Review

# A 'garbage can' for ribosomes: how eukaryotes degrade their ribosomes

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**Ribosome synthesis is a major metabolic activity that involves hundreds of individual reactions, each of which is error-prone. Ribosomal insults occur in *cis* (alteration in rRNA sequences) and in *trans* (failure to bind to, or loss of, an assembly factor or ribosomal protein). In addition, specific growth conditions, such as starvation, require that excess ribosomes are turned over efficiently. Recent work indicates that cells evolved multiple strategies to recognize specifically, and target for clearance, ribosomes that are structurally and/or functionally deficient, as well as in excess. This surveillance is active at every step of the ribosome synthesis pathway and on mature ribosomes, involves nearly entirely different mechanisms for the small and large subunits, and requires specialized subcellular organelles.**

## Ribosome synthesis is a multi-step, error-prone process

Ribosomes comprise two subunits of unequal size that carry out specialized functions in translation: mRNA decoding and peptidyl-transfer reaction for the small and large subunits, respectively [1]. Each eukaryotic ribosome consists of 4 rRNAs and ~80 ribosomal proteins. The synthesis, maturation and transport of individual ribosomal components and their assembly into ribosomal subunits requires the intervention of ~200 protein *trans*-acting factors, and numerous small nucleolar RNAs (snoRNAs) that are involved in hundreds of individual, error-prone, reactions [2,3] (Figure 1). Many ribosomal proteins perform additional non ribosomal functions [4]. Likewise, functions in processes not connected directly to ribosome biogenesis are currently being assigned to ribosome synthesis factors: connections to cell cycle progression, pre-mRNA splicing, the DNA damage response, nuclear organization and telomere maintenance are emerging. With so many reactions in the ribosome assembly pathway, the possibility to introduce mistakes with potential deleterious consequences for cell viability and human health is immense. As such, failure to bind, or the loss of, a synthesis factor could lead to the production of ribosomes that are structurally defective (e.g., lacking individual or subsets of ribosomal proteins or carrying misfolded rRNA) with functional consequences in translation. To circumvent such problems, cells have evolved multiple quality control mechanisms. For example, synthesis factors involved in late cytoplasmic ribosome assembly steps can bind pre-rRNAs at early nucleolar stages, thereby committing pre-ribosomes to productive synthesis pathways [5]. In other cases, *trans*-acting factors with partial homology to ribosomal proteins or translation factors might bind pre-ribosomes to monitor and tether the structural integrity of ribosomal protein-binding sites [6,7]. In wild-type cells, ribosome assembly defects can result from the delayed binding of a *trans*-acting factor. In these circumstances, and providing that the proper assembly reaction occurs within a defined timeframe, faithful assembly presumably resumes; otherwise, pre-ribosomes are identified as defective and targeted for rapid degradation by active surveillance mechanisms. In addition to alterations in *trans*, mutations can occur in *cis* either during RNA synthesis or, more frequently, as a consequence of exposure to genotoxic stress. The importance of 'clearing the system' of such mutations is that they could otherwise accumulate to pathological levels owing to the high stability (half-life of several days) and abundance (up to 80% of total cellular RNA) of the rRNAs. Indeed, human diseases, and in particular, neurodegenerative disorders, have recently been linked to the accumulation of such defective ribosomes [8]. Finally, there are situations where excess ribosomes must be degraded to recycle essential cellular building blocks; quite unexpectedly, this pathway also involves their specific recognition.

Recent research indicates that surveillance exerts its action at every step of the ribosome assembly line, as well as on the final product, that it involves dedicated subcellular structures, and that distinctive pathways prevail for monitoring the assembly and function of the small and large ribosomal subunits. This multi-faceted ribosome surveillance is the focus of this review (Figure 2).

## Mutations in *cis*: non-functional ribosomal RNA decay

Several surveillance pathways have been described that monitor the structural and functional integrity of mature RNA molecules [9]. One such pathway that monitors mRNAs is the 'no-go' decay pathway (NGD), wherein mRNAs that induce stalled ribosomes are degraded [10].

To test whether mutations in functionally relevant and conserved ribosomal sites affect rRNA stability, LaRivière *et al.* introduced substitutions in the decoding site (DCS; in 18S rRNA) and peptidyl transferase centre (PTC; in 25S rRNA) at positions that are essential for ribosome function in bacteria [11] (Table S1). This led to the identification of 'non functional rRNA decay' or NRD [11], a pathway that detects and eliminates functionally defective components of mature ribosomes (Box 1). Indeed, in each case, these

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0968-0004/$ – see front matter  doi:10.1016/j.tibs.2009.12.006 Available online 22 January 2010

