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Assembly of ribosomes and spliceosomes: complex ribonucleoprotein machines

Jonathan P Staley[1] and **John L Woolford Jr**[2]

1 *Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago IL 60637, Email: jstaley@uchicago.edu*

2 *Department of Biological Sciences, Center for Nucleic Acids Science and Technology, Carnegie Mellon University, Pittsburgh PA 15213, Email: jw17@andrew.cmu.edu, Phone: (412) 268-3193, FAX: (412) 268-7129*

Summary

Ribosomes and spliceosomes are ribonucleoprotein nanomachines that catalyze translation of mRNA to synthesize proteins and splicing of introns from pre-mRNAs, respectively. Assembly of ribosomes involves more than 300 proteins and RNAs, and that of spliceosomes over 100 proteins and RNAs. Construction of these enormous ribonucleoprotein particles (RNPs) is a dynamic process, in which the nascent RNPs undergo numerous ordered rearrangements of RNA-RNA, RNA-protein, and protein-protein interactions. Here we outline similar principles that have emerged from studies of ribosome and spliceosome assembly. Constituents of both RNPs form subassembly complexes, which can simplify the task of assembly and segregate functions of assembly factors. Reorganization of RNP topology, and proofreading of proper assembly, are catalyzed by protein- or RNA- dependent ATPases or GTPases. Dynamics of intermolecular interactions may be facilitated or regulated by cycles of posttranslational modifications. Despite this repertoire of tools, mistakes occur in RNP assembly or in processing of RNA substrates. Quality control mechanisms recognize and turnover misassembled RNPs and reject improper substrates.

Introduction

Ribosomes and spliceosomes are two of the best characterized large RNPs, multimolecular complexes that contain both RNA and protein constituents. Mature ribosomes in eukaryotes consist of two RNP subunits, the large subunit containing 47 different proteins and three rRNAs, and the small subunit containing 32 proteins and one rRNA. Assembly of these molecules into ribosomes begins with synthesis of rRNA in the nucleolus, followed by proper folding of nascent rRNA, to enable its modification (methylation or pseudouridylation), processing by exo- and endonucleases, and binding to ribosomal proteins. More than 180 assembly factors and 100 small nucleolar RNPs (snoRNPs) associate with pre-rRNA to catalyze ribosome assembly [1]. The snoRNPs catalyze posttranscriptional modifications of pre-rRNA, primarily at sequences that ultimately form or surround the active site of mature ribosomes. Each snoRNP contains four core proteins (including the modifying enzyme) plus a unique snoRNA, which targets the modifying enzyme via snoRNA-pre-rRNA basepairing

Corresponding author: Woolford, John L Jr (E-mail: jw17@andrew.cmu.edu).

[2]. After each assembly factor and snoRNP completes its function, it must somehow dissociate from pre-rRNPs, to be recycled for construction of other ribosomes. Ribosomal proteins also play a role in assembly of ribosomes, and remain integral components of ribosomes, together with rRNA, to also function in the dynamics of protein synthesis.

Preribosomes traffic from the nucleolus through the nucleoplasm to the cytoplasm, undergoing additional steps in maturation at each point, to finally assemble into functional subunits (Figure 1). In order to be exported to the cytoplasm, preribosomes must be sufficiently small to fit through nuclear pores. Presumably this is achieved by release of most of the assembly factors prior to nuclear export, as well as changes in pre-rRNP conformation [3,4]. Both pre-40S and pre-60S particles are directed specifically to and through nuclear pores by multiple export receptors, including Crm1/Xpo1 in concert with Ran-GTP [5–8]. Successful navigation through the hydrophobic environment of FG repeats of nucleoporins, by preribosomes likely to contain a hydrophilic exterior, may be aided by binding of the alpha-helical HEAT repeat protein Rrp12 to the surface of pre-rRNPs [9].

Spliceosomes contain five small nuclear ribonucleoprotein particles (snRNPs) plus more than 100 other splicing factors [for review, see 10 and references therein]. During each cycle of splicing, these factors assemble *de novo* on the pre-mRNA substrate. An intron is defined by sequences at the 5′ splice site, the branch site, and the 3′ splice site. These sequences participate in the two transesterification reactions in splicing—5′ splice site cleavage and exon ligation. After catalysis, the spliceosome dissociates the products and disassembles, so that the spliceosome can be reused in splicing of another pre-mRNA (Figure 2).

Here we outline common principles underlying construction of these complex RNPs, revealed by studies of the assembly of ribosomes and the assembly and function of spliceosomes.

Coupling transcription to assembly of RNPs and processing of RNAs

Pre-rRNA processing and ribosome assembly, as well as pre-mRNA processing and spliceosome assembly, occur co-transcriptionally and are physically linked to transcription of the respective RNAs. Proteins involved in 5′ mRNA capping, pre-mRNA splicing, and 3′-end formation of mRNA bind to RNA polymerase II, transcription factors or chromatin. Certain ribosome assembly factors bind not only to pre-rRNA but also to rDNA chromatin. These physical interactions between the transcription and RNA processing machinery enable reciprocal, functional coupling of transcription, RNA processing, and RNP assembly. These concepts have been reviewed recently [11,12], and will not be discussed further in this review.

