-4AII may act synergistically (Conroy et al., 1990).

Two proteins that show a striking homology to the translation initiation factor eIF-4A have been found in yeast (Linder and Slonimski, 1989). The gene products Tif 1 and Tif 2 show an identical amino acid sequence and, in contrast to the mammalian RNA helicases eIF-4AI and eIF-4AII, they exhibit the same level of expression. Loss of one of the two forms does not change the phenotype, but inactivation of both genes is lethal for the cell as would be expected for a translation initiation factor (Linder and Slonimski, 1989; Prat et al., 1990). This view is supported by the finding that in a yeast cell-free translation system the depletion of Tif 1 and Tif 2 leads to an inhibition of protein synthesis. The addition of purified mouse eIF-4A restores protein production, suggesting a direct implication of Tif 1 and Tif 2 in the translation process (Blum et al., 1989).

Three genes from *Nicotiana plumbaginifolia* coding for the RNA helicases NeIF-4A1, 2, and 3 have been isolated by heterologous hybridization with the yeast gene *TIF1* (Owttrim et al., 1991). NeIF-4A1 and NeIF-4A2 show a high sequence similarity with eIF-4A, while NeIF-4A3 has a lower homology and contains a DESD motif instead of the DEAD box. Whether NeIF-4A3 plays a different role in plant translation initiation or is involved in other processes is yet unknown (Owttrim et al., 1991).

Finally, by sequencing randomly selected cDNA clones, a human gene was isolated that probably encodes the human homologue of eIF-4AII (Sudo et al., 1995).

In addition to the eIF4A homologues, two other DEAD box helicases isolated from yeast may play a role in translation initiation. Initially the *DED1* gene had been identified as a suppressor of a mutation in the nuclear splicing protein Prp8, which is a major component of the U5 snRNP and mediates association with the U4/U6 snRNPs to form the tri-snRNP particle (see also the *Nuclear pre-mRNA Splicing* section). Therefore, it has been speculated that Ded1 is involved in unwinding U4/U6 snRNAs similar to the Prp28 RNA helicase (Jamieson et al., 1991). However, it has been reported recently that the *DED1* gene, which is essential for cell viability, may be required for translation (Chuang et al., 1997; de la Cruz et al., 1997). Two *ded1* cold-sensitive mutants show an inhibition of protein synthesis as well as an increase of 80S ribosomes and a decrease of

polyribosomes (Chuang et al., 1997; de la Cruz et al., 1997). This change in the polyribosome profile points to a direct role of Ded1 in translation initiation rather than a function in nuclear splicing (Chuang et al., 1997). Moreover, a genetic interaction between DED1 and CAF20 has been demonstrated (de la Cruz et al., 1997). Because CAF20 plays a negative role in translation initiation by competing with eIF4G for binding to eIF4E, it was proposed that Ded1 may have a function removing cap-proximal secondary structures from mRNAs (Altmann et al., 1997; de la Cruz et al., 1997). In this context it has been suggested that Ded1 might influence translation of nuclear splicing factors affecting pre-mRNA splicing.

The second RNA helicase, Dbp1, has been isolated as a high-copy-number suppressor of a *ded1*-mutation. Sequence comparisons revealed a 72% identity of the Dbp1 protein to the Ded1 RNA helicase. However, in contrast to the *DED1* gene, inactivation of the *DBP1* gene is not lethal (Jamieson and Beggs, 1991) and does not affect the polyribosomal profile (de la Cruz et al., 1997). Although genetic data point to an involvement in translation initiation, the physiological role of Dbp1 is yet not clear.

B. Ribosomal Biogenesis

Prokaryotic ribosomes consist of a small 30S subunit, which is composed of the 16S ribosomal RNA (rRNA) and 21 ribosomal proteins, as well as a large 50S subunit, that contains the 23S and the 5S rRNA and 34 additional proteins (reviewed by Noller, 1991). Ribosomal biogenesis requires the transcription of ribosomal RNA, which yields a precursor molecule. After several cleavage steps, leading to different pre-rRNA intermediates, the mature rRNA is obtained (Figure 4). This posttranscriptional process depends on a large number of ribosomal proteins as well as small nucleolar ribonucleoproteins (snoRNPs). It has been suggested that some of these proteins catalyze the cleavage of the rRNA precursors, while other proteins control rRNA processing through a conformational change of the secondary or tertiary structure of the rRNA intermediates (reviewed by Eichler and Craig, 1994). During ribosomal biogenesis, the association of ribosomal proteins and snoRNPs with the processed rRNA leads to the mature ribosomal subunits (Eichler and Craig, 1994).

In *Escherichia coli*, three RNA helicases are known that presumably participate in ribosomal biogenesis. For the SrmB protein, an RNA-dependent ATPase activity has been demonstrated. When expressed at high copy numbers, the *srmB* gene is able to suppress the effect of a temperature-sensitive mutation in the ribosomal protein L24 that is involved in the assembly of the large ribosomal subunit (Nishi et al., 1988). It is assumed that the mutated L24 no longer binds to the 23S rRNA and degradation of the rRNA is prevented by binding of the overexpressed RNA helicase to specific domains of the 23S rRNA (Nishi et al., 1988).

The second RNA helicase, DeaD, originally has been isolated as a high copy nuclear suppressor of a temperature-sensitive mutation in the *rpsB* gene (Toone et al., 1991). The *rpsB* gene encodes for the ribosomal protein S2 that binds to the 30S subunit late in the ribosomal assembly process and is required for incorporation of the S1 protein into the ribosome (Laughrea and Moore, 1978). Previous studies have shown that the S1 protein, which exhibits an RNA helicase activity, affects the affinity of ribosomes to different mRNA initiation sequences (Szer et al., 1976). It is possible that the level of ribosome associated protein S1 is reduced by a mutation in the S2 protein. An increased expression of a second RNA ribosomal helicase, such as the *DeaD* gene product, may compensate for the various defects. Another possible interpretation might be that the DeaD

