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## The SSU Processome in Ribosome Biogenesis – Progress and Prospects

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### Abstract

The small subunit (SSU) processome is a 2.2 MDa ribonucleoprotein complex involved in the processing, assembly and maturation of the SSU of eukaryotic ribosomes. The identities of many of the factors involved in SSU biogenesis have been elucidated over the past 40 years. However, as our understanding increases, so do the number of questions about the nature of this complicated process. Cataloguing the components is the first step towards understanding the molecular workings of a system. This review will focus on how identifying components of ribosome biogenesis has led to the knowledge of how these factors, protein and RNA alike, associate with one another into sub-complexes, with a concentration on the small ribosomal subunit. We will also explore how this knowledge of sub-complex assembly has informed our understanding of the workings of the ribosome synthesis system as a whole.

### Keywords

SSU processome; U3 snoRNA; Utp; ribosomal SSU; RNA processing; RNA chaperone

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The process of making a single ribosome is a herculean task, and is one of the most metabolically expensive activities of a cell. In vigorously growing yeast cells, it requires the activity of all three RNA polymerases, accounting for 70% of total transcription, 90% of pre-mRNA splicing and more than 25% of translation.<sup>1</sup> In *Saccharomyces cerevisiae*, nearly 7000 nucleotides of pre-rRNA must be accurately transcribed, cleaved, folded, chemically modified by 71 snoRNPs directing either 2'-*O*-methylation or pseudouridylation, and assembled with 78 ribosomal proteins (r-proteins) to form one mature ribosome. Despite the immensity of this task, about 2000 new ribosomes are produced each minute in yeast (~7500 subunits per minute in HeLa cells), leading to the presence of ~200 000 ribosomes in each cell (~10 million in each HeLa cell).<sup>1, 2</sup>

Because of its central importance, defects in ribosome biogenesis can have detrimental effects on cellular metabolism and vitality. Interestingly, a number of diseases have been found to be associated with defects in ribosome synthesis pathways. Several recent reviews contain details on the particular ribosomopathies known to date.<sup>3, 4</sup> In addition, ribosome biogenesis is a key component of the cell cycle where it regulates cell size and growth,<sup>5–7</sup> and is thus up-regulated in cancer.<sup>8</sup> Despite this critical linkage, ribosome biogenesis is

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understudied and its role in cancer is underappreciated. In addition, new evidence suggests that ribosome biogenesis proteins play critical roles in stem-cell differentiation in *Drosophila*.<sup>9</sup> Furthermore, ribosome synthesis may also be a mechanism through which HIV modulates host response.<sup>10</sup>

Tremendous efforts over the past 40 years or so have greatly increased our understanding of the molecular mechanisms of ribosome biogenesis. However, there are still many mysteries left to solve. A recent review in a different field outlined a particularly succinct way of describing how researchers can approach learning about a new biological system.<sup>11</sup> First, researchers must identify all the parts of the system. Over the past 10 years, great strides have been made in identifying the factors with roles in ribosome synthesis. Especially in the model organism *S. cerevisiae*, one can say that probably most of these factors have been catalogued. Once the parts list of a system is complete, or nearly complete, researchers can then investigate which parts interact with other parts. In the dynamic process of ribosome biogenesis, this can be a decidedly frustrating task. A number of large- and small-scale studies have identified sub-complexes involved in ribosome biogenesis. However, the scope and coverage of these interactions are limited. Additionally, there are numerous enzymes involved in maturation steps of pre-ribosomes. How these enzymes interact with pre-ribosomal particles and the specific substrates of their reactions is also a challenge for researchers to decipher. Once sub-complexes of the system are defined, a third central question that researchers can ask is how does this system of connected parts work as a whole? Our current understanding of the molecular mechanism by which eukaryotic ribosomes are assembled is largely speculative, owing mainly to the lack of an *in vitro* reconstitution system. Indeed, our knowledge of the assembly and function of the spliceosome has been greatly aided by the use of *in vitro* splicing assays (reviewed in<sup>12</sup>). An advantage of studying ribosome biogenesis in Bacteria is that the bacterial ribosome can be assembled, although inefficiently, *in vitro* from its protein and rRNA components.<sup>13</sup> Unfortunately, no system exists to study eukaryotic ribosome biogenesis *in vitro*, as coupling of transcription with pre-ribosome processing and maturation seems to be key to the complex journey of ribosome assembly.<sup>14</sup> 15

There are a number of excellent reviews on the details of ribosome biogenesis.<sup>16–20</sup> This review will focus mainly on biogenesis of the small subunit (SSU) of the ribosome in Eukaryotes, specifically on the current state of our understanding of ribosome biogenesis, and some anticipated future directions in this field. Therefore, we have included a brief overview of the process of SSU biogenesis to serve as a starting point for our commentary.

## Overview of ribosome biogenesis in Eukaryotes

Ribosome biogenesis takes place in the nucleolus, a specialized compartment (or compartments) of the nucleus where the rDNA loci are grouped in several hundred tandem repeats in nucleolar organizing regions (NORs). Although the size, number and structure of nucleoli vary according to cell type and metabolic state, the ultrastructural morphology of the nucleolus is quite conserved in Eukaryotes. Three basic nucleolar regions can be distinguished by EM: the fibrillar center (FC) contains inactive rDNA genes, the dense fibrillar component (DFC) where pre-rRNA synthesis and early processing events occur, and the granular component where late processing events and assembly of ribosomal particles occur (reviewed in<sup>21–23</sup>). In Eukaryotes, three of the four mature rRNAs are transcribed by RNA polymerase I as a polycistronic precursor (see Figure 1). In yeast, the 35S pre-rRNA contains the sequences for the mature 18S, 5.8S, and 25S rRNAs. The fourth rRNA, the 5S rRNA, is transcribed separately by RNA polymerase III. The 35S precursor must be cleaved at sites A<sub>0</sub>, A<sub>1</sub> and A<sub>2</sub> to generate the pre-18S rRNA, a process which requires a large RNP dubbed the SSU processome. A subset of ribosomal and non-ribosomal

proteins are assembled onto the pre-rRNA in the nucleolus. The rRNA precursor is also chemically modified by box C/D snoRNPs (43 in yeast), which are responsible for 2'-O-methylation, and box H/ACA snoRNPs (28 in yeast), which are responsible for pseudouridylation (reviewed in<sup>24–28</sup>). The 90S pre-ribosomal particles are separated into pre-40S and pre-60S particles via cleavage at site A<sub>2</sub>. Throughout the transcription and folding of the pre-rRNA, r-proteins are assembled into the pre-ribosomes as they migrate through the nucleoplasm. The final maturation steps, which include cleavage at site D, occur after the particles are exported into the cytoplasm. Details of these late steps can be found in several recent reviews.<sup>16–17, 20</sup>

## 1. WHAT ARE ALL THE PARTS OF THE SYSTEM?

### Ribosome biogenesis utilizes snoRNAs for varied functions

U3 was the first small nucleolar (sno) RNA identified and has since become the most extensively studied. It was discovered in 1968 by James L. Hodnett and Harris Busch during their investigations on the small RNAs of animal cells.<sup>29</sup> Although it was originally identified with the U1 and U2 small nuclear (sn) RNAs, the U3 snoRNA was found to be enriched in the nucleolus and to co-purify with ribosomes. However, its role in ribosome biogenesis remained enigmatic for several years. In the mid 1970's, the U3 snoRNA was conclusively implicated in pre-rRNA processing through the identification, and subsequent confirmation in the mid 1990's by chemical cross-linking and mutational studies, of regions of complementarity between the U3 snoRNA and the pre-rRNA/rRNA transcript.<sup>30–34</sup> Knockout of the U3 snoRNA genes was also found to arrest pre-rRNA processing at sites A<sub>0</sub>, A<sub>1</sub> and A<sub>2</sub>, resulting in the accumulation of unprocessed 35S pre-rRNA transcripts, loss of the mature SSU 18S rRNA and concomitant cell lethality.

