Trends in Biochemical Sciences

CellPress

Review

'View From A Bridge': A New Perspective on Eukaryotic rRNA Base Modification

Sunny Sharma,[1] and Denis L.J. Lafontaine[1,2,@,*]

Eukaryotic rRNA are modified frequently, although the diversity of modifications is low: in yeast rRNA, there are only 12 different types out of a possible natural repertoire exceeding 112. All nine rRNA base methyltransferases (MTases) and one acetyltransferase have recently been identified in budding yeast, and several instances of crosstalk between rRNA, tRNA, and mRNA modifications are emerging. Although the machinery has largely been identified, the functions of most rRNA modifications remain to be established. Remarkably, a eukaryote-specific bridge, comprising a single ribosomal protein (RP) from the large subunit (LSU), contacts four rRNA base modifications across the ribosomal subunit interface, potentially probing for their presence. We hypothesize in this article that long-range allosteric communication involving rRNA modifications is taking place between the two subunits during translation or, perhaps, the late stages of ribosome assembly.

Trends

All nine rRNA base methyltransferases have been identified in budding yeast.

An 18S rRNA acetyltransferase modifies tRNAs with a specific adaptor.

Four methyltransferases are stabilized by a common coactivator.

A eukaryote-specific bridge monitors base modifications across the subunit interface.

rRNAs Are Universally Modified

RNA modifications are present in all three kingdoms of life, in all classes of cellular RNA, and on some viral RNAs [1,2]. To date, more than 112 types of RNA modification have been identified. RNA modifications expand the naturally limited topological properties of RNAs and fine-tune their biological functions, stabilizing their structure and optimizing their interaction with ligands.

Ribosomes are cellular nanomachines essential for converting the information encoded in mRNAs to proteins in all living cells [3,4]. In human cells, each ribosome comprises four rRNAs and 80 RPs. Ribosome biogenesis involves the synthesis of ribosomal constituents and hundreds of *trans*-acting factors, their modification, assembly, and transport [5–7]. Ribosome biogenesis is initiated in the nucleolus, where precursor (pre-)rRNAs are synthesized and initial maturation occurs; it then progresses in the nucleoplasm and is finalized in the cytoplasm. Specific ribosomal structures, such as the beak and phospho-stalk, which protrude from the small subunit (SSU) and LSU, respectively, are formed only once subunits have reached the cytoplasm [8,9]. If they were formed earlier they would hinder the passage of the subunits through the nuclear pores. In the cytoplasm, the functionality of the subunits is proof-tested, and several assembly factors that mask important functional sites are displaced, making the ribosome competent for translation [10,11].

rRNA modifications are not randomly distributed on the ribosomal subunits. They are introduced on specific, highly conserved residues (which implies precise selection), cluster at functionally important areas of the ribosome, and are synthesized at specific stages throughout ribosome biogenesis. For specific modifications, there is a delay between the moment a modification enzyme binds to precursor ribosomes and the moment the modification is made. The early

[1]RNA Molecular Biology, FRS/FNRS, Université Libre de Bruxelles, B-6041 Charleroi-Gosselies, Belgium
[2]Center for Microscopy and Molecular Imaging, BioPark campus, B-6041 Charleroi-Gosselies, Belgium

*Correspondence: denis.lafontaine@ulb.ac.be (Denis L.J. Lafontaine).
@Twitter: @LafontaineLab

 http://dx.doi.org/10.1016/j.tibs.2015.07.008

binding of a modification enzyme to pre-rRNAs may serve as a quality-control step because it is essential for the progression of subunit maturation and will be reflected in faithfully modified mature subunits [12].

In budding yeast, the complete set of nine rRNA base MTases has been identified (Tables 1 and 2). Six of these proteins have specificity towards the LSU 25S rRNA, and have only been identified in the past 2 years [13–17]. An acetyltransferase (Kre33) with specificity towards both 18S rRNA and tRNAs has also been described [18,19], and important insights have been gained from recent structural work on previously identified components [20]. These developments are the subject of this review. We primarily focus on budding yeast, but provide parallel references to human cells where relevant.

The Repertoire of rRNA Modifications Found on Eukaryotic Ribosomes

During ribosome biogenesis, a significant fraction of rRNA nucleotides (approximately 2%, corresponding to 112 positions on yeast ribosomes and 201 on human ribosomes) is specifically modified, either by an enzyme guided by an antisense small nucleolar (snoRNA), or by a conventional protein enzyme. Despite this large number of modified nucleotides, the diversity of the rRNA modification repertoire remains limited to only 12 distinct types in budding yeast (Figure 1). By comparison, 25 different types of modification have been inventoried in yeast tRNAs [21].

Methylated sugars and pseudouridines constitute the majority of rRNA modifications. Methylated sugars are formed by the addition of one methyl group at the 2′-*O* position of the ribose on the nucleoside (Figure 1A). All four ribonucleosides are susceptible to 2′-*O*-methylation. Pseudouridine synthesis occurs through uridine isomerization, involving a 180° rotation of the pyrimidine ring about the N_3–C_6 axis (Figure 1C) [22]. 2′-*O*-methyls and Ψs are synthesized by box C/D and box H/ACA (snoRNPs, respectively [23]). These act as antisense guides for the associated MTase Fibrillarin (Nop1 in yeast) or pseudouridine synthetase Dyskerin (Cbf5 in yeast), which they present to the substrate residue. In budding yeast rRNAs, according to a revised count, there are 55 methylated sugars and 45 Ψs (see Table S1 in the supplementary material online); in human rRNAs, there are an estimated 94 2′-*O*-methyls and 95 Ψs.

In addition to uridine isomerization, a few rRNA bases are also modified. In budding yeast, there are six modified bases on the SSU and six on the LSU (Figure 2). These modifications occur through the addition of one, or sometimes two, methyl groups onto specific atoms, including positions 1, 6, and 7 on the purine rings (Figure 1B) and positions 1, 3, and 5 on the pyrimidine rings (Figure 1C). The pyrimidine ring can also be aminocarboxypropylated at position 3 and acetylated at position 4. Specifically, the SSU of budding yeast carries one hypermodified Ψ residue ($m^1acp^3\Psi_{1191}$), one N^7-methylated guanosine (m^7G_{1575}), two acetylated cytosines (ac^4C_{1280} and ac^4C_{1773}), and two N^6,N^6 dimethyladenosines ($m^6_2A_{1781}m^6_2A_{1782}$) (Figure 2, Table 1). The LSU bears two N^1-adenosines (m^1A_{645} and m^1A_{2142}), two C^5-cytosines (m^5C_{2278} and m^5C_{2870}), and two N^3-uridines (m^3U_{2634} and m^3U_{2843}) (Figure 2, Table 2). While the SSU rRNA base modifications are highly conserved in human ribosomes, only half of the LSU base modifications found in budding yeast have been maintained in human (Tables 1 and 2). However, there are indications that human rRNAs contain additional types of base modification that are not present in budding yeast [24].

The Location of rRNA Modifications on Eukaryotic Ribosomes

rRNA modifications are not randomly distributed on the ribosome. They are present at the inner cores of the subunits and at the interface between the SSU and LSU (Figure 2), but not at the solvent-exposed subunit periphery. They occur at highly conserved positions corresponding to important functional centers (Figure 2).

Glossary

A_3 cluster proteins: a group of ribosome-assembly factors involved in LSU maturation.
A-form helix: helix geometry adopted by RNA owing to a steric constraint imposed by the presence of 2′-OH. By contrast, DNA can form both the A- and B-forms, but favors the B-form under physiological conditions.
A-loop: an RNA element of the ribosome PTC that interacts with the 3′ CCA of the tRNA in the A-site.
A-site: the tRNA and mRNA binding site in the ribosome that binds the new aminoacyl tRNA (aminoacyl or A).
A-site tRNA: the aminoacyl (or A)-site tRNA presents to the ribosome the next amino acid to be incorporated, and functions as the acceptor of the growing polypeptide chain during protein elongation.
C3′-endo sugar conformation: predominant conformation of sugar puckers in RNA (and A-DNA), corresponding to the **A-form helix** conformation in a duplex. In B-DNA (the most common form), the sugar puckers adopt instead the C2′-endo conformation.
Decoding site (DCS): a region of the 40S subunit where the codon–anticodon interaction between mRNA and tRNA takes place. This region is important for translational fidelity.
E-site: the tRNA and mRNA binding site in the ribosome that holds the deacylated tRNA just before ejection from the ribosome (exit or E).
E-site tRNA: the deacylated exit (or E) site tRNA; it has been discharged and is ready to be expelled from the ribosome.
Gcn5-related N-acetyltransferases (GNATs): catalyze the transfer of an acetyl from acetyl-CoA to a primary amine on the acceptor protein.
P-loop: an RNA element of the ribosome that interacts with the 3′ CCA of the tRNA in the P-site.
P-site: the tRNA and mRNA binding site in the ribosome that binds the tRNA attached to the growing nascent peptide chain (peptidyl or P).
P-site tRNA: the peptidyl (or P) tRNA that carries the nascent polypeptide chain.
Peptidyl transferase center (PTC): the catalytic center of the ribosome. This is where peptidyl transfer

Each ribosome comprises two subunits: the SSU (40S in eukaryotes) containing one rRNA (the 18S) and 33 RPs, and the LSU (60S), comprising three rRNAs (5S, 5.8S, and 25S in yeast or 28S in humans) and 47 proteins. The mRNA is 'read' on the SSU, which bears the **decoding site** (DCS, see Glossary) and undergoes major structural rearrangements during translocation on the mRNA. The amino acids are linked together on the LSU, which carries the **peptidyl transferase center** (PTC), the translation factor binding sites, and the exit tunnel, from which the nascent protein emerges into the cell. Each translating ribosome carries three tRNAs lodged between the two subunits, the aminoacylated tRNA (**A-site tRNA**), the peptidyl tRNA (**P-site tRNA**, carrying the nascent polypeptide), and an empty tRNA (**E-site tRNA**, discharged and ready to be expulsed from the ribosome) [25] (Figure 2A). During translation elongation, an aminoacylated tRNA is selected in the **A-site** and progresses into the P-, and then the **E-site**, as the peptide bond is formed and translocation proceeds [26] (Figure 2). Concomitantly, the ribosome performs a ratchet-like movement, involving rotation of the subunits, and progresses on the mRNA [27].