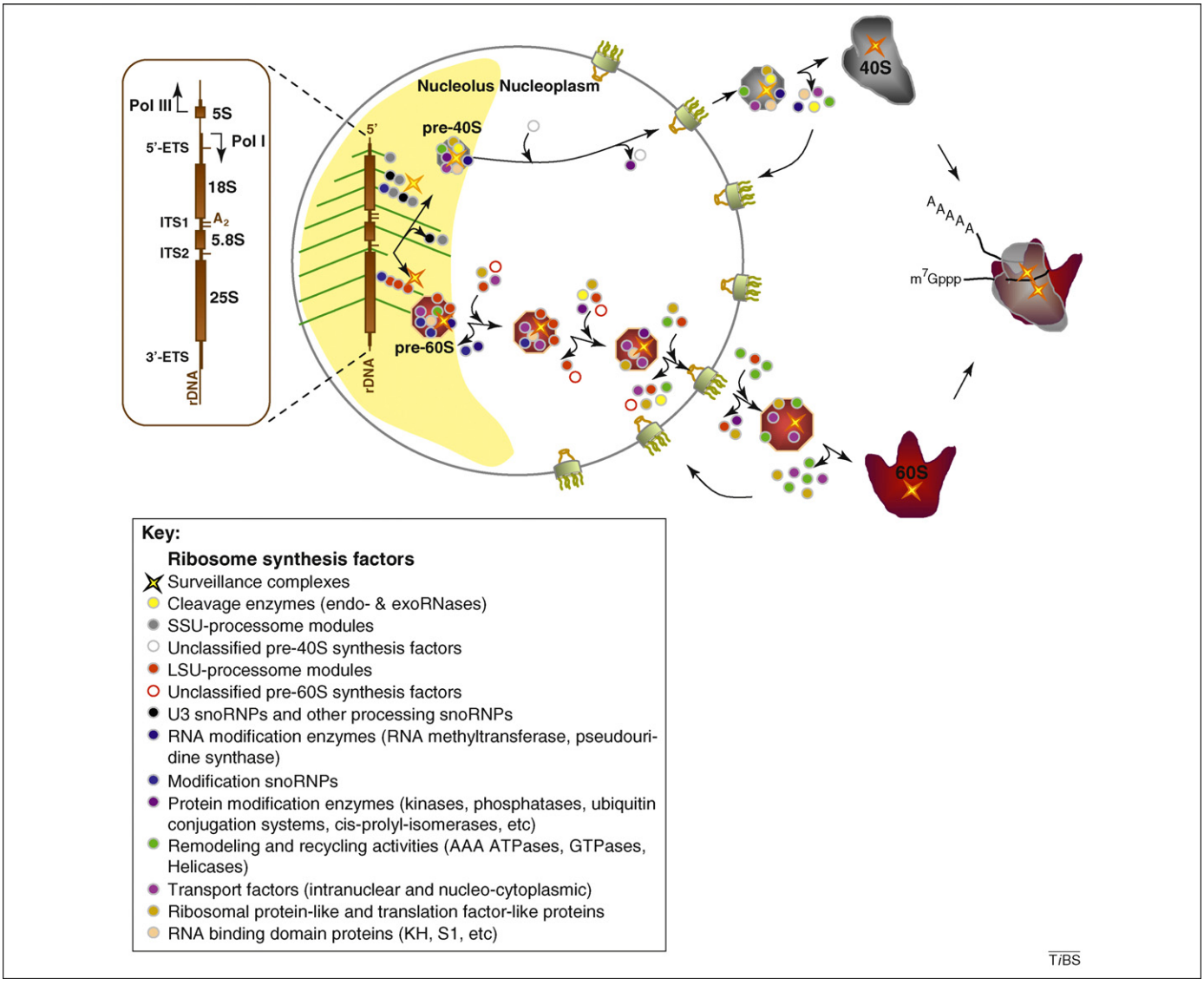


**Figure 1**. Eukaryotic ribosome synthesis is a multi-step, error-prone process. Ribosome synthesis starts in the nucleolus where the core, and catalytically active, pieces of the machinery, the rRNAs, are synthesized. Three out of the four rRNAs (18S, 5.8S and 25S rRNAs) are produced by RNA polymerase I (Pol I) from a single transcription unit. The fourth rRNA (5S) is synthesized by RNA Pol III with a 3′-extension (inset). The 18S, 5.8S and 25S rRNAs are interspersed with non-coding sequences: the 5′- and 3′-external transcribed spacers (5′-ETS and 3′-ETS) and internal transcribed spacers 1 and 2 (ITS1 and ITS2). An actively transcribed rDNA unit is depicted as a 'Christmas tree', reminiscent of its visualization by Miller chromatin spread, with the 'trunk' of the 'tree' (brown) representing the rDNA locus and the 'branches' (green) corresponding to nascent pre-rRNA transcripts. Pre-rRNA processing is initiated either post-transcriptionally (left of the 'tree') or co-transcriptionally (to the right). In fast-growing yeast cells, up to 50-70% of nascent transcripts are cleaved co-transcriptionally at site $A_2$ within ITS1, generating pre-40S and pre-60S ribosomes [57] (other pre-rRNA cleavage sites are described in Figure 3). In the remaining cases, a full-length transcript (35S) is generated and is assembled into a 90S pre-ribosome (not represented) that is cleaved post-transcriptionally at site $A_2$. Many ribosome synthesis factors escort pre-ribosomes from their initial site of synthesis in the nucleolus to their cytoplasmic site of function. Different ribosome synthesis factors display distinctive patterns of association with pre-ribosomes, resulting in a progressive reduction in protein complexity. The nuclear dwelling time for pre-40S and pre-60S subunits is distinctly different, with pre-40S subunits reaching the cytoplasm much faster than pre-60S. Translocation through the various nucleolar domains is facilitated by specific *trans*-acting factors. Nuclear export involves redundant pathways. About 2000 ribosomes are exported every minute in exponentially growing yeast cells, and control has been suggested to occur at the nuclear pore in response to nutrient availability [58,59]. There appears to be good timing between nucleo-cytoplasmic partitioning and the acquisition of prominent ribosomal structural features. For example, final shaping of the 'beak', a protruding structure of the small subunit, is thought to only occur once pre-40S has reached the cytoplasm [60]. Ribosome synthesis factors are available in limited quantities; therefore their recycling is essential. Recent work identified a mechanochemical process powered by a AAA-type ATPase distantly related to the motor protein dynein involved in this recycling [61]. Late sets of cytoplasmic *trans*-acting factors are released sequentially following further structural rearrangements fueled by a cascade of GTPase-mediated reactions and recycled to the nucleus and nucleolus, ready to engage further rounds of assembly. The stable incorporation of each resident ribosomal protein is then finalized. Major classes of *trans*-acting factors are listed. To illustrate the dynamics of ribosome synthesis and the plasticity of pre-ribosomes, several ribosome synthesis factors (arbitrarily color-coded) are represented.

changes resulted in a reduced accumulation (~5 to 10 fold) of the mature rRNA containing the mutation.

### *Small ribosomal subunit NRD: a process reminiscent of mRNA no-go decay*

During mRNA NGD, a stalled ribosome triggers initiating endonucleolytic cleavage events on the defective mRNA at the vicinity of the pause site, and this is followed by the exoribonucleolytic digestion of the 5'- and 3'-cleaved mRNA products by the RNA exosome and the 5'→3' exoRNase Xrn1, respectively [10]. The RNA exosome is a conserved multiprotein 3'→5' exoRNase complex active in the synthesis, degradation and surveillance of most classes of cellular RNAs, as well as some viral RNAs [12] (Box 2).

