

Hold on to your friends: Dedicated chaperones of ribosomal proteins

Dedicated chaperones mediate the safe transfer of ribosomal proteins to their site of pre-ribosome incorporation

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Eukaryotic ribosomes are assembled from their components, the ribosomal RNAs and ribosomal proteins, in a tremendously complex, multi-step process, which primarily takes place in the nuclear compartment. Therefore, most ribosomal proteins have to travel from the cytoplasm to their incorporation site on pre-ribosomes within the nucleus. However, due to their particular characteristics, such as a highly basic amino acid composition and the presence of unstructured extensions, ribosomal proteins are especially prone to aggregation and degradation in their unassembled state, hence specific mechanisms must operate to ensure their safe delivery. Recent studies have uncovered a group of proteins, termed dedicated chaperones, specialized in accompanying and guarding individual ribosomal proteins. In this essay, we review how these dedicated chaperones utilize different folds to interact with their ribosomal protein clients and how they ensure their soluble expression and interconnect their

intracellular transport with their efficient assembly into pre-ribosomes.

Keywords:

aggregation; chaperone; nuclear import; ribosomal protein; ribosome biogenesis; ribosomopathy

Introduction

Ribosomes are remarkably complex macromolecules that fulfill in all living cells, from bacteria to eukaryotes, the essential role of protein synthesis [1]. In eukaryotes, mature ribosomes are composed of a large 60S subunit and a small 40S subunit [1, 2]. In exponentially growing cells of the yeast *Saccharomyces cerevisiae*, the protein demand is such that each cell needs to produce ~2,000 ribosomes per minute [3]. Considering that each yeast ribosome is made up of four ribosomal RNAs (rRNAs) and 79 ribosomal proteins (r-proteins) [2], the cell has to spend a substantial amount of energy to create all these individual components and to accurately piece them together in order to produce fully functional ribosomes [3–5]. The assembly of a ribosome begins in the nucleolus, a subcompartment of the nucleus, where r-proteins associate with the pre-ribosomal RNA (pre-rRNA) in a co-transcriptional manner [2]. Due to the enormous complexity of this multi-step process in eukaryotes, ribosome assembly depends on more than 200 transiently acting and mostly essential biogenesis factors [4, 6, 7]. The first pre-ribosomal particle, called 90S, is in the course of maturation split into the pre-40S and pre-60S particles [4, 6, 7]. The nuclear maturation phase of the two subunits culminates in the acquisition of export competence [4, 6, 7]. Finally, following their transport across nuclear pore complexes (NPC) and after a series of cytoplasmic maturation events [2, 8–10], the ribosomal subunits are licensed to engage in subunit joining and translation. For a simplified scheme of the eukaryotic ribosome biogenesis pathway, see Fig. 1.

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Abbreviations:

ARM repeat, armadillo repeat; **HEAT repeat**, huntingtin, elongation factor 3, A subunit of PP2A, and TOR repeat; **NAC**, nascent polypeptide-associated complex; **NLS**, nuclear localization signal; **RAC**, ribosome-associated complex; **r-protein**, ribosomal protein; **rRNA**, ribosomal RNA; **TPR repeat**, tetratricopeptide repeat.

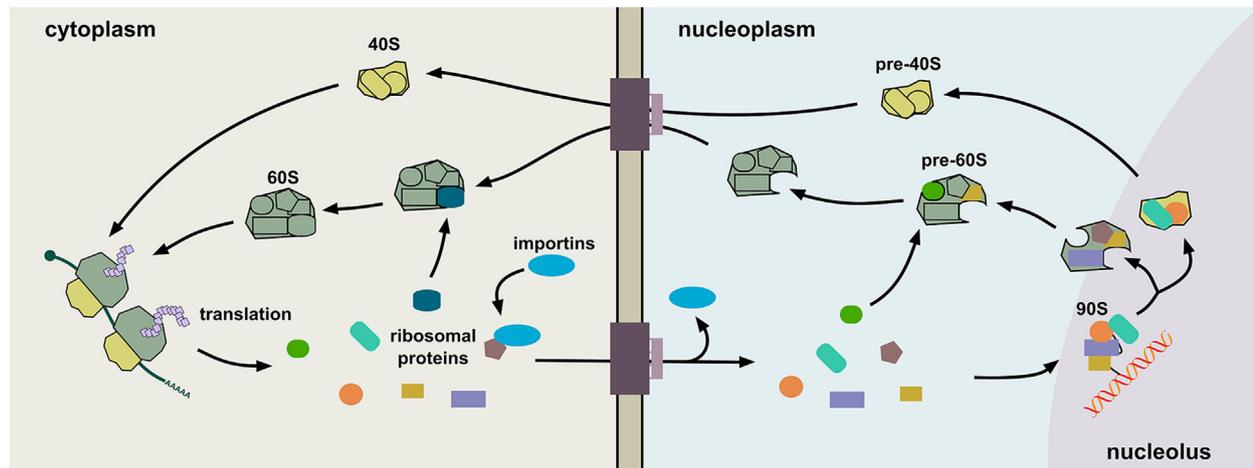


Figure 1. The assembly pathway of r-proteins. After their translation in the cytoplasm, most r-proteins are imported into the nucleus. In the nucleolus, the transcribed pre-rRNA assembles with r-proteins and non-ribosomal biogenesis factors (not shown) into a 90S particle. Upon pre-rRNA cleavage, pre-60S and pre-40S particles are released, and undergo separate maturation pathways. Final maturation steps, including the incorporation of the last r-proteins, occur after pre-ribosomal particles have been exported into the cytoplasm.

Recent progress has provided groundbreaking insights into the structures of mature ribosomes and diverse pre-ribosomal particles of eukaryotes [11–17]. However, much less is known about the processes that mediate the stable expression, nuclear import, and assembly of r-proteins. These are challenging tasks for a cell, not only because of the vast amounts of r-proteins ($\approx 150\,000$ per minute in yeast) that need to be synthesized and efficiently targeted into the nucleus [3], but also because of some unfavorable features intrinsic to r-proteins: they often contain basic regions and unfolded extensions that form interactions with rRNA within ribosomes [12], which, however, may undergo non-specific interactions when not embedded within their cognate rRNA environment, resulting in insolubility [18]. Consequently, mechanisms are needed to protect newly synthesized r-proteins before their arrival at their destination site on the ribosome.