On the SSU, three modified rRNA bases are present at or near the DCS. The other three line the mRNA channel or are directly adjacent to tRNA binding sites (Figure 2). $m_2^6A_{1781}$ and $m_2^6A_{1782}$ are at the top of helix 45, which stacks coaxially onto the base of helix 44 corresponding to the DCS. These two adjacent modified nucleotides are the most highly conserved rRNA modifications, present also in bacteria and Archaea [28]. One acetylated cytosine (ac^4C_{1773}) is also present in helix 45, creating a local environment that is particularly rich in modified bases. The second acetylated cytosine (ac^4C_{1280}) is on helix 34, which has been implicated in translation accuracy [29,30] and lies close to the mRNA channel (Figure 2B). $m^1acp^3\Psi_{1191}$ is at the P-site tRNA and m^7G_{1575} is at an evolutionarily conserved ridge between the **P-site** and the E-site tRNA [31] (Figure 2). When ratcheted and nonratcheted reconstruction views of the translating ribosome are compared, this ridge seems to accompany tRNAs from the P- to the E-site [31]. To us, this suggests that it may be involved in translocation.

On the LSU, three modified bases appear at or near the peptidyl transferase center (PTC) (m^1A_{645}, m^5C_{2870}, m^3U_{2634}), two at the subunit interface (m^1A_{2142}, m^5C_{2278}), and one at a more isolated position in the phospho-stalk (m^3U_{2843}, Figure 2).

Strikingly, five base modifications, two on the SSU (ac^4C and m_2^6A) and three on the LSU (m^1A, m^3U, and m^5C), are each present twice per yeast ribosome, together accounting for ten of the 12 modified bases. Given that, according to a recent conservative estimate, there may be up to 250 different modified nucleotides in nature, this suggests that the chemical nature of these five modifications, selected over billions of years of evolution, is particularly beneficial. It is likely that these modified bases optimize the basic functions of the ribosome (decoding, peptidyl transfer, and translocation), the landscape of its interaction with ligands (tRNAs, mRNAs, and translation factors), and its internal structure (modifications are present in the inner depths of the subunits and at the subunit interface). These modifications are likely to act by stabilizing otherwise unfavorable RNA folds either by influencing the local charge environment or by increasing the hydrogen-bonding capability [32] (Box 1). Although bacterial rRNAs are modified on different residues [33], their base modifications also cluster at functionally important areas of the ribosome, where they seem to have important roles in stabilizing distorted rRNA folds [34].

A Eukaryote-Specific Bridge Contacts rRNA Base Modifications on Both Sides of the Subunit Interface

Specific intersubunit bridges form upon joining of the SSU and LSU (80S formation). There are 14 bridges in the yeast ribosome, five of which are eukaryote specific [35]. These intersubunit bridges transmit important structural information between the subunits and help coordinate their action.

occurs. The PTC is built within the core of the 60S subunit.
Rossmann-fold-like: a class of SAM-dependent MTases involved in methylating a variety of substrates, including DNA, RNA, and proteins.
SPOUT: a class of SAM-dependent MTases including the SpoU and TrmD (SPOUT) RNA MTases. These enzymes contain a characteristic six-stranded parallel β-sheet flanked by seven α-helices and are known to form dimers.

Table 1. SSU rRNA Base Modifications in Yeast

Feature	Modification Name			
	$m^1acp^3\Psi_{1191}$	m^7G_{1575}	ac^4C_{1280}, ac^4C_{1773}	$m_2^6A_{1781}m_2^6A_{1782}$
Modification enzyme	Step 1: Ψ formation by snR35; step 2: N^1-methylation by EMG1/NEP1; step 3: addition of a acp group by an unknown enzyme	BUD23-TRM112; (TRM112 acts as a stabilizing co-activator for BUD23 and other MTases)	KRE33/RRA1 (also responsible for C_{12} acetylation of Ser and Leu tRNAs, assisted by TAN1)	DIM1
Requirement of factor for growth	snR35: non-essential; EMG1/NEP1: essential	BUD23 and TRM112 non-essential but their deletion leads to slow growth and cryosensitivity	Essential	Essential
Requirement of modification for growth	snR35-mediated pseudouridylation, not required; EMG1-mediated methylation, not required	Not required	Not required	Not required
Subcellular localization of modification activity (number of molecules per cell)	snR35 and EMG1, nucleolus (not reported)	Not reported [1620 (BUD23), 4800 (TRM112)]	Not reported	Nuclear with nucleolar enrichment (not determined)
Other cellular phenotypes	*emg1* mutants more sensitive to paromomycin	*bud23Δ* cells: decreased competitive fitness, decreased resistance to cycloheximide, paromomycin and neomycin; *trm112Δ* cells: decreased resistance to paromomycin	Decreased competitive fitness	Paromomycin and neomycin hypersensitivity of *dim1*-thermosensitive strains
Requirement for ribosome biogenesis	*emg1* mutants: SSU synthesis, processing at sites A_0, A_1, and A_2	*bud23Δ* cells: SSU synthesis (including export), processing at site A_2; *trm112Δ* cells: SSU and LSU synthesis, LSU stability	Processing inhibition at sites A_0, A_1, and A_2 (accumulation of aberrant 22S and 23S)	Processing inhibition at sites A_1 and A_2 (accumulation of aberrant 22S)
Position of modification in ribosome	At P-site tRNA, in loop 35	At a P-site/E-site tRNA ridge (1575–1578)	In helix 34 (translation accuracy) and helix 45 (near DCS)	On tip of helix 45, stacks on base of helix 44 (subunit interface), close to DCS and platform
Functional interactions	snR35 (Ψ1191), snR57 (Gm1572), eS19(RPS19), uS13A(RPS18A), uS13B (RPS18B), RRP8, EFG1, UBP3, BRE5, UBP6, DOM34 NOP6, NOP14, UTP30	BUD23: multiple SSU-processome components (including UTP2, UTP14, etc.), MRP, EMG1, uS15(RPS13), and uS19(RPS15); TRM112: UTP14	Not reported	Not reported
Effect of rDNA mutations in *cis*-	U1191A, U1191C, and U1191G all show significant growth impairment	G1575A viable	C1773G non viable	A1781G/A1782G non viable
Protein fold	EMG1: SPOUT, forms dimers	BUD23: Rossmann-like	GNAT	Rossmann-like
Equivalent in human	$m^1acp^3\Psi_{1248}$ made by ACA13 and hEMG1/hNEP1	m^7G_{1639} made by nucleoplasmic WBSCR22-TRMT112	ac^4C_{1337}, ac^4C_{1842} made by NAT10 and U13 (for 1842)	$m_2^6A_{1850}m_2^6A_{1851}$ made by nucleolar DIMT1L
Refs	[30,42,53,81–84]	[12,20,43,49,71,85–88]	[18,19,68]	[12,44,45,89]

Table 2. LSU rRNA Base Modifications in Yeast

Modification Name	m^1A_{645}	m^1A_{2142}	m^5C_{2278}	m^5C_{2870}	m^3U_{2634}	m^3U_{2843}
Modification enzyme	RRP8/BMT1	BMT2	RCM1/BMT3	NOP2/BMT4	BMT5	BMT6
Requirement of protein for growth	Non-essential but deletion is cryosensitive for growth	Non-essential	Non-essential	Essential	Non-essential	Non-essential
Requirement of modification for growth	Not required but catalytically deficient allele is cryosensitive for growth	Not required	Not required	Not required but absence of modification leads to slow growth	Not required	Not required
Subcellular localization of modification activity (number of molecules/cell)	Nucleolar-enriched (2780)	Nucleolar (623)	Nucleolar enriched (922)	Not reported (18 700 ± 2460)	Nucleolus (2960)	Cytoplasm (not reported)
Other cellular phenotypes	Decreased resistance to paromomycin of cells expressing point mutations and a C-terminal truncation	Anisomycin and peroxide sensitivity, extended hibernating lifespan and cold resistance	Decreased resistance to anisomycin; increased resistance to oxidative stress; downregulated in chronologically aged yeast cells	None reported	Not reported	Not reported
Requirement for ribosome biogenesis	Involvement in synthesis of both subunits; processing at site A_2	Not required	Not required	Catalytically deficient allele: A_0, A_1, and A_2 cleavages delayed (35S accumulation)	Not required	Not required
Position of modification in ribosome	Next to PTC, in helix 25.1, between domains I and II	Intersubunit surface, in helix 65, domain IV	Intersubunit surface, at eukaryotic-specific bridge eB14, in helix 70, domain IV	At PTC, in helix 89 (which together with helix 90 and 92 build the critical A-loop of the PTC)	Near PTC, helix 81, domain V	Peripheral, near P-stalk, in helix 89
Functional interactions	Co-purifies with snR190/U14; genetic interaction with GAR1, NEP1 and several 'A_3 cluster proteins' (NOP12, NOP15, NOP16)	Not reported	Not reported	Not reported	Not reported	Physical interaction with Nop2
Effect of rDNA mutations in *cis*-	A645U defective for 60S synthesis, severe cryosensitivity; marked paromomycin hypersensitivity	A2142U, A2142C, and A2142G all viable	C2278G and C2278U viable but anisomycin hypersensitivity	Not reported	Not reported	Not reported
Protein fold	Rossmann-like	Rossmann-like	Rossmann-like	Rossmann-like	Rossmann-like	Rossmann-like
Equivalent in human	m^1A_{1322} made by Nucleomethylin/NML/hRRP8 (eNOSC complex)	Not conserved	m^5C_{3782} made by NSUN5/WBSCR20	m^5C_{4447} made by NSUN1/hNOP2/NOL1/p120	Not conserved	Not conserved
Refs	[13,90,91]	[14]	[15,17,76]	[15]	[16]	[16]