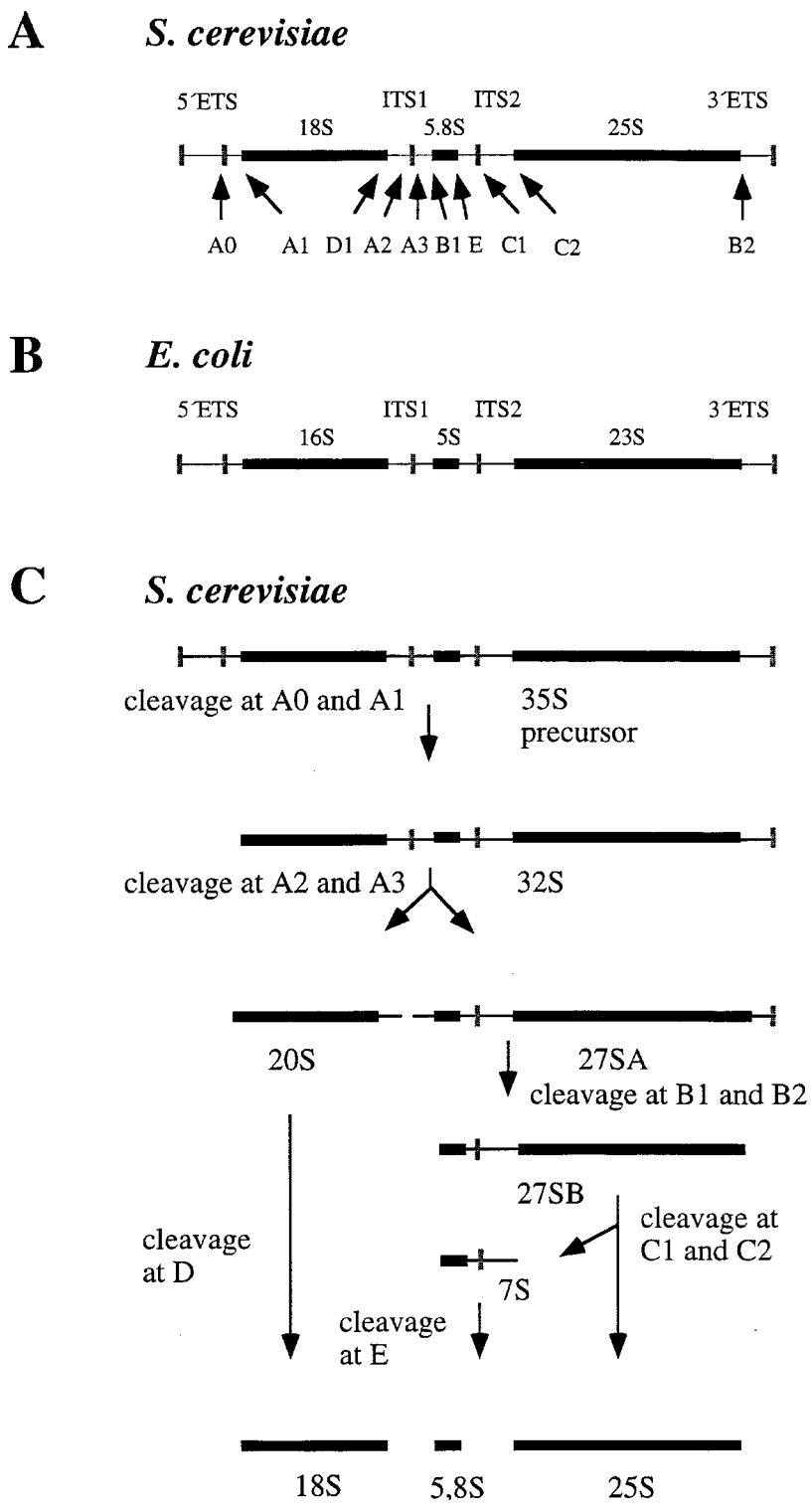

FIGURE 4. Representation of the rRNA processing pathway (Eichler and Craig, 1994). (A) Model of the precursor rRNA of *S. cerevisiae*. (B) Model of the precursor rRNA of *E. coli*. (C) Representation of rRNA processing pathway of *S. cerevisiae*. ETS and ITS are abbreviations used for external and internal transcribed spacers. Abbreviations A0, A1, A2, A3 D1, B1, B2, C1, C2, and E denote the different processing sites, where rRNA is cleaved. The size of the various intermediates and the end products of the process is indicated by their relative sedimentation coefficients.

protein stabilizes unstable intermediates in an early stage of ribosomal assembly similar to the *SrmB* product (Toone et al., 1991). Recent studies demonstrated that DeaD unwinds double-stranded RNA in the absence of ATP, indicating a helix-destabilizing activity instead of an RNA helicase activity. Moreover, the DeaD protein was found to become a major component of the 70S ribosomes at low temperatures. Considering that some *E. coli* mRNAs form a more stable secondary structure at low temperatures, DeaD may facilitate ribosomal translocation under these conditions by destabilizing mRNA structures (Jones et al., 1996).

The third RNA helicase of *E. coli*, DbpA (for DEAD box protein), appears to be an exception among the other known DEAD box proteins, because hydrolysis of ATP occurs only in the presence of bacterial 23S rRNA (Fuller-Pace et al., 1993). It has been shown that a single helix region of 93 basepairs of the 23S rRNA is sufficient to stimulate the ATPase activity of the DbpA protein (Nicol and Fuller-Pace, 1995). This region is part of the peptidyltransferase center and includes some bases that interact with the 3′ terminal adenosines of the tRNAs at both the aminoacyl (A) and the peptidyl (P) site (Moazed and Noller, 1991). From these data it has been speculated that DbpA may be involved in the peptidyltransferase reaction by supporting the correct folding of tRNAs or it may be involved in translocation. However, recent studies show that additional regions spread across the 23S rRNA can also stimulate this activity (Böddeker et al., 1997). These regions are either part of the functional center of the 50S ribosome or are related to other ribosomal proteins involved in the assembly process. Therefore, it is even possible that the DbpA RNA helicase might be responsible for the formation of the correct overall secondary structure of the 23S rRNA required for ribosomal assembly (Böddeker et al., 1997; Nierhaus, 1991). In addition, DbpA was shown to possess an unwinding activity that is independent of ATP hydrolysis. As shown for DbpA and the previously described helicase Dead, ATPase and helicase activity are not necessarily coupled in all DEAD box proteins. DbpA has an unusual carboxy-terminal region with 25% positively charged residues and 29% small amino acids (glycine and alanine) that might be responsible for nucleic acid binding independently from ATP hydrolysis (Böddeker et al., 1997). An additional putative cysteine-rich metal-binding sequence motif located between the SAT and ARGXD domain has been proposed to interact with nucleic acids (Klug and Schwabe, 1995). Therefore, dependence of ATP hydrolysis on 23S rRNA binding can be explained by a cysteine-rich domain structurally linked to the ATPase domain (DEAD box) (Böddeker et al., 1997).

To date, in *Saccharomyces cerevisiae* 11 putative RNA helicases are known to be involved in ribosomal biogenesis. The proteins Sbp4, Drs1, Dbp3, Dbp6, Dob1, and Dbp7 are implicated in the production of the 25S and 5.8S rRNAs (Daugeron and Linder, 1998; Kressler et al., 1998; de la Cruz, 1998, 1128; Ripmaster et al., 1992; Sachs and Davis, 1990; Weaver et al., 1997). The *SPB4* gene has been isolated by complementation of a cold-sensitive *spb-1* allele that suppresses a null mutation in the poly(A)-binding protein Pab1 (Sachs and Davis, 1990). Inactivation of the *SPB4* gene decreases the amount of the 60S ribosomal subunit and the ratio between 25S and 18S rRNA. The mutant exhibits 2.5-fold less mature 25S rRNA compared with the wild type. This suggests that Spb4 is involved neither in the early processing pathway of rRNA nor in the maturation process of 18S rRNA, whereas the processing of 27S to 25S rRNA requires the active RNA helicase (Eichler and Craig, 1994; Sachs and Davis, 1990).

Similar data have been described for the *DRS1* gene (for deficiency of ribosomal subunits). Mutations in the gene result in an accumulation of the 27S rRNA precursor of the 25S rRNA, and the level of the 60S ribosomal subunit decreases (Ripmaster et al., 1992). Because both gene products (Drs1 and Spb4) are essential for mitotic growth, it is thought that more than one RNA helicase is required for processing of the 27S rRNA and assembly of the large ribosomal subunit (Eichler and Craig, 1994; Ripmaster et al., 1992; Sachs and Davis, 1990).

The third RNA helicase Dbp3 was isolated by PCR using degenerated oligonucleotides (Chang et al., 1990). Disruption of the *DBP3* gene leads to a reduced growth rate of the cells and a deficiency of the 60S ribosomal subunit. Moreover, the production of mature 25S rRNA is delayed. The analysis of pre-rRNA intermediates suggested that cleavage efficiency at the A_3 site, and to a lower extent, also at the $A_0/A_1/A_2$ sites (see Figure 4A) is decreased (Weaver et al., 1997).

The Dbp6 protein is essential for cell viability. The *in vivo* depletion of Dbp6 results in a decreased production of the 27S and 7S rRNA precursors leading to a depletion of the mature 25S and 5.8S rRNA as well as to an accumulation of the 60S ribosomal subunit. It has been suggested that Dbp6 is essential for the assembly of preribosomal particles during biogenesis of the 60S ribosomal subunits (Kressler et al., 1998).

Similar data have been decribed for the Dob1 RNA helicase (de la Cruz et al., 1998). In addition, depletion of Dob1 exhibit a specific inhibition of the 3′ processing of the 5.8S rRNA precursor and the protein seems to prevent degradation of the 5′ external transcribed spacer region of the pre-rRNA. It has been suggested that Dob1 acts as a cofactor for the exosome complex by unwinding secondary structures in the precursor rRNA. Otherwise, the processing by exonucleases remains blocked.