Subcomplexes and hierarchical assembly of RNPs

The complexity of assembling the enormous ribosome and spliceosome RNPs is reduced in part by preassembling subcomplexes. More than a dozen subassembly complexes of preribosomal molecules have been discovered [reviewed in 1,13]. Five of these subcomplexes, t-UTP/UTP-A, UTP-B, UTP-C, the Mpp10 complex, and the U3 snoRNP, form independently of each other, then associate with the pre-rRNA in a hierarchical, stepwise fashion to complete the 90S preribosome [14••]. The tUTP complex proteins, which bind to rDNA and are necessary for transcription of pre-rRNA [15], are the first assembly factors to bind to pre-rRNA. The remaining complexes assemble via two independent pathways: the U3 snoRNP, UTP-B complex, and Mpp10 complex bind to rRNA after tUTP, to form a stable intermediate. The UTP-C complex assembles in a parallel pathway.

Many of the proteins in these SSU processome subcomplexes contain predicted protein-protein interaction domains or RNA binding motifs, and none contain predicted enzymatic motifs. Thus, interactions established through these motifs may enable formation of an RNP scaffold

upon which GTPases, ATPases, and nucleases can act to reconfigure the preribosome to catalyze rRNA modification and processing and particle maturation. Stepwise assembly of such large and dynamic RNPs may also provide the advantage of temporally and spatially separating different steps in their biogenesis [14••].

Identification of subcomplexes has provided clues about cofactors of assembly factors and substrates upon which they might act. For example, the discovery of the Rpf2 subcomplex containing the assembly factors Rpf2, Rrs1, ribosomal proteins rpL5 and rpL11, and 5S rRNA helped uncover the mechanism for assembly of 5S rRNA into preribosomes [16•]. Three of the four rRNAs within mature ribosomes are derived by processing of a single primary transcript, within the assembling particles. However, 5S rRNA is transcribed separately. Rpf2 and Rrs1 were shown to recruit 5S rRNA as well as ribosomal proteins rpL5 and rpL11 into 90S preribosomes. Failure to recruit these molecules blocks maturation of preribosomes several steps later in the assembly pathway. Because the abortive assembly intermediates lack 5S rRNA and rpL10, they cannot bind to nuclear export receptors Mex67-Mtr2 [8] or Nmd3 [5], respectively, and therefore accumulate in the nucleus (Figure 3). This example reminds us how assembly and export are coupled by creation of binding sites for receptors during biogenesis of the pre-rRNPs.

As with ribosome assembly, spliceosome assembly is reduced in complexity through the formation of subcomplexes. For example, the five snRNPs are observed as independent particles. These particles can be reduced even further; the SF3a and SF3b components of the U2 snRNP form stable subcomplexes. Conversely, the snRNPs also form higher order structures. Before interacting with pre-mRNA, the U4 snRNP interacts with the U6 snRNP via extensive base pairing between the snRNAs and then this complex binds to the U5 snRNP to form the U4/U6.U5 snRNP. Protein complexes also feature prominently. For example, a large protein complex, termed the Prp19p complex or NTC, has recently been found to be a major salt-stable component of purified, catalytically active spliceosomes [17••]. Experimentally, the U1, U2, U4/U6.U5 snRNPs and NTC were first observed to bind a pre-mRNA sequentially, but subsequently a U1.U2.U4/U6.U5 snRNP, or penta-snRNP, that includes the NTC was discovered, potentially a “holospliceosome” [18]. While compelling, the penta-snRNP has not yet been observed *in vivo* [19,20] and is not essential *in vitro* for early spliceosome assembly steps [21]. Regardless, preassembly of subcomplexes simplifies spliceosome assembly.

Several core splicing factors likely function as scaffolds both to recruit other splicing factors and to time their recruitment. For example, the C-terminal domain of the U5 snRNP protein and DExD/H box ATPase Brr2 interacts with at least five splicing factors that bind to the spliceosome at different stages [22]. Because some of these interactions with Brr2 are overlapping, Brr2 may necessitate sequential interactions with these factors. Additionally, Brr2 as an ATPase may assume alternative conformations during the splicing cycle that favor one interaction over another, and thereby time the recruitment and function of specific splicing factors. While assembly and processing of a genuine pre-mRNA appears to be a largely ordered process, the spliceosome can also traverse alternative pathways – especially in rejecting and discarding incorrect substrates (see below).

NTPases and dynamic rearrangements involving RNA and protein

A number of GTPases and ATPases are required for assembly of ribosomes and spliceosomes [23•,24]. Cycles of NTP binding and hydrolysis could drive assembly forward by several different mechanisms to recruit or release factors [23•]: (1) by direct binding to a protein to stabilize or destabilize its association with preribosomes, (2) by direct catalysis of conformational switches of RNA or RNP structures in preribosomes, or (3) by functioning as timers for assembly or acting as placeholders to prevent premature association of factors.

