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# **YbeY, *éminence grise* of ribosome biogenesis**

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## **Abstract**

YbeY is an ultraconserved small protein belonging to the unique heritage shared by most existing bacteria and eukaryotic organelles of bacterial origin, mitochondria and chloroplasts. Studied in more than a dozen of evolutionarily distant species, YbeY is invariably critical for cellular physiology. However, the exact mechanisms by which it exerts such penetrating influence are not completely understood. In this review, we attempt a transversal analysis of the current knowledge about YbeY, based on genetic, structural, and biochemical data from a wide variety of models. We propose that YbeY, in association with the ribosomal protein uS11 and the assembly GTPase Era, plays a critical role in the biogenesis of the small ribosomal subunit, and more specifically its platform region, in diverse genetic systems of bacterial type.

## Introduction

Some proteins evolve fast and look like *ad hoc* inventions with narrow, specialised roles, often restricted to a few related species. Others traverse myriads of generations and transcend deepest phylogenetic boundaries barely changing; they usually perform vital functions and constitute the legacy defining entire life forms [1]. The latter is the case for YbeY.

YbeY is found in nearly all bacteria and most eukaryotes, but not in archaea (Fig. 1). The two studied eukaryotic homologues – from *Arabidopsis thaliana* and human – localise in chloroplasts and mitochondria, respectively [2-4]. Additionally, the YbeY proteins of *Plasmodium falciparum* (Apicomplexa) and *Trypanosoma brucei* (Discoba) reside in the apicoplast and the kinetoplast, correspondingly [5, 6], supporting the notion that members of this protein family operate in bacterial-type genetic systems and have been carried over into the eukaryotic kingdom along with the ancestors of the modern plastids and mitochondria.

All studied YbeY homologues are critically important for cell physiology. Their loss may be lethal, as in *Haemophilus influenzae* or *Bacillus subtilis* [7-9], or result in pleiotropic phenotypes hardly compatible with survival in the wild (Table 1). YbeY appears to be of vital importance in Eukarya, too. Its loss in *Arabidopsis* is seedling-lethal due to the lack of photosynthesis [2]. It is also essential in *Plasmodium* [10]. In human, *YBEY* knockout severely impairs mitochondrial respiration [3, 4]; elevated *YBEY* expression has been associated with breast cancer, whereas a loss-of-function variant is a candidate driver of Lynch syndrome [11-13].

Surprisingly, after nearly two decades of research, our understanding of how YbeY works, why it is so important for bacteria and the derived eukaryotic organelles remains rather limited. Current evidence implicates YbeY in key gene expression processes, such as ribosome biogenesis, quality control and turnover, RNA processing, and posttranscriptional regulation. At the molecular level, structural and biochemical evidence strongly points at YbeY being an enzyme. However, existing assays struggle to bridge its *in vitro* activities with the observed phenotypes, making elaboration of detailed mechanistic models exceedingly difficult.

Here we attempt to provide a unifying view of YbeY biology based on available data from multiple species separated by billions of years of evolution (Fig. 1). We discuss the possible ‘aetiology’ of striking molecular deregulations caused by YbeY deficiency (Table 1). In quest for the genuine function of this protein, we dissect its structure, known activities and molecular partners and reflect on plausible molecular mechanisms of YbeY action.

## **Molecular phenotypes of YbeY deficiency: defective ribosomes with far-reaching consequences**

Due to the pleiotropy of phenotypes associated with YbeY loss (Table 1), this protein is sometimes called a multifunctional regulator. However, careful examination of their molecular underpinnings suggests that most of these phenotypes may stem from a unique, recurrently observed molecular cause, i.e., a critical biogenesis defect affecting the small ribosomal subunit (SSU) (Fig. 2).

Early studies demonstrated that ribosomes of *E. coli*  $\Delta ybeY$  strains are much less translationally active than their wild-type counterparts, and this handicap is specifically on the SSU side [14]. Indeed, one striking feature that accompanies YbeY loss in bacteria and chloroplasts is inefficient 16S rRNA processing, especially on the 3'-terminus [2, 9, 15-19]. This means that either the 3'-cleavage event itself or some critical upstream SSU biogenesis step cannot be properly completed in the absence of YbeY. The latter scenario is observed in its pure form in the human mitochondria. In this case, mitochondrial rRNA processing, ensured by two co-opted tRNA-processing enzymes (PRORP and ELAC2), occurs normally without YBEY. Nevertheless, the SSU assembly is impaired, with several head and platform proteins depleted or completely missing [3, 4, 20, 21].