Depletion of the putative ATP-dependent RNA helicase Dbp7 results in a decrease of 60S ribosomal subunit as well as an reduced amount of 27S and 7S precursor rRNAs. However, this reduction is not associated with an accumulation of preceding precursors or abnormal processing intermediates. Therefore, it has been suggested that the absence of Dbp7 leads to rapid degradation of the pre-rRNA. Possibly, Dbp7 is implicated in assembly of pre-ribosomal particles during biogenesis of the 60S ribosomal subunit (Daugeron and Linder, 1998).

The 18S rRNA biogenesis (see Figure 4A and C) is affected by the putative RNA helicases Fal1, Rok1, Rpr3, and Dbp4 (Kressler et al., 1997; Liang et al., 1997; O'Day et al., 1996a; Venema et al., 1997). Although the *FAL1* gene displays a high amino acid homology to the translation initiation factor eIF4A, it shows a completely different subcellular function and localization. The hemagglutinin epitope-tagged Fal1 protein could be localized in the nucleolus and depletion of the protein results in a decrease of both the 40S ribosomal subunit and the level of 18S rRNA (Kressler et al., 1997).

The RNA helicase Rok1 (for rescuer of *kem-1*) originally has been isolated as a high copy number supressor of a *kem1* null mutation in *S. cerevisiae* (Song et al., 1995). The gene product of *KEM1* is involved in various processes such as nuclear fusion during karyogamy, chromosome transmission and duplication, and separation of the spindle pole bodies during mitosis (Dykstra et al., 1991; Kim et al., 1990; Kipling et al., 1991; Larimer et al., 1992; Tishkoff et al., 1991). By indirect immunofluorescence using an epitope-tagged version of the Rok1, the helicase was predominantly localized in the nucleoli (Venema et al., 1997). Moreover, depletion of the *ROK1* gene inhibits 18S

rRNA synthesis, especially the cleavages at the sites A_0, A_1, and A_2. Therefore, it was suggested that Rok1 is required for the snoRNP complex that precedes the early pre-rRNA cleavages (Venema et al., 1997).

The *RPR3* gene, which was identified in a PCR screen, encoded a 60.9-kDA protein implicated in cleavages of the 35S primary precursor leading to the mature 18S rRNA (O'Day et al., 1996a).

The RNA helicase Dbp4 suppresses 18S rRNA processing defects resulting from mutations in the U14 snoRNA. It has been supposed that Dbp4 may function in assembly of the U14 snoRNP or interact with other ligands. Therefore, Dbp4 might mediate the interaction of different components such as the U14 RNP and the precursor 18S rRNA (Liang et al., 1997).

In all cases described above the molecular details of the function of the RNA helicases in ribosomal biogenesis are still a matter of speculation. It has been supposed that unwinding of pre-rRNA secondary structures may be essential for snoRNA/pre-rRNA interactions. Similarly, conformational changes of the precursor rRNA could be a prerequisite for a further processing leading to intermediates and the mature rRNA. Moreover, altering the secondary structure of the pre-rRNA may enable binding of ribosomal proteins or stimulate the dissociation of RNA/protein interactions established during the processing pathway (Eichler and Craig, 1994; Ripmaster et al., 1992).

C. Nuclear Pre-mRNA Splicing

The primary transcripts of eukaryotic genes are often discontinuously organized, alternating coding sequences (exons) and intervening sequences (introns). The introns have to be spliced from the precursor mRNA to produce a mature mRNA that can be translated into a specific protein. The splicing reaction takes place in the spliceosome, which, similar to the ribosome, consists of numerous proteins and small nuclear RNA (snRNA) molecules that exist in the cell as ribonucleoprotein particles or snRNPs (small nuclear ribonucleoprotein particles = snRNPs). At each individual intron a single spliceosome is formed *de novo* by the five snRNPs U1, U2, U4, U5, U6, and more than 50 additional proteins. The assembly of the spliceosome is a multistep process that requires, like the ribosome biogenesis, assisting proteins and energy in form of ATP. In the spliceosome the pre-mRNA interacts with snRNAs by complementary base pairing to fold the intron and adjacent exons sequences into the correct structure for splicing (for reviews see Lamond, 1993; Moore et al., 1993; Madhani and Guthrie, 1994a).

The processing of pre-mRNA starts during transcription. In the following two-step mechanism the introns are precisely removed from the primary transcripts and the exons are joined together (see Figure 5). In the first step of the splicing reaction the phosphodiester bond at the 5′ site of the pre-mRNA is cleaved by a nucleophilic attack of the 2′ hydroxyl group of an adenosine residue near the 3′ end of the intron. This cleavage generates two splicing intermediates: the free 5′ exon and an intron-3′ exon lariat. The lariat structure results from a 2′-5′ phosphodiester bond that links the 2′ OH group of the branchpoint adenosine to the free phosphate of the 5′ terminal, highly conserved guanosine residue of the intron. In the second step the 3′ hydroxyl group of the free 5′ exon attacks the phosphodiester bond at the intron-3′ exon junction in a nucleophile reaction. The two exons are joined together, and the intron is excised in a lariat form (Lamond, 1993; Moore et al., 1993). Removal of introns and the ligation of exons proceeds via two transesterification reactions, which require *trans*-acting splicing factors, including