The U3 snoRNA is evolutionarily conserved and has been identified in all Eukaryotes examined thus far, including the phylogenetically diverse protists.<sup>35–36</sup> The secondary structure of U3 snoRNA can be divided into three domains, each with specific sequence elements, protein components, and distinct functions: a 5' domain involved in base-pairing interactions with the pre-rRNA, a 'hinge' region consisting of the 5' and 3' hinge sequences, and a 3' terminal hairpin domain containing the box C/D motifs (see Figure 2).<sup>34–37</sup> The 5' domain contains the conserved sequence boxes GAC, A' and A. These sequence elements are complementary to the 5' end and to an internal region of the 18S rRNA.<sup>30–34, 38</sup> Base-pairing interactions between these anti-sense guide sequences in the U3 snoRNA and the pre-rRNA have been confirmed by chemical cross-linking,<sup>30</sup> mutational analysis,<sup>31–33</sup> *in vivo* structure probing<sup>34, 38</sup> and evolutionary conservation.<sup>39</sup>

The 'hinge' region of the U3 snoRNA is typically unstructured<sup>40–41</sup>, except for a small centrally located stem-loop structure found in many organisms (including yeast<sup>34</sup>). The Mpp10 sub-complex of the SSU processome is associated with this region.<sup>42–44</sup> The U3 snoRNA 5' and 3' hinge sequences have been proposed to provide proper spacing between the pre-rRNA-binding function of the 5' domain and the protein-complexed 3' domain of the U3 snoRNA.<sup>33–37</sup> Base-pairing interactions are also known to occur between the 'hinge' region of the U3 snoRNA and the 5' ETS.<sup>33–45–47</sup> In yeast and *Xenopus*, the 5' hinge was shown to base-pair with 5' ETS of the pre-rRNA.<sup>30–33</sup> The 3' hinge in yeast has been predicted to also base-pair with the pre-rRNA,<sup>33</sup> but this has not yet been tested.

The 3' domain of the U3 snoRNA forms an extended stem-loop punctuated by a number of short hairpins forming a cruciform structure in yeast.<sup>37</sup> This region is highly variable between different organisms and contains a number of dispensable non-conserved hairpins.<sup>35–37</sup> The 3' stem-loop contains the box C/D sequence boxes C', B, C and D, and is important in protein binding, RNA stability and nuclear retention.<sup>48–50</sup> This region also

contains two kink-turn (K-turn) RNA motifs within the juxtaposed box C/D and B/C elements.<sup>49</sup>

There are also other snoRNAs in addition to the U3 snoRNA involved in ribosome maturation. Many of these snoRNAs were identified based on co-immunoprecipitation with fibrillarin (Nop1 in yeast), a component of box C/D snoRNPs.<sup>51</sup> The U14 snoRNA was found to be able to base-pair with the 18S rRNA and is essential for cell viability.<sup>52</sup> U3 and U14 are both non-canonical box C/D snoRNAs, in that they are required for cleavage of the pre-rRNA.

Subsequent to the discovery of the box C/D snoRNAs, another class of snoRNAs was identified.<sup>53, 54</sup> These H/ACA snoRNAs are mainly involved in the chemical modification of uridine to pseudouridine.<sup>24, 25</sup> However, two H/ACA snoRNAs also have roles in processing the pre-rRNA. Deletion of snR10, an H/ACA snoRNA, results in a cold-sensitive phenotype – suggestive of defects in RNA folding and ribonucleoprotein assembly<sup>55</sup> – and also delays processing of the pre-18S rRNA.<sup>56</sup> snR30/U17 is an essential box H/ACA snoRNA, and is involved in processing of the pre-18S rRNA.<sup>57, 58</sup> snR30/U17 is unique in that it is the first box H/ACA snoRNA identified to have an evolutionarily conserved role in the cleavage of pre-rRNA.<sup>59, 60</sup> Although the U14, snR10 and snR30/U17 snoRNAs have been linked to pre-18S rRNA cleavages, it remains unclear whether or not these snoRNAs are incorporated into the SSU processome to perform their function.

Deletion of most modification-guide snoRNAs individually does not have a noticeable effect on ribosome biogenesis or ribosome stability. However, deletion of multiple snoRNAs in tandem, especially those with targets in the decoding region of the ribosome, the peptidyl-transferase center, or helix 69 of the LSU rRNA, results in slower growth rates, slower amino acid incorporation rates, decreased translational fidelity, as well as defects in pre-rRNA processing.<sup>28, 61, 62</sup> In addition to their roles as modifying enzymes, it has been postulated that snoRNAs could be instrumental in the correct folding and maturation of the rRNA, although this has yet to be tested directly.<sup>63, 64</sup>

### **The ribosome synthesis machinery includes many non-ribosomal factors**

The genetically tractable model organism *S. cerevisiae* has been used to identify many of the protein and RNA players in ribosome biogenesis. A majority of factors involved in ribosome biogenesis are both nucleolar and essential for growth. The characteristic processing intermediates visualized by northern blotting for pre-rRNAs have been useful as an assay to identify defects in ribosome biogenesis. Depletion of ribosome biogenesis factors results in processing defects, visualized by differing patterns of stalled pre-rRNA processing intermediates, and has allowed for the classification of proteins and non-coding RNAs into categories of pre-SSU and pre-LSU biogenesis factors. Sucrose gradients have been useful in identifying how individual proteins associate with pre-ribosomal particles via co-sedimentation experiments.

Biochemical purification of pre-ribosomal particles has largely been achieved through tandem affinity purification – mass spectrometry (TAP-MS) schemes, and has resulted in a large collection of ribosome biogenesis factors. The SSU processome, first purified by our laboratory, identified the so called Uthree proteins (Utps) that co-purified with the U3 snoRNA and its core proteins<sup>65, 66</sup> (discussed below). Not surprisingly, both the U3 snoRNA and nearly all SSU processome proteins are phylogenetically conserved in all Eukaryotes examined thus far,<sup>35, 36</sup> although they are not present in either Archaea or Bacteria.<sup>67</sup> Concomitant with the discovery of the SSU processome, Grandi *et al.* identified a 90S pre-ribosomal particle that had many overlapping components with the SSU processome.<sup>68</sup> Interestingly, these 90S particles were lacking many of the factors known to

be required for LSU biogenesis, implying that pre-SSU and pre-LSU biogenesis are two distinct activities. This finding was in line with previous work showing that the 35S pre-rRNA locus can be physically separated and that stand-alone pre-18S and 5.8/25S rRNA coding units can, when transcribed separately, be processed and assembled into mature, functional ribosomes.<sup>69</sup> Studies using TAP-MS or similar methods identified other components of 90S particles, many components of LSU biogenesis, as well as components of mitochondrial ribosomes.<sup>70–75</sup>