Trends in Biochemical Sciences

CellPress

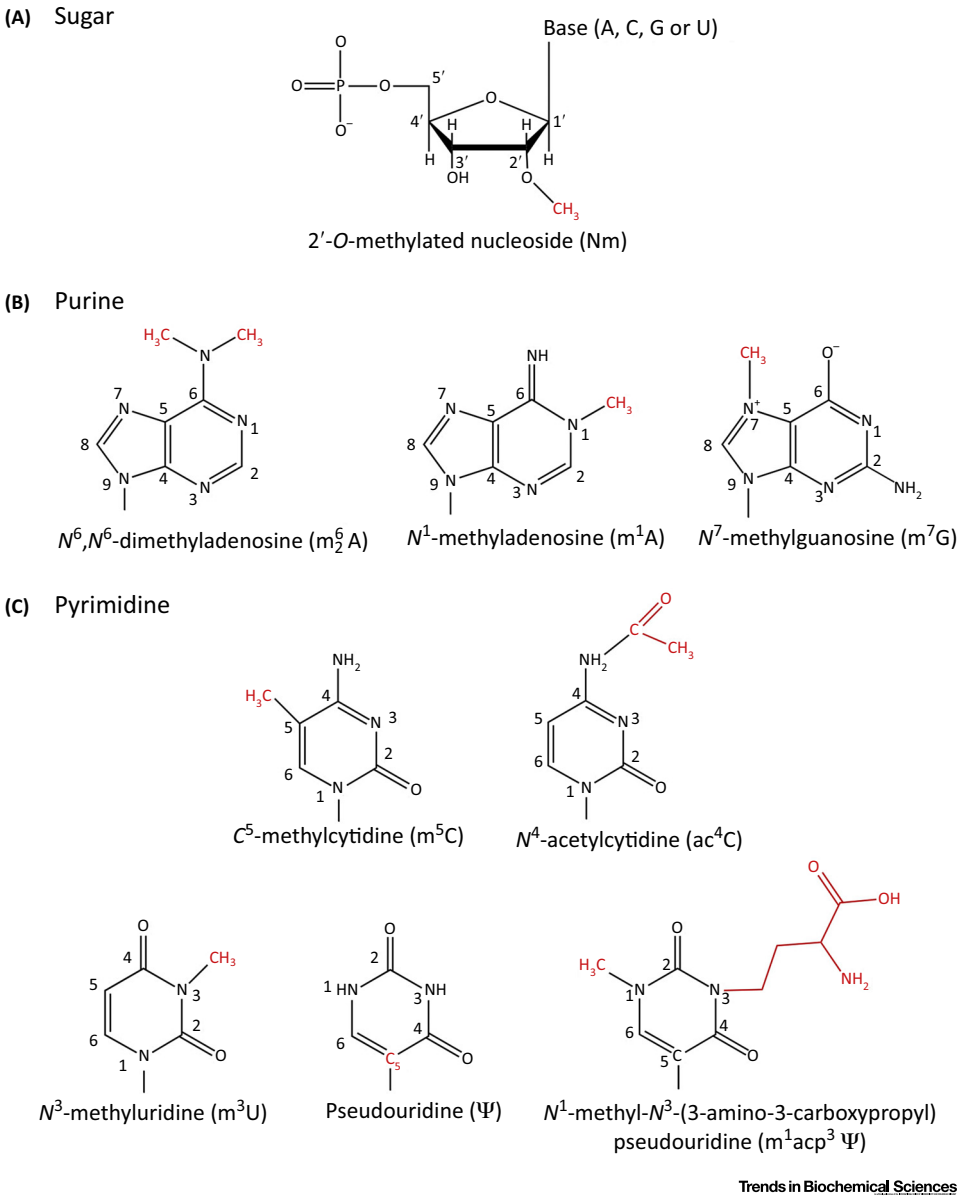
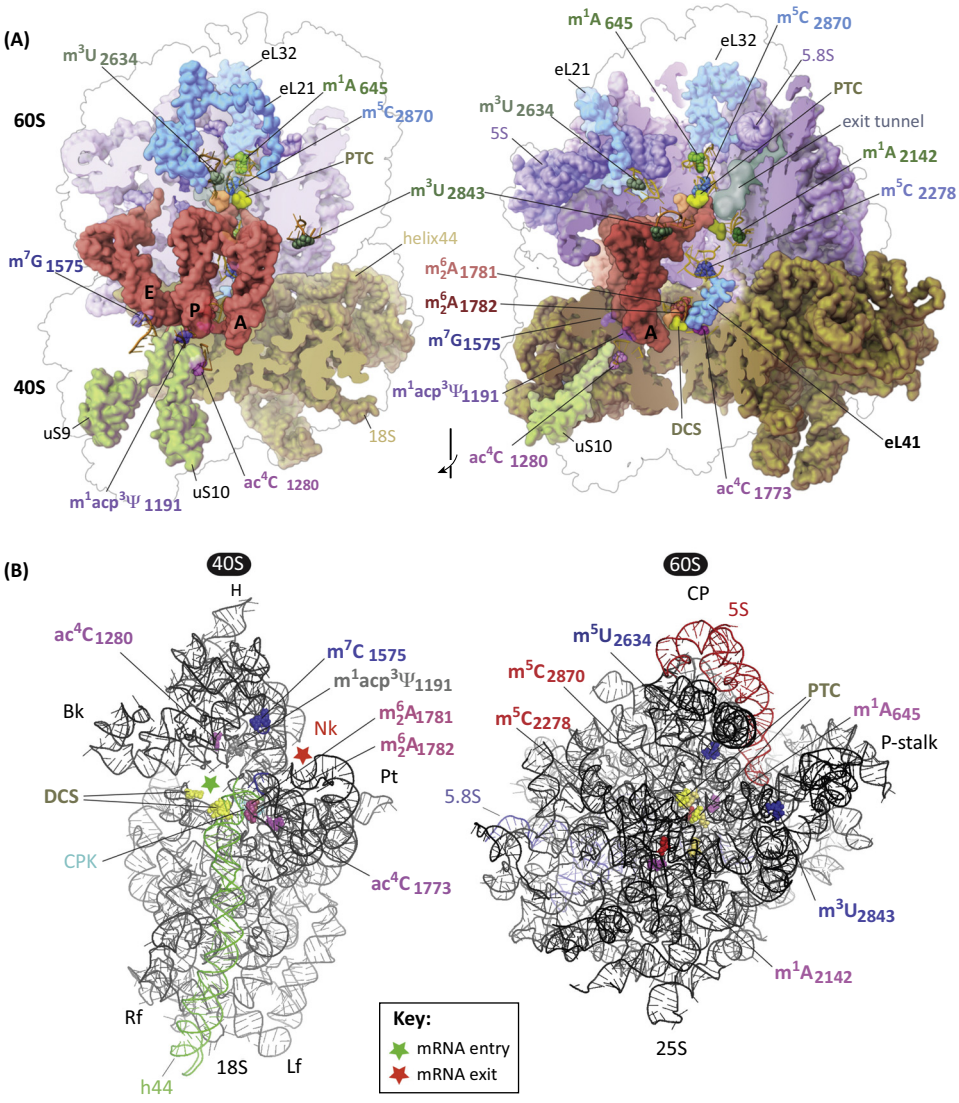


Figure 1. The Yeast rRNA Modification Repertoire. Twelve types of modification have been identified on yeast rRNAs: eight modified nucleobases (three purines and five pyrimidines) and all four 2′-*O*-methylated nucleosides (A, C, G, and U). Atoms in red indicate the modifications.

Upon mapping the rRNA base modifications on the 3D structure of the yeast ribosome, we unexpectedly observed that ribosomal protein eL41, which forms the centrally located eukaryote-specific bridge eB14, makes contact with four of the 12 modified bases: m^5C_{2278} on the LSU, and ac^4C_{1773} and $m_2^6A_{1781}m_2^6A_{1782}$ on the SSU (Figure 2A). On the basis of the available 3D structures [36], we predict that these contacts also occur in human ribosomes.

eL41 is a highly positively charged, 25-amino-acid protein that extends from the 60S into the 40S DCS. It protrudes from the 60S into a binding pocket in the SSU that is lined with helices 27, 44, and 45 [35]. Although eL41 is normally part of the LSU, it appears to be more strongly associated with 40S than with 60S in the complete 80S ribosome.