One example is the GTPase Bms1, which binds to the putative endonuclease Rcl1, and is required to deliver Rcl1 into preribosomes [23•,25,26]. Studies of the binding of Bms1 to its ligands in the presence of GTP or GDP led to the following model: binding of Bms1-GDP to Rcl1 could lead to exchange of GDP for GTP and subsequent association of Bms1-GTP-Rcl1 with pre-rRNPs containing U3 snoRNA. Entry into preribosomes or subsequent rearrangements of the pre-rRNPs, including dissociation of U3 snoRNA, may trigger the intramolecular GAP of Bms1 to activate GTP hydrolysis. This would enable release of Bms1 from Rcl1, strengthen association of Rcl1 with preribosomes, and trigger subsequent pre-rRNA processing.

A second example is Rea1, one of three AAA ATPases that catalyze release of assembly factors from preribosomes [27–29]. Rea1 is found in late nucleoplasmic pre-60S particles, and functions in late steps of pre-rRNA processing and subsequent nuclear export of pre-rRNPs [29]. Rea1 may remodel pre-rRNPs to expose the 3′ ends of 7S pre-rRNA to the exosome complex of nucleases for 3′ trimming. An important breakthrough was the demonstration of factor release activity by an ATPase *in vitro* [29]. When pre-60S particles were purified in the presence of ATP, Rea1 and another assembly factor Nug2 dissociated from preribosomes, but not when nonhydrolyzable analogues of ATP were used or ATP was omitted.

Nineteen DExD/H-box proteins (DBPs) are involved in ribosome biogenesis in yeast [24]. Likely substrates for these potential RNA helicases are the snoRNAs. Indeed, two DEAD-box proteins, Has1 and Dbp4, have been implicated in releasing snoRNAs from preribosomes [30,31]. Inactivation of Dbp4 or Has1 by mutation of residues necessary for ATP binding or hydrolysis prevents release of several different snoRNAs from pre-rRNPs. Depletion of each of the remaining 17 preribosomal DBPs results in defects at different steps in pre-rRNA processing. An important next step will be to discover the specific substrates of these 17 DBPs, either RNA helices to be unwound, RNP substructures to be remodeled, or proteins to be recruited into or released from pre-rRNPs.

At least eight DExD/H box ATPases are also required for spliceosome assembly and pre-mRNA processing. During splicing, the spliceosome dramatically rearranges RNA-RNA and RNA-protein interactions within the spliceosome or involving the substrate. The DExD/H box ATPases are strong candidates for, or known catalysts of, these key transitions. The DEAD box ATPase Prp5 promotes an intramolecular rearrangement of U2 stem IIc to stem IIa that is required for binding of the U2 snRNP to the pre-mRNA [32•]. After the first cleavage event, the DEAH box ATPase Prp16 has been implicated in toggling this switch again from the U2 stem IIc state to the stem IIa state, suggesting a role for Prp16 in modulating substrate-spliceosome interactions during the catalytic phase of splicing [32•,33•]. After exon ligation, the DEAH box ATPase Prp22, a 3′→5′ unwindase, promotes release of the mRNA product and appears to do so by binding downstream of the exon junction and then translocating upstream along the mRNA [34••]. Each of these ATPases has been implicated in promoting the fidelity of pre-mRNA splicing (see below). Suggesting an explicit parallel with ribosome assembly, the DEAH box ATPase Prp43 is required for both pre-mRNA splicing and pre-rRNA processing [35–37]. In splicing, the G-patch protein Ntr1 recruits and catalytically activates Prp43 to promote intron release [38,39•,40]. The G-patch, a short glycine-rich sequence, is found in a number of RNA binding proteins, including several that interact with DBPs [41]. In ribosome assembly, a related G-patch protein may similarly recruit and activate Prp43 to promote pre-rRNA processing and ribosome assembly. The dual role for Prp43 in splicing and ribosome assembly could provide a mechanism for coupling ribosome biogenesis with gene expression.

The only integral spliceosomal DExD/H box ATPase, Brr2, is regulated by the sole spliceosomal GTPase, Snu114. This ATPase promotes both spliceosome assembly, by

unwinding base-paired U4/U6, and spliceosome disassembly, perhaps by unwinding the mutually exclusive and catalytically essential U2/U6 interaction, and thereby likely necessitates tight regulation. Functioning as a classic G-protein switch, Snu114 in the GTP state promotes Brr2 function while in the GDP state, represses Brr2 function [42•]. While it is currently unclear what signals control this switch, they likely include the splicing substrate and the snRNAs, which together can specify the stage in the splicing cycle and thereby dictate a requirement for activating Brr2. In the future, it will be interesting to determine whether Snu114 also responds to signals outside of the spliceosome and/or whether Snu114 sends signals beyond the spliceosome. Intriguingly, the close paralog of Snu114 corresponds to the translation elongation factor EF-2, suggesting another connection between the spliceosome and the ribosome [43,44]. While EF-2 utilizes GTP hydrolysis to promote work, it is not yet clear if Snu114 also functions in this way.

