RNA Biology 10:1, 4–18; January 2013; © 2013 Landes Bioscience

DExD/H-box RNA helicases in ribosome biogenesis

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Keywords: RNA unwinding, snoRNA, RNP, translation, ATPase

Abbreviations: ATP, adenosine triphosphate; cDNA, complementary DNA; CRAC, UV crosslinking and analysis of cDNA; *E. coli*, *Escherichia coli*; ETS, external transcribed spacer; G-patch, glycine-rich domain; ITS, internal transcribed spacer; kDa, kilodalton; LSU, large subunit; MDa, megadalton; mRNA, messenger RNA; NMD, nonsense-mediated mRNA decay; NTP, nucleoside triphosphate; Pol, polymerase; rDNA, ribosomal DNA; RNAi, RNA interference; RNase, ribonuclease; RNP, ribonucleoprotein particle; RP, ribosomal protein; rRNA, ribosomal RNA; RT-PCR, real-time polymerase chain reaction; siRNA, small interfering RNA; snoRNA, small nucleolar RNA; snoRNP, small nucleolar ribonucleoprotein particle; snRNA, small nuclear RNA; snRNP, small nuclear ribonucleoprotein particle; SSU, small subunit

Ribosome synthesis requires a multitude of cofactors, among them DExD/H-box RNA helicases. Bacterial RNA helicases involved in ribosome assembly are not essential, while eukaryotes strictly require multiple DExD/H-box proteins that are involved in the much more complex ribosome biogenesis pathway. Here, RNA helicases are thought to act in structural remodeling of the RNPs including the modulation of protein binding, and they are required for allowing access or the release of specific snoRNPs from pre-ribosomes. Interestingly, helicase action is modulated by specific cofactors that can regulate recruitment and enzymatic activity. This review summarizes the current knowledge and focuses on recent findings and open questions on RNA helicase function and regulation in ribosome synthesis.

Introduction

Ribosomes mediate the translation of the nucleotide sequence of mRNAs (mRNAs) into the amino acid sequence of proteins and are essential in all forms of life. They sediment at 70S in bacteria and 80S in eukaryotes, and consist of a small and a large subunit of 30S and 50S in bacteria and of 40S and 60S in eukaryotes. Bacterial ribosomes contain 21 ribosomal proteins (RPs) and the 16S rRNA (rRNA) in the small subunit (SSU), and 34 RPs as well as the 5S and 23S rRNAs in the large subunit (LSU; reviewed in ref. 1). In eukaryotes, the composition, assembly and function of ribosomes in mitochondria and chloroplasts are thought to be similar to bacteria, while the biogenesis and function of cytoplasmic ribosomes is much more complex.[2,3] Ribosomes of the eukaryotic type contain additional RPs and sequence elements in the rRNAs, termed eukaryotic expansion segments, leading to the higher molecular weight of the ribosomal subunits. The SSU consists of the 18S rRNA as well as 32 or 33 RPs in yeast or mammalian cells, respectively, while the LSU contains 46 RPs and the 25S/28S, 5.8S and 5S rRNAs. In all domains of life, most (or in bacteria all) rRNAs are initially part of a large primary transcript, which is processed in multiple steps to derive the mature rRNAs that form a large part of the catalytic core of the ribosome.[1,3,4] The processing of the rRNAs and the assembly of the ribosomal subunits requires a number of non-ribosomal cofactors, such as nucleases, GTPases, RNA modifying enzymes and RNA helicases.

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Submitted: 07/05/12; Revised: 08/13/12; Accepted: 08/18/12
http://dx.doi.org/10.4161/rna.21879

RNA helicases bind and remodel RNA and RNP complexes in an ATP- (or NTP-) dependent manner (reviewed in refs. 5–7). The proteins possess a structurally conserved helicase core containing characteristic sequence elements and structural motifs. Additional domains are thought to confer specificity by recruitment to target RNPs, while the conserved helicase core interacts with RNA in a sequence-independent manner. Some DExH-box proteins have been suggested to act processively and translocate along RNA, whereas DEAD-box proteins are thought to mediate duplex unwinding by local strand separation or to act as RNA clamps in annealing (see refs. 8 and 9).

This review focuses on the DExD/H-box proteins involved in the biogenesis of ribosomal subunits in bacteria and eukaryotes, as RNA helicase function in the archaeal pathway has remained unexplored so far. It is believed that most of them act as ATP-dependent RNA helicases that function in unwinding of double-stranded RNA, assist dissociation of RNA-binding proteins or mediate structural remodeling of pre-ribosomal complexes. We discuss the current knowledge on DExD/H-box proteins involved in ribosome synthesis in bacteria and eukaryotes, with focus on major advances in recent years and on key questions regarding RNA helicase function and regulation in this pathway.

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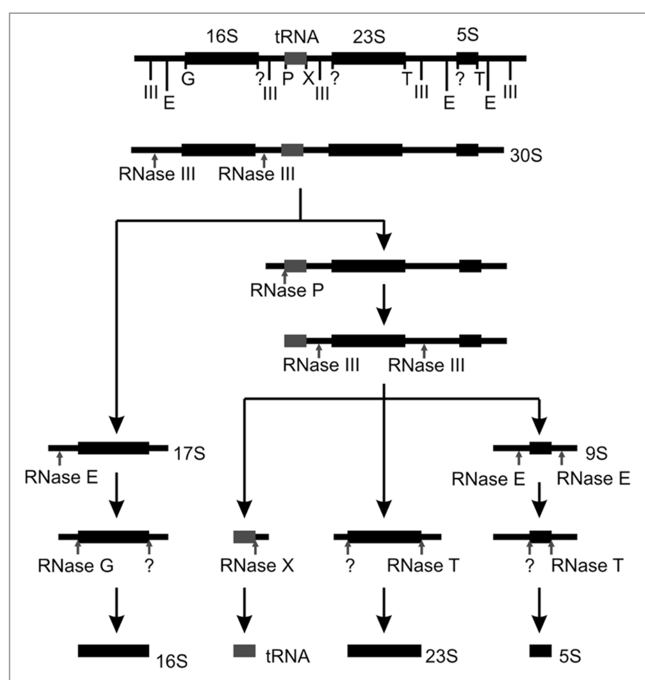

Figure 1. Processing of rRNA in bacteria. The schematic representation shows the rRNA processing pathway in *Escherichia coli*. Cleavage sites and the corresponding nucleases are indicated. Adapted with permission from Shajani, et al.[1]

RNA Helicases in Bacterial Ribosome Assembly

In bacterial ribosome assembly, a single primary transcript contains the sequences of all mature rRNAs (**Fig. 1**).[1,4] The transcript is cleaved by RNase III into the 17S, 23S and 9S pre-rRNAs and these are further processed. The mature 16S rRNA is obtained after cleavages by RNase E and G at the 5'-end and by an unknown RNase at the 3'-end. 5'-end processing of the 5S and 23S rRNAs is mediated by an unknown RNase (in case of 5S possibly by RNase E), while the 3'-ends are generated by RNase T (**Fig. 1**). A set of additional cofactors facilitates bacterial ribosome assembly in vivo (reviewed in ref. 1), but, interestingly, biologically active ribosomal subunits could be assembled in vitro from purified components.[10,11]

Bacterial ribosome assembly is best understood in *Escherichia coli*.[1,12] Although none of the five DEAD-box RNA helicases found in *E. coli* is essential, four of them have been implicated in ribosome assembly.