Trends in Biochemical Sciences

Figure 2. Positions of rRNA Base Modifications on the Yeast Ribosome. Complete 80S ribosome (A) or individual subunits (B), highlighting the positions of modified rRNA bases with respect to important functional areas (shown in yellow: DCS on 40S; PTC on 60S). (A) Cross-sectional views of the yeast ribosome [model based on Protein Data Bank (PDB) entry 4V88]. As an illustration, to position the A-, P-, and E-sites, *Thermus thermophilus* tRNAs (from PDB 4V5D) were modeled into the yeast structure. The 12 rRNA base modifications are highlighted. On the small subunit (SSU), decoding occurs at the A-site. On the large subunit (LSU), the A- and P-site tRNAs point towards the PTC (the A-loop, PTC, and **P-loop** residues are indicated in mustard, yellow, and orange, respectively). The peptide tunnel, from which the nascent polypeptide emerges, is shown in gray. Several ribosomal proteins are depicted for reference and to show their proximity to individual rRNA base modifications. Note that the A-loop and P-loop residues are 2′-*O*-methylated by Spb1 and the box C/D snoRNA snR67, respectively (see Table S1 in the supplementary material online). Highlighted residues are color-coded randomly. (B) Subunit interface view of individual subunits, with the RNA backbone only (models based on PDB 4V88). On the SSU: H, head; Nk, neck; Pt, platform; Lf, left foot; h44, helix 44; Rf, right foot; CPK, central pseudoknot; Bk, beak; green star, mRNA entry; red star, mRNA exit. On the LSU: CP, central protuberance (corresponding to 5S rRNA); P-stalk, phospho-stalk. The large rRNAs are shown in a gray-to-black gradient (18S on 40S and 25S on 60S). The 5.8S and 5S are shown in purple and red, respectively.

Box 1. Effects of rRNA Modification on RNA Structure and Reactivity

2′-*O*-Methylation (Nm): favors a **C3′-*endo* sugar conformation** (preferred by **A-form** RNA helix), promoting base stacking and resulting in higher RNA rigidity [92]. It also protects RNAs against alkaline and enzymatic (nuclease) hydrolysis.

Pseudouridine (Ψ): favors a C3′-*endo* sugar conformation (as do Nm and ac^4C). It provides added hydrogen bonding capability (by comparison to uracil; due to rotation, both N^1 and N^3 participate in hydrogen bonding) and increases thermal stability (by up to 2 °C) [93].

1-Methyladenosine (m^1A): gives the nucleobase a positive charge, making it a stronger base than adenosine (p*K*a increases from approximately 3.5 to approximately 8.25) and disrupting its participation in canonical Watson–Crick (W–C) base pairing [94,95]. Under physiological conditions, the positive charge on the base may also be involved in noncanonical hydrogen bonding and electrostatic interactions, thus strongly influencing RNA topology [96].

5-Methylcytidine (m^5C): increases the lipophilic surface of the base, promoting base stacking [93]. It also increases the thermal stability of hydrogen bonding with guanine and stabilizes RNA structurally and metabolically [97].

3-Methyluridine (m^3U): interrupts hydrogen-bond formation, blocking formation of canonical W–C base pairing [16]. It also increases the hydrophobicity of the base, altering its stacking properties, and promotes hairpin structure formation [98].

7-Methylguanosine (m^7G): introduces a positive charge in the imidazole ring under physiological conditions [95]; although no alteration of W–C base pairing is expected, the positive charge may affect the RNA conformation through its involvement in ionic interactions and promotion of RNA–protein interactions [95].

***N*^{6}, *N*^{6}-Dimethyladenosine ($m^6_2Am^6_2A$):** increases hydrophobicity and base stacking [77]. It also disrupts W–C base pairing, prevents duplex formation with complementary strands, and destabilizes the SSU rRNA terminal hairpin helix [99,100].

***N*^{4}-Acetylcytidine (ac^4C):** favors a C3′-*endo* sugar conformation (as do Nm and Ψ). It also increases hydrophobicity, the stability of hydrogen bonding with guanine (as does m^5C), and structural stability [101].

1-Methyl-3-(3-amino-3-carboxypropyl) pseudouridine ($m^1acp^3\Psi$): although Ψ formation results in increased polarity and extra hydrogen-bonding capability, the net effect of the additional N^1-methylation and N^3-acp results in disengagement of $m^1acp^3\Psi$ from canonical W–C base pairing and increased hydrophobicity as compared to Ψ [93].

To us, the contacts between eL41/eB14 and four rRNA base modifications located on both sides of the subunit interface suggest that rRNA base modifications are involved in long-range transmission of allosteric information. A straightforward hypothesis is that these contacts have a role during translation because this is when the bridge eB14 is formed and when it acts as 'a motion center' for 40S subunit rotation [37]. These contacts may also form during ribosome biogenesis, because the functionality of 40S subunit precursors is tested in a 'translation-like' cycle that involves their association with mature 60S subunits during the late stages of cytoplasmic maturation [38,39]. Interestingly, the binding sites of the translation factor eiF5B/Fun12, a small GTPase required for the formation of 80S-like particles (pre-40S associated with 60S), have been identified [38] and precisely flank the m^5C_{2278} modification.

In conclusion, we speculate that, for efficient and faithful translation, eukaryotes have evolved a sensing mechanism for monitoring the presence of several base modifications across the subunit interface, involving an intersubunit bridge. In support of this hypothesis, yeast cells lacking eL41 show deficient translation *in vitro*, with decreased peptidyltransferase activity and increased translocation [40].

The proximity of two other ribosomal proteins to rRNA base modifications is highlighted in Figure 2A: uS10 is close to ac^4C_{1280} on the SSU, and eL32 is nearby m^1A645 on the LSU. The functional implications of this proximity need to be addressed experimentally (see Outstanding Questions).

Proteins Involved in rRNA Base Modification

Strategies of Modification

RNA modifications are nearly all produced enzymatically. An exception is rRNA oxidation, which seems to have a role in the etiology of disease, such as Alzheimer's disease (discussed in [41]).

All nine rRNA base MTase [13–16,42–44] and an acetyltransferase [18,19] have recently been characterized in budding yeast. Four MTases (Bmt2, Rcm1, Bmt5, and Bmt6) are nonessential. They act on the LSU and three of them are not conserved in human (Tables 1 and 2). The other proteins, all conserved in humans, are either essential (Emg1, Kre33, Dim1, and Nop2) or important (Bud23-Trm112 and Rrp8) for growth. Surprisingly, this is not because of their RNA-modifying catalytic activity, as demonstrated by the analysis of catalytically deficient alleles, but because their presence in cells is strictly required for, or contributes substantially to, pre-rRNA processing [15,19,20,42–45]. Three of four rRNAs (5.8S, 18S, and 25S/28S) are produced as a single transcript synthesized by RNA polymerase I. This implies that extensive pre-rRNA processing is required to release the rRNAs and produce their mature 5′ and 3′ ends [46,47]. For this, a complex processing machinery is sequentially assembled onto nascent transcripts that folds the pre-rRNAs into a conformation that is compatible with cleavage and presents them to the relevant endo- and exoRNases [48]. This machinery comprises dozens of assembly factors, including modification enzymes. Some modification enzymes have a particularly important role in processing, as shown by the observation that cleavage is reduced, or simply does not occur, when they fail to assemble onto precursor ribosomes at the right time [15,19,20,42–45]. Thus, by making binding of the modification enzyme to pre-rRNA a prerequisite for cleavage, cells have selected a strategy through which, in principle, only modified molecules are produced. This suggests that these modifications benefit translation, although this remains to be formally demonstrated in most cases (see below). Such a quality-control mechanism seems to have been conserved, at least to some extent, in human cells, where the presence of DIMT1L and WBSCR22-TRMT112, the homologs of Dim1 and Bud23-Trm112, respectively, is required for pre-rRNA processing, but their RNA-methylating activity is not [12,49].

Protein Folds

Seven rRNA base MTases display a classical **Rossmann-like fold**, and one (Emg1) belongs to the **SPOUT** (alpha-beta knot fold) family (Tables 1 and 2). All use *S*-adenosyl-methionine (SAM) as a methyl donor. The acetyltransferase Kre33 has a classical **Gcn5-related *N*-acetyltransferase** (GNAT) domain, also found in the bacterial tRNA anticodon acetyltransferase TmcA [50], and uses acetyl coenzyme A (acetyl-CoA) as acetyl donor. To our knowledge, most rRNA base modification enzymes work as stand-alone enzymes. Exceptions are Bud23, Emg1, and NAT10 (the human homolog of Kre33). Bud23 acts in concert with the coactivator Trm112, which stabilizes it through formation of a β-zipper, which masks an important solvent-unfavorable hydrophobic surface on Bud23 [20]. Two-subunit enzymes, such as Bud23-Trm112, are frequent in tRNA modification [51], but this is the first example reported in eukaryotic rRNA modification. Emg1 assembles homodimers whose formations are important for RNA binding [52,53]. NAT10 works in conjunction with the box C/D snoRNA U13, which likely folds the pre-rRNA to expose the substrate residue to NAT10 [19].

Timing of rRNA Base Modifications

Most 2′-*O*-methylation and pseudouridylation are performed early during ribosome biogenesis, often co-transcriptionally [54,55]. By contrast, rRNA base modifications are expected to be formed later during the process, although the exact timing remains unknown in most instances.