Posttranslational modification: another mode to power dynamics

Cycles of posttranslational modifications of preribosomal or spliceosomal proteins add an extra layer of flexibility and complexity to the dynamics of RNP biogenesis. For example, an isoform of casein kinase I, Hrr25, is present in pre-40S and pre-60S ribosomal particles and is necessary for their maturation. Phosphorylation of assembly factor Tif6 by Hrr25 is required for production of 60S subunits [45]. It remains to be determined whether function of Tif6, or its association with or dissociation from preribosomes, depends on its state of phosphorylation. Hrr25-dependent phosphorylation followed by dephosphorylation of ribosomal protein S3, is necessary for proper integration of rpS3 into preribosomes, and induces remodeling of the structure of pre-40S particles, perhaps to enable export through the nuclear pore [3••].

Analysis of the SUMO proteome revealed that a number of ribosome assembly factors, especially those functioning in the nucleolus or nucleoplasm, are modified by sumoylation [reviewed in 46•]. In addition, screens for mutants defective in ribosome biogenesis, including those impaired in nuclear export of pre-rRNPs, identified mutants in the SUMO modification pathway [46•]. Interestingly, the *upl1-* mutant, defective in the SUMO deconjugating enzyme that is associated with nuclear pores, exhibited genetic interactions with the *mtr2-33* mutant defective in pre-60S subunit export. By preventing incorrect protein-protein interactions, sumoylation may enable orderly arrangement of molecules within assembling RNPs. Desumoylation may be important for efficient nuclear export of nascent ribosomes.

As with ribosome assembly and pre-rRNA processing, efficient spliceosome assembly and pre-mRNA processing require orderly progression through the splicing pathway. Posttranslational modifications are emerging as a mechanism to establish order. Cycles of phosphorylation and dephosphorylation have long been implicated in controlling the splicing cycle. Recently, direct evidence has revealed a requirement for PP1/PP2A phosphatases in the exon ligation step of splicing [47•]. Intriguingly, this requirement correlates with dephosphorylation of the GTPase U5-116 kDa (hSnu114) and the HEAT-repeat protein SAP155. In each case, dephosphorylation may regulate conformational rearrangements, given the switch like nature of G-proteins and the conformational flexibility of HEAT-repeat proteins. Ubiquitylation has also been implicated in splicing and the first direct evidence revealed conjugation of ubiquitin to Prp8 [48•], a central U5 snRNP component that interacts with all consensus sequences in the pre-mRNA [reviewed in 49]. The ubiquitylation state of Prp8, like the guanine nucleotide bound state of Snu114, regulates Brr2-dependent unwinding, suggesting that ubiquitylation also regulates conformation rearrangements. Ultimately, it will be important to determine what regulates these regulators, to understand how order is established in ribosome biogenesis and spliceosome assembly and function.

Quality control

In many mutants defective in ribosome assembly, pre-rRNAs do not accumulate to levels predicted if assembly intermediates were completely stable. Rather, these pre-rRNAs undergo significant turnover [reviewed in 1]. These results indicated the existence of a surveillance machinery that could recognize and destroy misassembled preribosomes. Indeed, the nuclear exosome, a complex of ten nucleases that can process or degrade nuclear RNAs, turns over pre-rRNAs when ribosome assembly is blocked [50,51••]. These RNAs targeted for turnover include aberrant processing intermediates that form when pre-rRNA cleavage is blocked [50]. Likewise, normal 27S pre-rRNAs are degraded by the exosome in mutants blocked in late steps of pre-60S maturation and nuclear export [51••], or in cells treated with 5-FU, an inhibitor of pre-60S subunit maturation [52].

The TRAMP nuclear polyadenylation complex, which includes the polyA polymerase Trf4, the zinc knuckle protein Air2, and the DEAD-box protein Mtr4, also is required for pre-rRNA degradation *in vivo* [53]. This purified complex can add polyA to RNA *in vitro*, perhaps to make it a better substrate for the exosome, and it also activates the processive activity of the exosome. Consistent with this role of TRAMP in pre-rRNA turnover, pre-rRNAs destined for destruction are polyadenylated *in vivo* [54].

Turnover of RNA in abortive ribosome assembly intermediates may occur in a discrete locale within the nucleolus. Pre-rRNPs destined for demolition, as well as components of the exosome and the TRAMP complex, are localized to subnucleolar foci called “No-bodies”[51••]. Formation of No-bodies requires the exosome and TRAMP complex. However, a distinct nucleolar body containing polyA+ RNA is observed in the absence of the exosome enzyme Rrp6 or Mtr4, but requires the presence of Trf4 [55]. It remains to be determined how abortive intermediates are recognized for destruction. Dez and collaborators speculate that assembly factors that fail to be released in a timely manner when assembly is aborted could recruit TRAMP or the exosome [51••].

As in pre-rRNA processing, exonucleases appear to promote resolution of stalled spliceosomes through turnover of the substrate; in addition, exonucleases turnover incorrect substrates. Substrates stalled by *cis* or *trans* mutations can be targeted for turnover by nuclear exonucleases, particularly the exosome [56]. Additionally, incorrect pre-mRNA substrates that fail to engage the spliceosome are exported to the cytoplasm where they are subject to nonsense-mediated decay. Remarkably, incorrect intermediates are also turned over by cytoplasmic nucleases, particularly Xrn1, implicating an energy-dependent mechanism for rejecting, dissociating and discarding incorrect substrates [57].