The RNA helicase SrmB was originally identified as a multicopy suppressor of a temperature-sensitive (ts) mutation in the essential RP L24.[13] It was further shown to directly and cooperatively interact with the RPs L4, L24 and with 23S rRNA, indicating a mode of early recruitment into 40S particles, which are considered as LSU precursors.[14] Deletion of the gene encoding the DEAD-box RNA helicase SrmB results in defects in LSU assembly, causing a reduced growth rate at low temperatures.[15] SrmB is recruited through interactions with L4, L24 and 23S rRNA (domain I), and the helicase is required for incorporation of the RP L13 in early stages of LSU assembly. Using rRNA mutants the binding site of SrmB on the rRNA was, however, mapped to sequences in domain II of the 23S rRNA,[16] indicating that SrmB might act in a different region of the pre-ribosome than it is originally recruited to.

The cold-shock DEAD-box protein A (CsdA/DeaD) was identified as a suppressor of temperature-sensitive mutations in rpsB, the gene encoding the ribosomal protein S2.[17] The expression of CsdA is specifically induced at temperatures below 20°C to facilitate LSU assembly.[18] At low temperatures, lack of CsdA leads to slow growth and accumulation of 40S precursor particles.[19,20] CsdA binds 50S precursors and was suggested to facilitate late steps of LSU assembly. It was further shown to possess RNA helicase activity in vitro.[18] Interestingly, overexpression of CsdA could rescue defects caused by deletion of SrmB, which is thought to act earlier in the pathway.[19] Conversely, increased levels of the RNA helicase RhlE can compensate for loss of CsdA at low temperatures, while RhlE has the opposite effect on srmB deletions.[21,22]

Deletion of the dbpA gene, encoding another RNA helicase, shows no growth defect,[20] while mutation of the arginine finger (R331A) in the active site strongly reduces growth and shows dominant negative effects that lead to accumulation of 45S LSU intermediates.[23,24] *E. coli* DbpA and the related *Bacillus subtilis* YxiN were extensively characterized in vitro and mutations, such as R331A in DbpA, were shown to strongly reduce ATPase and helicase activity (see for example refs. 23, 25 and 26). Interestingly, DbpA was shown to specifically recognize helix 92 in the 23S rRNA.[27] In turn, the rRNA sequence of this location specifically stimulates ATPase activity of the RNA helicase and mutations in the sequence reduce ATPase stimulation, suggesting a sequence-specific recognition of this RNA. To our knowledge, the related RNA helicases DbpA and YxiN are the only DEAD-box RNA helicases for which sequence-specific stimulation of helicase activity has been described so far.

Eukaryotic organelles, like mitochondria and chloroplasts, are thought to possess a ribosome assembly pathway similar to that in bacteria. To date, only few RNA helicases involved in organellar ribosome biogenesis have been identified and the proteins remain to be further functionally analyzed.[28,29]

RNA Helicases Involved in Ribosome Biogenesis in *Saccharomyces cerevisiae*

In cytoplasmic ribosomes of eukaryotes, three of the four rRNAs, the 18S, 5.8S and 25S/28S rRNAs, are derived from a common RNA Polymerase I (Pol I) transcript, the 35S pre-rRNA in yeast, while the 5S rRNA is transcribed separately by Pol III (reviewed in ref. 2, 3 and 30–32). Up to 2000 ribosomes are made per minute in exponentially growing yeast cells, and 60% of the cellular transcription activity was estimated to supply this pathway.[33]

Ribosome synthesis in eukaryotes involves a plethora of cofactors. The pathway is best understood in the yeast *Saccharomyces cerevisiae*, where more than 200 protein cofactors and 75 small nucleolar RNAs (snoRNAs) are recruited to various intermediates.[3,30] The biogenesis of ribosomes is initiated by the nucleolar

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transcription of the 35S pre-rRNA and co-transcriptional assembly of the 90S pre-ribosome, which contains a set of RPs and various cofactors. Besides the sequences of the 18S, 5.8S and 25S rRNAs, the 35S pre-rRNA includes the 5'- and 3'-external transcribed spacers (ETS) as well as the internal transcribed spacers (ITS) 1 and 2 (**Fig. 2**), which contain processing sites for multiple exo- and endonucleases.[3] Early cleavages occur at the sites A_0 and A_1 followed by cleavage at A_2, which leads to separation of the biogenesis pathways of the two ribosomal subunits. The A_2 cleavage releases the 20S pre-rRNA in the pre-SSU and can already occur cotranscriptionally.[34,35] Further maturation of the SSU involves the endonucleolytic cleavage of the 20S pre-rRNA to generate the 18S rRNA in the cytoplasm, while processing of the $27SA_2$ intermediate in the pre-LSU continues in the nucleus following either of two alternative pathways that lead to the generation of export-competent pre-60S particles containing the 25S and 5S mature rRNAs and the long or short form of the 6S pre-rRNA, which is matured to the $5.8S_{L/S}$ rRNA in the cytoplasm (**Fig. 2**; reviewed in refs. 2, 3 and 30). In addition to the complex processing pathway, the rRNA undergoes an extensive series of modifications, most of which are predicted to occur cotranscriptionally. The majority of modifications are mediated by small nucleolar RNPs (snoRNPs), which contain snoRNAs that basepair with the cognate sequence of the pre-rRNA flanking the modification site and thereby guide the enzymes to their target sites (reviewed in ref. 36). snoRNPs can catalyze 2'-hydroxyl methylation (box C/D snoRNPs) or pseudouridylation (box H/ACA snoRNPs) of specific residues, or snoRNA basepairing facilitates the processing of the pre-rRNAs.[37,38] The duplex of snoRNAs and pre-rRNA can involve more than 20 nucleotides, and due to the expected stability of these helices release of snoRNPs from pre-ribosomes was predicted to require the action of proteins, such as RNA helicases.[30,36,39,40] In addition, RNA helicases were suggested to mediate structural remodeling of ribosomes, including the rearrangement of RNA secondary structures and allowing access or release of RNA binding proteins.

Phenotypic classification of RNA helicases involved in ribosome biogenesis. First evidence for RNA helicase involvement in the process of ribosome biogenesis in yeast was found in 1992 by the analysis of cold-sensitive mutant strains, which revealed Drs1 as an RNA helicase that affects pre-rRNA processing.[39,41] To date, 19 putative RNA helicases are thought to directly participate in ribosome synthesis in yeast. With the exception of Mtr4, they all are members of the helicase superfamily 2 (see refs. 3 and 5). Initial description of pre-rRNA helicases mostly included localization or cell fractionation studies and mapped them predominantly to the nucleolus, the primary site of ribosome biogenesis and the nucleus. Further characterization involved deletion/depletion experiments and analysis of strains bearing mutations in conserved DExD/H motives, some of which displayed temperature dependent growth phenotypes (see **Table 1**; reviewed in refs. 3, 5 and 32). Results demonstrated that all RNA helicases involved in ribosome biogenesis are essential for cell viability, except for Dbp2, Dbp3 and Dbp7, where genetic deletion causes growth retardation (see **Table 1** and refs. 42–44). Density gradient centrifugation followed by polysome profiling as well as pulse-chase and northern blot analysis of RNA helicase depleted cells elucidated RNA helicase influence on precursor and mature rRNA formation and constituted the basis for classification. Seven RNA helicases (Dbp4, Dbp8, Dhr1, Dhr2, *Fal1*, Rok1, Rrp3) have been implicated in SSU biogenesis, while ten (Dbp2, Dbp3, Dbp6, Dbp7, Dbp9, Dbp10, Drs1, Mak5, Mtr4, Spb4) participate in synthesis of LSU precursors, and two RNA helicases (Prp43, Has1) contribute to the biogenesis of both subunits (see **Table 1**).