In bacteria, the kinetics of RNA modification largely parallels that of ribosomal protein assembly [33]. In other words, RNA modification is concomitant with binding of the modification enzymes

to the maturing subunits. By contrast, several eukaryotic base-modification enzymes bind at an early stage of subunit assembly, but do not modify their substrates until later. For instance, in a binding step required for early pre-rRNA processing (see above), Dim1 associates with nucleolar precursor ribosomes, but it only catalyzes the modification after the maturing SSU is exported to the cytoplasm [44,56,57]. The situation is similar for Bud23-Trm112, although in this case the modification likely takes place in the nucleus before pre-18S rRNA export [20,56]. 18S rRNA dimethylation in human cells was also reported to be a late 18S rRNA modification [24], but a recent study has shown that, in contrast to yeast, it occurs in the nucleus after WBSCR22-TRMT112-mediated N^7G methylation [12].

Synthesis of the hypermodified $m^1acp^3\Psi_{1191}$ is of particular interest because it requires three steps involving different factors, supposedly active in different subcellular compartments. First, U_{1191} is converted to Ψ by the H/ACA snoRNP snR35 [30], then Ψ_{1191} is N^1-methylated by Emg1/Nep1 [42,53], and finally, a 3-amino-3-carboxypropyl (acp) group is added at the N^3 position onto $m^1\Psi_{1191}$ by an enzyme yet to be identified, perhaps using SAM as the acp donor [58]. These three steps are expected to occur in the nucleolus, the nucleoplasm, and the cytoplasm, respectively [59,60]. For two steps out of three, the chemistry of the reaction imposes a strict order of events: N^1-methylation can occur only after U_{1191} has been isomerized to Ψ, because isomerization generates the substrate for methylation. By contrast, the addition of an acp group can occur independently of the other two reactions [30,60].

In human cells, several snoRNAs are associated with late forms of SSU precursors [61], indicating that at least some snoRNA-mediated modifications may occur late in the process of ribosome assembly. Interestingly, both NAT10 and U13 are associated with these precursors, suggesting that 18S rRNA acetylation is also a late event [19].

Catalysis Modes

The recently determined crystal structure of the Bud23-Trm112 MTase in a complex with SAM has provided novel insights into the catalysis and timing of modification [20]. Modeling of the substrate guanosine residue of Bud23 into its catalytic pocket, based on that of a xanthosine MTase of *Coffea canephora*, revealed that Bud23 can only modify precursor 40S ribosomes, not mature subunits, because of important steric clashes [20]. Incidentally, the model also showed that SAM is ideally positioned for an in-line S_N2-type reaction, which is the mechanism used by most class I SAM-dependent MTases [20,62]. The catalysis mode of bacterial m^5C rRNA MTases has been described, and the yeast mode is likely identical because key residues involved in the reaction are conserved [15,63].

In conclusion, rRNA modification occurs throughout subunit biogenesis from the initial steps in the nucleolus to the final ones in the cytoplasm. In most cases, the exact timing of modification remains to be established, and sometimes there is a regulated delay, implying specific activation, between the time of binding of a modification enzyme to precursor ribosomes and the time when it modifies the RNA.

How rRNA Base Modification Enzymes Access Their Substrates

Given that ribosomal RNAs are highly structured, another important question is how modification enzymes gain ready access to their substrate residues. Strikingly, several base-modification enzymes have been shown to interact physically with RNA-remodeling enzymes or to carry such an activity embedded within their own sequence.

In cells, the Bud23-Trm112 MTase interacts with the helicase Dhr1 [64], and *in vitro* these proteins form a trimeric complex [20]. Dhr1 is required for the efficient association of Bud23-Trm112 with pre-ribosomes [20]. Dhr1 associates with the box C/D snoRNA U3 [65], which it

displaces from pre-18S precursors to form the central pseudoknot (CPK) [66], a highly conserved tertiary interaction required for the overall folding of the SSU (Figure 2B). How N^7G_{1575} methylation relates to CPK formation is not yet clear. By contrast, the acetyltransferase Kre33/NAT10 contains its own RNA-remodeling activity. Kre33/NAT10 has an ATP-dependent RecD helicase domain that, in yeast, is required for rRNA and, to a lesser extent, tRNA acetylation [19]. Within maturing ribosomal subunits, local neighborhoods may be crowded because assembly factors may compete with each other for binding overlapping sites. In addition to its role in presenting the substrate residue, the helicase domain of Kre33 may also assist in the timely loading and/or release of other assembly factors. One such factor could be the box C/D snoRNA snR40 involved in the 2′-*O*methylation of G_{1271} in helix 34, which is also modified by Kre33.

In conclusion, the function of a few rRNA modification enzymes has been linked to remodeling activities with the suggestion that such activities assist in loading and/or releasing them from precursor ribosomes and help them to access their substrate residue. It is unclear at this stage whether other modification enzymes similarly rely on remodeling activities, but it is likely given that many of them act during the course of subunit assembly on well-preformed and already compact particles.

Crosstalk in the Modification of Different Classes of RNA

It is emerging that considerable crosstalk occurs between the processes that modify distinct classes of cellular RNA. This crosstalk involves shared modification factors, shared catalytic subunits working together with specific adaptors, and common coactivators for specific catalytic subunits.

For example, Kre33 is involved in both 18S rRNA modification and tRNA modification. Kre33 is responsible for N^4-acetylcytidine formation at two positions on the 18S rRNA (Figure 2, Table 1). These two modifications are conserved in budding and fission yeast [18,19,67], plants, and humans [19,68]. However, Kre33 interacts with the tRNA-binding protein Tan1 *in vivo* (in yeast) and together they are required for serine and leucine tRNA acetylation at position 12 [19]. Tan1 has long been known to be required for tRNA acetylation [69], but since it lacks an acetyltransferase domain, we suggest that it acts as a tRNA adaptor for Kre33. Such a function seems to be conserved in humans, because NAT10 (the Kre33 homolog) is required for both 18S rRNA and tRNA acetylation and interacts *in vivo* with THUMPD1 (the human homolog of Tan1) [19].

As discussed above, the 18S rRNA MTase Bud23 is protected from degradation in cells through formation of a heterodimer with Trm112. Trm112 interacts with at least three additional MTases: Mtq2, which methylates the eukaryotic translation release factor eRF1, and the tRNA-modifying enzymes Trm9 and Trm11 [70]. Trm112 is present in limited amounts in cells, and its partners compete with each other to interact with it to gain metabolic stability [71]. Strikingly, Trm112 interacts with four MTases whose substrates are all involved in protein synthesis. This positions it ideally to modulate translation.

Recently, transcriptome-wide mapping of RNA modifications revealed that mRNAs are abundantly 2′-*O* methylated and pseudouridylated, some modifications being inducible by stress and some synthesized by tRNA-modifying enzymes [72,73]. This sheds light on another case of RNA-modification crosstalk.

The exact extent of crosstalk in RNA modifications remains unclear, but more examples are likely to emerge in the near future from ongoing transcriptome-wide mapping efforts. The existence of RNA modification crosstalk implies that caution must be applied when attributing

a phenotype to any particular modification, and that several previous conclusions will have to be revisited.

Functions of rRNA Base Modifications

SnoRNA-based modifications have been shown to optimize translation, although in most cases no adverse effect is seen unless multiple modifications are knocked out concomitantly (e.g., [74,75]). To date, although they have been linked to disease on multiple occasions (Box 2), the functions of rRNA base modifications remain largely unknown. Yeast cells whose ribosomes lack specific base modifications are outcompeted by wild-type cells and show increased sensitivity or resistance towards antibiotics (e.g., aminoglycosides) and specific stresses (e.g., oxidative stress) (Tables 1 and 2). For instance, cells lacking Bmt2 or Rcm1, which are responsible for base modifications at the subunit interface (Figure 2, Table 2), show increased sensitivity towards anisomycin, which is consistent with subunit joining defects [14,15].

Several base modifications contacted by the bridge eB14 have been implicated in translation. In yeast, $m_2^6Am_2^6A$ is important for translation *in vitro* [45], and loss of m^5C_{2278} impairs translational fidelity, causing a twofold increase in stop codon read-through and increased recruitment of oxidative stress-responsive mRNAs to polysomes, with a concomitant increase in resistance to oxidative stress [76]. In bacteria, $m_2^6Am_2^6A$ is important for translation accuracy and RNA conformation in the vicinity of the DCS [77,78]. In yeast, m^5C_{2278}, together with Gm_{2288}, is required for 60S stability [17]. Thus, current evidence indicates that the modifications contacted by the bridge are important to ribosome function. This strengthens our hypothesis that monitoring their presence may be beneficial to the cells.

Box 2. rRNA Base Modifications and Human Disease

2′-*O*-methylation and pseudouridylation of ribosomal RNA have frequently been associated with disease, mostly cancers and autoimmune syndromes [102]. rRNA base modifications have also been linked, although more seldom, with cancer [103,104] and genetic diseases [105,106] (Table I). To date, however, it remains unclear in most cases whether the disease is due to loss of RNA-modifying activity, or failure to assemble sufficient ribosomes. Ribosomal RNA-modification enzymes are known to perform additional functions in diverse processes, including pre-rRNA processing (see section on 'Strategies of modification' in main text), rRNA synthesis regulation [90,106,107] and rRNA surveillance [108]. This is the case of human RRP8 (NML/nucleomethylin), which, as part of the energy-dependent nucleolar silencing complex (eNoSC) complex, is involved in rRNA transcription regulation during starvation [90]. Interestingly, in addition to being associated with Bowen–Conradi syndrome, Emg1/Nep1 interacts functionally with eS19 (RPS19) [81], which is mutated in 25% of patients with the ribosomopathy Diamond Blackfan anemia (DBA). Ribosomopathies are cancer predisposition syndromes associated with ribosome biogenesis dysfunctions due to mutations in assembly factors or ribosomal proteins [109].