Another group of proteins, histones, shares many common features with r-proteins. Histones are highly abundant nucleic acid binding proteins with a high content in positively charged amino acids, allowing them to engage in ionic interactions with the phosphate backbone of the DNA. However, before their incorporation into chromatin, their exposed basic regions make them prone to aggregation due to illicit interactions with non-specific polyanions [18]. To overcome these problems, they interact with so-called “histone chaperones.” Almost 40 years ago, nucleoplasmin, isolated from *Xenopus* eggs, was the first protein to be classified as a histone chaperone by virtue of its ability to prevent non-specific interactions between DNA and histones [19, 20]. Since then, a number of histone chaperones have

been identified, all complying to the broad definition of proteins that associate with histones and are involved in their traffic and assembly, but are usually not constituents of the active histone complex [21–23].

Considering their similar biochemical properties, it could be suspected that r-proteins, akin to histones, may also rely on specific chaperones. Indeed, in the last few years we have witnessed the identification of several proteins that interact with newly synthesized r-proteins and/or escort them to their ribosome assembly site. Given that each of these proteins selectively acts on a specific r-protein and that they are functionally distinct from the general chaperones, we introduced the term “dedicated chaperones” to refer to this class of proteins [24]. This review will familiarize the reader with this recent concept of dedicated r-protein chaperones and discuss the functions, mechanisms, common principles and differences between the so far identified r-protein chaperones.

Protection of r-proteins by the general chaperone network and importins

Before turning to the description of dedicated r-protein chaperones, we will briefly summarize the role of the general chaperone network and importins in the folding and protection of r-proteins. Like numerous other cellular proteins, r-proteins were shown to physically interact with various different general chaperones [25]. In contrast to the cytosolic heat shock chaperones, which mainly mediate response to stress conditions, a subset of the cellular chaperone machinery is associated with translating ribosomes and is engaged in *de novo* folding of newly synthesized proteins [26]. These ribosome-anchored chaperone systems consist of two complementary and partially redundant components, which contact nascent polypeptides as they emerge from the polypeptide exit tunnel. The nascent polypeptide-associated complex (NAC) captures nascent polypeptides and protects them from degradation and unintended interactions very early after their emergence from the exit tunnel [27]. The comrade of NAC, the chaperone triad SSB-RAC consists of the ATP-dependent Hsp70 SSB, which

binds the nascent polypeptides and ensures their proper co-translational folding, and its regulator, the two-component ribosome-associated complex (RAC) [26, 28–30]. The ribosome-anchored chaperone system is especially important for longer proteins that need assistance to fold properly. However, it has also been shown to play a crucial role in chaperoning the aggregation-prone species of r-proteins [31]. While deletion of NAC alone has no impact on r-protein solubility, the simultaneous inactivation of NAC and SSB/RAC leads to aggregation of 22 out of 33 r-proteins of the 40S subunit and 30 out of 46 r-proteins of the 60S subunit. Interestingly, the two r-proteins Rps31 (eS31 according to a recently proposed alternative r-protein nomenclature [32]) and Rpl40 (eL40), which are synthesized with an N-terminal ubiquitin moiety that is presumed to mediate a protective function [33–35], were not among these aggregated proteins [31]. This may be an indication that specific protection can bypass the need for the general ribosome-associated chaperone system. However, the r-proteins Rps3 (uS3), Rps26 (eS26), Rpl3 (uL3), and Rpl4 (uL4), which all have their own dedicated chaperones (see below), were also amongst the r-proteins that aggregated in the absence of SSB-RAC/NAC. A possible explanation might be that the inactivation of the co-translational chaperone system also leads to an impaired ribosome synthesis [31, 36]. Thus, the observed aggregation of r-proteins may partially arise from an accumulation of free r-proteins when cognate pre-ribosomal particles are only available in limiting amounts.

In addition to the general chaperone network, importins have been reported to function as chaperones both for histones and r-proteins [18]. Nuclear import is a highly selective process

in which nuclear localization signals (NLSs) of suitable cargoes are recognized by import receptors [37]. NLSs are typically composed of short, exposed stretches of basic amino acids, which are often contained within DNA or RNA binding regions [38–40]. Besides the well-known function in mediating nuclear import, importins were shown to have an additional function in protecting their r-protein and histone cargoes from aggregation and precipitation [18]. This protection is rather specific since only importins with highest affinity for a particular r-protein were able to fully protect their cargoes, while importins with lower affinity were not. Since importin-mediated protection ends in the nucleus upon RanGTP-induced cargo release, further mechanisms may be required to ensure protection of r-proteins until their incorporation into pre-ribosomal particles in the nucleoplasm or nucleolus.

Inventory of dedicated r-protein chaperones

In recent years, it has turned out that the general chaperone network and importins are not sufficient to protect r-proteins as obvious from the fact that at least some r-proteins additionally have their own specific chaperones. So far, six such dedicated chaperones for yeast r-proteins have been characterized (Table 1).

Two of these dedicated chaperones, Yar1 and Tsr2, interact with r-proteins of the small ribosomal subunit. While Yar1 binds to newly synthesized Rps3 already in the cytoplasm, thereby protecting it from aggregation, and likely accompanies Rps3 to its assembly site in the nucleus [24, 41–44], Tsr2 associates with

Table 1. Characteristics of yeast dedicated r-protein chaperones

Dedicated chaperone	Tsr2	Yar1	Acl4	Rrb1	Sqt1	Syo1
Associated r-protein	Rps26 (eS26)	Rps3 (uS3)	Rpl4 (uL4)	Rpl3 (uL3)	Rpl10 (uL16)	Rpl5 (uL18) Rpl11 (uL5)
Phenotype of null mutant	Strong slow growth	Strong slow growth at low temperature	Strong slow growth	Lethal	Lethal	Slow growth at low temperature
Steady-state localization	Nuclear	Mainly cytoplasmic	Nuclear	Nucle(ol)ar	Cytoplasmic	Mainly nuclear
Structure/fold	Unknown	Ankyrin repeats	TPR repeats	Predicted WD-repeat β -propeller	8-bladed WD-repeat β -propeller	ARM and HEAT repeats
Main binding region on r-protein (amino acids)	Unknown	N-terminus Rps3 (14-29)	Internal loop Rpl4 (72-114)	N-terminus Rpl3 (1-15)	N-terminus Rpl10 (2-13)	Rpl5 N-terminus (2-20) Rpl11: β -sheet (16-131)
Main binding region on chaperone	Unknown	Cradle formed by ankyrin-repeat core	TPR repeats 4-6	Unknown	Negatively charged top surface of β -propeller	Rpl5: HEAT domain Rpl11: helical segment at the end of acidic loop
Co-translational binding	Not tested	Yes	Yes	Yes	Yes	Rpl5: yes; Rpl11: no
Human orthologs	hTSR2	No obvious ortholog; hRPS3 interacts with I κ B α	No obvious ortholog	GRWD1 (Glutamate-Rich WD-Repeat Containing 1)	AAMP (Angio-Associated Migratory Cell Protein)	HEATR3 (HEAT repeat-containing protein 3)

Rps26 after its nuclear import and presumably assists its transfer to pre-ribosomal particles [45, 46].