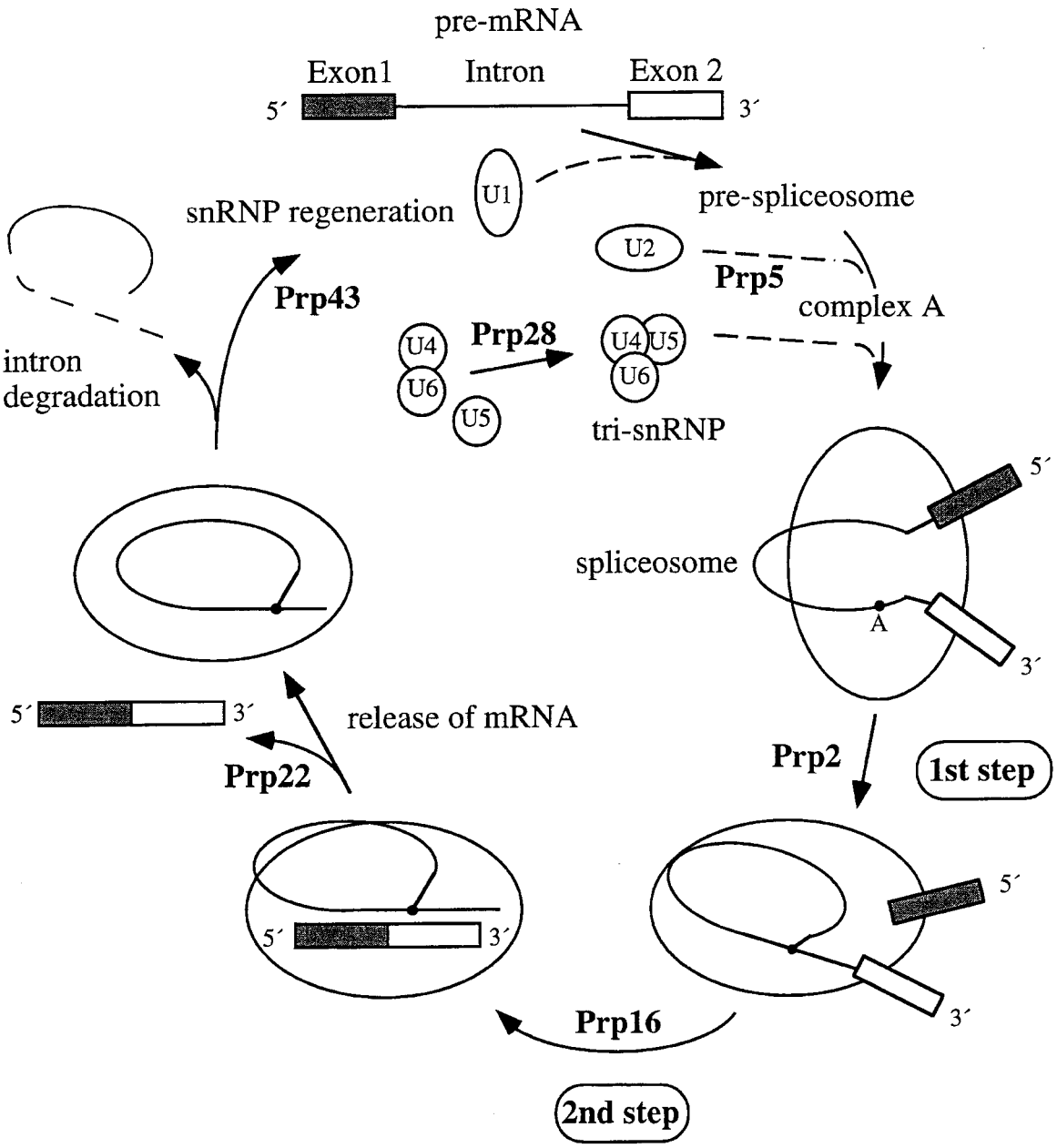

FIGURE 5. The nuclear splicesome cycle. The splicing process is characterized by two transesterification reactions indicated as 1st and 2nd step. The shaded and white boxes represent exon sequences. The intron is shown as a thin line and the branchpoint adenosine (A) as a black dot near the 3′ end of the intron. The snRNPs U1 and U2 are indicated as non-filled ellipses, and the snRNPs U4, 5, and 6 as non-filled circles. The postulated roles of the five DEAD/DEAH box proteins Prp2, 5, 16, 22, 28 and 43 in the splice cycle are indicated (see text for details) (Lamond, 1993; Staley and Guthrie, 1998).

snRNAs associated with proteins (snRNPs), additional proteins that are not an integral part of the spliceosome, and ATP as a co-factor (reviewed by Lamm and Lamond, 1993; Will and Lührmann, 1995; Krämer, 1996; Staley and Guthrie, 1998).

A number of yeast mutants have been isolated that show some defects in pre-mRNA

processing. These mutants are called *prp*-mutants (for pre-mRNA-processing) and genetic screening systems have led to the isolation of genes that act as suppressors of *prp* mutations. Approximately 30 *PRP* genes whose gene products affect pre-mRNA splicing have been identified (Ruby and Abelson, 1991; Lamm and Lamond, 1993). Six of the *PRP* genes encode putative RNA helicases. *PRP5* and *PRP28* belong to the class of DEAD box helicases, while *PRP2*, *PRP16*, *PRP22*, and *PRP43* represent DEAH box proteins (Staley and Guthrie, 1998). These proteins are involved in different stages of the nuclear splice cycle (Figure 5). Presumably, they are required to control the correct RNA base pairing during spliceosomal assembly and during the splicing reaction. *In vitro*, an RNA-dependent ATPase activity has been demonstrated for Prp2 (Kim et al., 1992), Prp5 (O'Day et al., 1996b), Prp16 (Schwer and Guthrie, 1991), Prp22 (Wagner et al., 1998), and Prp28 (Strauss and Guthrie, 1991). Until recently, no RNA helicase activity could be measured for those proteins and it was assumed that only highly specific RNA sequences induce this activity (Lamm and Lamond, 1993). However, an ATP-dependent RNA unwinding activity could be demonstrated for Prp16 that was found to be independent of specific sequences of the RNA substrate *in vitro* (Wang et al., 1998). In this case it was suggested that the spliceosomal context and additional auxiliary protein factors are required for full RNA-specific helicase activity *in vivo* (Wang et al., 1998). And also Prp22 was recently shown to unwind the U4/U6 duplex RNA and other intermolecular heteroduplex RNAs *in vitro* (Wagner et al., 1998). These data suggest that the protein might be essential for unwinding of non-specific inhibitory RNA/RNA base pairing. However, it cannot be ruled out that the specific substrate of Prp22 remains to be detected (Wagner et al., 1998).

The DEAD box proteins Prp5 and Prp28 act both prior to the cleavage of the 5′ splice site (Figure 5). Prp5 is involved in the first ATP-dependent step in spliceosome assembly. It is thought that this RNA helicase mediates the binding between snRNP U2 and the pre-mRNA (Dalbadie-McFarland and Abelson, 1990). Prp28 is required at a later stage of spliceosomal biogenesis. This DEAD box helicase has been shown to interact with Prp24, a protein associated with the U6 snRNP, as well as with Prp8, a protein found within snRNP U5 (Strauss and Guthrie, 1991). These findings indicate a relationship between Prp28 and the (U4/U6.U5) tri-snRNP. Possibly, the putative RNA helicase dissolves base pairing between snRNPs U4 and U6 that may precede the cleavage of the 5′splice site (Strauss and Guthrie, 1991). Interestingly, Prp28 has been demonstrated to be essential for the first step of the splicing reaction (Strauss and Guthrie, 1994).

In contrast, the DEAH box proteins are involved in three successive steps following spliceosome assembly (Figure 5). Prp2 associates temporarily with the spliceosome and is required for the first transesterification reaction at the 5′ splice site (King and Beggs, 1990; Lin et al., 1987). It has been demonstrated that Prp2 binds directly to the pre-mRNA. This interaction needs a fully assembled spliceosome and a spliceable pre-mRNA but is independent of ATP (Teigelkamp et al., 1994). Therefore, it has been suggested that after spliceosome assembly Prp2 interacts ATP-independently with the pre-mRNA and other splicing factors. As Prp2 exhibits an RNA-dependent ATPase activity, ATP hydrolysis is probably induced by RNA binding. Utilizing energy derived from ATP hydrolysis, the putative RNA helicase activity may cause conformational changes of the RNA binding region important for the first step of the splicing reaction (Kim et al., 1992; Plumpton et al., 1994). It has been speculated that the

RNA helicase activity needs additional cofactors or specific substrates, as described for the translation initiation factor eIF-4A (Linder and Prat, 1990; Pause et al., 1993). Indeed, a second protein, Spp2, that directly interacts with the DEAH box protein Prp2 is required for the first step of splicing reaction (Roy et al., 1995), and it has been proposed that Spp2 activates the putative RNA helicase activity of Prp2 (Roy et al., 1995).

The DEAH box protein Prp16 is required for the second step of splicing. Prp16 binds to the spliceosome after formation of the free 5′ exon and the lariat intron-3′ exon intermediates and dissociates from the splicing complex after hydrolysis of ATP (Schwer and Guthrie, 1991). As a result of a conformational change of the pre-mRNA induced by ATP hydrolysis, Prp16 can probably interact with the 3′ splice site. Indeed, binding of the 3′ splice site by Prp16 has been confirmed by UV cross-linking (Umen and Guthrie, 1995a). This conformational change causes interactions with two other proteins (Slu7 and Prp8) and requires ATP hydrolysis by Prp16 for the most efficient interaction with the 3′ splice site (Umen and Guthrie, 1995a). Prp16 could either activate the secondary structure of the 3′ splice site or it might modify the spliceosome conformation for the second step (Umen and Guthrie, 1995b). If the latter suggestion holds true, it should be possible to demonstrate an interaction between the DEAH box protein and the snRNPs. Interestingly, the snRNPs U2 and U6 are the strongest stimulators of the ATPase activity of Prp16 *in vitro* (Madhani and Guthrie, 1994b).