Our current view of the SSU processome is that of a massive 80S/2.2 MDa complex, of similar size to the ribosome, possibly containing as many as 72 protein components (see Table 1). Based on a strict inclusion criteria, ~46 proteins are able to (i) co-immunoprecipitate both U3 snoRNA and (ii) Mpp10, a known protein component of the SSU processome, and (iii) their genetic depletion results in loss of the 18S rRNA. Partial data (*i.e.*, ability to co-IP SSU processome components but pre-18S rRNA processing defects not tested, or *vice versa*) for an additional 26 proteins warrants their inclusion as probable SSU processome components. With a few exceptions, the exact molecular function of most SSU processome proteins is unknown. However, inspection of the list of protein components reveals a number of proteins with RNA binding and/or protein-protein interaction motifs. Undoubtedly, some of these proteins play structural roles in the SSU processome. In addition, the SSU processome also contains endonucleases, 10 RNA helicases and their co-factors, and ATPases, GTPases, kinases and other regulatory proteins.<sup>76–78</sup>

Currently, it would seem that the component list of the SSU processome is essentially complete. However, a caveat to the methods utilized thus far is that they may be excluding low abundance and/or low affinity proteins, regulatory components and transiently-associated factors, such as enzymes. Experiments that can cross-link proteins and/or RNA *in vivo* may still yield unexpected additions to the long (and expanding?) list of ribosome biogenesis players.

## 2. WHICH PARTS INTERACT WITH OTHER PARTS?

### The SSU processome enters the Systems Biology era

With a relatively complete, and extensive, parts list for the SSU processome, the next question then becomes: how do these many proteins assemble and interact together during SSU biogenesis? As a box C/D snoRNA, U3 was known, *de facto*, to associate with the four box C/D snoRNP proteins fibrillarin/Nop1, Nop56, Nop58 and Snu13/15.5K and with the U3 snoRNA-specific Rrp9/U3-55K thereby forming the so called U3 snoRNP. A yeast two-hybrid screen for proteins that physically interact with Mpp10 identified the second sub-complex of the SSU processome. Dubbed the Mpp10 sub-complex, it contains the Mpp10 protein along with Imp3 and Imp4.<sup>79</sup> Similarly, a yeast two-hybrid screen of the cyclase-like protein Rcl1 identified the regulatory GTPase Bms1 as a second member of this (as of yet) two component sub-complex.<sup>80</sup>

The breakthrough in defining the sub-complexes of the SSU processome came through a TAP-MS study of the RNA processing complexes in yeast.<sup>81</sup> This study identified three large sub-complexes of the SSU processome, namely the transcriptional Utps (t-Utps) or UtpA (t-Utp4, 5, 8, 9, 10, 15, 17 and Pol5), UtpB (Utp1, 6, 12, 13, 18 and 21) and UtpC (Utp22, Rrp7 and the four subunits of casein kinase II: Cka1, Cka2, Ckb1 and Ckb2).<sup>81</sup> While these sub-complexes were initially described by Krogan *et al.*, not all sub-complexes were present in their complete form.<sup>15</sup> <sup>82</sup> <sup>83</sup> Indeed, work is still needed to both verify existing sub-complex information and to identify other putative sub-complexes that were not observed in these previous studies.



The t-Utps/UtpA is believed to form one of the earliest sub-complexes in the assembly of the SSU processome.<sup>15, 84, 85</sup> This sub-complex, formed independently of the U3 snoRNA,<sup>15, 81</sup> is required both for the transcription of the pre-rRNA and its processing, whereas the remainder of the SSU processome components are only known to play a role in pre-rRNA processing.<sup>65, 66</sup> A protein-protein interaction (PPI) map of the t-Utp/UtpA sub-complex, determined by yeast two-hybrid assay, has recently been published,<sup>86</sup> and generally overlaps with a partial PPI map determined by protein-fragment complementation assay.<sup>87</sup> While the t-Utp/UtpA components are generally conserved in Eukaryotes, it is noteworthy that t-Utp8 and 9 are yeast-specific and have no known human homologues.<sup>65, 88</sup> This raises the intriguing possibility that currently unidentified components of the human t-Utp/UtpA sub-complex are the functional homologues of the yeast t-Utp8 and 9.<sup>88</sup>

Little is known of the function of the UtpB sub-complex. Our laboratory previously determined the architecture of the UtpB sub-complex by yeast two-hybrid methodology augmented by the validation of a key PPI by surface plasmon resonance.<sup>89</sup> From this PPI map, we obtained some insight into the assembly of the sub-complex from its individual components.

The UtpC sub-complex is the least studied of the sub-complexes. The presence of the casein kinase II complex within the UtpC sub-complex is unexpected and raises a number of intriguing possibilities. In a different yeast species, casein kinase II is known to phosphorylate SSU r-proteins,<sup>90</sup> suggesting the possibility that casein kinase II complex phosphorylates components of the SSU processome, and thereby temporally and/or spatially regulates their activities. It is noteworthy that the casein kinase II complex has also been identified as part of two additional complexes and is therefore not unique to the UtpC sub-component of the SSU processome.<sup>81</sup> Finally, a recently identified SSU processome component, Rrp36, may also be a member of the UtpC sub-complex based on TAP-MS studies,<sup>91</sup> though this has not been firmly established.

In all, six sub-complexes of the SSU processome have been described: (i) the U3 snoRNP “monoparticle,” (ii) the Mpp10 sub-complex, (iii) the Bms1/Rcl1 sub-complex and the (iv) t-Utp/UtpA, (v) UtpB and (vi) UtpC sub-complexes (see Table 1). However, the protein components of these various sub-complexes account for only 31 of the 72 (43%) protein components of the SSU processome; thus, more than half of the SSU processome proteins have yet to be ascribed to a sub-complex. A certain number of these proteins may individually associate with the SSU processome, as one would expect for regulatory proteins. The remainder of these proteins may form currently undescribed sub-complexes, be present in low-abundance, or engage in transient associations with components of the currently known sub-complexes. It is unknown whether the components of the SSU processome sub-complexes remain tethered to one another in some fashion during the ribosome assembly process. It is plausible that many of these sub-complexes exist as discrete functional units at different points of ribosome biogenesis. An example of one such complex is the t-Utp/UtpA subcomplex, which putatively assembles first onto the rRNA during its transcription.<sup>15</sup> The t-Utps/UtpA could then recruit other subcomplexes, resulting in the formation of the SSU processome as a whole. While the SSU processome, by definition,<sup>65</sup> contains at least the U3 snoRNA, Nop5/58, and Mpp10, the function of this large RNP may require that sub-complex association be somewhat dynamic. However, we know that components of the SSU processome, as originally purified, co-migrate on sucrose gradients in a large (80S) RNP.<sup>65</sup> Exploring the interaction profiles of these components will yield much information about the assembly and architecture of the SSU processome, along with the multiple dynamic interactions necessary for assembly of pre-ribosomes.