Table I. The Pathophysiology of rRNA Base MTases

Gene	Disease	Refs
EMG1/NEP1	Bowen–Conradi syndrome (neurodegenerative)	[105]
NAT10	Laminopathies, Hutchinson–Gilford progeria syndrome (premature aging)	[110]
WBSCR22	Cancer metastasis; multiple myeloma; Williams–Beuren syndrome (one of many genes associated); inflammation and neoplastic human lung pathology; plant dedifferentiation and wounding	[103,104,111–113]
DIMT1L	Chilling-induced chlorosis in plants (paleface1, Pfc1)	[114]
RRP8/Nucleomethylin (NML)	High-fat diet-induced obesity	[106]
WBSCR20/NSUN5	Williams–Beuren syndrome (one of many genes associated); animal lifespan	[76,111]
NOP2/NOL1/p120/NSUN1	Cancer; 'Cri du chat' syndrome	[115,116]

Concluding Remarks

In recent years, tremendous progress has been made in identifying the rRNA base-modification machinery, notably in budding yeast. Several human homologs have been described, and we anticipate that human ribosomes carry additional, lineage-specific rRNA modifications.

Important crosstalk between RNA modification processes has emerged. It remains unclear exactly how this affects overall cell homeostasis and, as far as rRNA, tRNA, and mRNA are concerned, translation. An attractive possibility is that rRNA modifications might interact functionally with modifications on mRNAs and tRNAs. For example, the geometry of the DCS, whose local environment is particularly rich in base modifications, might be influenced and possibly optimized by complementary modifications present on rRNA, tRNA, and even mRNAs. mRNAs are now known to carry numerous, and sometimes even stress-induced, modifications (including Ψ, m^6A, 2′-*O*, m^7G, m^3Um, m^6_2Am, and m^5C [1,2]). Such interactions might contribute to finely regulating translation, possibly of specific transcript subsets (e.g., stress-related).

By mapping rRNA base modifications on the 3D structure of the ribosome, a eukaryote-specific bridge at the center of the subunit interface, akin to a pivot and comprising a short conserved protein, has been shown to make contact with four rRNA base modifications, including three at the DCS. This suggests that long-range allosteric communication involving rRNA modifications occurs during translation and/or the late steps of ribosome biogenesis. Whether this underlies a probing mechanism is under investigation.

Special attention should be paid to substoichiometric (partial) RNA modification and stress-induced RNA modifications. It has recently become clear, at least for 2′-*O*-methylation and pseudouridylation, that not all rRNA positions liable to modification are fully modified at all times. This establishes *de facto* the existence of ribosome heterogeneity and specialized ribosomes [5]. For spliceosomal U snRNAs and mRNAs, it has further been demonstrated that partial modification at cryptic positions is inducible notably by nutritional stress [72,79]. How the occurrence of such stress-induced modifications is regulated and how, exactly, they affect RNA function is not yet known. Also unclear is whether rRNA bases can undergo cryptic and partial modification. Further structural work, notably on modification enzymes bound to precursor ribosomal species, is needed to gain important information on catalysis modes, substrate-binding strategies, and the timing of action of rRNA modification enzymes in the course of ribosome assembly.

Despite rapid advances in identifying the enzymes involved, the functions of rRNA base modifications in translation remain largely unknown. Yet their presence at functional sites, their conservation, and the frequently elaborated pathways involved in making them, all suggest that evolution has selected and retained them because they are important for optimizing translation. However, this remains to be demonstrated. High-resolution, high-throughput techniques, such as ribosome profiling and quantitative proteomics, are likely to enlighten us on this issue in the near future.

Acknowledgments

We are particularly indebted to Said Sannuga for producing Figure 2A and for carrying out background research for the alignment of the tRNAs. S.S. is supported by an EMBO long-term fellowship (ALTF644-2014). The Lab of D.L.J.L. is supported by the Communauté Française de Belgique ('Action de Recherches Concertées'), the Région Wallonne (DG06, CWALity), the Fonds National de la Recherche Scientifique (FRS/FNRS), the European Regional Development Fund (ERDF), through a secondary affiliation in the Center for Microscopy and Molecular and Imaging (Cmmi), and the Université Libre de Bruxelles (ULB).

Supplemental Information

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tibs.2015.07.008.

Outstanding Questions

What is the function of rRNA modifications? Do they become more important under certain circumstances, such as during developmental or differentiation pathways and stages, or in disease? Do they interact functionally with modifications on tRNAs or mRNAs to optimize the geometry of the translational machinery?

Do rRNA base modifications functionally communicate during translation or ribosome biogenesis? This is suggested by the observation that a eukaryote-specific bridge makes contact across the subunit interface with four modified bases.

How do RNA modifications modulate rRNA structure and function?

Can rRNA bases be partially (substoichiometrically) modified? Partial modification was reported recently for rRNA 2′-*O*-methylation and Ψ, which has important consequences for differential translation and disease etiology [5].

Does rRNA modification have a dynamic component? Are rRNA modifications reversible? Are there rRNA modification erasers (such as demethylases, deacetylases, etc.)? If so, what are the functional consequences of those dynamics?

Are rRNA base modifications, or their absence, specifically monitored by surveillance mechanisms? This is suggested by functional interactions between RNA modification enzymes and surveillance factors, and by the localization of MTases to specific subcellular structures.

What is the timing of rRNA modifications, and where in the cell do they take place? How interdependent are rRNA modifications? Do specific rRNA modifications affect the formation of others?

How are modification enzymes organized inside the cell? Are there physical interactions between rRNA modification enzymes, akin to aminoacyl-tRNA synthetases [80]?

rRNA modification profiling has great potential in clinical biology, if specific modifications can be developed as diagnostic or prognostic biomarkers. Human rRNA modification profiles should be thoroughly investigated in health and disease.