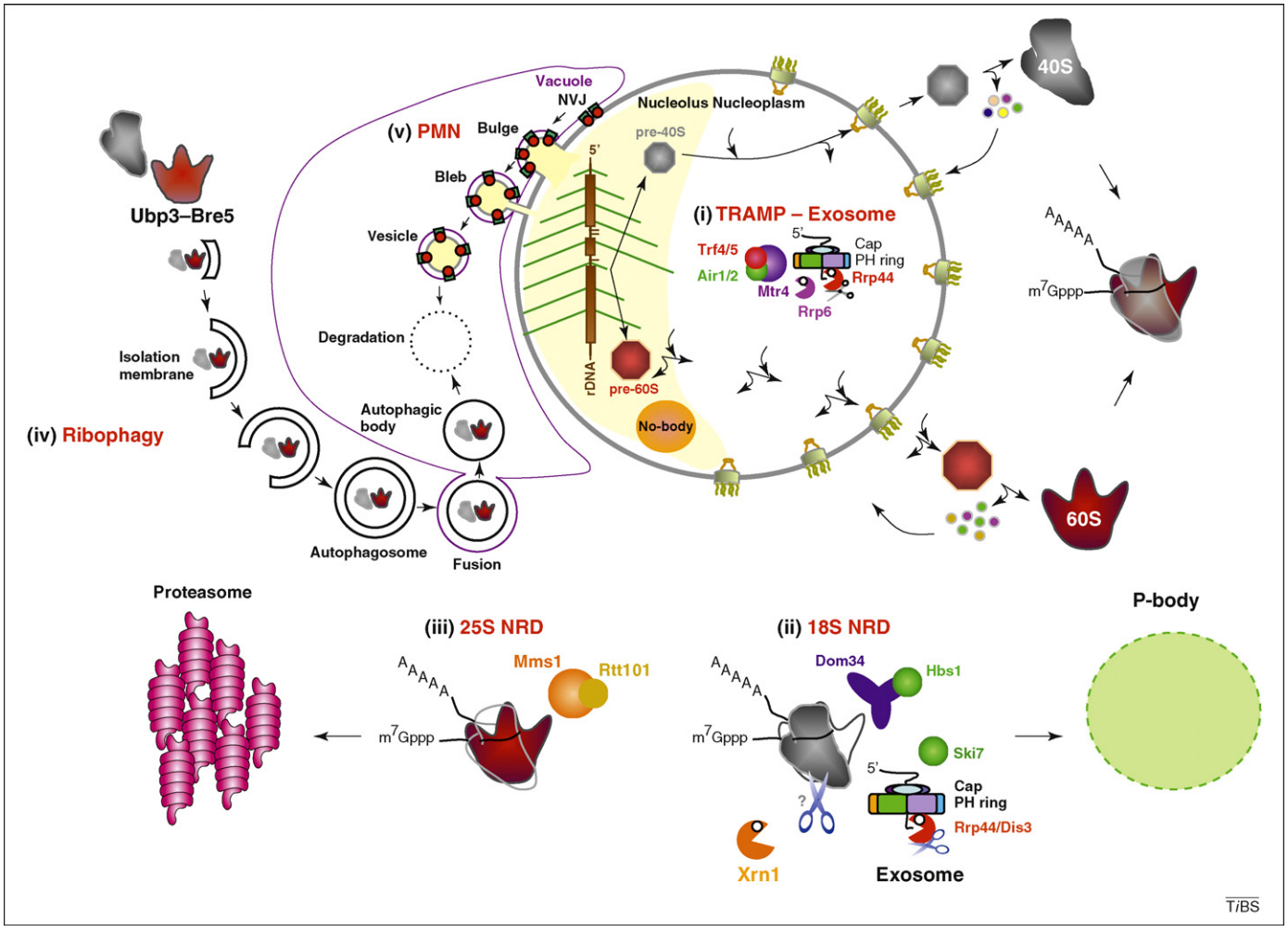


**Figure 2**. The major pathways of eukaryotic ribosomal RNA decay. The five pathways of rRNA decay described to date. (i) Nucleolar and nuclear pre-40S and pre-60S ribosomes are monitored actively by the 'TRAMP–exosome' pathway during which misfolded pre-ribosomes are identified by TRAMP binding followed by polyadenylation of the 3'-ends of defective precursor rRNAs in a step that stimulates both the recruitment and the decay activity of the exosome. It is not yet known how TRAMP detects defective ribosomes. Polyadenylation occurs both at normal and cryptic pre-rRNA processing sites. Alterations in RNA Pol I activity, as well as in pre-rRNA processing kinetics, can activate cryptic cleavage sites. Cytoplasmic mature subunits carrying *cis* mutations in functionally relevant ribosomal sites (and perhaps late cytoplasmic precursors, Box 4) are monitored by NRD, a process that involves different mechanisms for the small and large subunits. (ii) During 18S NRD, small subunits deficient in normal progression along the mRNA ('stalling subunits') are identified by the translation termination factor-related proteins Dom34 and Hbs1. They are probably cleaved endonucleolytically by an unknown activity (by analogy to mRNA NGD), the released products are digested by Xrn1 and the RNA exosome, assisted by its cofactor Ski7 (Box 2). (iii) During 25S NRD, defective 60S subunits are targeted for proteasomal degradation by Rtt101–Mms1-mediated ubiquitylation of unidentified, associated ribosomal component(s). During conditions of nutrient deficiency and stress, excess ribosomes and pre-ribosomes are turned over by ribophagy and PMN, respectively. (iv) Ribophagy is a specific form of macroautophagy that involves the engulfment and targeting of bulk cytosolic fractions to the vacuole by the *de novo* formation of an 'isolation membrane.' This membrane extends into an autophagosomal structure that fuses to the vacuolar membrane, and triggers the release of an autophagic body and its subsequent degradation by resident vacuolar hydrolases. For 60S subunits, ribophagy involves the specific deubiquitylation of unidentified ribosomal components by the Ubp3–Bre5 complex. The effector(s) for 40S ribophagy remain unknown. (v) In PMN, a specific form of microautophagy, a portion of the nuclear envelope is 'pinched off' by the vacuole, generating a specialized organelle termed a NVJ that comprises protein 'velcro-like' patches formed by the interaction between Nvj1 (small red circles) and Vac8 (small green squares). The NVJ matures sequentially into a bulge, a tethered bleb, and finally a vesicle that is degraded by resident hydrolases. The late steps (vesicle release) of PMN require several *ATG* genes also involved in macroautophagy [62]. Ribosome surveillance occurs at every step along the assembly pathway, starting in the nucleolus, where a specialized locale coined the 'No-body' concentrates polyadenylated pre-rRNA species and exosomal components, continuing in the nucleoplasm and ending in the cytoplasm. Cytoplasmic degradation involves several subcellular organelles, including P-bodies (where 18S NRD substrates, as well as mRNA NGD substrates, are localized), the autophagosome, the proteasome and a cytosolic perinuclear compartment enriched for 25S NRD substrates. Key *trans*-acting factors involved in each pathway are represented.

The possibility that stalling induced by defective ribosomes might elicit a similar pathway was quite appealing and, indeed, 18S NRD substrates are stabilized 2-fold in the absence of the translation termination factor-like proteins Dom34 and Hbs1 that are key components of NGD [13]. Strikingly, this is not the case for 25S NRD substrates, thus indicating that mechanistically, NRD comprises at least two distinct pathways.

During 18S NRD, Dom34 and Hbs1 act together in the same pathway (their simultaneous inactivation is not synergistic), and, consistently, the two proteins interact functionally *in vitro* [14] and *in vivo* [15]. Small molecule inhibitors of translation (cycloheximide and hygromycin B) specifically stabilized 18S but not 25S NRD substrates [13], thus providing further evidence that 18S NRD activation requires elongating ribosomes, and that 18S and 25S NRD are mechanistically different. The cytoplasmic 5'→3' exoRNase Xrn1 and Ski7 (a cytoplasmic exosome recruitment cofactor) have both been linked to NGD, and also contribute to 18S NRD. Hbs1 and Ski7 are GTPases homologous to the translation release factor eRF3. Deleting both *HBS1* and *SKI7* enhanced the stabilization of 18S

**Box 1. When and where does NRD occur?**

NRD does not depend upon rRNA synthesis, because mutations that affect the accumulation of 18S rRNA do not impact the stability of 25S rRNA, and vice versa (both RNAs are co-expressed; Figure 1 inset). NRD is believed to occur primarily in the cytoplasm on mature ribosomal subunits, and, perhaps, on late cytoplasmic precursors (Box 4), for the following reasons. First, neither of the mutations tested in the DCS or the PTC grossly affect the accumulation of pre-rRNA precursors. Second, NRD substrates colocalize on velocity gradients with mature ribosomal subunit-sized and/or polysomal fractions. Third, *trans*-acting factors involved in NRD (Dom34, Hbs1, Xrn1 and Ski7) are all involved in cytoplasmic processes, and neither the nuclear-specific exosomal cofactor Rrp6 (Box 2) nor the nuclear 5′→3′ exoRNase Rat1 impact 18S or 25S NRD. Fourth, NRD substrates accumulate strikingly in cytoplasmic structures (P-bodies for 18S NRD and cytosolic perinuclear foci for 25S NRD). Fifth, NRD-decay occurs with much slower kinetics (with half-lives ranging between ~50-100 min [11,13]) than nuclear decay (for example, in the absence of the export factor Nmd3, nuclear 25S rRNA decays with a half-life of ~4 min [65]). Finally, 18S NRD requires ongoing translation, and implies that the 18S rRNA is made, i.e. that the last and cytoplasmic pre-rRNA cleavage has occurred.