Most of our knowledge of the protein interactions within the sub-complexes of the SSU processome has been obtained by small-scale studies. Indeed, an examination of high-throughput genetic and binary PPI data has revealed a lack of information on the SSU processome protein components.<sup>87, 92–94</sup> In genetic interaction studies, either by epistatic mini-array profile (E-MAP) or by synthetic genetic array (SGA), the function of each gene in the genome is perturbed, often by deletion, and the phenotypic consequence is measured. The SSU processome is particularly recalcitrant to such approaches, most likely due to the fact that the overwhelming majority of its components (62/72) are essential proteins. Thus, alternative strategies for genetic perturbation, such as the decreased abundance by mRNA perturbation (DAMP)<sup>95</sup> or the creation of conditional or hypomorphic alleles, must be used. As a result, recent large scale E-MAP<sup>96</sup> and SGA<sup>97</sup> have included few SSU processome components in their studies. The need for such data is critical, and in its absence, important questions, such as the genetic and physical PPIs linking the various sub-complexes remain unknown.

### From PPI maps to structural studies of the sub-complexes

Biochemical studies to characterize sub-complexes of the SSU processome and other pre-40S particles have provided structural biologists with numerous problems to solve. Protein-protein and protein-RNA interaction data support the hypothesis that pre-ribosomes are assembled sequentially from multiple independently formed “modules.”<sup>15, 81, 85</sup> While limited information is available for how these proteins assemble in low resolution maps, even less is known about the atomic or near atomic resolution of these “modules.” Indeed, most of the high-resolution structures available for ribosome maturation factors is limited to snoRNPs. Even so, the majority of data that exists for the snoRNPs is from crystal structures of archaeal homologues (reviewed in<sup>27</sup>).

Although limited to lower resolution than can be achieved via X-ray diffraction, electron microscopy (EM) remains a valuable tool to use for structural studies. Recently, our laboratory solved the structure of an archaeal box C/D sRNP using single particle negative stain EM.<sup>98</sup> Importantly, it challenged the conventional view of box C/D sRNPs as monomeric moieties containing one sRNA and two copies of each of the three core proteins: L7Ae, Nop5, and fibrillarin. Instead, the structure showed that the archaeal box C/D sRNP is a di-sRNP with two sRNAs and four copies of each core protein. Formation of this dimeric structure correlates with enzymatic activity. Around the same time, Ye *et al.*<sup>99</sup> solved an atomic resolution structure of an archaeal box C/D sRNP. Their model conflicts with our di-sRNP reconstruction. This discrepancy could be a result of their sRNA construct, as they did not use a full-length sRNA in their structure, increasing the likelihood of artificial crystal packing interactions influencing their proposed model. Previously, results were limited by the available crystal structures of solely the individual core proteins, co-crystal structures containing the Nop5-fibrillarin dimer without RNA, or L7Ae bound to a kink-turn RNA.<sup>100–103</sup> In order to obtain pseudo-atomic resolution, it is possible to “fit” crystal structures into EM volumes (see Figure 3). This fitting allows more detailed information to be gleaned from lower-resolution structures, and is an alternative to using antibody or gold nanoparticle labeling to interpret individual protein placement in large protein complexes.<sup>104, 105</sup>

The other class of rRNA modifying enzymes, the box H/ACA s(no)RNPs, has also been a target of structural studies. In 2006, an X-ray crystal structure of a holo-archaeal box H/ACA sRNP was solved.<sup>106</sup> This work provided a glimpse into how its architecture affects the enzymatic activity of the sRNP, specifically how the guide sRNA is positioned by the core proteins that bind to it to position the guide sRNA to base-pair with the substrate. Recently, the structure of an archaeal box H/ACA sRNP, albeit lacking one of the four core proteins, Gar1, bound to a substrate was solved to atomic resolution.<sup>107</sup> The architecture of

this structure confirmed that the sRNA and associated proteins serve to position both guide and substrate RNA for correct enzymatic activity.

Neither high- nor low-resolution structures have been solved for protein complexes involved in folding of the eukaryotic pre-rRNA, nor for the enzymes responsible for the cleavages of the pre-rRNA, although some low-resolution PPI mapping has been done for the UtpA and UtpB sub-complexes.<sup>86, 87, 89</sup> In order to gain a better understanding of how different “modules” of the pre-ribosomes are assembled, both structural and biochemical studies need to be correlated to appreciate the concerted and coordinated efforts that are required for the assembly of mature ribosomes.

### 3. HOW DOES THIS SYSTEM OF CONNECTED PARTS WORK AS A WHOLE?

#### How is ribosome production regulated by cellular activity?

As emphasized above, ribosome synthesis is an extraordinarily metabolically expensive endeavor for a cell.<sup>1, 5-7</sup> Thus, when conditions for cell growth become favorable, mechanisms for up-regulating ribosome biogenesis become extremely important. Ribosome production is therefore tightly coordinated with environmental conditions and nutrient availability.<sup>108, 109</sup> In yeast, a cell will only divide once it reaches a critical size, called the “setpoint,” which is dependent on the cell’s protein synthesis capacity - otherwise stated as having a sufficient number of mature ribosomes. In addition, results from our laboratory suggest that yeast cells can, in addition to sensing mature ribosome levels, also sense ribosome biogenesis.<sup>110</sup> Genetic depletion of SSU processome components results in G1 arrest, implying that if ribosomes cannot be assembled at G1, progression through the cell cycle is arrested.<sup>111</sup> Thus, ribosome biogenesis directly promotes passage through the START phase of the cell cycle.<sup>5, 110</sup>

A number of signal transduction pathways, such as the target of rapamycin (TOR), have been implicated in linking nutrient availability to ribosome production.<sup>112</sup> Recent work is also starting to implicate a number of additional signaling pathways to have effects on ribosome synthesis, many of which have well established links to cancer<sup>8</sup>, such as the p53 pathway<sup>113, 114</sup> and the c-Myc pathway.<sup>115, 116</sup> Although in yeast the direct link of metabolism to ribosome biogenesis is well known, a number of disease states in humans are attributed to defects in ribosome synthesis pathways. It is unlikely that the whole story is clear surrounding this apparent linkage (for detailed reviews of ribosomopathies see 3, 4). In all, ribosome biogenesis is directly linked to the proliferative capacity of the cell, including cancer cells. Yet, this link remains understudied and underappreciated.

Similarly, regulation of the SSU processome’s activity is poorly understood. A number of SSU processome proteins have ATPase, GTPase or kinase activities, yet their role in regulating the activity of the SSU processome is just beginning to emerge.<sup>76, 77</sup> It is noteworthy that the UtpC sub-complex contains the casein kinase II components that are presumably regulating aspects of the SSU processome’s activity.

#### Pre-rRNA transcription is spearheaded by UtpA

The tUtps/UtpA form a complex that is interesting in that the proteins are required both for the transcription of the pre-rRNA and for its processing, whereas the other SSU processome components are only known to play a role in pre-rRNA processing. They were first discovered during the initial characterization of the SSU processome components through the observation that genetic depletion of a subset of Utps resulted in the loss of the mature 18S and an unexpected decrease in 25S rRNA levels,<sup>65</sup> suggesting a decrease in the levels



of the 35S pre-rRNA that is a precursor to both. Subsequent work found that the t-Utps/UtpA are required for optimal transcription of the pre-rRNA in both yeast<sup>15</sup> and humans<sup>88</sup>, as assayed by both transcription run-on assays and by the quantification of the number of rRNA transcripts in Miller chromatin spreads. Furthermore, the t-Utps are associated with rDNA and with the pre-18S rRNAs independent of the presence of the U3 snoRNA.<sup>15, 88</sup> Therefore, it was suggested that the t-Utps/UtpA form one of the earliest sub-complexes in the assembly of the SSU processome and thus link pre-rRNA transcription and processing.<sup>15, 84</sup> This finding was unexpected because it had been previously assumed that pre-rRNA transcription and processing were two separate processes. Unfortunately, the mechanism by which the t-Utp/UtpA sub-complex links pre-rRNA transcription by RNA polymerase I to processing of the pre-rRNA by the SSU processome remains unknown.