Indeed, the spliceosomal DExD/H box ATPases have been implicated in establishing fidelity through kinetic proofreading in which the DExD/H box protein utilizes the energy of ATP to compete with the pathway to splicing products (Figure 4, reviewed in 58). In this model, the spliceosome establishes specificity by favoring a correct substrate on the path to products and/or favoring incorrect substrates on the competitive branch, which leads to rejection. While the spliceosomal DExD/H box ATPases promote splicing of a correct substrate, they can antagonize splicing of an incorrect substrate if they act prematurely. In this way, Prp5 proofreads formation of the U2/branch site interaction [59•], Prp16 proofreads lariat intermediate formation [60,61] and Prp22 proofreads exon ligation [62••]. Perhaps the numerous ribosomal DExD/H box ATPases function similarly to proofread rearrangements and RNA processing steps in ribosome biogenesis. Given the role of spliceosomal DExD/H box ATPases in repressing incorrect splice sites, it will be interesting to determine whether these factors can also regulate splice site choice to control alternative splicing.

Neither Prp16 nor Prp22 reject incorrect substrates by dissociating the substrates, which requires additional factors [61,62••]. Indeed, the spliceosome appears to reject substrates in part by rearranging and thereby sequestering the substrates in effectively inactive conformations [33,61]. After rejecting substrates at the stage of 5′ splice site cleavage, the spliceosome rearranges to a conformation that resembles the exon ligation conformation [61]. Conversely, after rejecting substrates at the stage of exon ligation, the spliceosome can rearrange back to an intermediate state or further to the 5′ splice site cleavage conformation [33,61]. Moreover, consistent with the conservation of phosphodiester bonds in splicing, the spliceosome can reverse both steps of splicing [63•], suggesting that splicing could improve fidelity through self-correction. Unless pre-rRNA processing similarly conserves phosphodiester bonds, errors in pre-rRNA processing are likely catastrophic.

An evolutionary connection between the spliceosome and the ribosome?

The evidence that the catalytic function of the spliceosome evolved from self-splicing group II introns is compelling [64 and references therein]. In particular, there are strong similarities of group II intron domains V and VI with U6 and U2 snRNAs, respectively. However, the origins of the other snRNAs and the spliceosomal cofactors is less clear. An attractive hypothesis is that the evolving spliceosome borrowed activities already established to support the assembly and function of a mature ribonucleoprotein machine – the ribosome. For example, U4 snRNA shares intriguing similarities with the box C/D snoRNAs. Specifically, both bind Snu13 and while box C/D snoRNAs bind Nop56 and Nop58, U4 binds the highly homologous Prp31 [65 and references therein]. Perhaps in the evolution of the spliceosome, a snoRNA that functioned to modify rRNA evolved a separate function in base pairing with the catalytic domain of a group II intron to downregulate its activity, giving rise to base pairing of U4 with the catalytically central U6 snRNA. Given the role of Prp43 at the earliest stages of pre-rRNA processing when snoRNPs modify pre-rRNA [35–37], the recruitment of Prp43 to the evolving spliceosome may also be rationalized by an evolutionary connection between U4 snRNA and box C/D snoRNAs. Additionally, U5 snRNA shares similarities with tRNA [66], the GTPase Snu114 shares similarities with EF-2 [43], and the DExD/H box ATPase Brr2 shares similarities with Slh1, a protein involved in repressing translation of mRNAs lacking a polyA tail [67]. Deeper studies will reveal whether such parallels hold at a mechanistic level and provide support for a role for ribosomal factors in taming group II introns and evolving a mature spliceosome.

Conclusions

Many challenges lie ahead to develop a higher resolution view of the dynamics of RNP assembly. Further experiments will reveal additional interactions between components of assembling ribosomes and spliceosomes and the order in which these encounters occur. A critical next step is to determine how the order is defined – whether by obligatory, sequential steps and/or by the relative rates of different reactions that define a landscape of kinetically accessible pathways. To better understand how NTPases drive assembly or monitor substrate transitions, we will need to identify their cofactors and targets and define their enzymology, both outside of an RNP and within an RNP. We will need to define which DBPs are RNPases and which are helicases and determine if any function simply as ATP-dependent RNA-binding proteins. We will need to discover which GTPases perform work and which act as molecular switches and then elucidate their mechanisms. We are only beginning to appreciate the impact of posttranslational modifications on RNP assembly and we need to reveal their frequency, timing, and mechanistic roles. Studies of splicing have largely preceded those of ribosome biogenesis and in many cases will inform future studies. As research on ribosome biogenesis blossoms, it should in turn guide future studies in splicing.