The other four dedicated chaperones, Sqt1, Rrb1, Acl4, and Syo1, interact with large subunit r-proteins. Sqt1 binds to Rpl10 (uL16) in the cytoplasm where it is involved in the loading of Rpl10 onto almost mature 60S subunits [24, 47, 48]. Rrb1 associates with Rpl3 in the cytoplasm and probably ensures its efficient incorporation into pre-ribosomal particles in the nucleus [24, 49, 50]. Acl4 interacts with Rpl4 and accompanies this r-protein from its synthesis in the cytoplasm until its assembly into pre-ribosomes in the nucleus [51, 52]. Finally, Syo1 is an import adaptor and chaperone, which is unique in that it binds not just one r-protein, but has the ability to accommodate two different r-proteins at the same time, Rpl5 (uL18) and Rpl11 (uL5) [53–55].

In addition to these yeast r-protein chaperones, a dedicated chaperone of human RPL23 (hRPL23; uL14) has recently been described, the BRCA2 and CDKN1A interacting protein BCCIP isoform β , which forms a trimeric complex together with hRPL23 and the 60S biogenesis factor eIF6 [56]. The yeast homolog of BCCIP, Bcp1, has been implicated in ribosome biogenesis [57]; moreover, recent data from the Lo laboratory indicate that Bcp1 may indeed function as a dedicated chaperone for Rpl23 (Kai-Yin Lo, personal communication).

In contrast to typical ribosome biogenesis factors, r-protein chaperones were found mainly in complex with their r-protein binding partners, and are either only transiently or not at all bound to pre-ribosomal particles. In the following, we will summarize what is known about the seven chaperones with respect to their structures and modes of interaction with r-proteins, their timeline of interaction, and the phenotypes observed in their absence.

Dedicated chaperones counteract aggregation of unassembled r-proteins

A function common to most r-protein chaperones is that they protect their binding partners from aggregation. Indeed, the solubility of Rps3, Rpl4, and Rpl10 is reduced in *yar1*, *acl4*, and *sqt1* mutants [24, 42, 52]. Moreover, Tsr2 shields Rps26 from aggregation in vitro [46]. Likewise, BCCIP β depletion diminishes the soluble pool of hRPL23 and the solubility of yeast Rpl23 is reduced in *bcp1* mutant cells or after depletion of BCP1 (Ref. [56] and Kai-Yin Lo, personal communication). Furthermore, *RRB1* and *TSR2* depletion reduces the total cellular levels of Rpl3 and Rps26, suggesting degradation of the r-proteins [46, 49]. Considering this role in keeping r-proteins soluble, it is not surprising that ribosome biogenesis phenotypes of r-protein chaperone mutants resemble those observed upon depletion or mutation of their r-protein clients [42, 46–48, 52].

Despite this function in protecting r-proteins, four of the known r-protein chaperones, Yar1, Tsr2, Acl4, and Syo1, are non-essential; hence, ribosome assembly of their r-protein binding partners can also occur in their absence. Nevertheless, their knockouts cause growth defects suggesting that the chaperones ultimately increase the efficiency of r-protein assembly, thereby ensuring optimal growth rates [41, 42, 46,

51–53]. This view is supported by the fact that overexpression of Rps3, Rpl4, and Rpl5 can rescue the slow-growth phenotype of *yar1* Δ , *acl4* Δ , and *syo1* Δ mutant strains, respectively [41, 42, 52, 53]. Moreover, although Sqt1 is an essential protein, *sqt1* Δ cells are viable when Rpl10 is overexpressed, suggesting that also Sqt1 increases the efficiency of a process that can in principle occur in its absence [24].

Different folds to hold r-proteins

Gaining structural insights into r-protein recognition by dedicated chaperones is instrumental in order to eventually understand how dedicated chaperones protect r-proteins from aggregation and how they might facilitate their assembly into pre-ribosomal particles. For most of the chaperone/r-protein pairs the respective interaction domains have been mapped, and for many of them at least partial co-structures of the complexes have been solved (Fig. 2). Unfortunately, no structural or domain mapping data have so far been reported for the Tsr2/Rps26 and BCCIP β /hRPL23 complexes. Most notably, four of the chaperones, Sqt1, Rrb1, Yar1, and Syo1, interact with the very N-terminal region of their respective partners.

Sqt1 binds to the N-terminal residues (amino acids 2–13) of Rpl10 via the negatively charged top surface of its eight-bladed WD-repeat β -propeller structure (Fig. 2A) [24]. The conformation of the Sqt1-bound N-terminal extension of Rpl10 is very similar to its final fold within the 60S ribosomal subunit [12, 24]. Importantly, Sqt1 protects all N-terminal residues of Rpl10 that are later, in the context of the mature 60S subunit, involved in the binding of helix 89 (H89) of the 25S rRNA. Presumably, Rpl10 initially, while still being bound to Sqt1, forms contacts with pre-60S subunits via its other interaction sites and the final incorporation of its N-terminal extension is only possible after restructuring events, including the release of Sqt1 and the export adaptor Nmd3 [24, 48, 58].

Like Sqt1, Rrb1 is predicted to form a WD-repeat β -propeller structure and is also able to capture the very N-terminal residues (amino acids 1–15) of its r-protein partner Rpl3 [24]. Because of these two similarities, it is tempting to speculate that Rrb1 binds to Rpl3 in a similar manner as described above for the Sqt1-mediated recognition of Rpl10. Within mature 60S subunits, these 15 N-terminal amino acids of Rpl3 are engaged in interactions with helices H90–H92 and H96 of the 25S rRNA and with r-protein Rpl23 [12, 24].