In contrast to the other DEAD or DEAH box proteins, the putative RNA helicase Prp22 is not required for either spliceosome assembly or the two splicing steps. Mutations in Prp22 cause the accumulation of unspliced precursor RNA and spliced intron lariat. Furthermore, extracts of strains with a *PRP22* mutation are impaired in the release of fully spliced mRNA from the spliceosome (Company et al., 1991). Using polymerase chain reaction (PCR) a human gene, *HRH1* (for human RNA helicase 1), with high homology to PRP22 has been identified (Ono et al., 1994). Expression of *HRH1* in a *prp22*-deficient yeast strain resulted in a partial rescue of the temperature-sensitive phenotype (Ono et al., 1994). In mammalian cells, it could be shown that dominant-negative *hrh1*-mutations cause a permanent binding of the mutant protein to the spliceosome and affect the release of the spliced mRNA. Furthermore, expression of the altered protein in mammalian cells leads to an inhibition of the splicing reaction and to an increased export of pre-mRNA into the cytoplasm (Ohno and Shimura, 1996). It has been suggested that the regeneration of the spliceosomal components might be disturbed because accumulated spliceosomal complexes, in which the mutated Hrh1 protein remains associated with the spliced mRNA, may not be recycled. The lack of functional spliceosomes could then lead to an increased nuclear export of unspliced pre-mRNA (Ohno and Shimura, 1996).

The recently characterized DEAH box protein Prp43 seems to play a role in spliceosome disassembly. Mutations in the *PRP43* gene do not affect the two splicing step or the release of mRNA. However, various factors and snRNPs are depleted, leading to an increased level of pre-mRNA. Therefore, it is possible that Prp43 is involved in spliceosome disassembly after release of the fully spliced mRNA, ensuring an efficient recycling of splicing factors (Arenas and Abelson, 1997).

A new component of the spliceosome complex that belongs to the DEXH subgroup of RNA helicases could be identified very recently in yeast and in the mammalian system. The yeast protein Brr2 has been shown to mediate ATP-dependent dissociation of U4 and U6 in native RNPs

(Raghunathan and Guthrie, 1998) and the mammalian homolog U5-200kD is able to unwind U4/U6 snRNA duplexes *in vitro* (Laggerbauer et al., 1998). Both findings indicate that Brr2/U5-200kD might be the essential factor for dissociation of the U4 and U6 snRNAs in the spliceosome.

D. Mitochondrial RNA Splicing

Not only nuclear genes but also mitochondrial genes of lower eukaryotes are often discontinuously organized. Based on the conserved sequences and secondary structures, the introns have been classified as group I and group II introns (see reviews by Burke, 1988; Michel et al., 1989). Although some introns auto-catalyze their own splicing *in vitro*, there is strong genetic and biochemical evidence in *Saccharomyces cerevisiae* and *Neurospora crassa* that the splicing process *in vivo* depends on proteins. In addition to proteins that are intron-encoded, the so-called maturases (reviewed in Lambowitz and Belfort, 1993), also nuclear-encoded factors are involved in the splicing process (reviewed in Lambowitz and Perlman, 1990; Grivell, 1995; Lambowitz et al., 1998). In yeast three nuclear-encoded putative RNA helicases (Mss116, Suv3, and Nam7) have been found to be associated with the splicing reactions of group I or II introns (Ben Asher et al., 1989; Séraphin et al., 1989; Altamura et al., 1992; Stepien et al., 1992; reviewed in Margossian and Butow, 1996).

The complementation of a respiratory-deficient mutant that causes splicing defects in several group I and II introns led to the isolation of the *MSS116* gene encoding a typical DEAD box protein (Séraphin et al., 1987). The Mss116 protein could either be directly involved in the splicing process or act as a mitochondrial translation initiation factor. The protein might play a more general role in mitochondrial translation or could specifically support translation of intron encoded proteins (Séraphin et al., 1989). It could be demonstrated that overexpression of the Mss116 protein in mitochondrial lysates leads to an increased ATP-dependent splice reaction of the first intron of the cytochrome b gene (bI1) (Niemer et al., 1995). Keeping in mind that splicing of intron bI1 (an intron without a maturase-encoding ORF) takes place independently from the mitochondrial translation machinery, an indirect influence of Mss116 via stimulation of mitochondrial translation can be excluded. However, it cannot be ruled out that the DEAD box protein functions as a cytoplasmic translation factor that controls the synthesis of nuclear encoded splicing factors specific for bI1 and other mitochondrial introns (Niemer et al., 1995).

The *SUV3* gene encodes a putative RNA helicase that belongs to the DEXH subgroup (Stepien et al., 1992). In contrast to other known RNA helicases, the ATPase B motif is slightly degenerated. Instead of the D-E-X-H motif, the protein contains a D-E-I-Q box (Asp-Glu-Iso-Gln) but no S-A-T motif (Ser-Ala-Thr) (Stepien et al., 1992). Originally, the *SUV3* gene has been isolated as a dominant suppressor-allele, *SUV3-1*, that facilitates the translation of atypical *var1* transcripts with a deletion at the 3′ end of the gene (Zassenhaus et al., 1983). It could be demonstrated that the suppressor allele has pleiotropic effects regarding the processing of mitochondrial RNAs. Several group I introns and unspliced precursors that contain introns aI5 and bI3 accumulate. Interestingly, a reduced mRNA level of the cytochrome b gene (cob) and the gene encoding the first subunit of the cytochrome-c-oxidase (COXI) has been found. The rate of reduction appears to correlate with the number of introns (Conrad-Webb et al., 1990; Stepien et al., 1995; Zhu et al., 1989). Recent studies have shown that Suv3 is a functional part of a NTP-dependent exoribonuclease activity that is probably in-

volved in the degradation of the spliced intron (Margossian et al., 1996; Margossian and Butow, 1996). In this context a previously unknown gene, *DSS1*, has been isolated as a suppressor of a *SUV3* disruption, which shows homology to the bacterial RNaseII (Dmochowska et al., 1995). Based on these findings it has been hypothesized that Dss1 is part of the exoribonuclease activity and Suv3 is responsible for the RNA binding and unwinding. This would also explain the NTP dependence of the exoribonuclease activity (Stepien et al., 1992; Margossian et al., 1996).

The third putative RNA helicase Nam7 (for nuclear accommodation of mitochondria) was isolated as a high-copy suppressor of mitochondrial splicing defects of both group I and II introns (Ben Asher et al., 1989). Compared with most other RNA helicases, Nam7 exhibit a degenerated D-E-X-H motif (D-E-S-T), and surprisingly it has no a targeting sequence for import into mitochondria. Therefore, it has been suggested that the protein plays a more indirect role, such like modulating the expression of one or several genes encoding proteins implicated in mitochondrial splicing or biogenesis. Moreover, it has been shown that the adjacent ISF1 gene is essential for the efficient suppression of mitochondrial splicing defects by Nam7 (Altamura et al., 1994). As Nam7 was isolated as a *trans*-acting component of the degradation pathway of nonsense mRNA (see the *Degradation of Nonsense* mRNA section), it might be that the Isf1 protein influences Nam7 function at the level of mRNA turnover. Consequently, this may be affect expression of nuclear genes involved in mitochondria biogenesis (Leeds et al., 1991).

E. RNA Editing

Mitochondrial gene expression of flagellates such as *Trypanosoma*, *Leishmania*, or *Crithidia* requires posttranscriptional processing of mRNA. During this process, called kRNA (for kinetoplast RNA) editing, the sequence information of prior incomplete RNA molecules is modified by insertion and, less frequently, deletion of uridine residues at specific sites of the target RNA. The information for the editing process is provided by small mitochondrial transcripts, called guide RNAs (gRNAs). They are comple-mentary to the edited sequence and contain a 10 to 20 nucleotide long poly(U) extension at their 3′ end (Hajduk et al., 1993; Sollner-Webb, 1996). For some mRNAs up to several hundred uridine residues have to be added, which requires the interaction of a large number of gRNAs. Recent studies have shown that the editing process proceeds in 3′ to 5′ direction. During the interaction of pre-mRNA molecules with the gRNAs, short anti-parallel duplex structures are formed, and only after dissociation of the first gRNA the next duplex structure with the next gRNA can be formed further upstream. Taking the thermodynamic stability of double-stranded structures into account, it is likely that these helices must be actively melted to continue the process and to obtain the mature mRNA. Based on these notions an RNA helicase activity has been found in mitochondrial extracts from *Trypanosoma brucei* (Missel and Göringer, 1994). This activity was stronger for hybrids of gRNA and partially edited mRNA than for any other analyzed RNA double-stranded molecule. Recently, a novel mitochondrial DEAD box protein, Hel61, has been identified from *T. brucei*. A Hel61 null mutant shows a significant reduction of edited mRNAs, whereas unedited and nuclear mRNAs remain unaffected (Missel et al., 1997). These results may point toward a specific substrate specificity of the putative RNA helicase in the mitochondria of *Trypanosoma brucei* (Missel and Göringer, 1994).