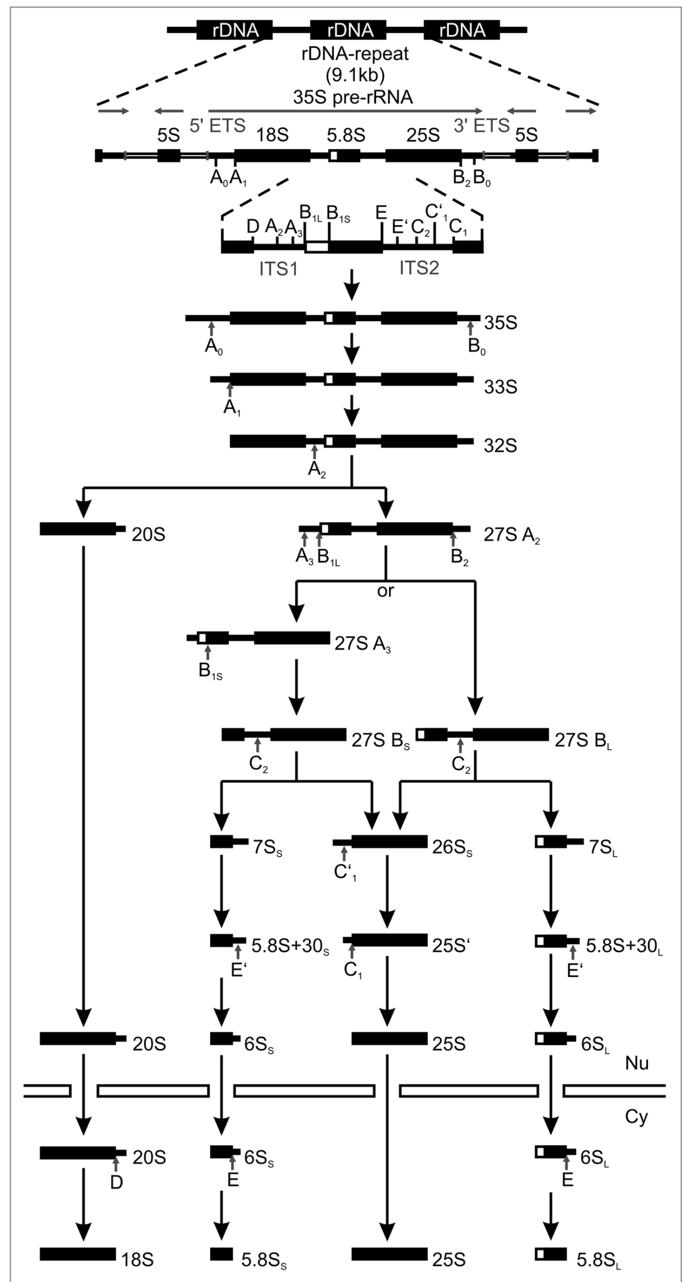

Figure 2. Processing of rRNA in *Saccharomyces cerevisiae.* The schematic representation shows the rRNA processing pathway in yeast. The direction of transcription is indicated for a rDNA repeat by horizontal arrows and processing sites are marked by small vertical arrows. The nuclear envelope is represented by horizontal bars, and nucleus (Nu) and cytoplasm (Cy) are indicated.

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Table 1. RNA helicases involved in ribosome biogenesis in *Saccharomyces cerevisiae*

Name				Phenotype		90S		(pre-)60S						(pre-)40S			Processing Inhibition						Ref.
Name	System name	Type	Alias	Polys. profile	Subunit	35S	32S	27SA2	27SA3	27SB	25S	7S	5.8S	23S*	20S	18S	A0	A1	A2	A3	C2	E	
DBP2	YNL112W	DEAD	-	hm	L	↑		↘			↓					↘							42, 43, 161
DBP3	YGL078C	DEAD	-	hm	L	↑		↑			←		-	↗			Y	Y	Y	X			54
DBP4	YJL033W	DEAD	ECM24 HCA4	-	S	↑		↓						↑	↓	↓	X	X	X				47, 49, 133
DBP6	YNR038W	DEAD	-	hm	L		↘	↓		↓	↓	↓	↓	↑	↘	↘	←	←	←				51, 53
DBP7	YKR024C	DEAD	-	hm	L	↗	↓	↓	↓	↓	↓	↓	↓	↑	↓	↘	←	←	←				52
DBP8	YHR169W	DEAD	-	red. 40S	S	↑	↓	↓						↑	↓	-	X	X	X				45, 76
DBP9	YLR276C	DEAD	-	hm	L	↗	↓	↓	↓	↓	↓		↓	↗	↓	↘	←	←	←				51, 113
DBP10	YDL031W	DEAD	-	hm, 66S	L	↑		↓		↓	↓	↓	↓	↑	↓		Y	Y	Y		Y		51, 58
DHR1	YMR128W	DEAH	ECM16	-	S	↗		↓						↑	↓	↓	Y	X	X				45, 46
DHR2	YKL078W	DEAH	-	-	S	↑	↓	↓						↑	↓	↓	X	X	X				45, 46
DRS1	YLL008W	DEAD	-	red. 60S hm	L						↓												51, 39, 41
FAL1	YDR021W	DEAD	-	red. 40S	S	↑		↓						↑	↓	↓	X	X	X				45, 48
HAS1	YMR290C	DEAD	-	red. 40S	S/L	↑		↓	↑	↑		↘		↑	↓	↓	X	X	X		Y		51, 63
MAK5	YBR142W	DEAD	-	hm	L	↑		←		←	←		←										51, 55
MTR4	YJL050W	DEVH	DOB1	hm	L	↑					↘	↑	↓	↑		↘	←	←	←			X	51, 126
PRP43	YGL120C	DEAH	JA1	-	S/L	↑		↓		↓	↓	↓	↓	↑	↓	↓		Y	Y				60, 61
ROK1	YGL171W	DEAD	-	-	S	↗	↓	↓						↑	↓	↓	X	X	X				80
RRP3	YHR065C	DEAD	-	-	S	↗		↓				↑		↑	↓	↓		X	X				45, 50
SPB4	YFL002C	DEAD	-	hm, 66S	L	↑		↓		↑	↓	↘	↘	↑	↓	↘	←	←	←		X		51, 56, 57, 59

↑	accumulation	↗	mild accumulation	-	reduction	↘	mild reduction
←	delay	X	processing defect	Y	mild processing defect		

Changes in precursor levels compared with wildtype are given if analyzed. If no analysis of steady-state levels was available, pulse-chase results were taken into account. Systematic names (System. name) are given according to the Saccharomyces Genome Database (SGD). S, small subunit; L, large subunit; hm, halfmer polysomes; red, reduction. The asterisk indicates that 23S is considered an aberrant intermediate.

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Involvement of RNA helicases in early stages (90S) and 40S biogenesis, for example of Dbp4, Dhr1, Dhr2, *Fal1* and Rrp3, typically coincides with defects in processing at the sites A_0, A_1 or in ITS1.[45-50] This results in a reduction in 18S rRNA and its direct precursor 20S as well as in enrichment of the 35S pre-rRNA and of aberrant intermediates, such as 23S and 21S pre-rRNAs. These aberrant pre-rRNAs are probably not processed further but rather targeted for degradation. 23S appears after cleavage at site A_3 under conditions where processing at sites A_0 to A_2 is compromised. Besides the 27SA$_2$ pre-rRNA, none of the LSU precursors is usually significantly affected by SSU helicase impairment, as cleavage at site A_3 rescues LSU biogenesis.