In either case, the lack of YbeY specifically affects the SSU platform which harbours the 3'-end of 16S rRNA. This announces two kinds of problems. First, the SSU becomes unstable and undergoes rapid degradation [3, 4, 9, 16, 22]. For example, in bacteria like *E. coli* aberrant, unspecific internal cleavages sever the 3'-minor domain of 16S rRNA [16, 23]. Second, the platform plays an important role in the presentation of the anti-Shine-Dalgarno sequence and/or the recruitment of initiation factors [24-28]. Problems at this level expectedly compromise translation initiation, as observed in *E. coli* and mitochondria [3, 15]. As a result, YbeY deficiency severely impairs protein synthesis, often presenting as the loss of 70S ribosomes and polysomes and the accumulation of free subunits (Table 1). This likely explains the recurrent growth phenotype (up to lethality) of  $\Delta ybeY$  strains and their characteristic sensitivity to translational inhibitors (Fig. 2). Interestingly, in *B. subtilis* inactivating RNase R, that is responsible for the degradation of defective ribosomes accumulating upon YbeY depletion, rescues the essentiality of the *ybeY* gene in this bacterium: the double mutant grows poorly but survives [9]. Therefore, in some cases the drastic ribosome biogenesis defect caused by YbeY

loss can be, albeit suboptimally, mitigated by readjusting other pathways controlling the ribosome production or turnover.

Being a ‘red button’ of the cellular protein synthesis machinery, YbeY appears to ‘regulate’ the expression of hundreds of genes at both RNA and protein levels [19, 29-33]. This influence occurs via both direct and indirect mechanisms (Fig. 2). Many proteins decrease their level in  $\Delta ybeY$  backgrounds due to insufficient activity of defective ribosomes. Some transcripts also get destabilised in response to low translation, which makes them vulnerable to RNase attack, as it happens with type 3 secretion system-encoding mRNAs in enteropathogenic *E. coli* [34].

The majority of observed gene expression changes, however, appear to be indirect. Indeed, while the interactions of YbeY with ribosomal components are extensively characterised across various species (see below), there is little evidence of its direct association with protein-coding or regulatory RNAs [33, 34]. Instead, a few studies exposed amazingly complex and meandering regulatory cascades underlying some of  $\Delta ybeY$  phenotypes [19, 32, 35]. Pervasive gene expression changes often arise when regulators, such as transcription factors or sRNAs, get involved. This is exactly what has been observed in various bacterial *ybeY* mutants [29, 30, 32, 33, 36]. In *Pseudomonas aeruginosa*, YbeY loss curtails the production of the key  $\sigma$ -factor RpoS, which is responsible for the expression of virulence and stress response genes [19]. In the same organism, a complex cascade involving the posttranscriptional regulators of the Rsm system mediates the upregulation of the type 3 and the downregulation of the type 6 secretion system upon *ybeY* deletion, resulting in high cytotoxicity and low biofilm formation [35]. Thus, by affecting the levels of just a few positive and negative regulators, YbeY deficiency can lead to impressive genome-wide gene expression changes and pleiotropic phenotypes (Fig. 2).

Besides this common ‘phenotypic core’, a few interesting species-specific alterations have been reported (Table 1). In *E. coli* and chloroplasts, the large subunit (LSU) rRNAs also show maturation defects [2, 15], whereas in *C. glutamicum* and the human mitochondria, the SRP RNA and a tRNA<sup>Ser</sup>, respectively, are significantly misprocessed in the absence of YbeY [4, 37]. Based on genetic and/or biochemical experiments, new roles for *E. coli* YbeY in rRNA transcription antitermination [38] and the turnover of defective or damaged ribosomes [16, 17, 23, 39] have been proposed. While some of these phenotypes may be indirect, they again contribute to the key theme of the  $\Delta ybeY$ -associated disorders – defective translation. Understanding the fundamental molecular causes thereof requires detailed analysis of the structure-function relationship and the molecular interactions involving YbeY.

## **YbeY structure and activities: a small, conserved yet elusive enzyme**

Apart from relatively rare decorations (e.g., extensions and additional domains, like in *A. thaliana*), the core YbeY domain is remarkably conserved both sequence- and structure-wise (Fig. 3). Moreover, most YbeY orthologues appear to be genetically interchangeable, i.e., they complement each other's deletion [15, 17, 18, 40, 41], supporting a deeply conserved molecular function transcending the specifics of individual biological contexts.