F. Nucleo-Cytoplasmic Export of RNA

The compartmentation of transcription in the nucleus and translation in the cytoplasm requires a nucleo-cytoplasmic export of RNA molecules. These macromolecules have to traverse the nuclear envelope via the nuclear core complexes (NPCs) to get into the cytoplasm. It has been shown that some proteins are complexed with mRNAs in the nucleus to form specific RNPs. The RNPs bind to nuclear export receptors using nuclear export signals (NES) present in most of the proteins of those complexes (Fornerod et al., 1997; Seedorf and Silver, 1997; Stade et al., 1997). The receptors mediate the interaction between RNPs and NPCs followed by their translocation into the cytoplasm, and the RNPs are subsequently released from the NES receptors (Ullman et al., 1997). During the transport through the NPCs, the mRNAs are expected to be packed in a highly complex structure in the RNPs raising the possibility that RNA helicases might play an important role in this process (Mehlin et al., 1992).

Recently, two DEXH box and one DEAD box protein have been shown to be involved in the export of nuclear mRNA. Temperature-sensitive mutants of the DEXH helicase Mtr4/Dob1 exhibit a nuclear accumulation of poly(A)$^+$ RNA in *S. cerevisiae* without a diminution in the cytoplasmic signal for poly(A)$^+$ RNA (Liang et al., 1996). Although the export of nuclear poly(A)$^+$ RNA is not blocked completely by these mutants, a role in nuclear RNA export cannot be ruled out. Additional data suggest that Mtr4/Dob1 is also required for the processing of pre-rRNA (see also the *Ribosomal Biogenesis* section; de la Cruz et al., 1998).

The human RNA helicase A was identified recently as a shuttle protein that co-localize with the constitutive transport element (CTE) of simian retrovirus (Tang et al., 1997). This CTE is a *cis*-acting RNA sequence that translocate from the nucleus to the cytoplasm by interacting with cellular nuclear export proteins. As described previously, helicase A is predominantly localized in the nucleus. However, overexpressed CTE containing RNA induces the distribution of helicase A in both the nucleus and the cytoplasm (Lee and Hurwitz, 1993; Tang et al., 1997), indicating that helicase A has a shuttle capability.

The ATP-dependent RNA helicase Dbp5 that belongs to the DEAD box proteins was shown to be essential for cell viability in *S. cerevisiae*. Temperature-sensitive *dbp5* mutants exhibit a loss of cytoplasmic mRNAs, causing a slow protein synthesis as well as a rapid nuclear accumulation of poly(A)$^+$ RNA (Snay-Hodge et al., 1998; Tseng et al., 1998). Performing confocal microscopy and indirect immunofluorescence, Dbp5 was localized surrounding the nucleus (Tseng et al., 1998). Additional data indicate a co-localization of Dbp5 with nucleoporins at the nuclear rim (Snay-Hodge et al., 1998). Thus, Dbp5 is possibly involved in unpackaging the RNPs. Dissolving the high complex mRNA structures by an RNA helicase might affect associated proteins in a way that leads to a release of the RNPs from the NES receptors (Tseng et al., 1998). However, it is also proposed that Dbp5 act as a shuttle protein. The amino acid sequence of the Dbp5 protein is characterized by some leucine-rich domains that might represent nuclear export signals (Snay-Hodge et al., 1998).

G. Degradation of Nonsense RNA

In both prokaryotes and eukaryotes nonsense mutations generating a premature translation termination signal result in a decreased steady-state level of the corresponding

mRNA, a process called nonsense-mediated mRNA decay (Cheng et al., 1990; Humphries et al., 1984; Takeshita et al., 1984). Several studies on the mechanism of mRNA turnover have led to the identification of *cis*-acting sequences and *trans*-acting protein factors. Furthermore, it is well established now that mRNA decay is tightly linked to the translation process (reviewed in Peltz et al., 1994). It has been hypothesized that the translating ribosome terminates at the premature stop codon, and that in a following step the 40S ribosomal subunit or a specific ribosomal factor scans in 3′ direction to a downstream located element that interacts with these *trans*-acting factors and promotes decapping and/or deadenylation of the mRNA. This allows the subsequent degradation of the nonsense RNA (Hagan et al., 1995). RNA helicases seem to be good candidates for these *trans*-acting factors that facilitate mRNA decay by unwinding RNA structures that may impede the degradation process.

The RNA helicase RhlB from *E. coli* that has been isolated previously by PCR was identified as a major component of the so-called RNA degradosome, a multienzyme complex that contains also the exoribonuclease PNPase (for polynucleotide phosphorylase) and the endoribonuclease RNAse E (Kalman et al., 1991; Py et al., 1996). It is possible that RNA helicase may unwind RNA secondary structures impeding the processive activity of ribonucleases. Indeed, the activity of the degradosome was shown to be dependent on ATP, but after depleting the degradosome of RhlB addition of ATP no longer promoted mRNA degradation. This finding strongly suggests that RhlB acts as an RNA helicase by unwinding double-stranded RNA structures in an ATP-dependent manner to enable the subsequent degradation of mRNA (Py et al., 1996).

So far, three different RNA helicases have been identified in yeast that are probably involved in RNA degradation.

It could be shown that the previously described product of the *SUV3* gene is a functional part of an NTP-dependent exoribonuclease activity and probably has a function in the degradation of spliced mitochondrial introns (Margossian et al., 1996; see also the *Mitochondrial RNA Splicing* section).

The second gene encoding an RNA helicase was isolated independently as *NAM7* (for nuclear accommodation of mitochondria; Altamura et al., 1992) and *UPF1* (for up frameshift; Leeds et al., 1991). The Nam7/Upf1 protein acts as a high-copy number suppressor of specific mitochondrial splicing mutations (see the *Mitochondrial RNA Splicing* section) and is also a *trans*-acting component of the degradation pathway of nonsense mRNAs. It could be demonstrated that Nam7/Upf1 contains an RNA-dependent ATPase activity, as well as an RNA and DNA helicase activity (Czaplinski et al., 1995). However, the function of Nam7/Upf1 in cytoplasmic mRNA turnover is yet not well understood. It is assumed that the RNA helicase may facilitate ribosomal scanning of the 5′ region upstream of the nonsense codon by displacing RNA duplex structures.

The RNA helicase Dbp2 from *S. cerevisiae*, originally isolated as a homologue of the human RNA helicase p68, was selected in a two-hybrid screen for genes that interact with Upf1, suggesting a function in targeting specific RNAs for degradation (He and Jacobson, 1995; Iggo et al., 1991).

H. The p68 Group

A well-characterized member of the DEAD box proteins is the human RNA helicase p68 (Ford et al., 1988). The protein

was isolated by immunological cross-reactions with the viral RNA helicase of simian virus. It has been found in dividing cells of different vertebrates, but not in quiescent cells (Lane and Hoeffler, 1980). Studies using immunofluorescence microscopy have shown that p68 possesses a distinct, granular distribution in the nucleus that undergoes dramatic changes during the cell cycle. During interphase p68 is found in the nucleoplasm, but during telophase the helicase is transiently associated with the nucleolus, suggesting that p68 has a role in the early step of nucleolar assembly (Iggo et al., 1991). p68 exhibits both an *in vitro* RNA-dependent ATPase activity and an ATP-dependent RNA helicase activity (Hirling et al., 1989; Iggo and Lane, 1989). Recent studies have shown that phosphorylation by protein kinase C (PKC) as well as addition of calmodulin (Ca^{2+}) inhibits ATPase activity (Buelt et al., 1994). The regulation of p68 is probably mediated by a domain consisting of 19 amino acids, showing homology to the overlapping binding sites of calmodulin and PKC. This domain has been named the IQ region (Buelt et al., 1994). Supporting the putative role of p68 in the cell cycle, it could be demonstrated that calmodulin acts as Ca^{2+}-dependent regulator in the cell cycle during the transition from DNA synthesis to mitosis and from metaphase to anaphase (Lu and Means, 1993).