The deletion or depletion of LSU helicases causes different phenotypes in pre-rRNA processing. Most LSU helicases, such as Dbp2, Dbp3, Dbp6 and Dbp7 seem to act at early or intermediate stages of 60S biogenesis. Besides reduction of mature rRNAs (25S and 5.8S), these helicases generally cause some accumulation of SSU precursors and 23S as well as a minor reduction in 18S rRNA.[43,51-54] These processing phenotypes are sometimes similar to defects observed for RNA helicases involved in formation of the SSU, though usually not as drastic. However, their primary implication into maturation of the LSU is in most cases clearly demonstrated by a decrease of mature 60S subunits in polysome profiles. In contrast to the other RNA helicases, deletion of Dbp3 induces accumulation of 27SA$_2$ precursors and it was shown to facilitate cleavage at position A_3 by the RNase MRP.[54]

Mak5 has been described to bind to both early and late intermediates.[51,55] Depletion of the RNA helicase causes similar defects as those described for the early LSU helicases, however, no significant effects on SSU intermediates were observed. A different phenotype of processing defects in LSU assembly is caused by depletion of Spb4 or Dbp10.[56-58] This causes the characteristic accumulation of the 27SB rRNA precursor, which indicates defects in ITS2 cleavage at site C_2 and leads to their classification as late acting LSU RNA helicases. Spb4 was shown to be associated with both early and late pre-60S particles, and proposed to dissociate after 27SB processing.[59] These findings might suggest that Spb4 already binds to earlier intermediates, but that activation of the helicase occurs later to allow 27SB processing and its dissociation from pre-ribosomes.

Finally, depletion of the essential RNA helicases Prp43 or Has1 causes changes in the levels of rRNA intermediates and mature RNAs of the SSU and the LSU, indicating a dual role of these proteins in both pathways.[60-63]

RNA helicase functions in yeast ribosome biogenesis. Besides the classification of the RNA helicases based on pre-rRNA processing defects and polysome profiles, information on the molecular function of these proteins is mostly lacking. Analyses of the composition of pre-ribosomal complexes and protein interaction studies have mapped many helicases to distinct pre-ribosomal intermediates (see for example refs. 64–66). Some of these particles contain several RNA helicases and in a few cases, a subcomplex containing RNA helicases or direct interaction partners has been identified.

One of the most prominent complexes in ribosome biogenesis is the SSU processome (see ref. 67 and reviewed in ref. 68). This 2.2 MDa particle assembles cotranscriptionally from pre-formed subcomplexes, such as the UTP-A/tUTP, the UTP-B and the UTP-C complexes, as well as snoRNPs and individual proteins (see refs. 64, 69–72). Associated snoRNPs include the essential snR30, U3 and U14 snoRNPs, which are required for early cleavages of the pre-rRNA (reviewed in refs. 36 and 37). Among a multitude of other cofactors, the RNA helicases Dbp4, Dbp8, Dhr1, Has1, Dhr2, *Fal1*, Prp43, Rok1 and Rrp3 were found in the SSU processome. While Dhr1 was found to associate with Mpp10 as well as the U3 snoRNP and suggested to remodel the U3-pre-rRNA pseudoknot structure,[46,70] the closely related Dhr2 was found to interact with Utp25 and Nop19.[73] Utp25 is an essential RNA helicase-like protein that shows only limited conservation of the helicase core domain.[74,75] This domain seems to be important for Utp3/Sas10 interaction in the SSU processome, however, RNA helicase activity is unlikely since even the highly conserved residues of motif Ia, usually implicated in substrate binding, seem to be dispensable for Utp25 function.

Dbp8 is another DEAD-Box protein involved in maturation of the SSU.[76] Its cofactor Esf2, a nucleolar RNA binding protein, was shown to be associated with the U3 snoRNP.[77] The C-terminal domain of Esf2 allows Dbp8 binding and stimulates its ATPase activity.[78] Since Esf2 is suggested to bind preferentially to pre-rRNA within the 5'-ETS,[77] it was proposed that Esf2 not only stimulates the enzymatic activity, but could in addition recruit Dbp8 to its substrate pre-rRNAs.[78]

Several RNA helicases are required for the release of snoRNPs from pre-ribosomes (see ref. 36 and references therein). Density gradient centrifugation of cellular material and subsequent analysis of snoRNA association with pre-ribosomal particles by northern blotting or quantitative RT-PCR identified snoRNAs that accumulate on pre-ribosomes when specific RNA helicases are depleted. Systematic analysis of RNA helicases involved in SSU biogenesis showed that especially the essential snoRNAs require RNA helicases for their release from pre-ribosomal particles.[79] Depletion of the helicase Rok1 leads to pre-ribosomal accumulation of the essential snoRNA snR30, suggesting a role of Rok1 in the release of the snR30-containing snoRNP.[79] Rok1 also genetically interacts with snR10 and Rrp5 and was reported to associate with the SSU processome in a Rrp5 dependent manner.[80-83] Interestingly, Rok1 was also identified as a high copy suppressor of Xrn1/Kem1 and to rescue the phenotype of Xrn1 deletions on pheromone induced nuclear fusion.[84] The cell cycle-dependent expression of Rok1 is regulated by an upstream open reading frame in the Rok1 5'-UTR.[85]

The RNA helicase Dbp4 was shown to be required for release of U14 and snR41 snoRNAs from pre-ribosomes.[47] This finding is in line with data that show suppression of U14 mutations in the Y-domain upon Dpb4 overexpression.[49] Interestingly, it was further shown that depletion of Has1 causes retention of U3, U14 and some additional modification guiding snoRNAs on pre-ribosomal subunits.[86] Quantitative RT-PCR analysis of snoRNA association to pre-ribosomal particles after Dbp4 or Has1 depletion, however, only confirmed an implication of both RNA helicases in association of U14 snoRNA.[79] Has1 was further suggested to play a role in U6 snRNP biogenesis, as depletion of Has1 leads to the reduction of cellular U6 snRNA levels.[86]

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One of the best studied RNA helicases acting in ribosome synthesis is the DEAH-box protein Prp43. It was originally identified as a helicase involved in spliceosome disassembly and intron lariat release.[87] Later on, its involvement in the biogenesis of both ribosomal subunits was shown,[60-62] but the molecular functions remained elusive. Recently, the application of the UV crosslinking and analysis of cDNA (CRAC) approach (see refs. 88 and 89) identified a number of putative interaction sites of Prp43 on pre-rRNAs and other RNA species.[90] Prp43 was found to crosslink to sites both in the SSU and in the LSU. Interestingly, Prp43 is required for the release of a subset of box-C/D snoRNAs (snR39, snR39b, snR50, snR59, snR60 and snR72) from pre-ribosomes, which guide the modification of the pre-rRNA at a cluster of methylation sites in helices 32 to 35 of the 25S rRNA sequence. Moreover, Prp43 crosslinks to snoRNAs guiding methylation in this cluster and to their basepairing sites in the pre-rRNA, suggesting a direct involvement of the RNA helicase in snoRNA unwinding and supporting the proposed role of Prp43 in snoRNA recycling.[62,90] In addition, depletion of Prp43 not only leads to accumulation of snoRNAs, but the protein also supports association of the snoRNAs snR64 and snR67 with pre-ribosomal intermediates. These findings are in line with a previously observed defect in methylation of the 25S rRNA guided by snR64 in the Prp43-Q423N mutant background (see ref. 62) and could suggest a role for Prp43 in rearranging secondary structures in the pre-rRNA to allow snoRNA basepairing and recruitment of the snoRNPs.