NRD substrates, indicating that the products of these two genes probably operate in parallel pathways, possibly competing for binding an empty-A site on a ribosome stalled at a sense codon [13]. The core exosome (tested for Rrp44, also called Dis3; Box 2) contributes, but is apparently not rate-limiting, for 18S and 25S NRD [13].

18S NRD and mRNA NGD substrates both accumulate in P-bodies that are conserved RNA–protein cytoplasmic granules containing untranslated mRNAs complexed with a set of translational repressors, the mRNA decapping machinery and Xrn1 [16]. Strikingly, 25S NRD substrates do not co-localize to P-bodies. Other differences between 18S and 25S NRD include the contribution of Xrn1 and Ski7 to 18S, but not 25S, NRD [13,17].

It is quite striking that 18S rRNA NRD and mRNA NGD operate with distinctively different kinetics (half-lives of up ~100 min and ~10 min, respectively [10,13]). This difference might reflect the higher complexity and compaction of mature ribosomal ribonucleoprotein particles (rRNP) versus mRNP substrates.

### *Large ribosomal subunit NRD: a role for ubiquitylation in ribosome turnover*

Fujii *et al.* conducted a genome-wide screen on the yeast knock-out collection aimed at the identification of mutations that stabilize 25S NRD substrates. This work led to the discovery of Mms1, a component of an E3 ubiquitin ligase that had previously been characterized as being involved in DNA repair [17]. There are two main intracellular protein degradation systems in eukaryotes [18]: the ubiquitin-proteasome system (UPS) that often targets short-lived proteins and involves ubiquitylation of the substrate and its targeting to the proteasome [18], and autophagy, a process that leads to the degradation of long-lived proteins and excess or aberrant organelles.

Originally identified as a protein involved in the repair of DNA damage induced by the alkylating agent methyl methanesulfonate (MMS), Mms1 belongs to the Mms22 module that comprises Mms1, Mms22, Rtt101 and Rtt107. Rtt101, but not the other members of the complex, is also required for 25S NRD [17]. Mutations in *MMS1* and *RTT101* are not synergistic, indicating that their gene products act together in the same pathway. Consistently, Mms1 and Rtt101 interact *in vitro* and *in vivo* [19,20]. Strikingly, neither Mms1 nor Rtt101 is required for 18S NRD, lending further credence to the idea that, mechanistically, NRD comprises distinct pathways.

Fujii *et al.* further demonstrated that the level of ubiquitylation observed in large ribosomal subunit fractions increased strikingly in strains that expressed 25S NRD rRNA, and that this modification required specifically Mms1 and Rtt101. Although the identity of the ubiquitylated components remains unknown, the overexpression of a ubiquitin variant that inhibits proteasomal function demonstrated clearly the requirement of ribosomal component ubiquitylation for 25S NRD.

25S NRD substrates accumulate in the cytoplasm in a previously undescribed perinuclear compartment that, the

**Box 2. The exosome and TRAMP**

The eukaryotic RNA exosome consists of a core of nine subunits, and one or two associated RNases (Rrp44, also called Dis3, and Rrp6) [12,26]. Six subunits of the core (homologs to bacterial phosphorolytic RNase PH and PNPase, as well as the archaeal exosome) form a structural inactive 'ring'. The remaining three core subunits are S1/KH-RNA binding proteins that provide a cap that bridges the ring subunits together. Rrp44 is homologous to bacterial RNase II/R and is endowed with both 3′→5′ exoRNase and endoRNase activities. Rrp44 endoRNase activity might contribute to the progression of the exosome on its substrates by resolving complex RNP structures. The exosome functions in concert with many cofactors that provide substrate specificity and modulate its functions. In the nucleus, the exosome functions together with Rrp6 (homologous to bacterial RNase T/D), another catalytically active 3′→5′ exoRNase subunit of the complex. Rrp44 (Dis3) and Rrp6 are hydrolytic enzymes that share complementary biochemical properties: Rrp44 is a processive enzyme, whereas Rrp6 is distributive. Rrp44 favors both structured and unstructured substrates and poorly degrades poly(A) and AU-rich sequences *in vitro*, whereas Rrp6 shows a marked preference for unstructured substrates and for poly(A) and AU-rich sequences *in vitro*. Trf4 and Trf5 are characterized by distinctive subcellular distribution (Trf5 is mostly nucleolar; Trf4 is nuclear), cellular abundance (Trf4 is >3x more abundant than Trf5), and requirement for normal growth (*TRF5* deletion has no associated phenotype whereas *TRF4* loss confers a slow-growth and cryo-sensitive phenotype). These differences probably reflect the subtle substrate preferences of the two proteins. TRAMP-mediated RNA polyadenylation stimulates nuclear RNA degradation; this contrasts with the role of canonical mRNA poly(A) tails that stimulate mRNA export, stability and translation. The role of short poly(A) tails as RNA 'degradation tags' has been compared to the role of RNA oligoadenylation in bacteria and proposed to reflect its ancestral origin [25,26]. The difference in function of the two types of poly(A) tails could reflect the difference in processivity of the two RNA polymerases: Pap1 is highly processive, adding long tails of ~250 adenines residues in human cells and ~60-90 in yeast, thus probably ensuring that no free 3′-end is available until a long tail has been generated and covered by poly(A) binding proteins, such as Pab1. By comparison, Trf4 and Trf5 display low processivity and are distributive (adding short tails of ~15-30 nucleotides) indicating that the 3′-ends of tails added by TRAMP could be available frequently as exosome substrates.

authors suggest, could be related to the recently described juxtanuclear quality control domain (JUNQ) that is enriched for both misfolded proteins and proteasomes [21]. Strikingly, localization of 25S NRD substrates to perinuclear foci relies upon Mms1, indicating that it might be the site of active degradation.

## Mutations in *trans*: the TRAMP-exosome pathway and nucleolar surveillance

It has long been appreciated that mutations in ribosome synthesis factors that specifically inhibit nuclear pre-rRNA processing reactions do not systematically lead to the expected accumulation of pre-rRNA precursors. This finding indicates that aberrant nucleolar and nuclear pre-rRNAs are degraded actively by surveillance mechanisms. This nuclear surveillance involves, at least in part, the addition of unstructured oligoadenylate tails at the 3'-end of flawed pre-rRNAs by a poly(A) polymerase activity residing in TRAMP complexes, followed by their degradation by the RNA exosome [22]. TRAMP complexes consist of a poly(A) polymerase (either of the paralogous proteins Trf4 or Trf5 in TRAMP4 and TRAMP5 complexes, respectively), a zinc-knuckle-containing and putative RNA-binding protein (either Air1 or Air2) and the DEVH helicase Mtr4 (also called Dob1) [23–25] (Box 2). In this pathway, the addition of short poly(A) tails and/or the actual binding of the TRAMP complex to the RNA commit aberrant molecules to degradation by both discriminating them from 'normal' RNA and by stimulating exosomal activity [26].

