An emerging mechanism for the maturation of the Small Subunit Processome

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Abstract (120)

The biogenesis of the eukaryotic ribosome is a tightly regulated and energetically demanding process involving more than 200 ribosome assembly factors. These factors work in concert to ensure accurate assembly and maturation of both ribosomal subunits. Cryo-electron microscopy (cryo-EM) structures of numerous eukaryotic ribosome assembly intermediates have provided a wealth of structural insights highlighting the molecular interplay of a cast of assembly factors. In this review, we focus on recently determined structures of maturing small subunit (SSU) processomes, giant precursors of the small ribosomal subunit. Based on these structures and complementary biochemical and genetic studies, we discuss an emerging mechanism involving exosome-mediated SSU processome maturation and disassembly.

Highlights (5 max)

- Recent cryo-EM structures reveal new molecular insights into the maturation and disassembly of the small subunit (SSU) processome.
- The RNA exosome drives SSU processome maturation by remodeling the 5' ETS.
- Extensive layers of control enforce a strict chronology during the maturation of the SSU processome.

Keywords (5 max)

Ribosome biogenesis; small subunit processome; RNA exosome

Introduction

Eukaryotic ribosome biogenesis involves the assembly of the small and large ribosomal subunit from approximately 80 ribosomal proteins and four ribosomal RNAs (18S, 5.8S, 25S/28S, and 5S rRNA). This process occurs across three subcellular compartments (nucleolus, nucleus and cytoplasm) and involves more than 200 ribosome assembly factors, which facilitate the formation of the small and large ribosomal subunit[1-3]. Large pre-ribosomal RNAs are generated for subsequent processing in the yeast *Saccharomyces cerevisiae* (**supplemental Fig. 1a**) and human cells (**supplemental Fig. 1b**). Within the nucleolus of yeast, RNA polymerase I transcribes a long precursor (35S) that contains the 5' external transcribed spacer (5' ETS) followed by the small subunit RNA (18S rRNA), internal transcribed spacer 1 (ITS1), 5.8S rRNA, internal transcribed spacer 2 (ITS2), 25S rRNA, and the 3' external transcribed spacer (3' ETS). Dedicated chaperones facilitate nuclear import and prevent aggregation of several ribosomal proteins that bind early during ribosome assembly [4-7]. Within the nucleolus, ribosome assembly factors and ribosomal proteins associate co-transcriptionally with pre-ribosomal RNA such that the first nucleolar assembly intermediates of both subunits can form. This process has been visualized by Miller Spreads before cleavage events within ITS1 at either site A2 or A3 separate large and small subunit biogenesis (**supplemental Fig. 1**) [8].

Advances in cryo-electron microscopy have led to numerous reconstructions of nucleolar, nuclear and cytoplasmic ribosome assembly intermediates that have been extensively reviewed previously [1,2]. In yeast, the first stable entity to form is the 5' ETS ribonucleoprotein (RNP) (**Fig. 1a**), a particle encapsulating the uncut 5' ETS rRNA. As transcription continues, each individual 18S rRNA subdomain (5', central, 3' major, and 3' minor) recruits distinct assembly factors and ribosomal proteins as it folds onto the 5' ETS RNP scaffold. Further recruitment of assembly factors and cleavage at site A0 result in the assembly of the SSU processome (**Fig. 1b,e**). Recruitment of the exosome and remodeling of the 5' ETS trigger the separation of the 5' ETS from the 18S rRNA through cleavage at site A1, leading to sequential disassembly of the SSU processome and transition into pre-40S particles (**Fig. 1c**). In humans, relatively few maturation steps of the SSU processome have been captured (**Fig. 1e**) and the 5' ETS RNP as well as the disassembly pathway have so far remained structurally unexplored (**Fig. 1d,f**). In both yeast and humans, the late stages of pre-40S biogenesis have been structurally characterized [9-12] (**Fig. 1g**). Recent complementary functional studies have highlighted the roles of deubiquitinases during late stages of small subunit assembly in human cells [13,14].

Since large ribosomal subunit assembly and late stages of small subunit assembly have been reviewed more recently [15-17], we focus on recent advances in small subunit maturation with a particular

emphasis on the controlled maturation of the SSU processome by the RNA exosome [18-21]. Based on these insights, we propose a mechanism that rationalizes recent structural, biochemical and genetic data.