The major interaction site of Prp43 in the SSU locates to helix 44, close to the 3'-end of the 18S rRNA.[90] Interestingly, genetic analysis linked Prp43 to the endonuclease Nob1, which mediates 3'-end formation of 18S in the cytoplasm, and is conserved in eukaryotes and archaea (see refs. 91–94 and references therein). Here, Prp43 has been suggested to remodel late pre-ribosomal particles in order to allow Nob1 cleavage, but both the Prp43 target site and the mechanism are controversially discussed and still remain to be elucidated.[91,92,94] The functions of Prp43 in ribosome synthesis thus not only involves its role in the release of snoRNPs, but probably also structural remodeling of pre-ribosomal particles to allow access of other snoRNPs and to facilitate pre-rRNA processing.

Another interesting aspect of Prp43 function is its interaction with various cofactors, mainly glycine-rich domain (G-patch) containing proteins. These proteins are thought to recruit Prp43 to its target RNPs and to regulate the activity of this versatile RNA helicase. In splicing, G-patch proteins were found to interact with Prp43 and another DEATH-box protein, Prp2.[95,96] Prp43 is recruited to spliceosomes by the G-patch protein Spp382/Ntr1 and the cofactor Ntr2, and its helicase activity can be regulated by interaction with Spp382.[97-99] Ribosome synthesis involves the two G-patch proteins Sqs1/Pfa1 and Pxr1/Gno1 and both were shown to interact with Prp43.[61,100,101] Sqs1 and Pxr1 are already recruited to 90S pre-ribosomes and Sqs1 was found in 40S as well as 60S precursors, indicating that this protein assists Prp43 in the maturation of both subunits. Sqs1 and Prp43 were also linked to Nob1 and the putative export adaptor Ltv1 for 20S to 18S processing and the final steps in SSU biogenesis.[92,101] In vitro binding and yeast two-hybrid analyses have shown that Sqs1 interacts with Prp43 via several domains and that it can stimulate the ATPase and helicase activity of Prp43.[101] Prp43 has recently been structurally analyzed, and its C-terminal oligonucleotide/oligosaccharide-binding domain was found to locate close to the RNA binding cavity and to interact with Sqs1.[102,103] Different G-patch proteins also seem to interact and regulate each other. Sqs1 was reported to interact with Spp382 in two-hybrid assays and lack of Pxr1 leads to a reduction in the levels of Sqs1.[101,104] Even though the regulatory network of interacting cofactors for Prp43 is not fully understood, it is tempting to speculate that Prp43 requires a number of cofactors to be able to act in such diverse functions as RNP disassembly after splicing, snoRNP release from pre-ribosomes and most likely the remodeling of pre-ribosomal particles. The cofactors seem to recruit Prp43 to its sites of action and regulate its catalytic activity and timing.

Besides Prp43, the RNA helicase Dbp2 has also been implicated in ribosome synthesis and mRNA metabolism.[43,105] Protein interaction analyses assigned Dbp2 as member of a protein complex involved in nonsense-mediated mRNA decay (NMD). Dbp2 was shown to directly interact with the RNA helicase Upf1 and suggested to act in the dissociation of the ribosome termination complex and thereby to facilitate decapping of mRNAs, besides a possible role in transcription regulation.[43,106]

As indicated before, the deletion of the DEAD-box protein Dbp3 leads to unusual rRNA processing defects, including the accumulation of the $27SA_2$ pre-rRNA.[54] The defects observed resemble those of RNase MRP inactivation, which could indicate that Dbp3 facilitates RNase MRP mediated pre-rRNA cleavage at site A_3 in ITS1.[54] Dbp3 was therefore suggested to modulate the secondary structure of a stable stem-loop near the A_3 processing site and to thereby allow the effective recruitment and function of the RNase MRP complex.[107]

Current knowledge on the three DEAD-box helicases Dbp6, Dbp7 and Dbp9 supports a model of interaction and partial cooperation of these RNA helicases in early steps of LSU biogenesis. Dbp6 is part of a 550 to 600 kDa subcomplex containing the ribosome synthesis cofactors Urb1/Npa1, Urb2/Npa2, Nop8 and Rsa3, which can associate independently of RNA.[108-111] Apart from these proteins, Dbp6 genetically interacts with Rsa1 and Urb2 and it is linked to Rpl3 and the RNA helicases Dbp7 and Dbp9.[52,108,112] Dbp6 and Dbp9 seem to be partially redundant, since Dbp9 can rescue several mutations in Dbp6.[113] Based on genetic interactions of Rpl3 with all components of the Dbp6-containing subcomplex as well as Dbp7 and Dbp9, the subcomplex was suggested to contribute to incorporation of Rpl3 into 60S intermediates.[108] As both Dbp6 and Dbp9 are essential, the two proteins can be expected to have distinct functions, which still remain to be elucidated.

Two of the helicases that were linked to ribosome synthesis, Sen1 and Mtr4, do not belong to the helicase superfamily 2. Sen1, an Upf1-like superfamily 1 helicase, is most likely not directly involved in the pathway, but affects ribosome synthesis by its roles in transcription termination and snoRNA biogenesis.[114-117] The RNA helicase Mtr4/Dob1 occurs free or as part of the TRAMP complex, which can add short 3'-oligoA tails to

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RNA and thereby target it for processing or degradation by the exosome.[118,119] Besides its function in unwinding of secondary structures in substrate RNAs, Mtr4 was also shown to regulate polyadenylation of substrates and even 3'-oligoA tail length.[120-122] Mtr4 and the TRAMP complex are required for multiple pathways in RNA/RNP biogenesis and turnover together with the exosome and in some cases other cofactors (see refs. 123–125). In ribosome biogenesis, the Mtr4 and the TRAMP complex are involved in nuclear steps of the 3'-end formation pathway of 5.8S rRNA as well as the degradation of aberrant intermediates and the 5'-A_0 spacer sequence.[126,127]

RNA Helicases in Human Ribosome Biogenesis

As in yeast, ribosome biogenesis in vertebrates is largely characterized by the sequential processing of the rRNA.[128] The genes coding for rRNA are present in multiple copies and in tandem arrays at the rDNA loci in the human genome.[129] Ribosome biogenesis is initiated by RNA Pol I mediated transcription of the 47S rRNA precursor from rDNA loci on the five acrocentric chromosomes in nucleoli, while the precursor of 5S rRNA is transcribed by Pol III in the nucleoplasm from a different genomic locus on chromosome 1. As described for yeast, the large primary transcript (47S pre-rRNA) contains 5'- and 3'-ETS sequences as well as ITS1 and ITS2 in addition to the sequences of the mature 28S, 18S and 5.8S rRNAs. The ITS and ETS sequences are removed during ribosome maturation by sequential nucleolytic cleavage steps (**Fig. 3**).[128] The nascent 47S pre-rRNA associates with a subset of ribosomal proteins, small nucleolar ribonucleoprotein particles (snoRNPs) and trans-acting factors to form a 90S pre-ribosomal particle. It is rapidly converted into the 45S pre-rRNA by cleavages at sites 01 and 02 that remove the 3'-ETS as well as part of the 5'-ETS. Further processing of the 45S pre-rRNA proceeds via alternative pathways generating different rRNA intermediates. Common to both pathways is the separation of the pre-40S particle from the pre-60S particle by cleavage at site 2 located in ITS-1.[128] The pre-rRNA component of the small ribosomal subunit is converted into the 18SE intermediate that gives rise to mature 18S rRNA after export from the nucleus into the cytoplasm. The 32S pre-rRNA species of the large ribosomal subunit is processed into the mature 28S and 5.8S rRNA (**Fig. 3**), where 5.8S is formed via 12S and 7S rRNA intermediates. Incorporation of the 5S rRNA into the 60S particle occurs in the nucleus.