Yar1 is composed of four ankyrin repeats and interacts with the N-terminal part (amino acids 14–29) of Rps3 (Fig. 2B) [43, 44]. The N-terminus of 40S-bound Rps3 folds into a long α -helix (amino acids 6–29) and is engaged in interactions with 18S rRNA helix H41 and r-proteins Rps20 (uS10), Rps10 (eS10), and Rps29 (uS14). In the Yar1-bound state, however, the N-terminal α -helix of Rps3 is shorter (amino acids 14–29), leaving the very N-terminal residues unstructured [43]. Rps3 is composed of two globular domains (N- and C-domain), followed by an unstructured C-terminal extension [12]. Unexpectedly, when associated with Yar1, Rps3 dimerizes via domain swapping of two β -strands in its C-domain [43, 44]. Apart from its main contact with the N-terminal part of Rps3, which is both necessary and sufficient

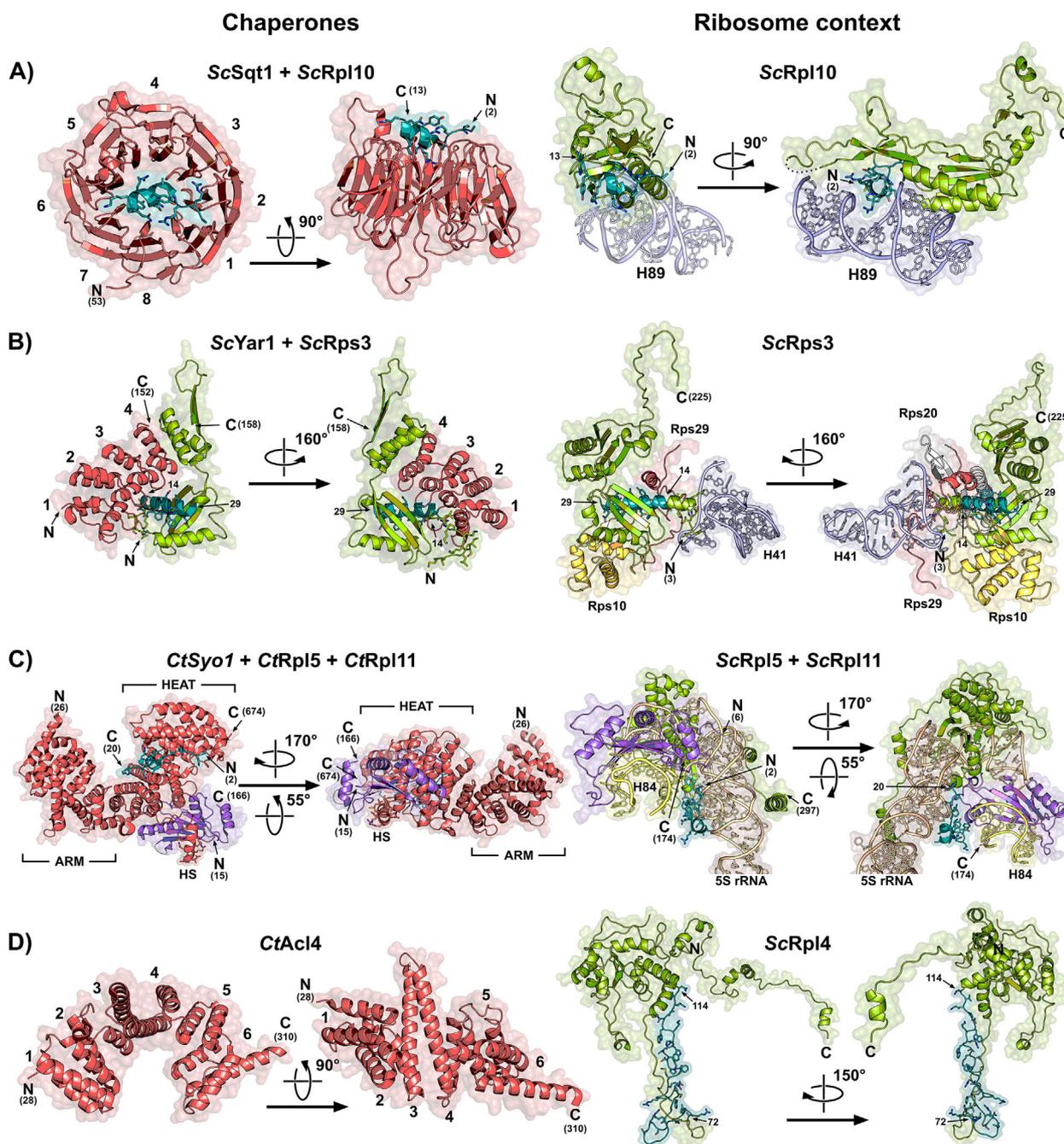


Figure 2. Interactions of r-proteins with chaperones and within ribosomal subunits. **A:** Structure of the eight-bladed WD-repeat β -propeller of ScSqt1 (red) in complex with the N-terminal residues of ScRpl10 (blue) (left panel; based on PDB 4ZOX [24]). Numbers indicate the individual β -propeller blades of ScSqt1. Side chains of amino acids 2–13 of ScRpl10 are shown with oxygen atoms in red, nitrogen atoms in blue, and sulfur atoms in yellow. Structure of ScRpl10 (bright green, with Sqt1-interacting region in blue) in the context of the mature 60S subunit, highlighting helix 89 (H89) of the 25S rRNA (light blue) (right panel; based on PDB 4V88 [12]). **B:** Structure of ScYar1 (red) in complex with ScRps3 (bright green) (left panel; based on SAXS model [44] generated using PDB 4BSZ [43]). Numbers indicate the individual ankyrin repeats of ScYar1. Note that the Yar1-interacting region of Rps3 (amino acids 14–29) is displayed in blue and that the side chains of amino acids 1–29 are shown. Structure of ScRps3 (bright green, with Yar1-interacting region in blue) in the context of the mature 40S subunit, highlighting Rps10 (yellow), Rps29 (red), Rps20 (light grey), and helix 41 (H41) of the 18S rRNA (light blue) (right panel; based on PDB 4V88 [12]). **C:** Structure of CtSyo1 (red) in complex with CtRpl11 (purple) and the N-terminal residues of CtRpl5 (blue) (left panel; based on PDB 5AFF [55]). The ARM- and the HEAT-repeat domain of CtSyo1 are indicated. Side chains of CtSyo1's helical segment (HS) and of amino acids 2–20 of CtRpl5 are shown. Structure of ScRpl5 (bright green, with Syo1-interacting region in blue) and ScRpl11 (purple) in the context of the mature 60S subunit, highlighting the 5S rRNA (wheat) and helix 84 (H84) of the 25S rRNA (yellow) (right panel; based on PDB 4V88 [12]). **D:** Structure of CtAcl4 (red) (left panel; based on PDB 4YNW [51]). Numbers indicate the individual TPR repeats of CtAcl4. Structure of ScRpl4 (bright green) within the mature 60S subunit (right panel; based on PDB 4V88 [12]). Note that the Acl4-interacting region of Rpl4 (amino acids 72–114) is displayed in blue and its residues are shown with side chains.