### The SSU processome as a pre-rRNA chaperone

The pre-rRNA, containing roughly 7000 nucleotides, must be correctly cleaved and assembled into a functional ribosome. With such a large molecule, one can imagine a nearly infinite number of thermodynamic traps into which the pre-rRNA can misfold. Both the protein and RNA components of the SSU processome have been hypothesized to be involved in ribosome chaperone activities.<sup>39, 43, 44, 117</sup> Indeed, the SSU processome is mainly regarded as a chaperone for the RNA folding that must occur prior to the multiple sequence-specific pre-rRNA cleavage events that eventually liberate the mature 5' end of the 18S rRNA. A recent study by the Correll laboratory used *in vitro* FRET-based assays to demonstrate the assembly of the Imp3 and Imp4 'chaperone complex.' This complex was shown to possess RNA chaperone activities that stabilize the U3 snoRNA:pre-rRNA duplex.<sup>44</sup> In addition to the Imp3 and Imp4 proteins playing a role in promoting the correct U3:pre-rRNA interactions, the U3 snoRNA has been hypothesized to be a chaperone for the folding of the pre-rRNA.<sup>16, 39</sup> The role of the U3 snoRNA as an RNA chaperone stems from the observation that sequences within the conserved box A of U3 snoRNA are complementary to, and base-pair with, highly conserved regions of the mature SSU rRNA which fold into the universally conserved 5' end pseudoknot (see Figure 2). Base-pairing interactions between the U3 snoRNA and the 5' end pseudoknot of the 18S rRNA form a secondary structure that is incompatible with that of the mature 18S rRNA.<sup>39, 117</sup> This interaction may serve to chaperone the folding of the SSU rRNA by sequestering the 5' domain of the 18S rRNA, thereby preventing it from folding prematurely. A chaperone role has also been proposed for the U14 snoRNA.<sup>63</sup>

In addition to avoiding incorrect folding around the central pseudoknot, the interactions between the U3 snoRNA and the pre-18S rRNA also guide, by a complex and poorly understood mechanism, the multiple sequence-specific pre-rRNA cleavage events at sites A<sub>0</sub>, A<sub>1</sub> and A<sub>2</sub>.<sup>31, 32, 34, 39, 117</sup> The chaperone activity may serve to fold the pre-rRNA into a structure that can be recognized as a substrate by the putative endonucleases.<sup>118</sup> Directly testing the chaperone roles of the SSU processome and its components, in particular the U3 snoRNA, is important for understanding their roles in ribosome biogenesis.

### Is the SSU processome an RNA "untanglese"?

A remarkably large number of RNA helicases participate in ribosome biogenesis, with 10 such helicases participating in SSU assembly.<sup>78, 119</sup> Most of these are members of the DEAH/D box families of RNA helicases, aptly named for their conserved D-E-A-H/D sequence. Their exact role in SSU biogenesis remains a matter of speculation, however, one can presume that RNA helicases mediate the many highly dynamic pre-rRNA folding and conformational rearrangements, rRNA duplex annealing and unwinding, and rRNA/protein remodeling and displacement events (such as the removal of non-ribosomal proteins from the pre-rRNA) that occur during ribosome biogenesis. Intriguingly, it has also been

proposed that helicases may play a role in the unwinding of snoRNAs from their pre-rRNA/rRNA target. This is seen by the retention of snoRNAs on the pre-rRNA in the absence of the helicases Dbp4, Has1, Rok1 or Prp43, the latter in the pre-LSU rRNA,<sup>120–123</sup> though this activity has not been directly demonstrated. This concept has recently been bolstered by the finding that Prp43 interacts with both snoRNAs and their corresponding substrate regions in the pre-LSU rRNA, as seen by the cross-linking and analysis of cDNA (CRAC) approach.<sup>123</sup> Lastly, it has also been suggested that RNA helicases may act as molecular clamps, thereby holding their RNA substrate until its release by ATP hydrolysis.<sup>124</sup> This is in line with the finding that RNA helicases can display both duplex unwinding and annealing activities.<sup>125</sup>

RNA helicase biochemical activities have been fairly well characterized.<sup>126–127</sup> However, the mechanism by which they temporally and spatially reach their target substrate(s) during ribosome biogenesis remains generally unclear and represents one of the most pressing questions in RNA helicase biology. It is generally believed that protein co-factors recruit, through PPIs, the helicase to its site of activity,<sup>128</sup> as a few reported examples suggest. The SSU processome helicase Dbp8 is known to interact with Esf2, another SSU processome component. Esf2 can bind to pre-rRNAs, presumably providing substrate specificity, and can stimulate the activity of Dbp8, possibly providing temporal specificity.<sup>128</sup> Similarly, the helicase Prp43 associates with the glycine-rich motif (G-patch) containing proteins Pfa1 and Gno1, both components of the pre-SSU particle.<sup>70–124, 129–130</sup> A G-patch motif is a short conserved domain, consisting of seven highly conserved glycines, found in a number of RNA-binding proteins.<sup>131</sup> The binding of Pfa1 to Prp43 was found to stimulate its helicase activity in both yeast<sup>124</sup> and human cells,<sup>130</sup> thus possibly providing temporal and spatial specificity. These examples involve only 2 of the 10 helicases involved in SSU biogenesis. It remains unknown which co-factors provide temporal and spatial specificity to the 8 remaining helicases. Also intriguing is whether these co-factors all play a stimulatory role, or do some also play an inhibitory role? Furthermore, it will be interesting to investigate the target specificity of these co-factors.

### On a road to understanding function via structure

A monumental breakthrough occurred nearly a decade ago when atomic resolution structures of the bacterial and archaeal ribosomes were solved.<sup>132–133</sup> An atomic model of eukaryotic ribosomes, however, has been more elusive. Most recently, a pseudo-atomic model of the eukaryotic ribosome has been achieved through docking and modeling yeast rRNA and r-proteins into a cryo-EM volume of the *Thermomyces lanuginosus* ribosome.<sup>134</sup> Understanding the movement of ribosomes during the process of translation has been immensely aided by deciphering the structures of both bacterial and eukaryotic ribosomes with different accessory factors.<sup>135–141</sup>

If one considers the problem of understanding the structure of a mature ribosome a difficult puzzle to solve, an equally or perhaps more daunting problem is delineating the stages of assembly and deconstructing the associated molecular machines involved in producing this massive molecular motor *in vivo*. For these steps, practically all of the current structural knowledge relies on negatively stained and cryo EM reconstructions. Work from the Hurt laboratory on the yeast late pre-40S subunit identified a maturation step involving the r-protein Rps3 and the protein kinase Hrr25.<sup>142</sup> Based on their EM models, as well as biochemical experiments testing the stability of Rps3 association with the 40S in Hrr25 depleted cells, they determined that Hrr25-dependent phosphorylation and subsequent dephosphorylation was required for beak formation in the mature 40S subunit.