Several RNA helicases have been linked to ribosome biogenesis in vertebrates, but only few have been characterized in more detail so far. Since the ribosome biogenesis pathway per se is expected to be highly conserved among eukaryotes, various mammalian RNA helicases have been determined as homologs of yeast helicases and are generally expected to fulfill similar functions.[130] This has been experimentally supported in some cases, such as DDX10 (homolog of Dbp4 in yeast), DDX24 (Mak5), DDX47 (Rrp3), DHX37 (Dhr1) and SK2L2 (Mtr4) (see **Table 2**).[131-134] The concept has to be taken with a grain of salt, however, as in other cases localization or knockdown experiments with human proteins could not confirm the yeast function

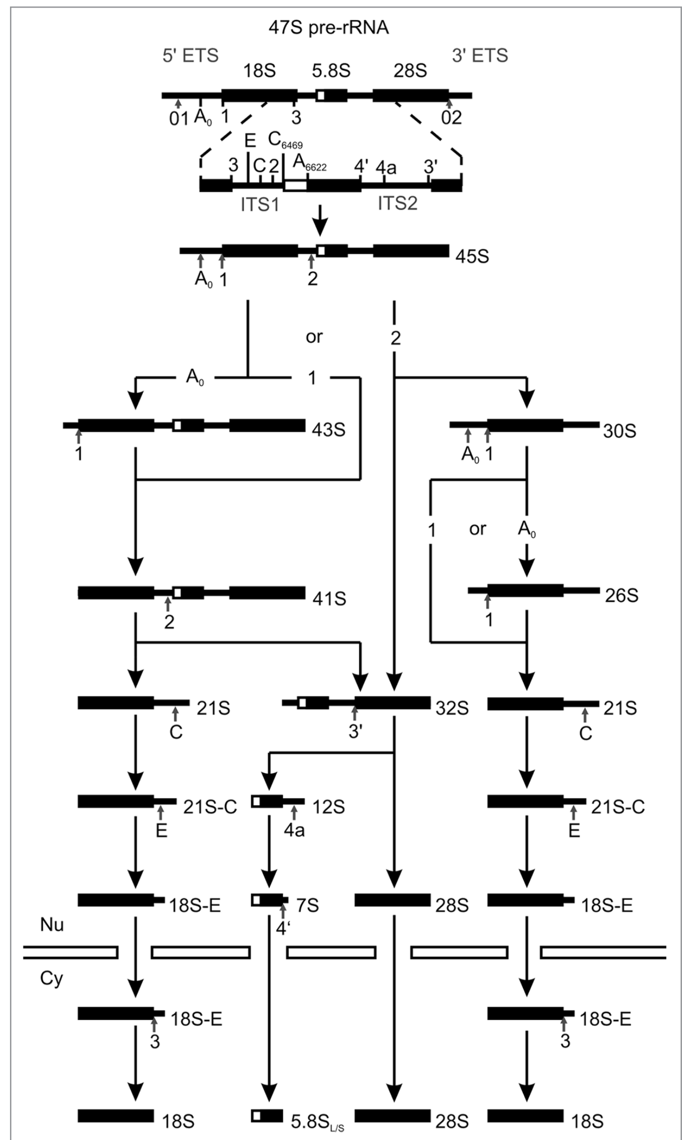

Figure 3. Processing of rRNA in human cells. The schematic representation shows the rRNA processing pathway in human cells. Processing sites are marked by vertical arrows. The nuclear envelope is represented by horizontal bars, and nucleus (Nu) and cytoplasm (Cy) are indicated. Modified with permission from Millineux et al.[128]

for the potential human homologs (see for example refs. 131 and 135). Conversely, lack of evidence using a single assay certainly does not prove that a protein is not involved in the pathway. In addition, several yeast RNA helicases have more than one homolog in mammals, and there are also mammal-specific RNA helicases implicated in ribosome biogenesis that have no counterpart in yeast.[130,136]

Proteomic analysis identified more than 30 putative RNA helicases in the human nucleolus.[137-139] Among a multitude of other proteins, 23 putative RNA helicases were recently screened for involvement in ribosome biogenesis by RNA interference (RNAi) in stable cell lines expressing GFP-tagged ribosomal proteins or a ribosome biogenesis cofactor and using localization of the reporters as readout.[131] Seven helicases were found to have an impact on

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Table 2. RNA helicases implicated in ribosome biogenesis in human cells

Name	Type	Aliases	Yeast homol.	Loc.	(Proposed) function	Putative cofactors	Ref.
DDX3X	DEAD	DBX; DDX3; HLP2; DDX14	Dbp1	Nu Cy	rRNA transcription		135, 138, 176
DDX5	DEAD	p68; HLR1; G17P1	Dbp2	No	rRNA transcription; 5.8S maturation; U8 snoRNA release during pre-32S cleavage	NPM	138, 160-162, 164
DDX10	DEAD	HRH-J8	Dbp4	No	rRNA transcription; component of SSU processome; SSU biogenesis		131, 133, 135, 138
DDX17	DEAD	P72; RH70	Dbp2	No	rRNA transcription; 5.8S maturation; U8 snoRNA release during pre-32S cleavage		135, 138, 162, 164
DDX18	DEAD	MrDb	Has1	No	rRNA transcription; SSU biogenesis		131, 133, 135, 138, 177
DDX21	DEAD	GUA; GURDB; RH-II/ GU; RH-II/GuA	-	No	28S rRNA stabilization; assembly of ribo-somal proteins; 18S / 28S production	RPL4; c-Jun; NS	138, 152-158
DDX23	DEAD	prp28; PRPF28; U5-100K; U5-100KD	Prp28	Nu	rRNA transcription		135, 138
DDX24	DEAD	-	Mak5	No	LSU biogenesis		131, 138
DDX46	DEAD	Prp5; PRPF5	Prp5	Nu	rRNA transcription		135, 138
DDX47	DEAD	RRP3; E4-DBP	Rrp3	No	rRNA transcription, 18S and 28S maturation		135, 138
DDX50	DEAD	GU2; GUB; RH-II/GuB; MGC3199	-	No	rRNA transcription, 18S and 28S maturation (antagonist of DDX21)		135, 138, 152, 154
DDX51	DEAD	DKFZp686N2081; MGC42193	Dbp6	No Nu	rRNA transcription; 3´ end maturation of 28S rRNA; release of U8 snoRNA from pre-rRNA	Nog1	135, 138, 159
DDX56	DEAD	DDX21*; DDX26; NOH61	Dbp9	No Nu	rRNA transcription; LSU biogenesis		131, 135, 165
DHX9	DEAH	LKP; RHA; DDX9; NDH2; NDHII	-	No Nu Cy	rRNA transcription		135, 140, 141
DHX15	DEAH	DBP1; HRH2; DDX15; PRP43; PRPF43	Prp43	Nu	SSU biogenesis		131, 138
DHX16	DEAH	DBP2; PRP8; Prp2; DDX16; PRPF2	Prp2	Nu	rRNA transcription		135, 178
DHX33	DEAH	DDX33	-	No	rRNA transcription; facilitating conformational change of rDNA	UBF	135, 138
DHX37	DEAH	DDX37	Dhr1	No	SSU biogenesis		131, 133, 138
EIF4A3	DEAD	DDX48; NUK34; NMP265; eIF4AIII	Fal1	No Nu	rRNA transcription; 18S rRNA maturation; SSU biogenesis	NOM1	131, 135, 138, 148
SKIV2L	DExH	HLP; SKI2; DDX13; SKI2W; SKIV2; THES2	Ski2	No Cy	further analysis required to clarify		138, 179
SKIV2L2	DExH	Dob1; Mtr4	Mtr4	No	3´-end processing of 5.8S rRNA	MPP6	134