for the interaction, Yar1 also makes contacts with the C-domain of Rps3; hence, it is possible that Yar1 contributes to the formation or stability of the dimeric Rps3 arrangement. Moreover, in this complex, the Rps3 N-domain is rotated by $\sim 180^\circ$ relative to the C-domain compared to its conformation in the mature 40S subunit [43, 44]. This N-domain rotation is reasonable since the same small subunit surface, which is in contact with the Rps3 N-domain in mature 40S subunits, is occupied by the assembly factor Ltv1 in pre-40S particles [59–61]. Hence, the rotated conformation of Rps3 permits that, initially, only the Rps3 C-domain is assembled with the pre-40S particles at its correct position, allowing for important structural changes to occur before the Rps3 N-domain is stably incorporated [43, 44].

Syo1 has an overall α -solenoid shape that is made up of an N-terminal ARM domain, consisting of four complete ARM (armadillo) repeats, and a C-terminal HEAT domain, containing six HEAT (huntingtin, elongation factor 3, A subunit of PP2A, and TOR) repeats [53]. Structural analyses revealed that the inner, concave surface of the HEAT-repeat domain of Syo1 accommodates the N-terminal residues (amino acids 2–20) of Rpl5 (Fig. 2C) [53, 55]. The main Rpl11-binding determinant on Syo1 consists of a helical segment (HS) at the end of the acidic loop, which connects HEAT repeats 1 and 2 [53, 55]. On the Rpl11 side, the interaction with Syo1's HS is mediated by the groove of a five-stranded β -sheet contained within amino acids 16–131 of Rpl11 [55]. However, since these structural studies were carried out with the proteins of the thermophilic ascomycete *Chaetomium thermophilum* (*Ct*) and since the acidic loop is not well conserved among eukaryotic species, it remains to be determined whether the same binding mode between Syo1 and Rpl11 applies in other organisms, including *S. cerevisiae* and *Homo sapiens*. Nonetheless, the reported structural, biochemical, and crosslinking data reveal interesting insights into 5S RNP assembly and its subsequent incorporation into pre-60S subunits [53, 55]. Notably, binding of the 5S rRNA to the ternary Syo1-Rpl5-Rpl11 complex leads to structural rearrangements that suggest the formation of a pre-5S RNP containing Syo1 [55]. Importantly, Syo1 binds to the same surface on Rpl11 that interacts with helix H84 of the 25S rRNA, suggesting that Syo1 protects this binding site before being outcompeted by H84, which likely corresponds to a primary docking site during recruitment of the 5S RNP to the pre-60S ribosome [55]. At present it is, however, not clear whether Syo1 is still associated with the 5S RNP when it is transferred to the pre-60S ribosome. Even though Syo1 is fulfilling an important function, especially at lower temperatures, it is dispensable for yeast growth [53]; hence, import of Rpl5 and Rpl11, nuclear 5S RNP formation, and assembly of the 5S RNP into pre-60S subunits can proceed in its absence. Recent structural and biochemical analyses indicate that the two essential biogenesis factors Rpf2 and Rrs1, which can form a stable complex with Rpl5, Rpl11, and the 5S rRNA [62], may mediate the recruitment of the 5S RNP into early pre-60S particles [16, 63, 64]. However, future studies are required to elucidate whether the Rpf2-Rrs1 complex contributes, either on its own or with the help of Syo1, to the assembly of the 5S RNP.

Acl4, in contrast to the above-described dedicated chaperones, does not bind to the N-terminal part of Rpl4. Instead, the minimal binding site of Acl4 consists of amino

acids 72–114 within Rpl4's long internal loop (amino acids 43–114), which dives deep into the ribosomal core, with its tip region being located at the polypeptide exit tunnel, of mature 60S subunits [12, 51, 52]. The partial crystal structure of Acl4 reveals that its central α -helical region is composed of 6.5 TPR (tetratricopeptide) repeats exhibiting a crescent-shaped topology (Fig. 2D) [51]. Moreover, the second half of the α -helical domain, starting with TPR 4, is the structural element mediating the interaction with Rpl4 [51]. It will be interesting to learn how exactly Acl4 binds to the long internal loop of Rpl4 in order to obtain insights into the transfer of Rpl4 into pre-60S subunits at a molecular level.

Taken together, dedicated chaperones utilize the surfaces of different folds, such as those of WD-repeat β -propellers, ankyrin repeats, ARM/HEAT repeats, and TPR repeats, to interact with exposed regions of their r-protein clients. Notably, despite the differences in the binding modes, dedicated chaperones shield, prior to r-protein assembly, r-protein residues that engage in interactions with rRNAs or r-proteins within the mature ribosome (see Fig. 2). In addition, as exemplified by Yar1 and Syo1, dedicated chaperones can assist restructuring events. To answer whether any of the other dedicated chaperones also contribute to the restructuring of their substrates, it will be necessary to solve their structures in complex with their full-length r-protein partners.