References

1. Cantara, W.A. *et al.* (2011) The RNA Modification Database, RNAMDB: 2011 update. *Nucleic Acids Res.* 39, D195–D201
2. Machnicka, M.A. *et al.* (2013) MODOMICS: a database of RNA modification pathways–2013 update. *Nucleic Acids Res.* 41, D262–D267
3. Melnikov, S. *et al.* (2012) One core, two shells: bacterial and eukaryotic ribosomes. *Nat. Struct. Mol. Biol.* 19, 560–567
4. Ramakrishnan, V. (2014) The ribosome emerges from a black box. *Cell* 159, 979–984
5. Lafontaine, D.L.J. (2015) Noncoding RNAs in eukaryotic ribosome synthesis and function. *Nat. Struct. Mol. Biol.* 22, 11–19
6. Woolford, J.L., Jr and Baserga, S.J. (2013) Ribosome biogenesis in the yeast *Saccharomyces cerevisiae*. *Genetics* 195, 643–681
7. de la Cruz, J. *et al.* (2015) Functions of ribosomal proteins in assembly of eukaryotic ribosomes in vivo. *Annu. Rev. Biochem.* 84, 93–129
8. Schafer, T. *et al.* (2006) Hrr25-dependent phosphorylation state regulates organization of the pre-40S subunit. *Nature* 441, 651–655
9. Lo, K.Y. *et al.* (2010) Defining the pathway of cytoplasmic maturation of the 60S ribosomal subunit. *Mol. Cell* 39, 196–208
10. Panse, V.G. and Johnson, A.W. (2010) Maturation of eukaryotic ribosomes: acquisition of functionality. *Trends Biochem. Sci.* 35, 260–266
11. Karbstein, K. (2013) Quality control mechanisms during ribosome maturation. *Trends Cell Biol.* 23, 242–250
12. Zorbas, C. *et al.* (2015) The human 18S rRNA base methyltransferases DIMT1L and WBSCR22-TRMT112 but not rRNA modification are required for ribosome biogenesis. *Mol. Biol. Cell* 26, 2080–2095
13. Peifer, C. *et al.* (2013) Yeast Rrp8p, a novel methyltransferase responsible for m1A 645 base modification of 25S rRNA. *Nucleic Acids Res.* 41, 1151–1163
14. Sharma, S. *et al.* (2013) Identification of a novel methyltransferase, Bmt2, responsible for the N-1-methyl-adenosine base modification of 25S rRNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 41, 5428–5443
15. Sharma, S. *et al.* (2013) Yeast Nop2 and Rcm1 methylate C2870 and C2278 of the 25S rRNA, respectively. *Nucleic Acids Res.* 41, 9062–9076
16. Sharma, S. *et al.* (2014) Identification of novel methyltransferases, Bmt5 and Bmt6, responsible for the m3U methylations of 25S rRNA in *Saccharomyces cerevisiae*. *Nucleic Acids Res.* 42, 3246–3260
17. Gigova, A. *et al.* (2014) A cluster of methylations in the domain IV of 25S rRNA is required for ribosome stability. *RNA* 20, 1632–1644
18. Ito, S. *et al.* (2014) A single acetylation of 18 S rRNA is essential for biogenesis of the small ribosomal subunit in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 289, 26201–26212
19. Sharma, S. *et al.* (2015) Yeast Kre33 and human NAT10 are conserved 18S rRNA cytosine acetyltransferases that modify tRNAs assisted by the adaptor Tan1/THUMPD1. *Nucleic Acids Res.* 43, 2242–2258
20. Létoquart, J. *et al.* (2014) Structural and functional studies of Bud23-Trm112 reveal 18S rRNA N7-G1575 methylation occurs on late 40S precursor ribosomes. *Proc. Natl. Acad. Sci. U.S.A.* 111, E5518–E5526
21. Machnicka, M.A. *et al.* (2014) Distribution and frequencies of post-transcriptional modifications in tRNAs. *RNA Biol.* 11, 1619–1629
22. Ge, J. and Yu, Y.T. (2013) RNA pseudouridylation: new insights into an old modification. *Trends Biochem. Sci.* 38, 210–218
23. Watkins, N.J. and Bohnsack, M.T. (2012) The box C/D and H/ACA snoRNPs: key players in the modification, processing and the dynamic folding of ribosomal RNA. *Wiley Interdiscip. Rev. RNA* 3, 397–414
24. Salim, M. and Maden, B.E. (1973) Early and late methylations in HeLa cell ribosome maturation. *Nature* 244, 334–336
25. Steitz, T.A. (2008) A structural understanding of the dynamic ribosome machine. *Nat. Rev. Mol. Cell Biol.* 9, 242–253
26. Voorhees, R.M. and Ramakrishnan, V. (2013) Structural basis of the translational elongation cycle. *Annu. Rev. Biochem.* 82, 203–236
27. Zhou, J. *et al.* (2014) How the ribosome hands the A-site tRNA to the P site during EF-G-catalyzed translocation. *Science* 345, 1188–1191
28. Rife, J.P. (2009) Roles of the ultra-conserved ribosomal RNA methyltransferase KsgA in ribosome biogenesis. In *DNA and RNA Modification Enzymes: Structure, Mechanism, Function and Evolution* (Grosjean, H., ed.), pp. 512–526, Landes Bioscience
29. Chernoff, Y.O. *et al.* (1996) The translational function of nucleotide C1054 in the small subunit rRNA is conserved throughout evolution: genetic evidence in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 93, 2517–2522
30. Liang, X.H. *et al.* (2009) Loss of rRNA modifications in the decoding center of the ribosome impairs translation and strongly delays pre-rRNA processing. *RNA* 15, 1716–1728
31. Ben-Shem, A. *et al.* (2010) Crystal structure of the eukaryotic ribosome. *Science* 330, 1203–1209
32. Chawla, M. *et al.* (2015) An atlas of RNA base pairs involving modified nucleobases with optimal geometries and accurate energies. *Nucleic Acids Res.* Published online June 27, 2015. http://dx.doi.org/10.1093/nar/gkv606
33. Popova, A.M. and Williamson, J.R. (2014) Quantitative analysis of rRNA modifications using stable isotope labeling and mass spectrometry. *J. Am. Chem. Soc.* 136, 2058–2069
34. Polikanov, Y.S. *et al.* (2015) Structural insights into the role of rRNA modifications in protein synthesis and ribosome assembly. *Nat. Struct. Mol. Biol.* 22, 342–344
35. Ben-Shem, A. *et al.* (2011) The structure of the eukaryotic ribosome at 3.0 A resolution. *Science* 334, 1524–1529
36. Anger, A.M. *et al.* (2013) Structures of the human and *Drosophila* 80S ribosome. *Nature* 497, 80–85
37. Khatter, H. *et al.* (2015) Structure of the human 80S ribosome. *Nature* 520, 640–645
38. Lebaron, S. *et al.* (2012) Proofreading of pre-40S ribosome maturation by a translation initiation factor and 60S subunits. *Nat. Struct. Mol. Biol.* 19, 744–753
39. Strunk, B.S. *et al.* (2012) A translation-like cycle is a quality control checkpoint for maturing 40S ribosome subunits. *Cell* 150, 111–121
40. Dresios, J. *et al.* (2003) A dispensable yeast ribosomal protein optimizes peptidyltransferase activity and affects translocation. *J. Biol. Chem.* 278, 3314–3322
41. Lafontaine, D.L.J. (2010) A 'garbage can' for ribosomes: how eukaryotes degrade their ribosomes. *Trends Biochem. Sci.* 35, 267–277
42. Leulliot, N. *et al.* (2008) The yeast ribosome synthesis factor Emg1 is a novel member of the superfamily of alpha/beta knot fold methyltransferases. *Nucleic Acids Res.* 36, 629–639
43. White, J. *et al.* (2008) Bud23 methylates G1575 of 18S rRNA and is required for efficient nuclear export of pre-40S subunits. *Mol. Cell. Biol.* 28, 3151–3161
44. Lafontaine, D.L.J. *et al.* (1995) The 18S rRNA dimethylase Dim1p is required for pre-ribosomal RNA processing in yeast. *Genes Dev.* 9, 2470–2481
45. Lafontaine, D.L.J. *et al.* (1998) Yeast 18S rRNA dimethylase Dim1p: a quality control mechanism in ribosome synthesis? *Mol. Cell. Biol.* 18, 2360–2370
46. Mullineux, S.T. and Lafontaine, D.L.J. (2012) Mapping the cleavage sites on mammalian pre-rRNAs: where do we stand? *Biochimie* 94, 1521–1532
47. Henras, A. *et al.* (2015) An overview of pre-ribosomal RNA processing in eukaryotes. *Wiley Interdiscip. Rev. RNA* 6, 225–242
48. Fernandez-Pevida, A. *et al.* (2014) Processing of preribosomal RNA in *Saccharomyces cerevisiae*. *Wiley Interdiscip. Rev. RNA* 6, 191–209