The YbeY fold consists of five  $\alpha$ -helices arranged around a mixed four-strand  $\beta$ -sheet (Figure 3B). The presumed active centre is formed by nearly invariant residues embedded in  $\alpha 2$ ,  $\beta 3$ ,  $\alpha 4$  and the short  $\alpha 4$ - $\alpha 5$  loop. The latter two elements are of paramount importance because they harbour the emblematic signature HGxLHLxGYDH, whose three histidines coordinate a  $Zn^{2+}$  ion [18, 42, 43]. This zinc is likely architecturally important, since *H. influenzae* YbeY, purified in the presence of EDTA, showed an exploded fold with displaced  $\alpha$ -helices (Fig. 3B), which could be restored by addition of  $Zn^{2+}$  [42].

First structural studies noted a striking similarity between YbeY and matrix metalloproteinases (MMPs) of the met-zincin family [43, 44]. Not only do met-zincins feature a closely related fold (Fig. 4), but they also possess a conspicuously similar consensus motif (HExxHxxGxxH) and a characteristic methionine residue (so-called 'Met-turn'), which structurally supports the  $Zn^{2+}$ -coordinating histidines [45] and has its exact counterpart (M133) in the helix  $\alpha 5$  of YbeY (Fig. 3A and 5B).

More recently, YfcM/EpmC, an enzyme involved in post-translational modification of the elongation factor P in *E. coli*, has been recognised as a structural homologue of YbeY (Fig. 4). In its active centre, two histidines, forming a helix-embedded HExxH motif, together with a more distant glutamate coordinate a cobalt ion, much in the same way as in YbeY and classical MMPs [46]. Interestingly, the metal ions of MMPs and EpmC are directly involved in catalysis, suggesting that YbeY is a  $Zn^{2+}$ -dependent enzyme, too. However, because the critical glutamate residue in the second position of the MMP consensus is replaced in YbeY by a glycine, it is unlikely to participate in proteolytic reactions [44, 47].

Despite the kinship with enzymes involved in protein metabolism, YbeY, both in bacteria and eukaryotes, was found to possess endoribonuclease activity. This activity is relatively weak (often requiring micromolar concentrations to observe cleavages), promiscuous, with a strong preference for single-stranded RNA [2, 3, 16-19, 31, 33, 40, 41, 48]. Interestingly, while YbeY does not cleave double-stranded transcripts, it can completely degrade complex RNA

molecules, such as rRNAs, provided they have single-stranded ‘entry points’ [2, 3, 16, 18, 33, 40]. This property may rationalise the potential role of YbeY in ribosome quality control and turnover [16, 23, 39].

The chemical nature of the RNase activity demonstrated by YbeY preparations raises some questions. Zinc plays a critical role in this activity because its removal with EDTA or replacement with a much less reactive  $\text{Ni}^{2+}$  significantly inhibits cleavage [2, 16-18]. But unlike classical  $\text{Zn}^{2+}$ -dependent RNases (e.g., PRORP and other NYN-domain enzymes), YbeY produces 5'-hydroxyl and 3'-phosphate groups, which is more characteristic of metal-independent enzymes (e.g., RNase A) or of in-line cleavage catalysed by free divalent metals [16, 49-52]. This suggests that YbeY may simply present the catalytic  $\text{Zn}^{2+}$  in an orientation compatible with in-line attack, without being directly involved in the reaction.

The biological significance of the RNase activity is not fully clear either. Analysis of point mutations within the active centre of YbeY yielded discordant and sometimes surprising results across species (Fig. 5). For instance, the three  $\text{Zn}^{2+}$ -coordinating histidines are obviously not equivalent: mutation of the first one, H114, appears to be loss-of-function in most species, whereas the other two residues can be substituted without compromising the ability of the protein to complement *ybeY* deletion [3, 9, 15, 19]. While mutating H114 kills the RNase activity in the *E. coli* and *P. aeruginosa* enzymes [16, 19], an equivalent change in the human homologue has only a minor effect [3]. The consequences of another frequently analysed mutation, R59A, on the RNase activity and the functionality of YbeY *in vivo* are also highly species-specific (Fig. 5). This conserved residue seems to be important in *E. coli* and *P. aeruginosa* but not in *B. subtilis* or human [3, 9, 15, 16, 19].

These discrepancies likely stem from subtle variations in the structure and the stability of different YbeY homologues, making them more or less tolerant to specific alterations. They may also reflect differential requirement for interactions with functionally important partners, whose complement varies between evolutionarily distant groups.