The early steps of nucleolar pre-rRNA processing (cleavage at sites $A_0$, $A_1$ and $A_2$ in yeast, Figure 3), that separate the primary RNA polymerase I (Pol I) transcript into precursors destined to the small and large subunits, respectively, require a large RNP known as the SSU-processome that consists of the box C+D snoRNA U3 and ~40 associated proteins referred to as UTP (for U Three-associated Proteins) [27,28]. The SSU-processome comprises autonomous protein building blocks that are loaded onto nascent pre-rRNAs and assemble into catalytically active pre-rRNA processing complexes in a stepwise and highly hierarchical process following alternative pathways (Figure 4). At least some of these autonomous modules are evolutionarily conserved [29,30]. It is currently understood that failure to assemble the SSU-processome with proper kinetics activates nucleolar surveillance, as depletion of individual SSU-processome components leads to early nucleolar pre-rRNA processing inhibition (cleavage at sites $A_0$-$A_2$), the concomitant activation of a cryptic cleavage at site $A_3$ (further downstream in internal transcribed spacer 1; ITS1, Figure 3) by the endoRNase MRP, and the synthesis of the aberrant 23S RNA. This 23S RNA is polyadenylated, mostly by TRAMP5, and targeted for rapid degradation by the exosome (Figure 3) [31,32]. As TRAMP is a distributive enzyme that adds short poly(A) tails, it is thought that flawed RNAs undergo multiple rounds of TRAMP-mediated polyadenylation, followed by exosomal digestion to achieve greater degradation efficacy. Trf5 co-localizes with the SSU-processome at the rDNA in living yeast cells, indicating that this surveillance starts co-transcriptionally on nascent pre-ribosomes [31]. Interestingly, low levels of normal pre-rRNA intermediates are polyadenylated and can be detected in strains defective for the exosome component Rrp6 (Box 2) [33,34]. This indicates that every available 3'-end is probably polyadenylated by TRAMP, and that physiological pre-rRNA processing sites are also used as entry points for rRNA surveillance. Moreover, stabilized pre-rRNA fragments that terminate at multiple positions within the coding sequence of the 18S rRNA can be detected in surveillance-defective strains [31,32]. Thus, several cryptic pre-rRNA processing sites appear to be activated under conditions of defective ribosome assembly. The accumulation of such fragments is also observed in RNA polymerase I mutants defective for transcription elongation, pointing to a possible regulatory role for RNA polymerase elongation rates and pausing in ribosome assembly [35].

The assembly of the large ribosomal subunit includes many more nuclear steps than that of the 40S subunit, and involves many more *trans*-acting factors (Figure 1). Similar to UTP sub-complexes (Figure 4), several autonomous structural neighborhoods have been characterized within pre-60S ribosomes, and emerging evidence indicates that at least some of them correspond to functional modules (reviewed in [2]). Precursor rRNAs for large ribosomal subunits are also monitored by the TRAMP-exosome surveillance pathway; however, for the large ribosomal subunit, it is mostly TRAMP4 that tags the RNAs. In some instances, surveillance has been suggested to occur in a specialized nucleolar domain termed the 'Nobody' that is enriched in both TRAMP and exosomal components [36].

Ribosome surveillance exerted by TRAMP–exosome complexes initiates 3' to 5' RNA degradation at multiple entry points; however, pre-rRNAs are long and highly structured molecules, and it is expected that 5'→3' nuclear and nucleolar exoRNAse activities also contribute to pre-rRNA decay.

## Bulk ribosome and pre-ribosome decay by ribophagy and PMN

Under limiting growth conditions, ribosome synthesis is shut down at multiple levels. *De novo* synthesis is blocked and nascent pre-ribosomes and mature ribosomes are targeted to bulk degradative pathways. Pre-ribosomes and mature ribosomes are turned over to recycle essential cellular building blocks to cope with stress, and adapt to a novel environment. It is quite striking that ubiquitin, which plays an important role in 25S NRD, is also involved in bulk ribosome degradation.

### *Ribophagy: a role for deubiquitylation in specific 60S macroautophagy*

Under starvation conditions, bulk portions of the cytosol, including protein aggregates and entire organelles, such as mitochondria and ribosomes, are recycled via two forms of autophagy (micro- and macroautophagy). Macroautophagy entails the formation of a double-membrane-bound vesicle in the cytoplasm: the 'autophagosome' that sequesters cytoplasmic material and delivers it for breakdown and eventual recycling to the lysosome, or the vacuole in yeast