Timeline of interaction

Co-translational capturing of nascent r-proteins by dedicated chaperones

Free r-proteins are prone to aggregation and degradation [18, 65, 66], suggesting that protection by chaperones is needed as early as possible after their synthesis. Indeed, at least five r-proteins, Rps3, Rpl3, Rpl5, Rpl10, and Rpl4, are bound by their dedicated chaperones already while they are being translated [24, 52]. The fact that dedicated chaperones recognize the N-terminal parts of Rps3, Rpl3, Rpl5, and Rpl10 suggests that they capture their respective r-protein clients as soon as their first few amino acid residues emerge from the translating ribosome [24]. In contrast, Rpl11 is only bound post-translationally by Syo1 [52], which is not surprising considering that the main Syo1 binding site on Rpl11 consists of a surface made up of a five-stranded β -sheet, indicating that folding into the correct tertiary structure has to occur before the interaction can take place. Although it has not been tested so far, co-translational capturing of Rps26 by Tsr2 is unlikely given that Tsr2 is believed to only bind to Rps26 after its import into the nucleus (see below).

Nuclear import of r-protein/chaperone complexes

With the exception of Rpl10, which is, with the help of Sqt1, incorporated into almost mature 60S subunits in the cytoplasm (Fig. 3A) [8, 48], presumably all other r-proteins that interact with dedicated chaperones assemble into pre-ribosomal particles within the nucleus. Even though

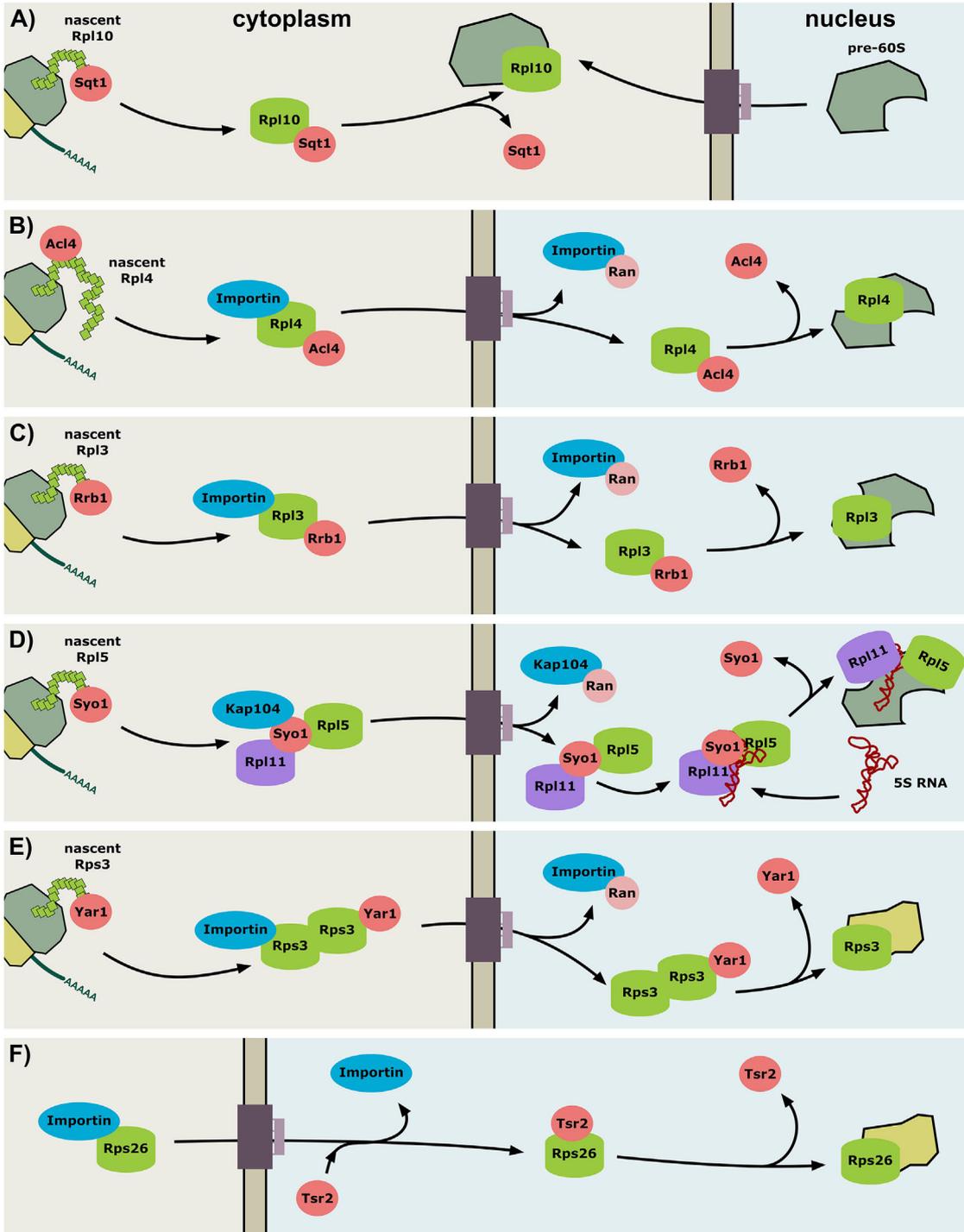


Figure 3. The different functional roles of dedicated chaperones on the path of their r-protein clients to their pre-ribosomal assembly sites. **A:** Sqt1 binds the N-terminus of Rpl10 co-translationally and assists Rpl10 incorporation into pre-60S subunits in the cytoplasm. **B:** Acl4 recognizes the C-terminal part of the long internal loop of Rpl4 in a co-translational manner. Acl4 then accompanies Rpl4 into the nucleus and dissociates upon incorporation of Rpl4 into pre-60S subunits. **C:** Rrb1 binds Rpl3 co-translationally and accompanies the r-protein to its pre-60S assembly site in the nucleus. **D:** Syo1 captures Rpl5 co-translationally and binds Rpl11 post-translationally. The importin Kap104 then binds to Syo1 and mediates nuclear import of the complex. After recruitment of the 5S rRNA to the Syo1-Rpl5-Rpl11 complex in the nucleus, the 5S RNP is incorporated into pre-60S subunits and Syo1 is released. **E:** Rps3 is co-translationally recognized by Yar1 and then dimerizes with a second Rps3 molecule. After import of the Rps3-Rps3-Yar1 complex into the nucleus, the Rps3 dimer is dissociated and each Rps3 is incorporated into a pre-40S particle, while Yar1 is released. **F:** The escortin Tsr2 associates with Rps26 after its nuclear import, thereby dissociating the Rps26-importin complex in a RanGTP-independent manner, and guides its safe transfer to the 90S pre-ribosome.

importins might in part fulfill the protective function during transport into the nucleus, there is evidence that most dedicated chaperones that bind to r-proteins in the cytoplasm also accompany their r-protein clients to their nucle(ol)ar assembly site on pre-ribosomal particles. This is not surprising, since the r-protein cargoes are dissociated from these transport receptors in the nucleoplasm, and r-proteins still require guidance to safely reach their assembly site on nucle(ol)ar pre-ribosomes.