Formation of the SSU processome

The association of ribosome assembly factors with nascent pre-ribosomal RNAs of both subunits has been elucidated by using pre-ribosomal RNA mimics that contain 3' end truncations [22-25]. For small subunit assembly, these studies have revealed the following key principles: First, that the 5' ETS acts as a structural blueprint for the SSU processome. Second, that the RNA chaperone U3 small nucleolar RNA (snoRNA), which base-pairs with both the 5' ETS and the 18S rRNA, is incorporated into the 5' ETS RNP. Third, that each of the 18S rRNA subdomains can recruit distinct assembly factors before the SSU processome is formed. In yeast, biochemical studies have revealed essential elements of the 5' ETS and highlighted that parallel maturation of the 5' ETS and individual domains of the 18S drive initial stages of SSU processome formation [26,27]. Subsequently, all domains are required to form the SSU processome, in which cleavage at site A0 and recruitment of the RNA helicase Dhr1, the dimethyladenosine transferase Dim1, and the RNA exosome can occur thereafter [27]. The architecture of the 5' ETS RNP further indicated that cleavage at site A0 is necessary for the recruitment of Utp14, a key activator of Dhr1 helicase activity [27-29].

While the mechanisms involved in the formation of the SSU processome remain poorly characterized, significant structural data is now available for the SSU processome in which site A0 is cut and site A1 is uncut, a state that we will refer to as state pre-A1 (**Fig. 1b**). Following an initial set of architectural models of state pre-A1 [30-32], subsequent high-resolution structures [33-35] have led to the complete assignment of all pre-A1 constituents (reviewed in [36]). More recently, structures of later states of the SSU processome have been determined from yeast and human cells [18-21]. Studies in yeast have provided an overview of the transition from the SSU processome to pre-40S particles under normal growth conditions as well as in the absence of the RNA helicase Dhr1, thereby highlighting some of the dependencies within the process [18-20]. Very recent data from human cells provide the so far highest resolution information on SSU processome maturation, revealing key regulatory mechanisms including exosome-mediated recognition, the mechanism of cleavage at site A1, and the inhibition of Dhr1 RNA helicase activity [21]. Separately, X-ray crystallographic studies of Dhr1 in different states together with associated functional data have revealed extensive layers of control of this key enzyme that later separates 18S from the RNA chaperone U3 [21,37-39].

Together these studies now allow us to present universal principles of SSU processome maturation. Below, we will outline 1) a mechanism for Rrp6-mediated recruitment of the exosome to the SSU

processome and 2) a model for exosome-mediated remodeling of the SSU processome which rationalizes all existing structural data and explains A1 cleavage and SSU processome disassembly as a function of exosome-mediated 5' ETS unwinding.

Rrp6-mediated recruitment of the RNA exosome to the SSU processome

The RNA exosome can bind to the SSU processome in two ways: either via the exosome-associated nuclease Rrp6 and/or the exosome-associated helicase Mtr4. In yeast, the SSU processome component Utp18 can use a so-called arch-interacting motif (AIM) to interact with Mtr4 and in humans presumably TDIF2 can fulfil a similar role [21,40]. Recently, the C-terminus of Rrp6/EXOSC10 (yeast/human nomenclature), termed "lasso" was identified as a universal second bridging element between the SSU processome and the exosome in yeast and human cells [21,41].

A key function of the SSU processome is to integrate pre-ribosomal RNA folding with the recruitment of the RNA exosome. Different structural snapshots now illustrate the sequence of events leading to the recruitment of the RNA exosome on the SSU processome (**Fig. 2**). The transition from state pre-A1 to pre-A1* is associated with the dissociation of Lcp5/NGDN and the conformational switch of the Rrp9/U3IP2 N-terminus that vacates a space for eS4 (**Fig. 2a,b**). Further remodeling steps, including stabilization of 18S helix 21 by the newly positioned eS4 and compaction of Utp20/UTP20, create multiple bindings sites for the Rrp6/EXOSC10 C-terminus, which contains three or four binding motifs in yeast and humans, respectively [21]. These motifs can thus engage SSU processomes starting from state pre-A1* (**Fig. 2c**) and remain bound until after cleavage at site A1 in state post-A1 (**Fig. 2d**).

This model, which includes redundant connections involving either Mtr4 or Rrp6-mediated recruitment of the RNA exosome, is consistent with the synthetic lethality observed between Utp18 AIM motif mutants and the deletion of Rrp6 [40] as the loss of these two links would prevent exosome recruitment and hence SSU processome maturation. However, further biochemical and structural studies will be required to decipher the detailed chronology of these events.