Electron microscopy has also been effectively used to observe LSU biogenesis. Nissan *et al.*<sup>105</sup> took advantage of the TAP method to investigate pre-60S subunits that contain the Rix1

complex. They were able to use antibody cross-linking in combination with electron microscopy to localize the AAA-type ATPase Rea1 to the “tail” region of the “tadpole-like” pre-60S structure. Interestingly, antibodies against ribosomal proteins Rpl3 or Rpl10 yielded predominantly “head-to-head” cross-links, suggesting that the Rea1 protein is distal to ribosomal proteins incorporated into the pre-60S particles. A more recent study on this particle shows that the positioning of Rea1 may enable the protein to use its metal ion-dependent adhesion site (MIDAS) as a mechanochemical lever to release non-ribosomal factors from the pre-60S particle.<sup>143</sup> The combination of EM imaging with the biochemical and genetic experiments in this study provided strong and compelling evidence for their pre-60S maturation model.

### Low resolution structural data remains informative

Despite the relative dearth of atomic or lower-resolution structural information available for the early pre-SSU, techniques used since the 1970s have yielded useful information about when, temporally, events in processing and assembly occur. Chromatin spreads, first described by Miller and colleagues,<sup>144</sup> have long been used to study ribosome biogenesis in many organisms, including the yeast *S. cerevisiae*.<sup>145</sup> The rDNA is arranged on chromosomes in tandem repeats, and is one of the most actively transcribed loci in the genome. Miller chromosome spreads have been dubbed “Christmas trees,” because the same repeat is transcribed at the same time by multiple molecules of RNA Pol I (see Figure 4). The pre-rRNA transcripts (the “branches” of the trees) have different lengths depending on the location of the polymerase, and, under the Miller spreading conditions, are splayed out from the “trunk” of the tree, corresponding to the rDNA. These pre-rRNA transcripts were also observed to have knobs of high electron density, which are involved in rRNA processing.<sup>146</sup> Our laboratory, in collaboration with the Beyer laboratory, showed that depletion of any essential SSU processome component resulted in loss of these terminal knobs. Indeed, Miller spreads showed that depletion of any of the components of the UtpA sub-complex of the SSU processome resulted in reduced transcription at the rDNA loci.<sup>15</sup> Interestingly, pre-rRNA processing can also be observed using Miller chromatin spreads. The Beyer laboratory also used EM chromatin spreads to visualize release of terminal knobs, as well as compaction of the pre-rRNA during SSU biogenesis.<sup>147</sup> <sup>148</sup> Recently, the Tollervey laboratory has confirmed these co-transcriptional events using new techniques for harvesting and analyzing metabolically labeled pre-rRNA.<sup>14</sup>

### FUTURE DIRECTIONS

A great deal of thought and experimental effort has been devoted to understanding the process of ribosome biogenesis, and ongoing experiments hope to address the remaining questions in the field. In *S. cerevisiae*, probably most of the factors involved in ribosome synthesis have been identified, addressing our first concern of the parts of the system. However, a great deal of information remains missing about the connectivity of the system. Despite a plethora of data generated by high-throughput studies,<sup>87</sup> <sup>92</sup>–<sup>94</sup> <sup>96</sup>–<sup>97</sup> most genetic and PPI linkages are still missing when describing which parts of the system interact with other parts. More effort needs to be applied to understand the interactome of the yeast nucleolus, as many proteins in the SSU processome are absent from these studies.<sup>149</sup> These putative interactions could give insight into how the SSU processome is assembled on a larger scale, yielding structural information that may aid in understanding how the sub-complexes chaperone and regulate ribosome assembly. Recent work from the Tollervey laboratory has given the field an interesting look into where RNA helicases and other ribosome biogenesis factors bind to RNA.<sup>50</sup> <sup>123</sup> However, it remains to be seen and validated how these helicases are directed to their site(s) of action and how their activities are regulated. Finally, we would like to know how the system of connected parts works as a whole. Gaining structural insight into both large and small functional units of the pre-

ribosome could both answer and inspire new questions of how ribosome biogenesis is regulated through its many interacting factors. Although much is known about the SSU processome's components, and some is known about the function of this massive molecular machine, there are assumptions in the field that have yet to be tested and proven. The SSU processome is predicted to mediate the correct folding of the pre-18S rRNA, however this attractive hypothesis has not yet been experimentally validated. An *in vitro* reconstitution system to study eukaryotic ribosome biogenesis could lead to an understanding of how the U3 snoRNA directs the cleavages of the 35S pre-rRNA. Availability of *in vitro* assays for ribosome biogenesis could also facilitate single-molecule experiments that may answer questions about the base-pairing interactions of the U3 snoRNA to the pre-rRNA. Are the multiple U3:pre-rRNA interactions simultaneous or sequential? Also, can these interactions occur within a single molecule of U3 snoRNA or from multiple molecules, as might be predicted if one imagines evolutionary conservation of the dimeric architecture of archaeal box C/D snoRNPs.<sup>98</sup> While yeast is an incredibly versatile model organism, more focus should be applied to studying ribosome biogenesis in vertebrates in order to flesh out the differences and highlight the similarities between humans and yeast. Above all, despite the predictions that current results allow, it is important to experimentally validate hypotheses, because conclusions are strongest when supported by data.

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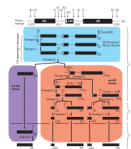


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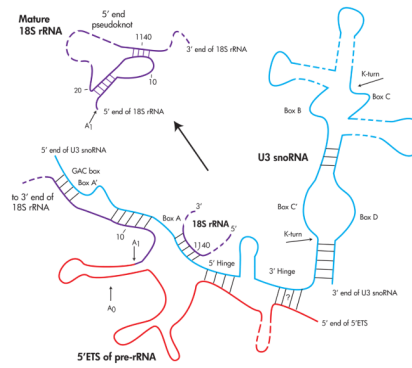
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**Figure 1. *S. cerevisiae* pre-rRNA processing scheme**

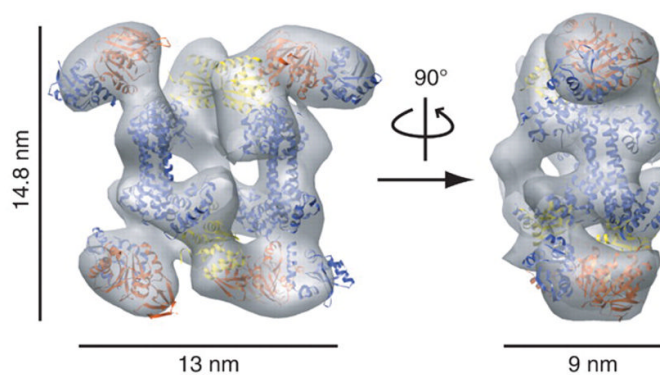
In yeast, the 35S polycistronic precursor rRNA is processed to the mature 18S, 5.8<sub>(S+L)</sub> and 25S rRNAs. The colored boxes signify pre-ribosomal particles: Blue – SSU processome, Purple – pre-40S particles, Red – pre-60S particles. See text for details on processing steps.