Acl4 (Fig. 3B) shows a mainly nuclear steady-state localization, but lacks predicted nuclear localization signals (NLS). Rpl4 on the contrary contains at least five distinct NLSs [52], including a PY-NLS, which is typically bound by the importin Kap104 [67]. Acl4 can form a trimeric complex together with Rpl4 and Kap104 *in vitro*, suggesting that it is imported into the nucleus in complex with Rpl4 [51]. However, Kap104 does not seem to be the only importin responsible for nuclear import of Rpl4, since Rpl4 lacking the C-terminal extension, which harbors the Kap104 binding site, is still targeted to the nucleus and since the C-terminal extension (amino acids 263–362) still confers nuclear localization to a GFP reporter construct in *kap104* mutant cells [52].

Rrb1 (Fig. 3C), which is predominantly located in the nucleolus, was shown to interact with Rpl3 in the nucleus [49]. Moreover, while overexpression of Rrb1 leads to nuclear accumulation of Rpl3, Rrb1 relocalizes to the cytoplasm upon inhibition of translation [49], altogether indicating that Rrb1 accompanies Rpl3 into the nucleus.

The symportin Syo1 (Fig. 3D), which localizes predominantly to the nucleus at steady state, not only accompanies Rpl5 and Rpl11 into the nucleus but it even plays an active role in their import by functioning as an import adaptor containing an N-terminal PY-NLS that is recognized by the importin Kap104 [53]. Upon arrival in the nucleoplasm, binding of RanGTP to Kap104 promotes the release of the trimeric Syo1-Rpl5-Rpl11 cargo complex, which can then associate with the 5S rRNA to form a Syo1-containing pre-5S RNP [53, 55].

Although Yar1 (Fig. 3E) has a cytoplasmic steady-state localization, it transiently enters the nucleus, as suggested by the nuclear accumulation of Yar1-GFP upon inhibition of the exportin Xpo1/Crm1 [42]. The NLS of Rps3 is located directly adjacent to the Yar1 binding site [42–44], and there is competition between importins and Yar1 for binding to the Rps3 N-domain [68]. Nevertheless, considering that Rps3 is dimeric in the Yar1-bound state and that Yar1 associates with only one of the two Rps3 molecules *in vivo*, the NLS within the N-domain of the second Rps3 molecule is available for binding to an importin [44, 68]. Indeed, complexes containing Rps3 and both Yar1 and the importin Kap60 are formed *in vivo*, suggesting co-import of Rps3 and Yar1 [68].

After having delivered their r-protein binding partners at their pre-ribosomal destination sites in the nucleus, these four dedicated chaperones need to travel back to the cytoplasm where they can engage in another round of r-protein recognition. Free Syo1, but not the Syo1-Rpl5-Rpl11 complex, has the capability to directly interact with the phenylalanine glycine (FG) repeats of nucleoporins and to translocate through nuclear pores, indicating that it shuttles back to the cytoplasm on its own [53]. Although the exact mechanism is not known, the nuclear accumulation of Yar1 upon Xpo1/Crm1

inhibition suggests that Yar1 export is Xpo1/Crm1 dependent [42]. The export pathways of Acl4 and Rrb1 still remain to be determined.

Escortin-mediated nuclear release from importins

Tsr2 (Fig. 3F) exhibits a predominantly nuclear localization and does not join its r-protein client in the cytoplasm but rather only after its import into the nucleus [46]. Notably, Tsr2 actively participates in nuclear import of Rps26 by displacing Rps26 from its transport receptors Kap123, Kap121/Pse1, and Kap104 in a RanGTP-independent manner [46]. Upon capturing Rps26 in the nucleoplasm, Tsr2 then guides Rps26 to its assembly site within 90S pre-ribosomes [46]. In anticipation of a possibly more widespread role of dedicated chaperones in promoting the nuclear transfer of r-proteins from their transport receptors to the pre-ribosomal particles, the term “escortin” has been proposed to refer to this class of dedicated chaperones [46].

BCCIP β shows a nuclear localization in HeLa cells and its overexpression leads to nucleoplasmic accumulation of hRPL23 and eIF6, indicating that it stabilizes a nuclear population of hRPL23 [56]. While this is suggestive of an escortin function, it remains to be determined whether BCCIP β captures hRPL23 already in the cytoplasm or indeed only after its nuclear import. In support of such a scenario, unpublished data indicate that the yeast ortholog Bcp1 acts as an escortin as it is able to release Rpl23 from importins in a RanGTP-independent manner (Kai-Yin Lo, personal communication).

Taken together, it seems to be a prevalent theme that dedicated chaperones are associated with their r-protein clients from the time of their synthesis until the moment when they are assembled into pre-ribosomal particles. An exception are the escortins, which only bind to their r-protein partners in the nucleus and guide the nuclear transfer to their pre-ribosomal destinations.

Conservation of dedicated chaperones between yeast and humans

R-proteins are highly conserved, suggesting that similar mechanisms may exist to protect the orthologous yeast and human r-proteins. Up to now, most dedicated chaperones have only been investigated in yeast. An exception is BCCIP β , the only so far characterized human r-protein chaperone. Its yeast ortholog Bcp1 was found to interact with Rpl23 in several large-scale studies (see for example [69–71] and functions as a dedicated chaperone for Rpl23 (Kai-Yin Lo, personal communication). Tsr2 clearly has a human ortholog, and the fact that hTSR2 was found to interact with hRPS26 in a large-scale yeast two-hybrid screen suggests that it may also act as RPS26 chaperone in human cells [72]. Moreover, human proteins with homology to Syo1, Rrb1, and Sgt1 exist (Table 1), and, even though experimental proof is still missing, it is very likely that these function as dedicated chaperones of the corresponding human r-proteins. Although the Acl4 binding site within the

long internal loop of Rpl4 is highly conserved and Acl4 orthologs can be found in many eukaryotic species, there are notably no obvious orthologs in reptiles, birds, and mammals; thus, suggesting that different mechanisms may have developed to protect this r-protein on its journey to its nuclear assembly site in distinct evolutionary branches [51, 52]. Finally, also for Yar1, no obvious human homolog can be identified. Interestingly however, another ankyrin-repeat protein, the NF κ B-inhibitor I κ B α directly interacts with hRPS3 [73]. It remains to be clarified whether and how the extra-ribosomal, NF κ B/I κ B α -bound hRPS3 and the pool of hRPS3 destined to be incorporated into pre-ribosomal particles communicate.