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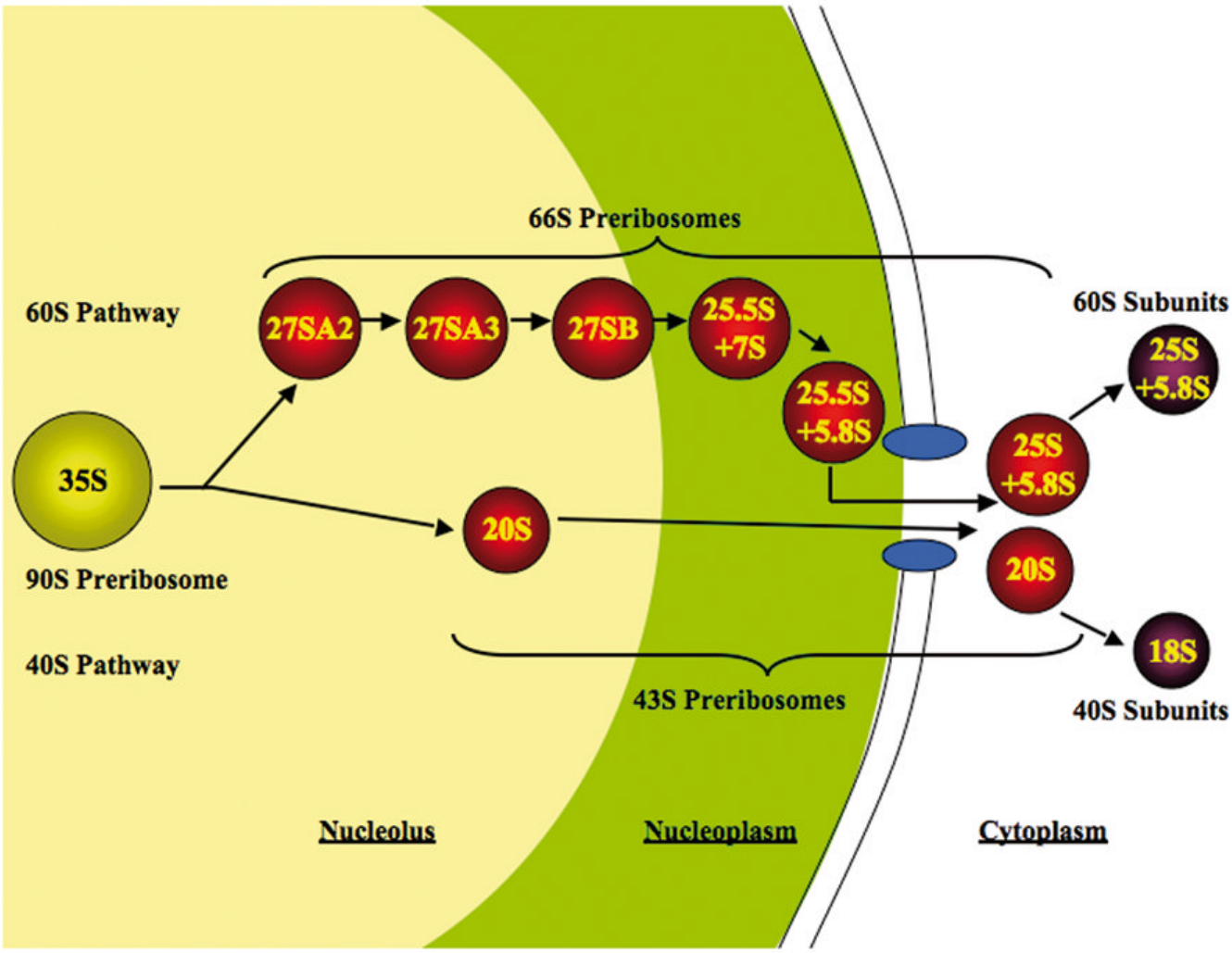

Fig. 1.

Maturation of preribosomes in *Saccharomyces cerevisiae*. Ribosome biogenesis begins in the nucleolus, where pre-rRNA is transcribed and packaged into the 90S pre-rRNP, together with a subset of ribosomal proteins and ribosome assembly factors. Subsequent steps of maturation occur in the nucleolus, nucleoplasm and cytoplasm. The 90S pre-rRNP is converted into the 66S and 43S particles by cleavage within the pre-rRNA. There are at least six consecutive 66S precursors to mature 60S ribosomal subunits, distinguished by the consecutive pre-rRNA processing intermediates contained within them. The 43S pre-rRNP containing 20S pre-rRNA is exported to the cytoplasm where mature 40S subunits are formed.