A model for exosome-mediated maturation of the SSU processome

Key requirements for exosome-mediated maturation of the SSU processome are a 3' end resulting from cleavage at site A0, the presence of the RNA helicase Mtr4, and tethering of the RNA exosome to the SSU processome via Rrp6 and/or Mtr4. Here we propose a mechanism for exosome-mediated 5' ETS degradation and concomitant maturation of the SSU processome, which includes cleavage at site A1 and subsequent SSU processome disassembly. Key to our model is the 3' to 5' unwinding of the 5' ETS starting from site A0, a premise that predicts a series of conformational changes and assembly factor dissociation events for which experimental evidence is now available. The initial unwinding of helices 9,8

and 7 of the 5' ETS by Mtr4 presumably occurs without major conformational changes (**Fig. 3a-c**). The first key transition is the unwinding of helix 6, which eliminates the binding sites of assembly factors Krr1 and Faf1, thereby liberating the previously constrained Box A and exposing the endonuclease Utp24 (**Fig. 3c,d**). As the presence of Dhr1 is required for cleavage at site A1, the absence of this factor causes a particular arrest in which Pno1 and the associated helix 45 are trapped near Pwp2. In addition, parts of the 5' ETS are still located between Utp7 and Pwp2 [19]. In a critical subsequent step, this part of the 5' ETS is pulled between Utp7 and Pwp2, resulting in a "Ring opening" step after which the relative orientations between Utp7 and Pwp2 have changed (**Fig. 3e-f**). This change in orientations is observed in both yeast and human SSU processomes [18,20,21]. As Utp14 occupies the same location previously occupied by the 5' ETS [21] , an opening between Utp7 and Pwp2 must occur for maturation to proceed. In state post-A1, Utp14 and the N-terminus of Dhr1 stabilize a Dim1-H45-Pno1 complex that is flanked by the C-terminus of Utp7 that has been repositioned in response to the remodeling of the 5' ETS. Here Box A' is stabilized in an upward conformation and cleavage at site A1 has been catalyzed by Utp24 (**Fig. 3f**). This state shows how the presence of noncatalytic elements of Dhr1 are essential for SSU processome maturation prior to A1 cleavage and the separation of U3 from the 18S rRNA by Dhr1. Our model further predicts that the continued unwinding of the 5' ETS by the RNA exosome will cause the disassembly of the SSU processome starting with the release of Utp7 and Sof1 from the SSU processome, resulting in state Dis-A, a particle in which the beginning of the 5' ETS is still anchored within the UtpA complex (**Figs. 1c, 3g,h**). Subsequent unwinding and removal of the 5' ETS will eventually release the 5' ETS-bound UtpA complex, resulting in state Dis-B where the UtpA complex and associated proteins have dissociated (**Fig. 3i**). With the dissociation of the UtpC complex and the controlled repositioning of Dhr1 towards its substrate, the 18S-U3 RNA duplex, state Dis-C is reached and further maturation of the pre-40S particle can occur (**Fig. 1c**). Starting from state pre-A1, Dhr1 and its cofactor Utp14 are controlled through a series of changing binding sites that result from the maturing and disassembling SSU processome [18,21].

Recently, yeast genetics was used to reveal a network of functional interactions between late SSU processome factors and Bud23, a methyltransferase which associates with subsequent particles [42,43]. The combination of yeast genetics with structural biology highlights how seamlessly the different stages of SSU processome maturation are coordinated in a temporal fashion.

Conclusions

The nucleolus has emerged not only as the site of ribosome assembly but also as a phase separated entity in which co-transcriptional ribosome assembly can be concentrated [44,45] and revealed by cryo-electron

tomography in situ [46]. In human cells, genome editing and new biochemical approaches for the isolation of human pre-ribosomal particles have laid the foundation for structural studies of early particles together with associated functional data [21,47,48]. The availability of extensive structural and biochemical data in yeast and human cells now facilitates more mechanistic descriptions of nucleolar assembly events with relevance to human diseases.

At a structural level, major progress has been made during the past few years to decipher the underlying mechanisms of SSU processome formation, maturation and disassembly. The exosome has moved into the focus of recent work and as outlined by the proposed mechanism in this review, SSU processome maturation and disassembly can be described as a function of the removal of the 5' ETS by the RNA exosome. Another key theme that has emerged is how the structural plasticity of the SSU processome allows this giant particle to coordinate multiple maturation events and communicate the resulting structural changes through the creation of binding sites for assembly factors, a concept that was previously already associated with the 5' ETS RNP [27]. However, despite these advances, the precise mechanisms and functional interplay of numerous ribosome assembly factors involved in SSU processome formation, maturation and disassembly remain poorly characterized at a functional and mechanistic level. The combined used of genetic, biochemical and structural approaches will therefore be essential to tease apart the more dynamic steps associated with formation, maturation and disassembly of the SSU processome.