Protein localization (Loc.) is indicated as nucleolus (No), nucleus (Nu) or cytoplasm (Cy). Putative cofactors suggested for ribosome biogenesis are shown. SSU, small subunit; LSU, large subunit. The asterisk indicates that the name DDX21 was also used for DDX56, besides for DDX21 itself.

the biogenesis of the SSU (DDX10, DDX18, DHX15, DHX37, eIF4A3) or the LSU (DDX24, DDX56). In a lentiviral RNAi screen with 28 putative DEAD/H-box RNA helicases known to localize to the nucleolus or nucleus and whose yeast homologs have been implicated in ribosome biogenesis, Zhang and colleagues (2011) addressed protein involvement in Pol I-mediated transcription of the 47S rRNA precursor.[135] They observed an almost 10-fold decrease of the pre-rRNA transcript after knockdown of DHX9 and DHX33, while the 47S rRNA level was reduced by more than 50% after knockdown of 12 other candidate proteins, namely DDX3X, DDX10, DHX16, DDX17, DDX18, DDX23, DDX46, DDX47, DDX48, DDX50, DDX51

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and DDX56. Further functional and mechanistic analysis of DHX33 revealed that it localizes to rDNA loci and regulates RNA Pol I recruitment by interacting with the upstream binding factor (UBF), a well-known rDNA chromatin remodeling protein.[135] Since both the NTPase and DNA binding activities of DHX33 were necessary to regulate 47S rRNA transcription, DHX33 might assist in conformational changes of rDNA loci through ATP hydrolysis. Based on the observation that knockdown of DHX33 did not result in accumulation of any downstream rRNA precursor or a change in the ratios of rRNA species, it was proposed that DHX33 is not involved in pre-rRNA processing per se but might rather act as a DNA helicase in transcription.[135] However, to learn more about the substrate specificity and enzymatic activity of DHX33, further in vitro characterization of the protein will be necessary.

The involvement of DHX9, also known as nuclear DNA helicase II (NDH II) and RNA helicase A (RHA), in rRNA transcription is also suggested by studies of Zhang and colleagues who found that DHX9 localizes to the nucleolus and that nucleolar localization depends on active rRNA transcription.[140,141] Since DHX9 can unwind RNA-RNA, DNA-DNA and DNA-RNA duplexes in vitro as well as more complex DNA-RNA hybrids containing forks, displacement loops (D- and R-loops) and also G-quadruplexes that can form during transcription (see ref. 142), further studies are necessary to clarify by which mechanism DHX9 regulates rRNA transcription and whether this requires RNA or DNA helicase activity. However, DHX9 contains an intrinsic transcriptional activation domain,[143] which could also permit functions independent of helicase activity.[144] In addition, DHX9 has been proposed to act in the translation of specific mRNAs.[145]

While no yeast homologs were identified for DHX9 and DHX33, the human DEAD-box helicase eIF4AIII (DDX48) is homologous to the yeast protein *Fal1*, which is essential and required for 18S rRNA biogenesis in *S. cerevisiae.* Although eIF4AIII is well described as a component of the exon junction complex (see for example refs. 146 and 147), expression of human eIF4AIII rescued the lethal phenotype and the 18S rRNA biogenesis defect of *fal1* deletion in yeast.[148] Mutations in the Walker A and Walker B motifs of the human protein prevented complementation of the *fal1* deletion, indicating that the ATPase activity is essential. NOM1 was identified as an eIF4G-like interaction partner of human eIF4AIII, while its yeast homolog Sgd1 interacts with *Fal1*.[148] In human cells, siRNA-induced knockdown of eIF4AIII decreased the levels of the 41S and 21S pre-rRNAs as well as the mature 18S rRNA, and lead to an increase in the ratio of 45S pre-rRNA to 30S/32S pre-rRNA, indicating that eIF4AIII participates in ribosome biogenesis.[148] A function of eIF4AIII in SSU biogenesis is further supported by reporter assays, in which knockdown of eIF4AIII affected RPS2-YFP and ENP1 localization.[131]

DDX21, also named RNA helicase II or Gu(α) protein, was originally cloned from HeLa cells and has been linked to watermelon stomach disease.[149,150] DDX21 and its *Xenopus* homologs xGu-1 and xGu-2 were shown to be required for ribosome synthesis in *Xenopus* oocytes and human cells, suggesting its conservation in vertebrates.[151,152] The protein localizes to nucleoli in both *Xenopus* and mammalian cells. Antisense oligonucleotide mediated knockdown of xGu-1/2 in *Xenopus* oocytes resulted in decreased 18S and increased 20S levels as well as reduced amounts of 28S rRNA. In addition, faster migrating RNA species, other than 18S rRNA appeared in the absence of xGu-1/2 and were confirmed to be degradation products of 28S rRNA, suggesting that the reduction in 28S rRNA levels was due to reduced stability.[151] Although microinjection of wildtype xGu-1 mRNA into oocytes rescued the antisense effect, a mutant deficient in RNA unwinding due to a mutation in the DEVD motif did not, indicating that helicase activity is required for efficient production of 18S and 28S rRNA. In human cells, knockdown of DDX21 reduced 18S and 28S rRNA levels accompanied by a minor decrease in the 32S rRNA precursor.[152] In contrast to the *Xenopus* study, neither an accumulation of 20S intermediates nor the degradation of 28S rRNA was observed in mammalian cells. Surprisingly, a helicase-deficient mutant of human DDX21 (SAT mutant) rescued 28S rRNA production, but did not restore 18S rRNA levels, pointing to an involvement of DDX21 RNA unwinding activity in 18S but not in 28S rRNA biosynthesis in mammalian cells.[152] However, a DDX21 DEVD mutant could neither restore 18S nor 28S rRNA levels.[153] Interestingly, the overexpression of the DDX21 paralog DDX50 (RHII/Guβ) inhibited rRNA maturation, even in the wildtype background with normal levels of DDX21,[152] suggesting antagonistic roles of the two paralogues. DDX50 was also shown to colocalize with the splicing factor SC35 in nuclear speckles.[154]

Several interaction partners of DDX21 have been identified and suggested to recruit DDX21 and regulate its function in ribosome biogenesis. DDX21 was shown to directly interact with RPL4.[153] Another report identified DDX21 as a component of a > 700 kD protein complex containing several ribosomal proteins and the cofactors Pes1, EBP2 and nucleostemin.[155] The association with components of the PeBoW complex (see ref. 156) is supported by the identification of DDX21 in pulldowns together with parvulin and Bop1.[157] The C-terminal tail of DDX21 has been shown to interact with the transcription factor c-Jun that stimulates the intrinsic rRNA binding activity of DDX21 and is required for targeting DDX21 to the nucleolus.[158]

DDX51 was identified as an interaction partner of the mammalian ribosome synthesis cofactor Nog1 in a yeast two-hybrid screen and shown to colocalize with Nog1 to the nucleolus of a murine fibroblast cell line.[159] RNAi against DDX51 resulted in decreased levels of the 32S and 12S rRNA intermediates, while the early 47S/45S precursors accumulated. Further, accumulation of 3'-ETS-extended rRNA precursors indicated an impaired processing at site 6 located downstream of mouse 28S rRNA, which was also observed upon overexpression of a dominant negative helicase mutant. These findings indicate that efficient rRNA processing requires helicase activity. Indeed, inactivation of DDX51 results in pre-ribosomal accumulation of the U8 snoRNA, which is required for 3'-processing of the 28S rRNA (see ref. 159 and references therein).