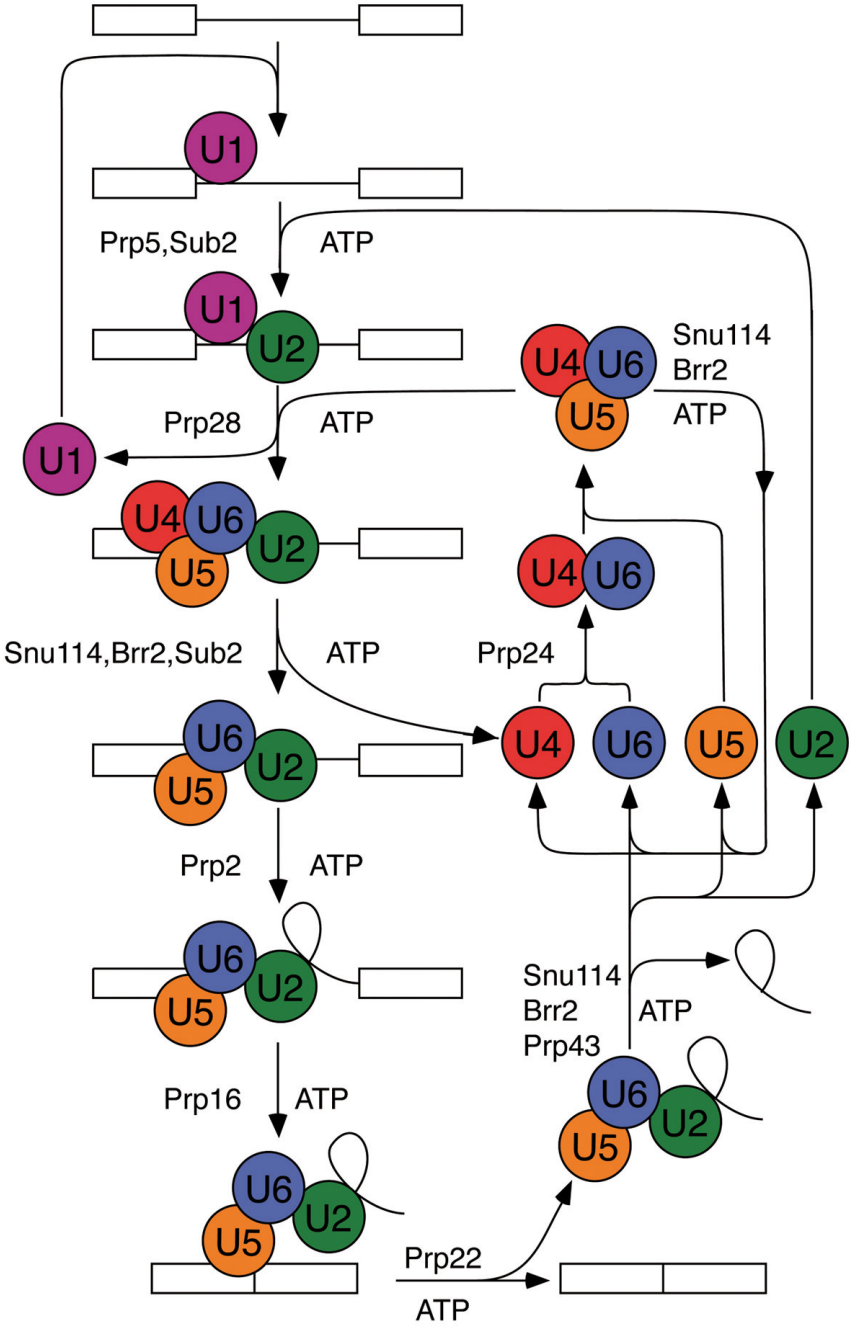

Fig. 2.

The spliceosome cycle. The spliceosome assembles de novo on a pre-mRNA transcript, catalyzes intron removal, dissociates the products and disassembles to permit recycling for subsequent rounds of splicing. Numerous ATP-dependent steps require factors belonging to the DExD/H box family of proteins. Revised from *Cell* 1998 **92:**315–326, with permission from Elsevier.

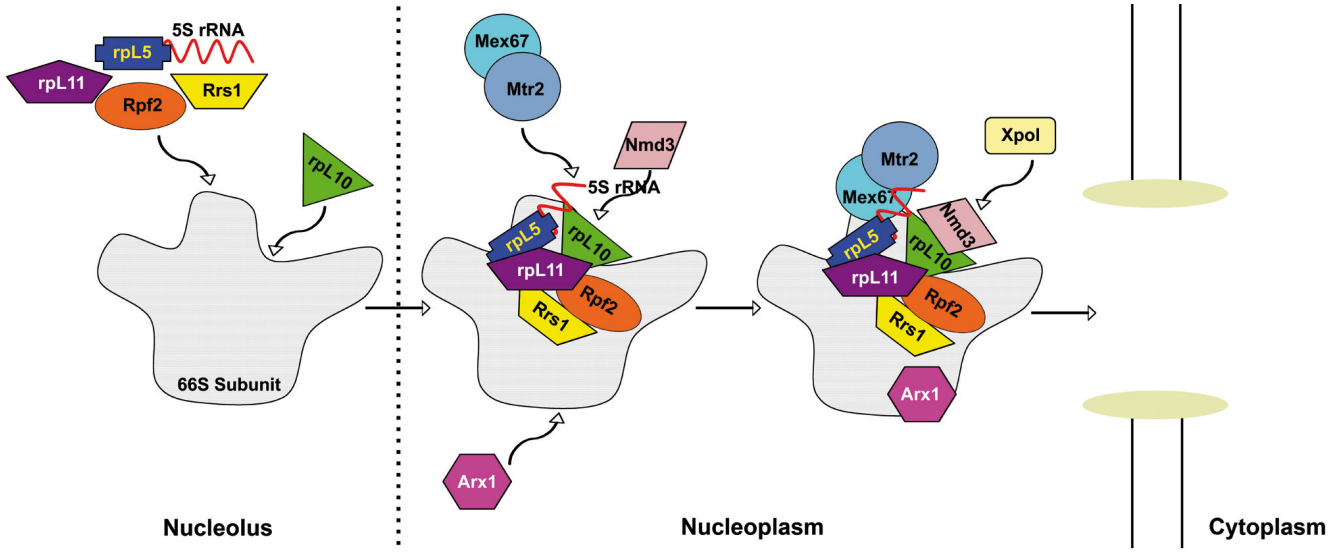

Fig. 3.