Declaration of Interest

The authors declare no conflict of interest.

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Figure 1. Assembly and maturation pathway of the eukaryotic small ribosomal subunit.

(a) Structure of the yeast 5'ETS RNP, a first key assembly intermediate. **(b)** Structures of maturing SSU processomes in yeast: state pre-A1 (before A1 cleavage), exosome-associated state pre-A1* (primed for exosome binding), and exosome-associated state post-A1 (after A1 cleavage). SSU processome structures obtained from Dhr1-depleted yeast cells remain uncleaved at site A1 and are not depicted for clarity. **(c)** Structures of SSU processome disassembly intermediates in yeast: state Dis-A, state Dis-B and state Dis-C. The maturation steps of the yeast pre-40S were omitted for clarity. **(d)** No structure of the human 5'ETS particle has been reported to date. **(e)** Structures of maturing SSU processomes in human: state pre-A1, state pre-A1*, exosome-associated post-A1. **(f)** No structures of the human SSU processome disassembly complexes have been reported to date. **(g)** Structures of maturing pre-40S in human: states A-H and mature 40S. The inset on the top right shows the relative orientation of views between the Dis-C complex and pre-40S particles. Proteins or proteins complexes joining or leaving the particles are color-coded. Otherwise, they are depicted as transparent outlines. PDB accession codes are indicated for each structure and separately listed in supplementary Table 1.

Figure 2. Structural remodeling of the SSU processome mediates RNA exosome recruitment.

Different structural snapshots illustrate the sequence of events leading to the recruitment of the RNA exosome and the subsequent cleavage of the rRNA at site A1. **(a)** State pre-A1 is characterized by the presence of Lcp5/NGDN. **(b)** The transition from states pre-A1 to pre-A1* is associated with the dissociation of Lcp5/NGDN and the conformational switch of the Rrp9/U3IP2 N-terminus. **(c)** Further remodeling steps, including stabilization of helix 21 by the newly positioned eS4 and compaction of Utp20/UTP20, create multiple bindings sites for the Rrp6/EXOSC10 C-terminus ("lasso") and leads to the recruitment of the RNA exosome. The exosome recognizes the SSU processome through the C-terminus of Rrp6/EXOSC10 and the SSU processome recruits Mtr4/MTR4 by way of arch-interacting motifs (AIMs) present in either Utp18 (yeast) or presumably TDIF2 (human). Note that the yeast Rrp6 EAR domain and human exosome were added to the models. **(d)** Degradation and remodeling of the 5'ETS by the exosome through its 3'-5' exonuclease activity, together with the dissociation of the Kre33/NAT10 module, facilitates the cleavage of the rRNA at site A1. The insets show the yeast and human Mtr4/MTR4 helicases bound to an AIM motif.

Figure 3. Mechanism for RNA exosome-mediated SSU processome maturation.

The remodeling of the 5'ETS is driven by the RNA exosome 3'-5' exoribonuclease activity leading to the cleavage of the rRNA at site A_1 and disassembly of the yeast *Saccharomyces cerevisiae* SSU processome. An important aspect of the mechanism postulates that the U3 snoRNA remains steady during the remodeling events with the 5'ETS being pulled by the exosome to slide along the fixed 5' and 3' hinges. This model is consistent with the remaining presence of the 5' and 3' hinges in the state post-A1, Dis-A or Dis-B, in which most of the 5'ETS has been degraded by the exosome. **(a)** Structures of RNA elements in state pre-A1. 18S, green; U3, pink; 5′ ETS, from light yellow to red. The bottom insets show U3 snoRNA forming intermolecular helices with the 5'ETS (5' and 3' hinges) and with the 18S rRNA (Box A and Box A'). The exosome is modeled. **(b)** The 3' end of the 5'ETS cleaved at site A_0 is inserted into the central cavity of the exosome through the Mtr4/MTR4 helicase and starts to be degraded by the exoribonuclease Dis3/RRP44. **(c)** 5'ETS helix 9 (H9) and 8 (H8) have been pulled into the exosome and degraded. **(d)** The removal of helix 7 (H7) is the first remodeling event forcing the 5'ETS to slide along the hinges. The zoomed view shows the tight interactions between of the pre-A1 assembly factors Krr1, Faf1 and H6. Pno1 and H45 (18S) are in an immature conformation. **(e)** Removal of helix 6 (H6) is a key event coupled to the dissociation of Krr1 and Faf1. The zoomed view shows the absence of H6, Krr1 and Faf1, creating an open space for Pno1 and H45 to advance to a more mature conformation. The next portion of 5'ETS to be remodeled comprises helix 5 (H5) and the connection to helix 4, which together form a ring-like structure at the interface of Utp7 and Pwp2. A 'ring opening' step, which requires the passage of the 5'ETS through the Utp7 and Pwp2 interface, is necessary and induces important remodeling of their interface. The dissociation of Krr1 and Faf1 and the local disorder caused by the movement of Utp7 liberate the Box A and Box A', thereby liberating the previously constrained Box A and exposing the endonuclease Utp24. **(f)** The cleavage at site A_1 has occurred. Boxes A and A' have moved to an upward position and are stabilized by the newly recruited methyltransferase Dim1. Pno1 and H45 are in their mature position, also stabilized by Dim1 and the Utp7 C-terminus. **(g)** The remaining helices 2-4 (H2-H4) are degraded by the exosome. Helix 1 is still observed in state post-A1 and will be removed together with UtpA during SSU processome disassembly **(h,i)**.