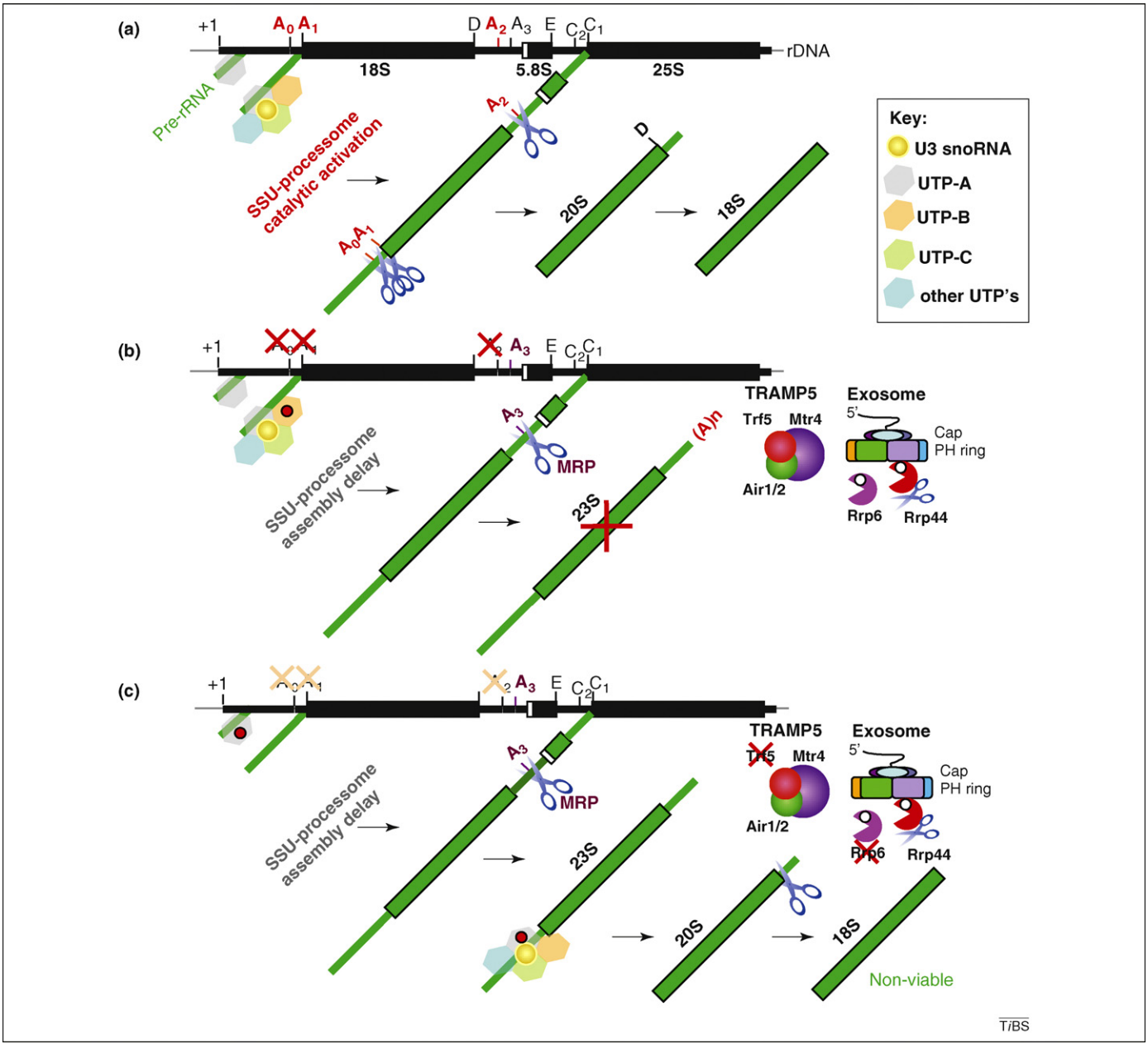


**Figure 3**. Nucleolar surveillance, the TRAMP-exosome pathway. Failure to assemble pre-ribosomes with proper kinetics leads to activation of nucleolar surveillance and subsequent rRNA degradation. **(a)** In wild-type yeast cells, an early step in SSU-processome assembly is the binding of the UTP-A subcomplex (gray) to nascent pre-rRNA. This is followed by the recruitment of other subcomplexes (UTP-B: orange; and UTP-C: green), additional individual ribosome synthesis factors (blue), the box C+D snoRNA U3 (gold) and other snoRNAs, leading to SSU-processome catalytic activation in RNA cleavage. The nascent pre-rRNA is cleaved at sites $A_0$, $A_1$ and $A_2$ (red) leading to the production of the 20S pre-rRNA (the immediate precursor to the 18S rRNA) that is exported to the cytoplasm, dimethylated and cleaved at site D to produce mature 18S rRNA. For simplicity, only co-transcriptional cleavage in ITS1, that occurs in ~50-70% of the cases in fast-growing yeast cells, is illustrated here. Co-transcriptional cleavage in ITS1 at site $A_2$ occurs when RNA polymerase I has reached the 5′-end of the 25S gene [57]. At this position, pre-40S compaction has brought the SSU-processome at its closest position relative to the rDNA; in living yeast cells, this correlates with a strong RNA-dependent ChIP interaction of SSU-processome components [31]. In the remaining cases, cleavage in ITS1 is delayed until transcription has reached the 3′-end of the gene, and a full-length pre-rRNA precursor (35S) has been generated (not represented). **(b)** Mutations in any components of the SSU-processome (illustrated here as a red dot on UTP-B) inhibits SSU-processome assembly and/or function, thereby resulting in inhibition of cleavage events at sites $A_0$-$A_2$, and activation of a downstream cleavage in ITS1 at site $A_3$ (purple) by endoRNase MRP. Cleavage of the nascent transcript at site $A_3$ generates an aberrant 23S RNA that is rapidly polyadenylated by TRAMP5, followed by its digestion by the nuclear exosome; no mature 18S rRNA is made. Upon surveillance inactivation (deletion of Trf5, Rrp6 or both), the 23S RNA is stabilized and not further matured (not shown). **(c)** The UTP-A subcomplex of the SSU-processome is dispensable for cleavage at sites $A_0$-$A_2$. As described in panel B for UTP-B, a mutation in a component of UTP-A leads to alterations in pre-rRNA processing kinetics ($A_3$ occurs prior to $A_0$-$A_2$). However, for UTP-A and by ensuring that the newly generated 23S RNA is stabilized by surveillance inactivation, this RNA is further matured into 20S pre-rRNA and 18S rRNA. Despite this pre-rRNA processing restoration, cells are unable to survive, thus providing a strong indication that surveillance is essential [31].

and plants (Figure 2). Autophagy can degrade nearly any biomolecule, as the lysosome/vacuole contains non-specific hydrolases, including proteases, nucleases, lipases, and glycosylases. By contrast, microautophagy entails the direct uptake of cytosolic components through invaginations of the lysosome's limiting membrane. Both macro- and microautophagy contribute to cell survival during starvation, and the two processes involve common and specific sets of *trans*-acting factors [18,37]. Moreover, although both micro- and macroautophagy are primarily bulk degradative processes, selective forms of both pathways have been uncovered.