The *DBP2* genes from *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* were isolated via heterologous hybridization using the p68 gene as a probe (Iggo et al., 1991). The two genes show 55% similarity to p68 with the largest deviation in the carboxy-terminal region. The Dbp2 proteins contain neither an IQ region nor the sequence that is recognized by monoclonal antibodies against the SV40 antigen (Buelt et al., 1994; Iggo et al., 1991). These differences to the p68 RNA helicase might reflect that both Dbp2 proteins are involved in different functions during the cell cycle. Interestingly, there is evidence that Dbp2 from *S. cerevisiae* may be involved in the mRNA degradation pathway (He and Jacobson, 1995; Iggo et al., 1991). Inactivation of the genes in both organisms show that they are essential, but further studies are required to elucidate their specific functions (Iggo et al., 1991).

I. Cell Differentiation and Development

The genetic information of multicellular organisms is passed from generation to generation through germ cells. Based on several studies, RNA helicases appear to be involved in the formation of germ cells and may induce the development of the embryo. In *Drosophila*, for example, absence of the maternal *vasa* causes gene defects in the abdomen and in the pole cells, which are the precursors of the germ cells (Hay et al., 1988; Lasko and Ashburner, 1988). Furthermore, egg production is affected and oogenesis terminates in early vitellogenic stages (Lasko and Ashburner, 1988). During oogenesis, *vasa* is expressed in the perinuclear region of the nurse cells and is later transferred to the oocyte, where it is localized at the posterior end of the pole cells (Hay et al., 1988; Lasko and Ashburner, 1988). In the later stages of oogenesis, RNA molecules and proteins associate to ribonucleoprotein particles forming the granular pole plasma (Mahowald, 1971). It is possible that *vasa* is directly implicated in pole plasma assembly (Lasko and Ashburner, 1990). Additionally, *vasa* may act as a translational regulator of downstream located genes controlling development of the germ cells (Mahowald, 1992; Strome, 1992). A gene homologous to *vasa* has been detected in *Xenopus laevis* (*XVLG1* for *Xenopus vasa*-like gene), which

is specifically expressed in the early stages of nearly all germ line cells except sperm cells. Based on this germ line-specific expression, it is thought that Xvlg1 is involved in oogenesis, spermatogenesis, or in the determination and development of germ cells (Komiya et al., 1994; Komiya and Tanigawa, 1995).

In addition to the protein *vasa*, several other RNA helicases have been identified in *Drosophila* that might have a function in cell development and differentiation. The gene *ME31B* encodes a DEAD box protein that is strongly expressed during oogenesis, but no exact role can be attributed to this RNA helicase (de Valoir et al., 1991). *Schizosaccharomyces pombe* contains a gene sharing high homology to *ME31B*, which was identified by complementation of a mutation in the *STE13* gene. When starved for nitrogen, a *ste13*-mutant is not able to undergo the G1-arrest, followed by the initiation of the sexual cycle. The expression of the *ME31B* cDNA in *S. pombe* suppresses the mutation in the *STE13* gene (Maekawa et al., 1994). Me31B and Ste13 share more similarities with the translation initiation factor eIF-4A (see the *Translation Initiation* section) than with *vasa*, suggesting that these RNA helicases enhance translation of specific mRNAs that are expressed during cell differentiation (de Valoir et al., 1991; Maekawa et al., 1994).

An expression pattern similar to *vasa* has been demonstrated for the *Drosophila* gene *DBP73D*. This gene is expressed in males and females, but its expression in ovaries is restricted to germ cells. Its function has yet to be unveiled (Patterson et al., 1992).

The RNA helicase Mle (for *maleless*) has been isolated as one of four regulatory genes that are required for dosage compensation in male cells of *Drosophila* (Golubovsky and Ivanov, 1972; Kuroda et al., 1991). In female cells both X chromosomes remain active, a situation that is mimicked in male cells by an increased transcription of the X chromosomal genes (Lucchesi and Manning, 1987). The loss of function of the Mle protein is lethal for males, whereas neither viability nor fertility of females is affected. Although the Mle RNA helicase is expressed in both sexes, it has been shown that only in male cells it is predominantly associated with the X chromosome, suggesting that Mle regulates the expression of genes linked to the X chromosome (Kuroda et al., 1991). *In vitro* studies show that a mutant of Mle (*mle*-GET) with abolished NTPase and helicase activity is still able to bind ssRNA and ssDNA. *In vivo*, the mutated protein *mle*-GET is still localized on the male X chromosome, but complementation of mle^{1} mutant males failed. Thus, NTPase and helicase activity of the Mle protein are not necessary for chromosomal association, but are required for dosage compensation perhaps by altering the structure of the nascent RNA, which could enhance transcription (Lee et al., 1997). Interestingly, polyclonal antibodies against the *Drosophila* Mle protein recognize RNA helicase A from *HeLa* cells. In addition to the immunological cross-reaction, RNA helicase A shares a 85% homology with Mle (Lee and Hurwitz, 1993). Based on this extensive similarity a comparable role of RNA helicase A has been assumed. In contrast to *Drosophila*, only one copy of the X chromosome transcriptionally is active in mammalian females. The remaining X chromosomes are localized in the Barr bodies where most of the X chromosome-linked genes are inactive due to a facultative heterochromatic structure of Barr bodies. Interestingly, it has been shown that the gene *XIST*, which is localized on the putative inactivation center of the X chromosome, is expressed exclusively from the inactive X chromosome (Brockdorff et al., 1992; Brown et al., 1992). As the gene encodes no open reading frame, it has been suggested that the *XIST* transcripts directly

inhibit the X chromosome. Because RNA helicase A is able to dissolve as well as to generate intra- and intermolecular double-stranded structures, it might be involved in X chromosome inactivation by interacting with the *XIST* transcripts (Lee and Hurwitz, 1992; Lee and Hurwitz, 1993).

In *Xenopus laevis* the mRNA of the RNA helicase An3 has been detected exclusively in the pole cells of oocytes and in the early embryo where the mRNA is uniformly distributed. In adult tissues only a reduced expression could be demonstrated (Gururajan et al., 1991). The subcellular localization of the An3 protein by immunohistochemical analysis indicates that the RNA helicase may be involved in the processing or production of mature rRNA during oogenesis (Gururajan et al., 1994). Recently, An3 was shown to possess AtPase activity, although full acitivity could not be stimulated by any of the various RNAs tested, indicating the requirement for a specific RNA substrate (Gururajan and Weeks, 1997).

RNA helicase Pl10 from mouse is expressed exclusively in male tissues. High expression of the *PL10* transcript has been detected during meiotic and haploid stages of spermatogenesis (Leroy et al., 1989). Interestingly, Pl10 is able to replace the DED1 gene in yeast that plays a role in translation initiation (Chuang et al., 1997). These data and the sequence homology to the translation initiation factor eIF-4A gave rise to the speculation that the helicase Pl10 supports translation of specific transcripts that are expressed during spermatogenesis (Leroy et al., 1989; Chuang et al., 1997).

The gene *GLH-1* (for germ-line helicase), isolated from the nematode *Caenorhabditis elegans* by PCR, displays a high similarity to the genes *vasa* and *PL10*. According to expression patterns, RNA helicase Glh-1 plays a role in cell cycle development. The *GLH-1* transcript could not be detected in the embryo or the first two larval stages, but expression occurs in the third and fourth larval stages. The highest expression has been found in adult tissues, but so far no precise function could be attributed to Glh-1 (Roussell and Bennett, 1993).