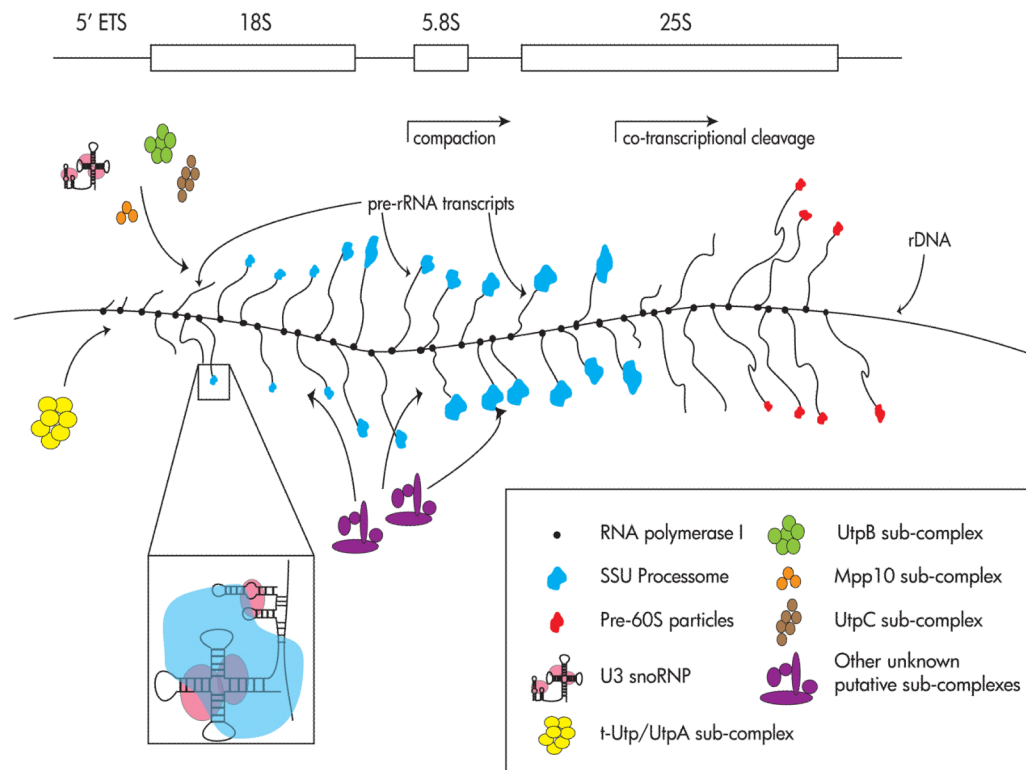
**Figure 2. The U3 snoRNA base-pairs with the pre-18S rRNA to direct cleavages at A<sub>0</sub>, A<sub>1</sub>, and A<sub>2</sub>**

The U3 snoRNA has been proposed to act as a chaperone for the formation of the conserved 5' end pseudoknot by base-pairing with the 18S rRNA. Colored lines correspond to different RNAs - Blue – U3 snoRNA, Purple – mature 18S rRNA sequences, Red – 5' ETS of the pre-rRNA that is cleaved during pre-rRNA processing. See text for details.



**Figure 3. X-ray crystal structures can be docked into EM reconstruction volumes to give pseudo-atomic resolution of large complexes**

Shown here is a docking of the crystal structures of *Pyrococcus furiosus* Nop5-fibrillarin [PDB 2nnw]100 and *Methanococcus jannaschii* L7Ae [1xbi]101 in the isodensity map of the *M. jannaschii* di-sRNP [EMBD accession code EMD-1636].98



**Figure 4. Miller chromatin spreads, so-called “Christmas trees,” can be used to visualize pre-rRNA transcription and steps of pre-rRNA processing**

This diagram represents a Miller spread, and indicates how the SSU processome is assembled via sub-complexes onto the pre-rRNA transcript. See text for details. One repeat of the rDNA tandem chromosomal repeats is shown above the Miller chromatin spread diagram to indicate the relative positions of the 35S pre-rRNA.

**Table 1**  
**The known and putative protein components of the yeast SSU processome**

The proteins are enumerated by their membership in the known sub-complexes of the SSU processome. Proteins that are confirmed SSU processome components but which have not been ascribed to a specific sub-complex are listed as unclassified. Candidate proteins, for which partial data suggests that they may be SSU processome components, are listed as unknown. The five SSU r-proteins (Rps4, Rps6, Rps7, Rps9 and Rps14) that are confirmed components of the SSU processome66 were not tabulated. Commonly used yeast names are listed, along with their alias when applicable. The name of the human orthologue, along with its GenBank accession number, is also noted.

Name	Alias	KDa	Human Orthologue	Sub-Complex	Comments	References
Nop1	Lot3	34.5	Fibrillarin; NP_001427	Box C/D	2'-O-methyltransferase; N-terminal glycine/arginine-rich (GAR) domain	150- 151
Nop56	Sik1	56.9	NOP56; NP_006383	Box C/D	KKE/D repeats	152
Nop58	Nop5	57.0	NOP58; NP_057018	Box C/D	KKE/D repeats	153
Snu13		13.6	15.5K/NHP2-like protein 1; NP_001003796	Box C/D	binds to the helix-bulge-helix (K-turn) motifs of U3 snoRNA; also part of the U4/U6-U5 tri-sRNP	154
Rtp9		65.1	U3-55K; NP_004695	U3 snoRNP	binds to the B/C motif of U3 snoRNA; contains WD40 repeats	155- 156
Imp3		21.9	IMP3; NP_060755	Mpp10	S4 RBD	79
Imp4		33.5	IMP4; NP_219484	Mpp10	contains a $\sigma^{70}$ -like motif	79
Mpp10		67.0	MPHOSPH10; NP_005782	Mpp10	coiled-coils; associated with the hinge region of U3 snoRNA	157
t-Utp4		87.8	Clrthin; NP_116219	UtpA	WD40 repeats	15- 65- 81
t-Utp5		72.0	WDR43; NP_055946	UtpA	WD40 repeats	15- 65
t-Utp8		80.2	?	UtpA	also involved in nuclear tRNA export	15- 65- 158
t-Utp9		65.3	?	UtpA	WD40 repeats	15- 65- 81
t-Utp10		200.1	BAP28/HEATR1; NP_060542	UtpA	HEAT repeats	15- 65- 81
t-Utp15		57.7	UTP15; NP_115551	UtpA	WD40 repeats	15- 65- 81
t-Utp17	Nan1	101.2	WD repeat domain 75; AAH40567	UtpA	WD40 repeats; also part of RENT complex	15- 65- 81
Poi5		115.9	Myb-binding protein 1A; AAF33021	UtpA	required for rRNA synthesis; not required for DNA replication	15- 81- 159
Utp1	Pwp2	104.0	PWP2; NP_005040	UtpB	WD40 repeats, $\beta$ transducin family; Pwp2 domain	65- 81- 82- 89
Utp6		52.4	UTP6; NP_060898	UtpB	half-a-tetratricopeptide repeat motif [half-a-TPR (HAT)]	65- 81- 82- 89
Utp12	Dip2	106.3	WDR3; NP_006775	UtpB	WD40 repeats, Dip2 domain	65- 81- 82- 89