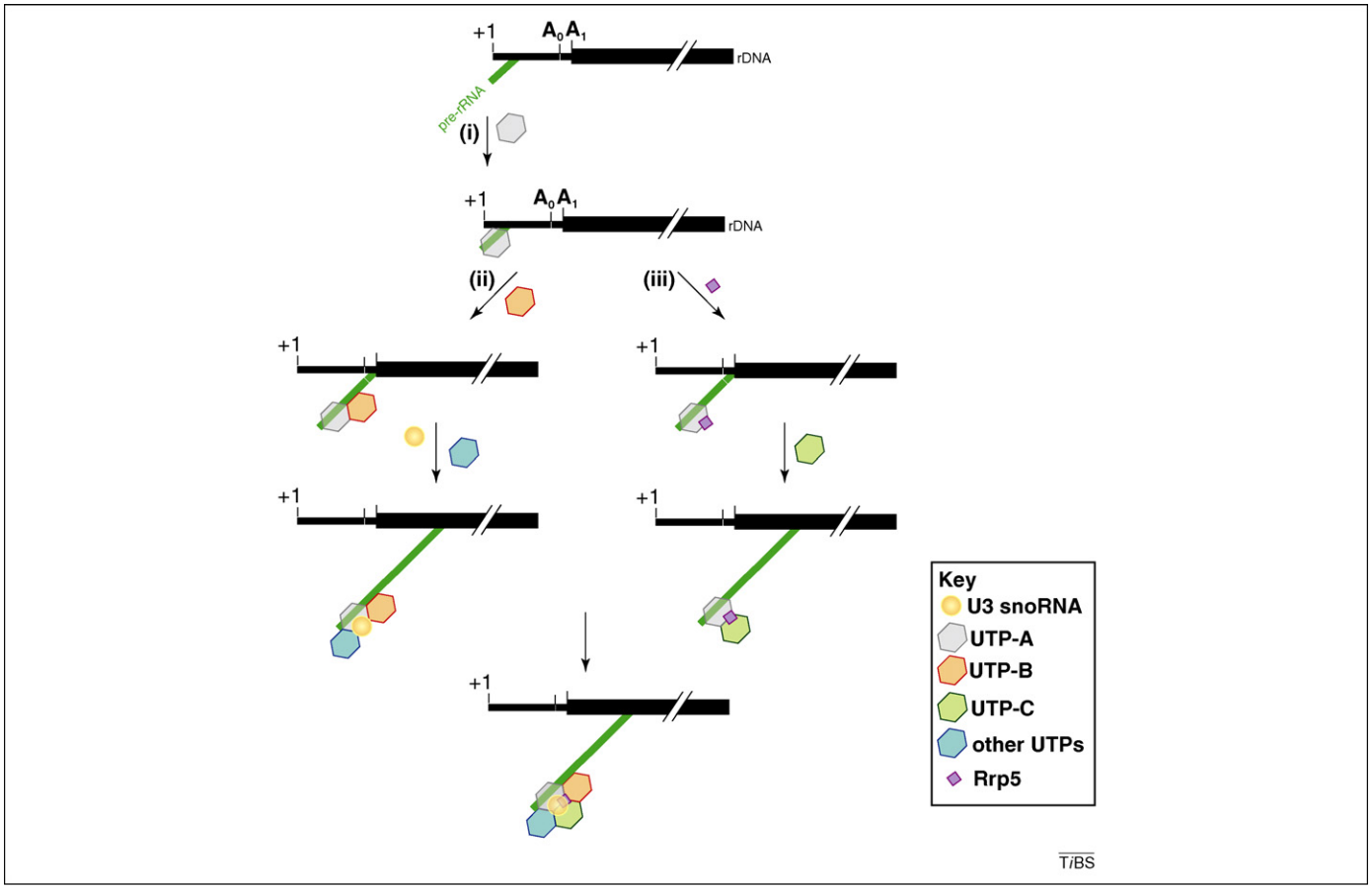


**Figure 4**. Small Subunit (SSU)-processome assembly involves concurrent parallel pathways. The SSU-processome comprises individual autonomous preassembled building blocks that are sequentially loaded onto nascent pre-rRNA following multiple concurrent pathways, rather than via a single route [28,63]. This strategy, which minimizes complexity, is likely to be the rule rather than the exception in ribosome assembly, and probably prevails in LSU-processome (pre-60S) formation. The currently described SSU-processome subcomplexes include the UTP-A (gray), UTP-B (orange) and UTP-C (green), each comprising ~6-7 proteins, as well as additional ribosome synthesis factors (blue), including the Mpp10–Imp3–Imp4 and the Rcl1–Bms1 subcomplexes (reviewed in [2]). Components of UTP-A (also referred to as t-UTPs) influence efficient Pol I activity in yeast and humans but are not strictly required for pre-rRNA synthesis in yeast [28,30–32]. UTP-C (also known as the CURI complex) integrates pre-rRNA processing and ribosomal protein production [64]. Specific functions have not yet been assigned to UTP-B. (i) Binding of UTP-A to nascent transcripts is an early step that initiates assembly. (ii) In one of two currently described alternative pathways, binding of UTP-A is followed by the sequential recruitment of UTP-B, U3 snoRNA and additional ribosome synthesis factors. (iii) In an alternative route, UTP-A binding initiates the sequential recruitment of Rrp5 and UTP-C.

In yeast, prolonged nitrogen starvation leads to preferential targeting of both small and large ribosomal subunits to the vacuole by selective macroautophagy [38]. This process, specific to ribosomal subunits, is termed 'ribophagy'. For the large subunits, the increased turnover kinetics rely upon the Ubp3 ubiquitin protease and its activator Bre5. Mutations in *UBE3* or *BRE5* do not affect detectably the increased turnover rates of small subunit reporter constructs, indicating that, much as for 18S and 25S NRD, small and large ribosomal subunit ribophagy relies on distinctly different pathways; notably, however, the effector(s) for small ribosomal subunit ribophagy remain unknown. Ubp3 deubiquitylation activity is required for 60S ribophagy, and the ubiquitylation level of several, still unidentified, ribosome-associated proteins is increased in cells lacking Ubp3 [38]. It is thought that deubiquitylation of Ubp3–Bre5 target(s) assists in the packaging of ribosomes in the autophagosome, or enables its maturation and/or fusion to the vacuole [38]. Recent data indicate that the ubiquitin ligase Rsp5 might help to regulate 60S ribophagy as cells harboring mutant versions of both Rsp5 and Ubp3 show enhanced synthetic sickness and reduced ribosome turnover [39].

### *PMN: the involvement of a specialized nucleo-vacuolar junction*

Piecemeal microautophagy of the nucleus (PMN) is a selective autophagic pathway in which the vacuole 'pinches off' and degrades non-essential portions of the nucleus. This process involves the formation of a specific inter-organelle contact dubbed the nucleo-vacuole junction (NVJ) which acts as true 'Velcro-like' patches formed by interactions between the vacuolar membrane protein Vac8 and the outer nuclear membrane protein Nvj1 [40]. PMN is a constitutive process that is induced to high levels upon nutrient starvation. It bears relevance to ribosome degradation, because there are cases where the site of vacuole docking to the nucleus map to a position juxtaposing the nucleolus [41] (Figure 2). In these captures, granular material of nucleolar origin transfers directly from the nucleolus to the vacuolar lumen; this event is indicative of bulk destruction of pre-ribosomes.

## Ribosome surveillance in human health

RNA damage occurs under normal cell growth as well as during stress and in disease situations. Chemical modifications to nucleobases, as well as RNA–RNA and RNA–

protein crosslinks, are introduced into RNA and RNPs as a result of exposure to ultraviolet light, oxidation, chlorination, nitration and alkylation [42]. These alterations all constitute potential physiological triggers to ribosome degradation pathways. Some cases of ribosomal RNA damage induced by UV irradiation and oxidation have been documented, notably in conjunction with human neurodegenerative diseases such as Alzheimer's and Parkinson's, as well as atherosclerotic plaques [8] (Box 3). It has been suggested that RNA oxidation could be involved in disease progression and that RNA susceptibility to oxidative damage is probably influenced by various factors, including the degree of association with proteins (protection) or iron (sensitization). Mature 5.8S and 25S rRNA are fragmented heavily in yeast cells exposed to elevated levels of reactive oxygen species (ROS) generated by oxidative stress (including exposure to hydrogen peroxide and menadione), chronological aging, and other apoptotic cues [43–45]. Yeast cells treated with the anti-metabolite and chemotherapeutic agent 5-fluorouracil (5-FU) accumulate polyadenylated pre-rRNAs, and this accumulation is exacerbated in exosome mutants, that are hypersensitive to the drug. These findings suggest that the degradation of 5-FU containing RNAs occurs via nucleolar surveillance [34]. Nucleolar dysfunction has been associated with numerous human diseases, including cancer [46]. Various 'nucleolar stresses', such as drug-mediated interference of rRNA synthesis, pre-rRNA processing (e.g. actinomycin D, 5-FU), or inhibition of ribosome synthesis factor function, lead to nucleolar disruption, p53 stabilization and, ultimately, cell cycle progression defects and/or apoptosis [47–50]. Of interest to the general public is the observation of an increased abundance of polyadenylated rRNA fragments in the gut of western honey bees originating from populations infected with colony collapse disorder (CCD) [51]. The insect gut serves as a primary interface with the environment, as it is the principal site of pesticide detoxification and an integral component in the immune defense against pathogens. CCD has been linked to picorna-like viral infections (known to 'hijack' cellular ribosomes) and the extensive use of pesticides; both stimuli could potentially trigger a ribosome degradation response.

**Box 3. RNA damage in human health**

Environmentally relevant doses of UVA and UVB are sufficient to induce lesions in nucleic acids. UV is a source of photoproducts, crosslinking and oxidative damage. UV crosslinking is dependent on the geometry (distance, angle) and photoreactivity of the nucleotide and amino acids, suggesting that certain cellular RNAs are more likely to be protected from alterations by UV crosslinking than others (e.g. double stranded RNA region). UV-mediated rRNA damage has been reported in cultured mammalian cells, consistent with the formation of photoproducts [66]. The lesions clustered around the active sites of the large ribosomal subunit (domains V and VI near the PTC), and were correlated with reduced translation and the activation of a kinase-mediated stress response. Under oxidizing conditions, ribosomes undergo two types of damage: formation of 8-oxo-7,8-dihydroguanosine (8-oxoG) and cross-linking of rRNA and ribosomal proteins (shown in yeast cells treated with $H_2O_2$ [67]). Most oxidative damage results from the action of ROS that arise from metabolic reactions. Increased levels of oxidatively damaged RNAs, as revealed by the presence of 8-oxoG, have been reported in neurons from patients with human neurodegenerative diseases, including Alzheimer's (AD; where it has been detected in nucleoli) and Parkinson's, as well as dementia, and in patients with atherosclerosis [68–72]. Studies in neurons from patients with AD revealed higher levels of oxidized rRNA [68,69], and ribosomes purified from AD patients showed elevated levels of associated redox-active iron [73]. Advanced human atherosclerotic plaques have been linked to severe loss of rRNA integrity and accumulation of elevated levels of 8-oxoG-containing RNAs, a probable consequence of oxidative stress, ROS production and intraplaque hemorrhage that is accompanied by iron deposition [70,71].