J. Viral RNA Helicases

RNA helicases of superfamily II have been also identified in several viral genomes. They have been found in double-strand DNA viruses such as vaccinia virus, as well as in the group of positive-stranded RNA viruses such as the Hepatitis C virus and the family of poty- and togaviruses (reviewed by Koonin, 1991; Kadaré and Haenni, 1997). Sequence analyses showed that the isolated RNA helicases of superfamily II belong to the DEXH group. Comparison with other RNA helicases points to a closer relationship with the DEAH box proteins Prp2, 16, and 22 than to eIF-4A (Koonin, 1991).

In the vaccinia virus, which is a double-stranded DNA virus, only a single RNA helicase has been identified. The 18R gene encodes the nucleoside triphosphate phosphohydrolase II (NPH-II) with an NTPase activity that is stimulated by both single-stranded DNA and RNA (Paoletti et al., 1974; Shuman, 1992). NPH-II exhibits an NTP-dependent RNA helicase activity as well as a DNA helicase activity (Bayliss and Condits, 1996; Shuman, 1993). Temperature-sensitive mutants of 18R are neither affected in protein synthesis nor in DNA replication or morphogenesis, which makes it less likely that 18R has a function in DNA replication. Interestingly, the formed virions are not infectious. This phenotype indicates a defect in the early stages of virus propagation such as early transcription (Fathi and Condit, 1991a,b). Because of its RNA helicase activity, NPH-II is thought to be involved in the export of mRNA from the virus core during early transcription (Bayliss and

Condits, 1996; Shuman, 1993). Because the RNA helicase has an additional DNA helicase activity, it is also possible that the 18R protein enhances initiation of transcription by melting double-stranded DNA. Whether DNA or RNA helicase activity or both activities are essential remains to be seen (Bayliss and Condits, 1996). Vaccinia virus has three other genes, A18R, D6R, and D11L, containing helicase-typical motifs such as the DEXH box (Gorbalenya and Koonin, 1989; Koonin, 1992), but no RNA helicase activity could be demonstrated for these proteins (Koonin and Senkevich, 1992). Based on the homology of A18R to the DNA helicases ERCC3 and RAD25, the A18R product is suggested to unwind DNA duplex structures (Koonin and Senkevich, 1992; Pacha et al., 1990). D6R and D11L show a DNA-dependent ATPase activity and, like NPH-II, seem to be implicated in early transcription. D6R forms a subunit of the early transcription factor VETF and might be responsible for the DNA-stimulated ATPase activity of the transcription factor (Gershon and Moss, 1990). D11L is associated with a RNA polymerase complex that is involved in the transcription of early expressed genes (Broyles and Moss, 1987).

In another DNA virus, the African swine fever virus (ASFV), two genes have been identified that encode putative helicases. Both genes are expressed during the later stages of infection. Sequence comparisons show that the gene termed D1133L is homologous to the vaccinia genes D11L and D6R. In contrast, the open reading frame of the second gene, B962L, resembles 18R of vaccinia as well as the yeast PRP genes 2, 16, and 22 of the DEAH subgroup (Yánez et al., 1993). In view of the different expression patterns of the ASFV proteins compared with the vaccinia helicases, the role of the putative ASFV-helicases is still unclear.

Among the positive-strand RNA viruses, RNA helicases have been identified in hepatitis C virus (HCV), bovine viral diarrhoea virus (BVDV), plum pox potyvirus (PPV), and rubella virus. The genome of the hepatitis C virus codes for a single polypeptide that is subsequently cleaved by cellular and viral proteases. The amino-terminal region contains structural proteins, while the carboxy-terminal domain accommodates proteins that have a function in viral replication (Houghton et al., 1994). This includes the NS3 protein, which contains, in addition to the protease domain, a carboxy-terminal ATPase/RNA helicase region. The carboxy-terminal part of the NS3 protein exhibits an ATP-dependent RNA helicase activity as demonstratred by *in vitro* studies (Jin and Peterson, 1995; Kim et al., 1995). Similarly, the NS3 protein of the animal pathogenic virus BVDV shows an RNA helicase activity *in vitro* (Warrener and Collett, 1995). The potyviral CI protein (for cylindrical inclusion) has a helicase domain at the amino-terminal end. The complete CI protein as well as a recombinant fragment containing deletions in the carboxy-terminal region exhibit RNA helicase activity (Fernández et al., 1995; Lain et al., 1990). Also for the above-mentioned rubella virus an RNA-dependent ATPase activity has been described (Gros and Wengler, 1996). As positive-strand RNA viruses contain a single-stranded RNA genome, it has been suggested that RNA helicases are generally essential enzymes for these organisms (Fernández et al., 1995; Jin and Peterson, 1995; Kim et al., 1995; Lain et al., 1990).

VII. SUMMARY

RNA helicases are ubiquitously distributed over a wide range of organisms. Com-

parisons of the conserved sequences of the DEAD and DEAH box proteins reveal a close relationship between them and suggest that these proteins might be derived from a common ancestor. Although an RNA helicase activity has only been demonstrated for a few examples, yet, it is generally assumed that all members of the DEAD and DEAH box family exhibit this activity. Biochemical studies revealed a strong dependence of RNA helicase activity on ATP hydrolysis. Based on similar results for different RNA helicases and the high sequence homology of the proteins, it is believed that their action can be principally characterized by the following general mechanism: The initial binding of ATP induces a conformational change of the RNA helicase that allows subsequent binding of RNA. Following RNA binding the hydrolysis of ATP provides the energy required for unwinding the double-stranded RNA.

The RNA helicases characterized so far seem to be involved in a variety of cellular processes such as spliceosomal assembly and disassembly, nuclear and mitochondrial splicing processes, and RNA editing. They interact with RNA during ribosomal biogenesis, rRNA processing, translation initiation, nuclear mRNA export, and mRNA degradation. Furthermore, RNA helicases are involved in the regulation of maternally expressed mRNA, as well as in mRNA expression during cell development and differentiation. Some viral helicases play a role in transcription and in the replication of single-stranded RNA genomes. Although RNA helicases share a high degree of sequence homology, they differ in size as well as in their amino- and carboxy-terminal sequences. Those sequences most likely seem to control the specificity of RNA helicases. Based on this specificity for a certain RNA substrate and the wide distribution of RNA molecules in cellular processes, one can imagine that the family of RNA helicases is still growing, and a large number of new members will be added over the next few years. For instance, searching the complete *Saccharomyces cerevisiae* genome data base reveals more than 50 genes encoding putative RNA helicases. But so far only about half of them have been analyzed for their biological functions and only five were shown to express specific RNA unwinding acitivty.

Many of the known RNA helicases appear to be essential for their organism. Some mutations are lethal, as with the translation initiation factor eIF-4A, or they lead to phenotypical changes, as with proteins involved in processing of ribosomal, nuclear, or mitochondrial RNA. These results indicate a key role for RNA helicases in the RNA metabolism.

For many of the RNA helicases described so far, a specific function was initially proposed predominantly based on their high sequence homology to other often only partly characterized helicases. However, in many cases, when a detailed molecular analysis was performed the proposed function turned out to be different or more complex. For example, RNA helicase A from *HeLa* cells, a homologue of the *Drosophila* Mle protein (see the *Cell Differentiation and Development* section) was also assumed to be involved in X chromosome inactivation (Lee and Hurwitz, 1993). However, recent data clearly revealed a role in nuclear RNA export (Tang et al., 1997). Therefore, in future work it will be important to identify and characterize specific substrates, interacting proteins, and cofactors of RNA helicases to precisely elucidate their specific function in RNA metabolism. It might be also possible to identify specific RNA structures and dynamic conformational changes essential to their reactions. Last but not least, the observation that mutations in a viral RNA helicase gene (18R; see the *Viral RNA Helicases* section) inhibit the infectivity of vaccinia virus

suggests that one or other member of the DEAD box proteins family might also have some therapeutic potential.

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