Coupling assembly of ribosomes with their export to the cytoplasm. Assembly factors Rpf2 and Rrs1 are necessary for incorporation of 5S rRNA and ribosomal proteins rpL5, rpL10, and rpL11 into 90S preribosomes in the nucleolus. Subsequently, nuclear export receptor Mex67-Mtr2 can bind to 5S rRNA in preribosomes, Nmd3 can bind to rpL10 in pre-rRNPs, and Arx1 can associate with nascent ribosomes, in the nucleoplasm. Mex67-Mtr2, Nmd3, and Arx1 then function to direct preribosomes to and through nuclear pores into the cytoplasm. Nmd3 does so via binding to Xpo1/Crm1.

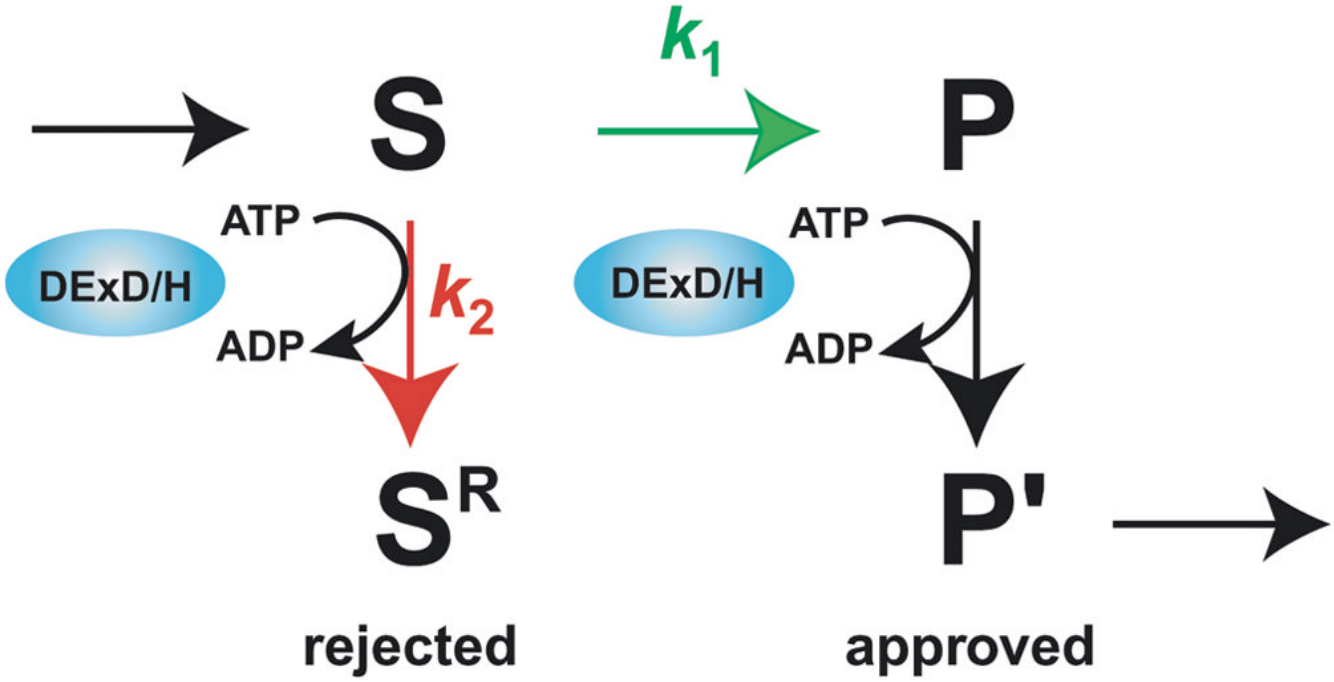

Fig. 4.

A general mechanism for proofreading RNP transitions by DExD/H box ATPases. In the kinetic proofreading scheme, k_1 (for S→P, shown in green) represents the rate of a chemical reaction, such as exon ligation, a binding event, such as binding of U2 to the branch site consensus, or potentially a conformation change; k_2 (for S→S^R, shown in red) represents the rate of rejecting a substrate. Specific discrimination against an incorrect substrate can be established by a slower k_1 and/or a faster k_2. Note that the DExD/H box ATPase expends energy to reject an incorrect substrate but also expends energy to promote a genuine product (P→P′), if the DExD/H box ATPase functions after, rather than before, the step under inspection. It is currently unclear what determines whether the DExD/H box ATPase acts before or after the proofread step and how the ATPase antagonizes splicing before while promoting splicing after the proofread step.