Are there still more r-protein chaperones to be discovered?

So far, dedicated chaperones have been identified for one human and six different yeast r-proteins. It is unlikely that with these proteins the inventory of dedicated chaperones of r-proteins is already complete, although it is difficult, at this point, to predict how many of the ~80 r-proteins have their own dedicated chaperones.

The so far known r-protein chaperones have been identified by various different biochemical and genetic methods, and only some of these may be suitable for the systematic search for novel dedicated chaperones. Rps26 and Rpl3 were uncovered as main interaction partners of Tsr2 and Rrb1, respectively, by affinity purification of these biogenesis factors [45, 49, 50]. However, the identification of dedicated chaperones by affinity purification of r-proteins is complicated by the fact that the bulk of r-proteins in a cell is contained in mature ribosomes, while the proportion of r-proteins that are employed in the assembly process and could potentially be bound by dedicated chaperones is relatively low. Hence, specific strategies will have to be implemented to enrich the chaperone-bound pool of an r-protein. Such enrichment may fortuitously occur due to the reduced efficiency of ribosome incorporation of the tagged r-protein, as observed in the case of Rpl5-TAP, thus allowing the identification of Syo1 as a specific interaction partner of free Rpl5 [53]. Additionally, the purification of truncated versions of r-proteins with reduced or abolished assembly capability may be an option, as employed in the case of Rpl4 to identify Acl4 [52]. A very promising, recently developed method, based on the coupling of a non-radioactive pulse-chase labeling with affinity purification [74, 75], enabled the identification of Acl4 as an interaction partner of Rpl4 [51]. This method might prove to be especially useful for the systematic discovery of dedicated chaperones of newly synthesized r-proteins. Alternatively, yeast genetic methods, such as yeast two-hybrid (Y2H) and high-copy suppressor screens, may be employed to uncover novel r-protein/chaperone pairs. For example, a Y2H screen with Yar1 permitted the identification of Rps3 as its interaction partner [41]. However, the utilization of r-proteins as baits for Y2H screens may be hampered by their efficient and stable incorporation into pre-ribosomes; therefore, as already described above, it might be necessary to conduct the screens

with truncated r-proteins with reduced assembly efficiency. Moreover, by searching for high-copy suppressors of a dominant-negative *rpl10* mutant, Sqt1 could be successfully identified as a direct binder of Rpl10 [47].

A few more proteins have been discussed in the literature to be potential chaperones for r-proteins. The adenylate kinase Fap7 is a ribosome assembly factor that directly interacts with Rps14 (uS11) and it was suggested to act as a dedicated chaperone for this r-protein [76, 77]. This hypothesis is supported by the observation that the solubility of Rps14 expressed in bacteria is increased upon co-expression of Fap7 [76]. Moreover, Fap7 binds pre-ribosomal particles only very transiently [78, 79]. Interestingly, the interaction of Fap7 with Rps14 blocks important rRNA interaction sites within Rps14, entailing that Rps14 cannot be bound to 40S subunits in its final conformation as long as it is associated with Fap7 [76, 77]. All these features are reminiscent of other r-protein chaperones discussed in this review. However, there is so far no evidence that Fap7 binds Rps14 already before its initial association with pre-40S particles. For this reason, a definite conclusion as to whether Fap7 acts as a dedicated chaperone for Rps14 cannot be drawn at this point.

In addition, it has to be mentioned that human RPS9 and *Arabidopsis thaliana* RPS6 were reported to interact with histone chaperones B23/NPM (nucleophosmin) and AtNAP1, respectively [80, 81]. These findings may be a first indication for a functional overlap between dedicated histone and r-protein chaperones.

Conclusions and prospects

The identification of dedicated r-protein chaperones has taught us that the complex process of ribosome biogenesis starts well before the association of r-proteins with the nascent pre-rRNA in the nucleolus. At this point, most r-proteins have already traveled a long way in an adverse environment to reach their assembly site on pre-ribosomal particles. Although the seven so far known dedicated chaperones have the common function to protect their r-protein clients on this path, they differ in many other aspects. While most of them bind to their respective r-protein partners already as soon as they are being translated and guard them on the entire journey to their pre-ribosomal destination, a subgroup termed escortins does only the nuclear shift of the work. Several of them even perform additional jobs such as assisting nuclear import, promoting restructuring events, or facilitating incorporation of their r-protein clients. From structural studies we have learnt that dedicated chaperones utilize different classes of protein-repeat folds to selectively and firmly interact with mostly unstructured regions, in many cases involving the N-terminal residues, of their r-protein binding partners. Given that the individual r-proteins are exclusively associated with a specific dedicated chaperone, it can be concluded that the cell has developed custom-made strategies to optimally cope with the specific demands of each r-protein. Most of the assistance-requiring r-proteins have been implicated in the development of human diseases (see Table 2), notably including the “classical” ribosomopathy Diamond-Blackfan anemia (DBA) [82–84]; hence, it is possible that disease formation

Table 2. Human diseases linked to r-proteins or their dedicated chaperones

Disease (ribosomopathy)	Mutated r-protein/ chaperone	Type of mutation	References
Diamond-Blackfan anemia (DBA)	RPS26	Deletion, nonsense, missense, frameshift, start codon, splice-site	[85–88]
	hTSR2	Missense	[85]
	RPL3/RPL3L	Missense	[89, 90]
	RPL5	Missense, nonsense, frameshift, start codon, splice-site	[91–93]
	RPL11	Missense, nonsense, frameshift, splice-site	[91–93]
T-cell acute lymphoblastic leukemia (T-ALL)	RPL5	Missense, nonsense	[94]
	RPL10	Missense	[94]
Autism	RPL10	Missense	[95]

may also be linked, as already shown for hTSR2 [85], to mutation of the corresponding dedicated chaperone. It will be exciting to watch new dedicated r-protein chaperones emerge in the future and to study their functions and mechanisms of actions, in order to finally gain a deeper understanding of the important “pre-assembly” aspects of ribosome biogenesis.

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