49. Haag, S. *et al.* (2014) WBSCR22/Merm1 is required for late nuclear pre-ribosomal RNA processing and mediates N7-methylation of G1639 in human 18S rRNA. *RNA* 21, 180–187
50. Chimnaronk, S. *et al.* (2009) RNA helicase module in an acetyltransferase that modifies a specific tRNA anticodon. *EMBO J.* 28, 1362–1373
51. Guy, M.P. and Phizicky, E.M. (2014) Two-subunit enzymes involved in eukaryotic post-transcriptional tRNA modification. *RNA Biol.* 11, 1608–1618
52. Taylor, A.B. *et al.* (2008) The crystal structure of Nep1 reveals an extended SPOUT-class methyltransferase fold and a pre-organized SAM-binding site. *Nucleic Acids Res.* 36, 1542–1554
53. Wurm, J.P. *et al.* (2010) The ribosome assembly factor Nep1 responsible for Bowen-Conradi syndrome is a pseudouridine-N1-specific methyltransferase. *Nucleic Acids Res.* 38, 2387–2398
54. Kos, M. and Tollervey, D. (2010) Yeast pre-rRNA processing and modification occur cotranscriptionally. *Mol. Cell* 37, 809–820
55. Turowski, T.W. and Tollervey, D. (2015) Cotranscriptional events in eukaryotic ribosome synthesis. *Wiley Interdiscip. Rev. RNA* 6, 129–139
56. Brand, R.C. *et al.* (1977) Secondary methylation of yeast ribosomal precursor RNA. *Eur. J. Biochem.* 75, 311–318
57. Schafer, T. *et al.* (2003) The path from nucleolar 90S to cytoplasmic 40S pre-ribosomes. *EMBO J.* 22, 1370–1380
58. Noma, A. *et al.* (2006) Biosynthesis of wybutosine, a hypermodified nucleoside in eukaryotic phenylalanine tRNA. *EMBO J.* 25, 2142–2154
59. Brand, R.C. *et al.* (1978) Biosynthesis of a hypermodified nucleotide in Saccharomyces carlsbergensis 17S and HeLa-cell 18S ribosomal ribonucleic acid. *Biochem. J.* 169, 71–77
60. Hector, R.D. *et al.* (2014) Snapshots of pre-rRNA structural flexibility reveal eukaryotic 40S assembly dynamics at nucleotide resolution. *Nucleic Acids Res.* 42, 12138–12154
61. Sloan, K.E. *et al.* (2015) The association of late-acting snoRNPs with human pre-ribosomal complexes requires the RNA helicase DDX21. *Nucleic Acids Res.* 43, 553–564
62. Schubert, H.L. *et al.* (2003) Many paths to methyltransfer: a chronicle of convergence. *Trends Biochem. Sci.* 28, 329–335
63. Foster, P.G. *et al.* (2003) The first structure of an RNA m5C methyltransferase, Fmu, provides insight into catalytic mechanism and specific binding of RNA substrate. *Structure* 11, 1609–1620
64. Sardana, R. *et al.* (2014) Physical and functional interaction between the methyltransferase Bud23 and the essential DEAH-Box RNA helicase Ecm16. *Mol. Cell. Biol.* 34, 2208–2220
65. Colley, A. *et al.* (2000) Dhr1p, a putative DEAH-box RNA helicase, is associated with the box C+D snoRNP U3. *Mol. Cell. Biol.* 20, 7238–7246
66. Sardana, R. *et al.* (2015) The DEAH-box helicase Dhr1 dissociates U3 from the pre-rRNA to promote formation of the central pseudoknot. *PLoS Biol.* 13, e1002083
67. Taoka, M. *et al.* (2014) RNA cytidine acetyltransferase of small-subunit ribosomal RNA: identification of acetylation sites and the responsible acetyltransferase in fission yeast, *Schizosaccharomyces pombe*. *PLoS ONE* 9, e112156
68. Ito, S. *et al.* (2014) Human NAT10 is an ATP-dependent RNA acetyltransferase responsible for N4-acetylcytidine formation in 18S rRNA. *J. Biol. Chem.* 289, 35724–35730
69. Johansson, M.J. and Bystrom, A.S. (2004) The *Saccharomyces cerevisiae* TAN1 gene is required for N4-acetylcytidine formation in tRNA. *RNA* 10, 712–719
70. Graille, M. *et al.* (2012) Methylation of class I translation termination factors: structural and functional aspects. *Biochimie* 94, 1533–1543
71. Figaro, S. *et al.* (2012) Trm112 is required for Bud23-mediated methylation of the 18S rRNA at position G1575. *Mol. Cell. Biol.* 32, 2254–2267
72. Carlile, T.M. *et al.* (2014) Pseudouridine profiling reveals regulated mRNA pseudouridylation in yeast and human cells. *Nature* 515, 143–146
73. Schwartz, S. *et al.* (2014) Transcriptome-wide mapping reveals widespread dynamic-regulated pseudouridylation of ncRNA and mRNA. *Cell* 159, 148–162
74. Liang, X.H. *et al.* (2007) rRNA modifications in an intersubunit bridge of the ribosome strongly affect both ribosome biogenesis and activity. *Mol. Cell* 28, 965–977
75. Jack, K. *et al.* (2011) rRNA pseudouridylation defects affect ribosomal ligand binding and translational fidelity from yeast to human cells. *Mol. Cell* 44, 660–666
76. Schosserer, M. *et al.* (2015) Methylation of ribosomal RNA by NSUN5 is a conserved mechanism modulating organismal lifespan. *Nat. Commun.* 6, 6158
77. van Knippenberg, P.H. (1986) Structural anf functional aspects of the N6, N6 dimethyladenosines in 16S ribosomal RNA. In *Structure, Function, and Genetics of Ribosomes* (Hardesty, B. and Kramer, G., eds), pp. 412–424, Springer-Verlag
78. Demirci, H. *et al.* (2010) Modification of 16S ribosomal RNA by the KsgA methyltransferase restructures the 30S subunit to optimize ribosome function. *RNA* 16, 2319–2324
79. Wu, G. *et al.* (2011) U2 snRNA is inducibly pseudouridylated at novel sites by Pus7p and snR81 RNP. *EMBO J.* 30, 79–89
80. Havrylenko, S. and Mirande, M. (2015) Aminoacyl-tRNA synthetase complexes in evolution. *Int. J. Mol. Sci.* 16, 6571–6594
81. Buchhaupt, M. *et al.* (2006) Genetic evidence for 18S rRNA binding and an Rps19p assembly function of yeast nucleolar protein Nep1p. *Mol. Genet. Genomics* 276, 273–284
82. Meyer, B. *et al.* (2011) The Bowen-Conradi syndrome protein Nep1 (Emg1) has a dual role in eukaryotic ribosome biogenesis, as an essential assembly factor and in the methylation of Psi1191 in yeast 18S rRNA. *Nucleic Acids Res.* 39, 1526–1537
83. Thomas, S.R. *et al.* (2011) Structural insight into the functional mechanism of Nep1/Emg1 N1-specific pseudouridine methyltransferase in ribosome biogenesis. *Nucleic Acids Res.* 39, 2445–2457
84. Schilling, V. *et al.* (2012) Genetic interactions of yeast NEP1 (EMG1), encoding an essential factor in ribosome biogenesis. *Yeast* 29, 167–183
85. Liger, D. *et al.* (2011) Mechanism of activation of methyltransferases involved in translation by the Trm112 'hub' protein. *Nucleic Acids Res.* 39, 6249–6259
86. Sardana, R. and Johnson, A.W. (2012) The methyltransferase adaptor protein Trm112 is involved in biogenesis of both ribosomal subunits. *Mol. Biol. Cell* 23, 4313–4322
87. Sardana, R. *et al.* (2013) The rRNA methyltransferase Bud23 shows functional interaction with components of the SSU processome and RNase MRP. *RNA* 19, 828–840
88. Bellemer, C. *et al.* (2010) Genetic interactions show the importance of rRNA modification machinery for the role of Rps15p during ribosome biogenesis in *S. cerevisiae*. *PLoS ONE* 5, e10472
89. Lafontaine, D.L.J. *et al.* (1994) The DIM1 gene responsible for the conserved m6(2)Am6(2)A dimethylation in the 3′-terminal loop of 18 S rRNA is essential in yeast. *J. Mol. Biol.* 241, 492–497
90. Yang, L. *et al.* (2013) Regulation of SirT1-nucleomethylin binding by rRNA coordinates ribosome biogenesis with nutrient availability. *Mol. Cell. Biol.* 33, 3835–3848
91. Bousquet-Antonelli, C. *et al.* (2000) Rrp8p is a yeast nucleolar protein functionally linked to Gar1p and involved in pre-rRNA cleavage at site A2. *RNA* 6, 826–843
92. Prusiner, P. *et al.* (1974) Effect of ribose O(2′)-methylation on the conformation of nucleosides and nucleotides. *Biochim. Biophys. Acta* 366, 115–123
93. Hayrapetyan, A. *et al.* (2009) Function of modified nucleosides in RNA stabilization. In *Structure, Mechanism, Functions, Cellular Interactions and Evolution* (Grosjean, H., ed.), pp. 550–563, Landes Bioscience
94. Macon, J.B. and Wolfenden, R. (1968) 1-Methyladenosine. Dimroth rearrangement and reversible reduction. *Biochemistry* 7, 3453–3458
95. Agris, P.F. *et al.* (1986) Transfer RNA contains sites of localized positive charge: carbon NMR studies of [13C]methyl-enriched

Escherichia coli and yeast tRNAPhe. *Biochemistry* 25, 5126–5131

96. Mikhailov, S.N. *et al.* (2002) Chemical incorporation of 1-methyladenosine into oligonucleotides. *Nucleic Acids Res.* 30, 1124–1131
97. Motorin, Y. *et al.* (2010) 5-methylcytosine in RNA: detection, enzymatic formation and biological functions. *Nucleic Acids Res.* 38, 1415–1430
98. Micura, R. *et al.* (2001) Methylation of the nucleobases in RNA oligonucleotides mediates duplex-hairpin conversion. *Nucleic Acids Res.* 29, 3997–4005
99. Heus, H.A. *et al.* (1990) Conformational and thermodynamic effects of naturally occurring base methylations in a ribosomal RNA hairpin of *Bacillus stearothermophilus*. *Eur. J. Biochem.* 188, 275–281
100. Heus, H.A. *et al.* (1983) High-resolution proton magnetic resonance studies of the 3′-terminal colicin fragment of 16 S ribosomal RNA from *Escherichia coli*. Assignment of iminoproton resonances by nuclear Overhauser effect experiments and the influence of adenine dimethylation on the hairpin conformation. *J. Mol. Biol.* 170, 939–956
101. Kawai, G. *et al.* (1989) Conformational characteristics of 4-acetylcytidine found in tRNA. *Nucleic Acids Symp. Ser.* 21, 61–62
102. McMahon, M. *et al.* (2015) Small RNAs with big implications: new insights into H/ACA snoRNA function and their role in human disease. *Wiley Interdiscip. Rev. RNA* 6, 173–189
103. Nakazawa, Y. *et al.* (2011) The novel metastasis promoter Merm1/Wbscr22 enhances tumor cell survival in the vasculature by suppressing Zac1/p53-dependent apoptosis. *Cancer Res.* 71, 1146–1155
104. Jangani, M. *et al.* (2014) The methyltransferase WBSCR22/Merm1 enhances glucocorticoid receptor function and is regulated in lung inflammation and cancer. *J. Biol. Chem.* 289, 8931–8946
105. Armistead, J. *et al.* (2009) Mutation of a gene essential for ribosome biogenesis, EMG1, causes Bowen-Conradi syndrome. *Am. J. Hum. Genet.* 84, 728–739
106. Oie, S. *et al.* (2014) Hepatic rRNA transcription regulates high-fat-diet-induced obesity. *Cell Rep.* 7, 807–820
107. Tessarz, P. *et al.* (2014) Glutamine methylation in histone H2A is an RNA-polymerase-I-dedicated modification. *Nature* 505, 564–568
108. Jobert, L. *et al.* (2013) The human base excision repair enzyme SMUG1 directly interacts with DKC1 and contributes to RNA quality control. *Mol. Cell* 49, 339–345
109. Narla, A. and Ebert, B.L. (2010) Ribosomopathies: human disorders of ribosome dysfunction. *Blood* 115, 3196–3205
110. Larrieu, D. *et al.* (2014) Chemical inhibition of NAT10 corrects defects of laminopathic cells. *Science* 344, 527–532
111. Doll, A. and Grzeschik, K.H. (2001) Characterization of two novel genes, WBSCR20 and WBSCR22, deleted in Williams-Beuren syndrome. *Cytogenet. Cell Genet.* 95, 20–27
112. Ounap, K. *et al.* (2013) The human WBSCR22 protein is involved in the biogenesis of the 40S ribosomal subunits in mammalian cells. *PLoS ONE* 8, e75686
113. Ohbayashi, I. *et al.* (2011) Genetic identification of *Arabidopsis* RID2 as an essential factor involved in pre-rRNA processing. *Plant J.* 67, 49–60
114. Tokuhisa, J.G. *et al.* (1998) Chloroplast development at low temperatures requires a homolog of DIM1, a yeast gene encoding the 18S rRNA dimethylase. *Plant Cell* 10, 699–711
115. Sato, G. *et al.* (1999) Prognostic value of nucleolar protein p120 in patients with resected lung adenocarcinoma. *J. Clin. Oncol.* 17, 2721–2727
116. Wu, Q. *et al.* (2005) Determination of the 'critical region' for cat-like cry of Cri-du-chat syndrome and analysis of candidate genes by quantitative PCR. *Eur. J. Hum. Genet.* 13, 475–485