Supplemental Figure 1. Ribosomal RNA processing in yeast and human cells.

Yeast and human mature rRNAs are generated from long polycistronic pre-rRNA precursors that are processed through different pathways. The figure focusses on processing of the small subunit pre-rRNA. **(a)** In yeast, most of the nascent transcripts are cleaved co-transcriptionally at sites A_0, A_1, and A_2, yielding the 20S and 27S-A_2 pre-rRNAs (black arrows). Alternative pathways involve cleavage at either A_3 or A_2 sites followed by cleavage at A_0 and A_1 sites (red, green or blue arrows). Processing of the large subunit pre-rRNA is simplified for clarity. **(b)** In human cells, cleavage of the nascent 45S transcript (already pre-processed at sites A' and 02) cleavage at site 2, yielding 30S and 32S pre-rRNAs. The rRNAs precursors present in forming, maturing or disassembling SSU processomes are highlighted in light blue, cyan, and purple, respectively.

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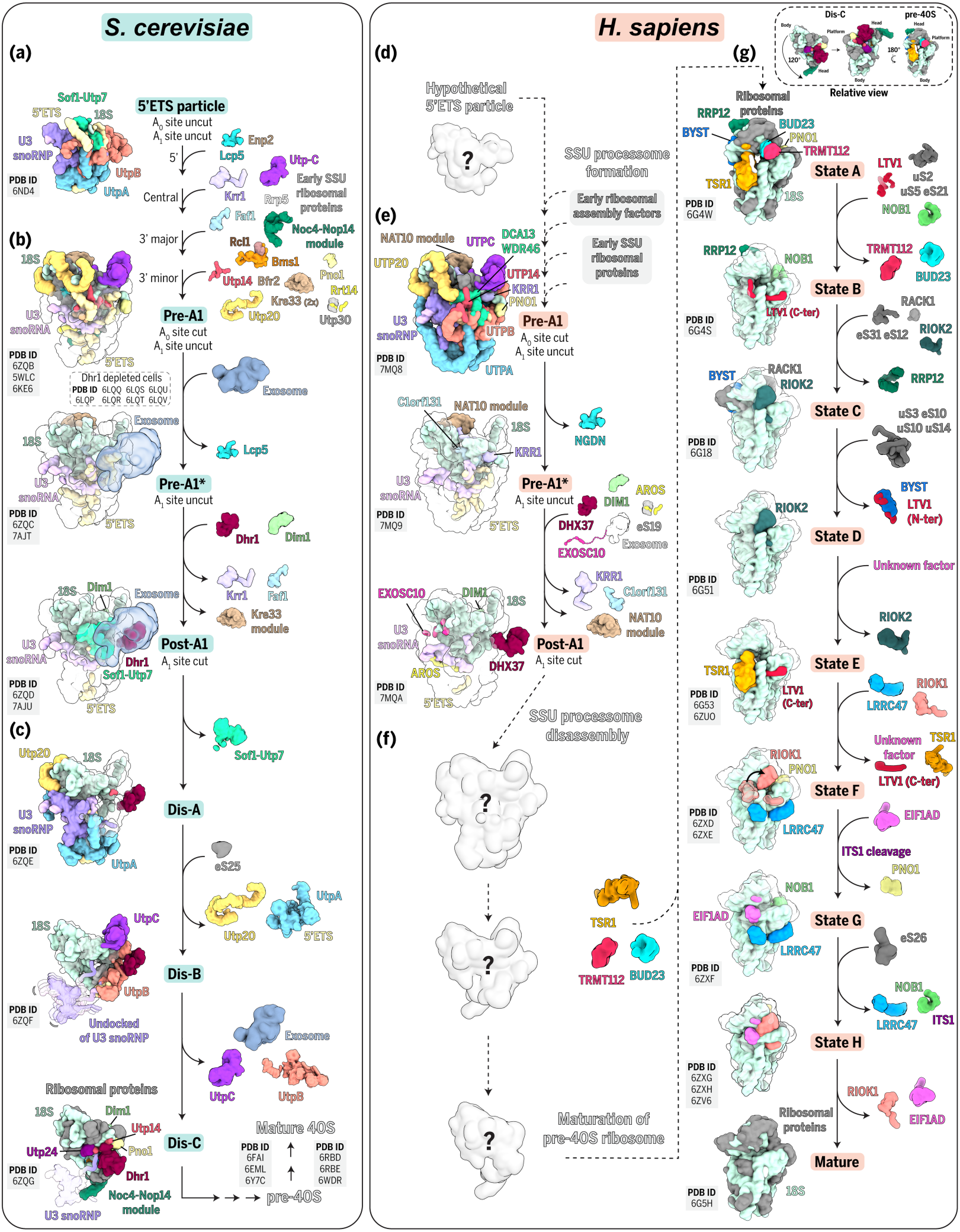
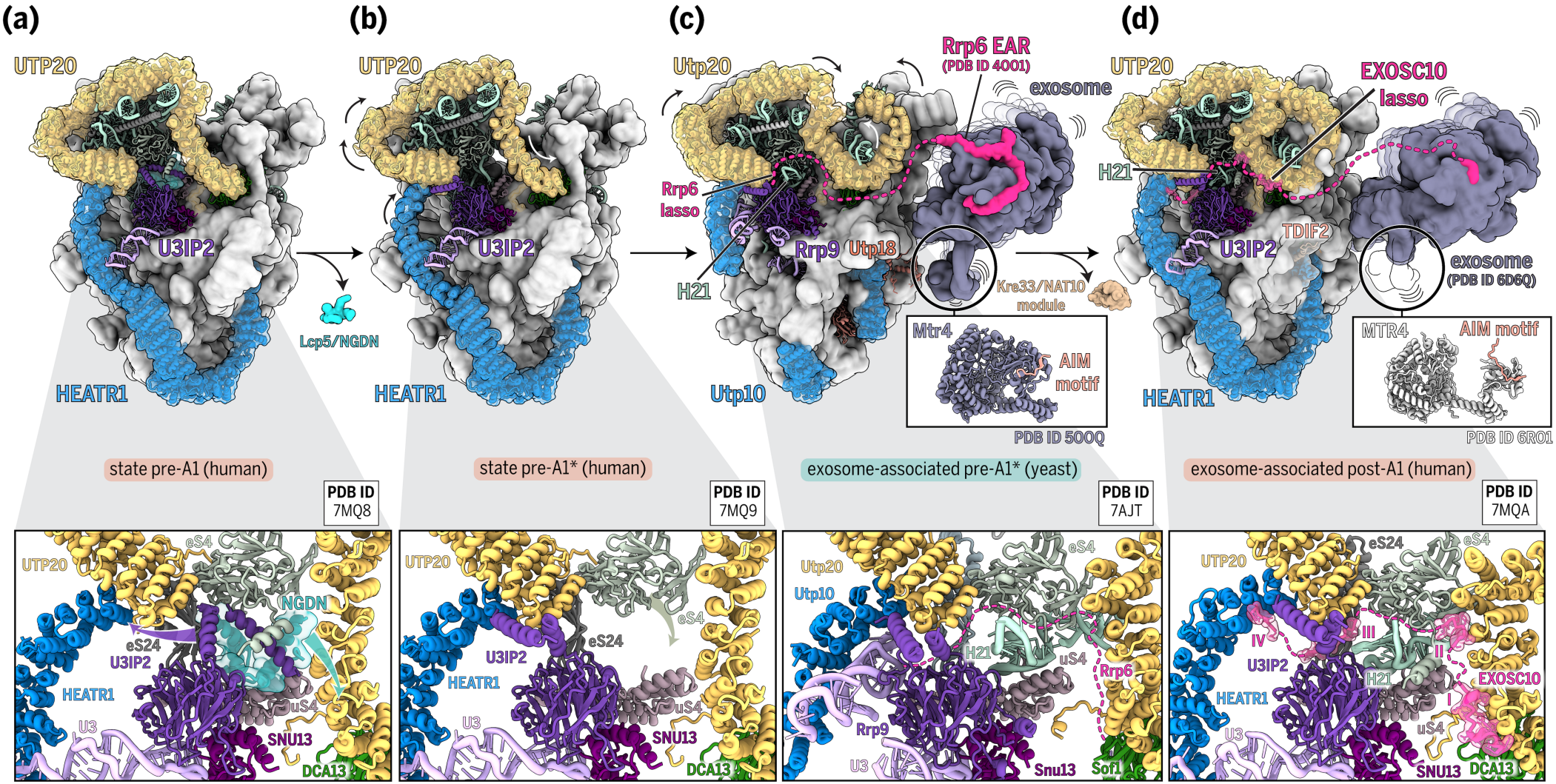
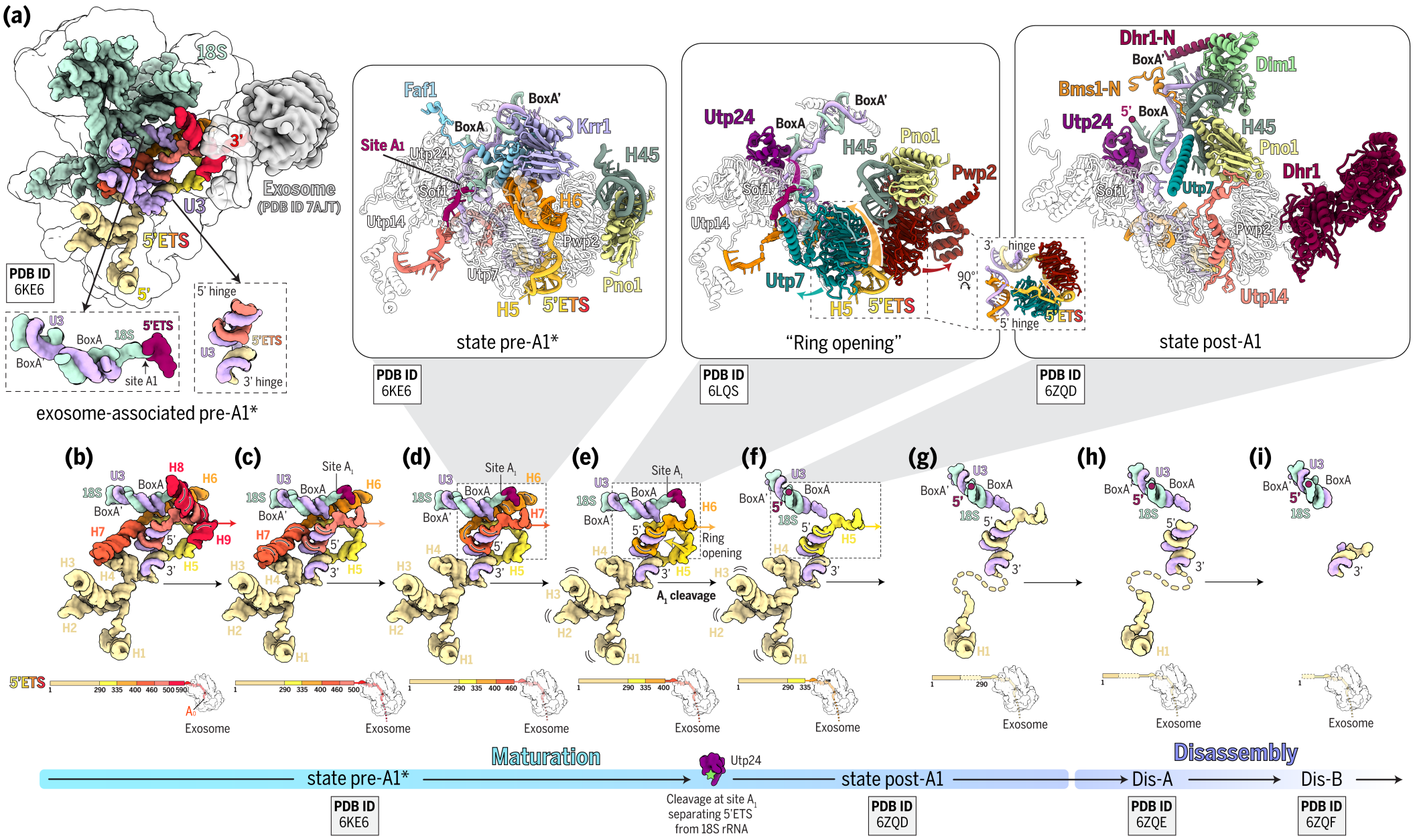