DDX5 (p68) was shown to localize to nuclei and nucleoli and associate with the rDNA promoter in a cell cycle-dependent and p19Arf-regulated manner.[160,161] Arf can prevent interaction

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of DDX5 with nucleophosmin, which is required for nucleolar DDX5 recruitment. DDX5 is closely related to another member of the DEAD box family, DDX17, which is expressed in the two isoforms p72 and p82. The two helicases can form a heterodimer and have been implicated in several pathways of RNA metabolism.[162,163] DDX5 negatively regulates expression of DDX17 and both helicases have a redundant function in cell proliferation and viability.[164] Detailed functional analysis revealed that co-silencing of both genes disturbed nucleolar structure and impaired processing of the 32S rRNA precursor. Mutant mice lacking either of the helicases showed reduced levels in mature 5.8S rRNA.[162] Further studies indicated that the redundant roles of DDX5 and DDX17 were based on the catalysis of RNA rearrangements. Interestingly, U8 snoRNA slightly accumulated upon co-silencing of DDX5 and DDX17 in the 60S/90S preribosomal fractions.[164] It remains to be shown, whether this is due to involvement of the helicases in snoRNA release, or an indirect effect due to a lack of structural remodeling in early preribosomal complexes.

DDX56, previously named nucleolar helicase of 61 kDa (NOH61), is considered as the homolog of yeast Dbp9.[136] DDX56 was shown to possess ATPase activity in vitro, to localize to the nucleolus and to associate with pre-60S particles.[165] Its involvement in LSU biogenesis is further supported by nuclear accumulation of RPL29-GFP upon knockdown of DDX56.[131]

Similarly, the yeast homolog of DHX15, Prp43, acts both in pre-mRNA splicing and ribosome biogenesis (see above), while human DHX15 was only found to localize to splicing factor containing nuclear speckles and not to nucleoli.[166] Dhx15 knockdown was observed in the fluorescent reporter screen to affect RPS2-YFP localization, indicating a role in SSU biogenesis,[131] but no direct evidence for a function in ribosome synthesis has been published so far.

DDX10, DDX18 and DHX37 were found in the human SSU processome.[133] Both DDX10 and DDX18 were also picked up in the reporter screen using RPS2-YFP to study SSU maturation.[131] DDX10 was found together with nucleolin, RRP5 and the U3 snoRNP in a 50S particle, which accumulated in response to inhibition of Pol I transcription by actinomycin D treatment or upon depletion of tUTP proteins.[133] Based on these results, the authors proposed that the 50S U3 snoRNP represents a SSU processome assembly intermediate and that its incorporation into the processing complex requires the presence of pre-rRNA and the tUTP proteins. However, the detailed functional characterization of human DDX10 and the functional comparison to its yeast homolog Dbp4 will be interesting topics of future research.

Outlook

Since DExD/H-box RNA helicases play key roles in all major pathways of RNA metabolism, it is not surprising that a number of these proteins are involved in ribosome synthesis. While basic concepts of helicase function apply to ribosome synthesis in all organisms, the current state of knowledge and the major questions differ significantly between model organisms. In bacteria, the players are known and the timing of their action as well as ribosomal proteins that depend on helicases for their incorporation into nascent ribosomal subunits have been analyzed.[1] Some of the proteins, for example DbpA/YxiN, have been studied extensively in vitro, including kinetics, RNA binding and structural rearrangements during the ATPase cycle.

In yeast, the best understood eukaryotic model system, the molecular function of the majority of proteins involved in ribosome biogenesis has remained elusive so far and the question remains, why so many putative RNA helicases are required for this pathway. As one possible answer to this question, it was suggested that snoRNAs in general might require RNA helicases for their unwinding and release from pre-rRNA.[30] Stable basepairing of snoRNAs with pre-rRNA has been further supported by the recent identification of additional basepairing sequences,[40] and by the fact that snoRNA-guided modifications cluster in certain functional regions of the rRNA leading to overlapping basepairing sites. Indeed, several RNA helicases are required for the release of specific snoRNPs from pre-ribosomes (reviewed in ref. 36), even though they have not been formally shown to unwind snoRNA-pre-rRNA basepairing. The model of helicase-mediated unwinding is supported by recent evidence for Prp43, where the DEAH-box protein is required for release of a number of snoRNPs that modify a cluster of sites in the 25S rRNA. Here, Prp43 was found to crosslink to snoRNAs and to their basepairing sites on the pre-rRNA, suggesting direct contact and involvement of the RNA helicase in snoRNA unwinding.[90]

Despite the extended basepairing, most snoRNPs do not seem to rely on (individual) helicases for their release, as was systematically investigated for SSU biogenesis.[79] It is well possible that several helicases are recruited to the same part of the pre-ribosome and will turn out to be partially redundant, if they act in release of overlapping sets of snoRNAs. It needs to be kept in mind, however, that most of the helicases implicated in ribosome synthesis in yeast are essential, suggesting at least one unique cellular function for each of these proteins. Also, only few RNA helicases have been implicated in other cellular pathways in addition to ribosome biogenesis so far, but more such cases can be expected.

Many pre-rRNA helicases are functionally not as well understood as some other RNA helicases, such as proteins involved in splicing. This is probably due to the high complexity and dynamics of the pathway and the relative inaccessibility of the nucleolus. A major aim in the next few years will be the identification of recruitment and target sites of RNA helicases within pre-ribosomal complexes, and in RNPs in general. UV crosslinking in combination with deep sequencing has turned out to be a powerful tool to identify interaction sites of RNA binding proteins (see for example refs. 88 and 167). This will, on the one hand, allow the use of substrate RNAs in in vitro studies, which have so far been done using model substrates (for example see refs. 50, 168–170). The kinetics might turn out to be different when the authentic substrate RNAs are used in these assays. On the other hand, the identification of interaction sites will allow a more detailed functional analysis of RNA helicases, including their regulation, the roles of cofactors and their molecular function. These studies are supported by the recent structures of the eukaryotic ribosome (see for example refs. 171 and 172), which

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allows the mapping of putative interaction sites in 3D. Structural analysis of pre-ribosomal complexes (for example see ref. 173), however, will be required to better understand the context in which RNA helicases act in pre-ribosomes that might be structurally different than mature ribosomal subunits.

Ribosome biogenesis is much less understood in multicellular eukaryotes. Only two RNA helicases have been shown to be involved in ribosome synthesis in plants,[174,175] with many more to be identified. In general, the genes of most yeast helicases are conserved in many eukaryotes and the corresponding proteins thus expected to fulfill similar if not equivalent functions. As detailed above, further helicases add to the complexity of the pathway in mammalian cells, and it can be expected that more proteins involved in ribosome synthesis will be found. For most of the proteins identified so far, their binding sites on pre-ribosomes, molecular functions and regulation also remain to be analyzed. The regulation of RNA helicases is a very interesting topic that has started to be addressed in recent years. Remarkably, it was observed that some helicases seem to bind early in a pathway but only to be specifically activated later on, probably by protein cofactors (e.g., Prp43). A further level of regulation is helicase expression, which is often deregulated in cancers. In addition, it will be interesting to see how helicase activity is modulated by signaling networks and how these are cross-regulated in case of helicase function in several pathways of RNA metabolism.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Nicholas Watkins, Sara Müller and Matthias Leisegang for critical comments on the manuscript and the Deutsche Forschungsgemeinschaft (BO 3442/1-1 and SFB860) as well as the Göttingen University Medical Department for funding. We would like to apologize to all colleagues whose work could not be cited due to limitations in space and the number of references.

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