## Concluding remarks

Ribosome synthesis is a major cellular metabolic activity that can enforce a rapid 'energy drain' in the absence of tight regulation [52,53]. Control is exerted at the level of the synthesis and assembly of the pieces, as well as in the function of the final product. Ribosome synthesis has evolved to be fully integrated with complex nutrient-sensing cascades, such that defective precursor ribosomes, as well as damaged and excess mature ribosomes, are targeted specifically for rapid breakdown and recycling. Several surveillance pathways that either select defective or excess pre-ribosomes or mature particles have been identified; they display strong specificity towards small and large ribosomal subunits, involve specialized subcellular locales, and, altogether, are active at each step of a ribosome's life. The question is now to know whether and how these pathways interconnect (Box 4).

**Box 4. Outstanding questions**

1. How tightly is pre-RNA synthesis coupled to pre-rRNA processing and pre-rRNA surveillance? Do polymerase elongation rates and/or pausing influence nucleolar surveillance?
2. How does TRAMP discriminate aberrant from normal RNAs? Are other exosome cofactors involved in nuclear ribosome surveillance?
3. Do any nucle(ol)ar mechanisms of pre-ribosome surveillance initiate from the 5′-end of the pre-RNAs? There are suitable exoRNase activities in the nucle(ol)us.
4. To what extent is (pre-)rRNA modification monitored by surveillance?
5. Does PMN contribute quantitatively to nucleolar and nuclear ribosome surveillance?
6. How does ubiquitin serve both a stabilizing and destabilizing function in ribosome turnover? Which ribosome-associated components are ubiquitylated in the 25S NRD and ribophagy pathways? A ribosomal protein from each subunit (Rps31 and Rpl40a/b) is produced as a fusion with ubiquitin [74] and Rpl28 ubiquitylation is required for optimal ribosome function [75]. What is the involvement of ubiquitin in nuclear pre-ribosome turnover? What are the effector(s) of 40S ribophagy?
7. How are defective large ribosomal subunits functionally monitored by 25S NRD?
8. Which endoRNase(s) initiate the cleavage events in mRNA NGD and in 18S NRD? What is the fate of associated mRNAs in NRD and, reciprocally, that of associated ribosomes in NGD? Late cytoplasmic pre-40S ribosomes might enter translation, and therefore be actively monitored by NRD [76–78].
9. Do ribosome repair mechanisms exist? *A priori*, this seems quite unlikely, owing to the compaction of mature subunits. A demethylase is, however, known to repair alkylated RNAs in bacteria and eukaryotes [79,80], suggesting that some rRNA repair could occur during assembly. Evidence for ribosome rejuvenation by ribosomal protein replacement is suggested by *in vitro* translational reactivation of chemically-damaged bacterial ribosomes [81].
10. How does ribosome surveillance failure contribute to human disease etiology and progression?

*How are defective (pre-)ribosomes recognized?*

The exact molecular basis for recognition is not yet known; however, the huge diversity of potential ribosome insults makes it extremely unlikely that each one is monitored by individual surveillance mechanisms. It is thought that each reaction in the ribosome assembly pathway is provided with a time frame for completion and that surveillance is triggered by inefficient kinetics. Work with mature ribosomes indicates that, in this case, it is the overall dynamics of the translation process that is monitored. Thus far, this property is best demonstrated for small subunits where stalling ribosomes are identified by 'translation-factor like' proteins that are also active in mRNA NGD. For 25S NRD, the kinetics of interaction with translation factors might be the key parameter that is monitored. It can thus be anticipated that rRNA sequences that have been the most highly conserved throughout evolution are probably prime targets for monitoring by surveillance. Likewise, pre-rRNA modifications that cluster around functional ribosomal sites and contribute to translation efficacy, are also expected to be monitored. The specialized functions of the two ribosomal subunits in translation is reflected in their overall 3-D structure and shape, with a flexible 'Y-shape' for the small subunit and a monolithic block for the large subunit that might require additional 'effort' (i.e., ubiquitylation, and possible dissociation, of some of the protein components) to expose naked RNAs to salient cellular endo- and exoRNase activities. The selective cellular compartmentalization of aberrant pre-ribosomes (No-body), and mature ribosomes (P-bodies), in specific organelles might also contribute to the processes of discrimination and degradation. Functional compartmentalization could be particularly relevant to 25S NRD substrates that accumulate in cytosolic perinuclear foci and primarily colocalize with 60S subunits in velocity gradients. These findings indicate that defective large subunits might be segregated away from the translationally active pool of ribosomes. By contrast, 18S NRD RNAs co-migrate with monosomes, 80S particles and polysomes; these findings are consistent with their monitoring during translation [11,13,17].

*How are defective ribosomes tagged for degradation?*

Thus far, two mechanisms have been identified that involve either the addition of short poly(A) tails at the 3'-ends of the RNA in the nucleus or the ubiquitylation/deubiquitylation of unknown associated proteins in the cytoplasm. Strikingly, ubiquitin plays both a stabilizing and destabilizing function; clearly, its role in ribosome turnover is only starting to unfold. That ubiquitylation also plays a role in nuclear pre-ribosome degradation is suggested by the severe pre-rRNA processing inhibition, and production of aberrant and truncated rRNA fragments that are observed in yeast upon conditional inactivation of the ubiquitin ligase Rsp5 [54]. Mammalian proteasome inhibition also leads to pre-rRNA processing defects as well as striking nuclear accumulation of ribosomal proteins; such findings are consistent with the existence of further quality control systems [55,56].

*Do we need ribosome surveillance?*

The physiological relevance of ribosome surveillance is illustrated by emerging connections to human diseases (Box 3), and observations that cells harboring an inactivated nucleolar pathway, 25S NRD or ribophagy, accumulate defective or excess ribosomes, and are either unable to survive or show a much decreased lifespan [17,31,32,38].

Proteomic analyses of purified nucleoli and pre-ribosomes have left us with a myriad of *trans*-acting factors to which function(s) and structure must be assigned. The field is entering a new era in which deciphering the post-translational modification of synthesis factors and ribosomal proteins will dictate future progress in understanding the regulated assembly and turnover of ribosomes.

**Acknowledgements**

Makoto Kitabatake (Kyoto University), Natalia Shcherbik and Dimitri Pestov (University of Medicine and Dentistry of New Jersey), Martin Kos and David Tollervey (University of Edinburgh) are acknowledged for communicating unpublished observations prior to publication. I apologized to my friends and colleagues whose work could not be listed here owing to space limitation. The laboratory is funded by the FRS-FNRS, Région Wallonne (Cibles) and Communauté Française de Belgique (ARC).

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tibs.2009.12.006.

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