Name	Alias	KDa	Human Orthologue	Sub-Complex	Comments	References
Utp13		91.0	TBL3/transducin $\beta$ -like 3; NP_006444	UtpB	WD40 repeats, Utp13 domain	65· 81· 82· 89
Utp18		66.4	UTP18/WDR50/CGI-48; NP_057085	UtpB	WD40 repeats	66· 81· 82· 89
Utp21		104.8	WDR36/TA-WDRP; NP_644810	UtpB	WD40 repeats, coiled-coil domains, Utp21 domain	66· 81· 82· 89· 160
Rtp7		34.5	RRP7A; NP_056518	UtpC	Also part of the CURI complex	81· 83· 161
Utp22		140.5	NOL6/NRAP; NP_075068	UtpC	Also part of the CURI complex	66· 81· 83· 160· 162
Cka1		44.7	CSNK2A1; NP_808227	UtpC	Co-purifies with UtpC; $\alpha$ catalytic subunit of casein kinase 2; also part of the CURI complex; non-essential	81· 83
Cka2		39.4	CSNK2A1; NP_808227	UtpC	Co-purifies with UtpC; $\alpha'$ catalytic subunit of casein kinase 2; also part of the CURI complex; non-essential	81· 83
Ckb1		32.3	CSNK2B; NP_001311	UtpC	Co-purifies with UtpC; $\beta$ regulatory subunit of casein kinase 2; also part of the CURI complex; non-essential	81· 83
Ckb2		29.8	CSNK2B; NP_001311	UtpC	Co-purifies with UtpC; $\beta'$ regulatory subunit of casein kinase 2; also part of the CURI complex; non-essential	81· 83
Rtp36		36.1	C6orf153; NP_149103	UtpC?		91
Utp2	Nop14	94.3	Nop14; NP_003694	unclassified	also involved in SSU nuclear export	65· 109· 163
Utp3	Sas10	70.3	UTP3; NP_065101	unclassified	contains a Utp3 domain; gene silencing	65
Utp7	Kre31	62.3	WDR46/BING4; NP_005443	unclassified	WD40 repeats; adenylate binding site; kinetochore component required for chromosome segregation	65· 164
Utp11		29.7	UTP11/CGI-94; NP_057121	unclassified	coiled-coils	65
Utp14		103.0	UTP14A; NP_006640	unclassified	coiled-coils; ATP/GTP binding site (P-loop)	65
Utp16	Bud21	24.4	yeast-specific	unclassified	coiled-coils; non-essential	65
Noc4	Utp19	63.6	NOC4L/MGCS162; NP_076983	unclassified	Noc domain; also involved in SSU nuclear export	66· 165
Utp20		287.6	1A6/DRIM; NP_055318	unclassified	HEAT repeats, ARM-type fold	66· 160
Utp23		28.8	UTP23; NP_115710	unclassified	PINc nuclease domain not required for function	118
Utp24	Fef1	21.6	FCF1; NP_057046	unclassified	PINc nuclease domain required for function	118· 166
Utp25		84.0	DEF/C1orf107; NP_055203	unclassified	DEAD-box helicase-like; DUF1253 domain	167· 169
Utp30		31.6	RSL1D1; NP_056474	unclassified	non-essential	160
Bms1		135.6	BMS1; NP_055568	unclassified	GTPase, stimulated by Rcl1	77· 80
Dbp8		47.9	DDX49; NP_061943	unclassified	DEAD-box RNA helicase, stimulated by Est2	119· 128

Name	Alias	KDa	Human Orthologue	Sub-Complex	Comments	References
Dhr1	Ecm16	145.0	DHX37; NP_116045	unclassified	DEAH-box RNA helicase	119; 170
Dhr2		82.7	DEAH box polypeptide 8; AAH47327 (?)	unclassified	DEAH-box RNA helicase	119; 170
Eng1	Nep1	27.9	EMGI/C2F; NP_006322	unclassified	member of $\alpha/\beta$ knot fold methyltransferase (SPOUT) superfamily; displays pseudouridine methyltransferase	66; 171
Krr1		37.2	KRR1/HRB2; NP_008974	unclassified	contains a KRR-R motif activity and a KH domain	66
Rcl1		40.2	RCL1; NP_005763	unclassified	RNA terminal phosphate cyclase-like protein; stimulates	80-172
Rok1		63.7	DDX52; NP_008941	unclassified	DEAD-box RNA helicase Bms1	119
Rrp3		56.0	DDX47; NP_057439	unclassified	DEAD-box RNA helicase	119
Rrp5		193.1	PDCD11/NFBP; NP_055791	unclassified	12 S1 RNA-binding motifs; HAT motif; binds single stranded tracts of U's; participates in A3 cleavage in 5.8S processing	65-173
Sof1		56.8	WDSOF1/DCAF13; NP_056235	unclassified	WD40 repeats; similar to $\beta$ subunit of trimeric G-proteins	174
Dbp4	Hca4/Ecm24	87.2	DDX10; NP_004389	unknown	DEAD-box RNA helicase	175
Enp1	Meg1	55.1	bystin/BYS1; NP_004044	unknown	contains a bystin domain and coiled-coil domains	66-176
Esf1		72.4	ESF1; NP_057733	unknown		177
Esf2	Abt1	36.4	ABT1; NP_037507	unknown	binds to RNA and stimulates ATPase activity of Dbp8	128; 178
Fall1		45.2	DDX48/EIF4A3; NP_055555	unknown	member of the eIF4A subfamily of DEAD-box RNA helicases	179
Fyv7		18.2	yeast-specific	unknown	contains coiled-coil motifs; non-essential	162
Gno1	Pxr1	31.3	PINX1; NP_060354	unknown	also involved in snRNA maturation; contains a G-patch RNA interacting domain and KKE/D repeats	180
Has1		56.7	DDX18; NP_006764	unknown	DEAD-box RNA helicase; also required for LSU synthesis	181
Kre33		119.3	NAT10; NP_078938	unknown	contains an N-acetyltransferase and a putative ATPase domain	68-169
Lcp5		40.8	Neuroguidin/NGDN; NP_001036100	unknown	contains a Utp3 domain	182
Ltv1	Ykl2	53.4	LTV1; NP_116249	unknown	also required for SSU nuclear export; non-essential	108
Mrd1		101.1	RBM19; NP_057280	unknown	contains 5 RNA-binding domains	183
Nop9		77.7	C14orf21; NP_777573	unknown	multiple pumilio-like RNA binding repeats	184
Nsr1	She5	44.5	Nucleolin/NCL; NP_005372	unknown	contains 2 RNA recognition motifs and a GAR domain	185; 186
Pta1	Sqs1	86.9	GPATCH2; NP_060510	unknown	stimulates the ATPase and helicase activities of Prp43p; contains a G-patch RNA interacting domain; non-essential	124
Prp43	JAI	87.6	DHX15; NP_001349	unknown	DEAH-box RNA helicase; also involved in mRNA splicing and LSU biosynthesis	187



Name	Alias	KDa	Human Orthologue	Sub-Complex	Comments	References
Sen1	Ctk3/Nrd2	252.5	Senataxin/SETX/ALS4; NP_055861	unknown	Upf1-like RNA helicase; also required for LSU synthesis	188
Sgd1		102.9	NOM1; NP_612409	unknown	contains armadillo-type fold	169
Slx9		24.1	DBF4-type zinc finger-containing protein 2; NP_065974	unknown	non-essential	189
YGR251W		22.3	BAF82397 (?)	unknown		162

(?) denotes uncertain human orthologue identification or unclear membership in an SSU processome sub-complex.