S. cerevisiae
H. sapiens
(a)
(b)
(c)
(d)
(e)
(f)
(g)
5'ETS particle
Pre-A1
Pre-A1*
Post-A1
Dis-A
Dis-B
Dis-C
pre-40S
Mature 40S
Hypothetical 5'ETS particle
SSU processome formation
SSU processome disassembly
Maturation of pre-40S ribosome
Early ribosomal assembly factors
Early SSU ribosomal proteins
Ribosomal proteins
Relative view
State A
State B
State C
State D
State E
State F
State G
State H
Mature
Dhr1 depleted cells
Undocked of U3 snoRNP
ITS1 cleavage
Unknown factor


(a)
(b)
(c)
(d)
UTP20
U3IP2
HEATR1
Lcp5/NGDN
Utp20
Rrp6 EAR
(PDB ID 4001)
exosome
Rrp6
lasso
H21
Rrp9
Utp18
Utp10
Kre33/NAT10
module
Mtr4
AIM
motif
PDB ID 500Q
EXOSC10
lasso
TDIF2
exosome
(PDB ID 6D6Q)
MTR4
AIM motif
PDB ID 6R01
state pre-A1 (human)
state pre-A1* (human)
exosome-associated pre-A1* (yeast)
exosome-associated post-A1 (human)
PDB ID
7MQ8
PDB ID
7MQ9
PDB ID
7AJT
PDB ID
7MQA
eS4
eS24
NGDN
uS4
U3
SNU13
DCA13
Rrp6
Rrp9
Snu13
Sof1
IV
III
II
I


(a)
18S
3'
Exosome
(PDB ID 7AJT)
U3
5'ETS
5'
PDB ID
6KE6
5' hinge
U3
BoxA
18S
5'ETS
BoxA'
site A1
5'ETS
U3
3' hinge
exosome-associated pre-A1*
Faf1
BoxA'
BoxA
Krr1
Site A1
Utp24
H45
Sof1
H6
Utp14
Pwp2
Utp7
Pno1
H5
5'ETS
state pre-A1*
PDB ID
6KE6
Utp24
BoxA'
BoxA
H45
Pno1
Pwp2
Sof1
Utp14
Utp7
H5
5'ETS
90°
3'
hinge
5' hinge
5'ETS
"Ring opening"
PDB ID
6LQS
Dhr1-N
BoxA'
Dim1
Bms1-N
Utp24
5'
BoxA
H45
Pno1
Sof1
Utp7
Dhr1
Pwp2
Utp14
state post-A1
PDB ID
6ZQD
(b)
U3
H8
BoxA
H6
18S
BoxA'
H7
H9
5'
3'
H3
H4
H5
H2
H1
(c)
Site A1
U3
BoxA
H6
18S
BoxA'
H7
5'
3'
H5
H3
H4
H2
H1
(d)
Site A1
U3
BoxA
H6
18S
BoxA'
H7
5'
H4
3'
H5
H3
H2
H1
(e)
Site A1
U3
BoxA
18S
H6
BoxA'
5'
Ring
opening
H4
3'
H5
A1 cleavage
H3
H2
H1
(f)
U3
BoxA
BoxA'
5'
18S
5'
H5
H4
3'
H3
H2
H1
(g)
U3
BoxA
BoxA'
5'
18S
5'
3'
H1
(h)
U3
BoxA
BoxA
5'
18S
5'
3'
H1
(i)
U3
BoxA
BoxA'
5'
18S
3'
5'ETS
1
290
335
400
460
500
590
A1
Exosome
1
290
335
400
460
500
Exosome
1
290
335
400
460
Exosome
1
290
335
400
Exosome
1
290
335
Exosome
1
290
Exosome
1
Exosome
Maturation
Disassembly
state pre-A1*
PDB ID
6KE6
Utp24
Cleavage at site A1
separating 5'ETS
from 18S rRNA
state post-A1
PDB ID
6ZQD
Dis-A
PDB ID
6ZQE
Dis-B
PDB ID
6ZQF