### **YbeY interactions: ribosomal *leitmotiv* with clade-specific variations**

In line with the recurrent translational phenotypes, YbeY was found to interact with ribosomal components in bacteria [33, 34, 53], chloroplasts [54], and mitochondria [3, 4], indicating that this association is deeply conserved and mechanistically important. The privileged contact site appears to be the protein uS11 residing in the SSU platform. The YbeY-uS11 interaction is direct, strong and can occur outside the ribosomal context, as shown by B2H assays for the *E.*



*coli* proteins and by reconstitution of a stable stoichiometric complex between their mitochondrial orthologues [3, 53]. The YbeY-uS11 link is of paramount importance. In *E. coli*, certain mutations in the  $\beta$ -sheet of YbeY (Fig. 5) abolish uS11 binding and induce phenotypes similar to those of the  $\Delta ybeY$  strain (with the double R44D/D85R substitution behaving as a complete loss-of-function) [53]. In human *YBEY* knockout cells, uS11m is strongly depleted from the mitochondrial SSU and destabilised, suggesting that it cannot be properly assembled. A few other SSU proteins, whose recruitment hierarchically depends on the presence of uS11m (e.g., the platform proteins bS21m and mS37), follow suit. Importantly, uS11m knockdown phenocopies *YBEY* deficiency, confirming an intimate mechanistic connection between the two proteins [3].

While YbeY does not interact stably with mature ribosomes [3, 4, 14, 55-57], it seems to recognise and transiently associate with certain SSU biogenesis intermediates [33, 54], most prominently those containing the highly conserved assembly GTPase Era. YbeY and Era interact closely in mitochondria and bacteria [3, 4, 53, 58], up to the point that the two proteins may be fused in one polypeptide in certain Firmicutes and Acidobacteria. This interaction is meaningful: Era associates with immature SSUs in the immediate vicinity of the platform and plays a critical role in its shaping [59, 60]. Era depletion phenotypically resembles YbeY loss: 16S rRNA processing is impaired, 70S ribosomes fail to assemble, and translation stops [58, 60]. Additionally, the SSU platform gets disordered, uS11 fails to assume a fixed position while bS21 is virtually absent, which resembles the outcome of *YBEY* knockout in mitochondria [3, 60]. The loss of its human orthologue ERAL1 also compromises the SSU stability and mitochondrial translation, provoking the Perrault syndrome [62-64]. Importantly, overexpression of Era in  $\Delta ybeY$  *E. coli* significantly improves the 16S rRNA processing and the 70S ribosome assembly [22], further highlighting the tight mechanistic connection between the two proteins.

The YbeY-uS11-Era trio seems to constitute a core functional unit conserved from bacteria to eukaryotic organelles. By contrast, a few other YbeY partners are restricted to certain phylogenetic groups [53]. For example, the putative ATPase YbeZ/PhoL, found in many bacterial clades and often encoded in one operon with YbeY (Fig. 6), interacts with the latter physically and shows similar phenotypes upon deletion [19, 35, 53, 65]. The functional significance of this highly conserved association is currently unknown. In mitochondria, the top-scoring and apparently obligate *YBEY* interactor is the highly conserved eukaryotic protein p32. *YBEY* and p32 form a stable stoichiometric complex that can additionally recruit uS11m

[3]. Like YBEY, p32 interacts with mitochondrial ribosomes and is essential for mitochondrial translation and cellular respiration, although its molecular mechanism remains elusive [66-68]. Mutations in p32 result in severe mitochondrial diseases, whereas its upregulation is a hallmark of aggressive cancers [69-74]. While its contribution to YBEY function is not entirely clear, it seems to play an important structural role. Unlike bacterial YbeY homologues which are mostly negatively charged, mitochondrial YBEY proteins are usually neutral and, therefore, poorly soluble in isolation. Association with the highly negatively charged p32 endows human YBEY with sufficient solubility and may facilitate its interactions with positively charged ligands, such as uS11m [3].

### **The molecular mechanism of YbeY: learning from eukaryotes?**

While the exact molecular mechanism of YbeY is a mystery, the mist surrounding it is getting thinner. Based on interactomic and phenotypic data from phylogenetically diverse models, one can now with reasonable confidence situate its intervention at some relatively advanced SSU maturation stage taking place at the level of the platform. Late biogenesis events are conceptually similar in different types of ribosomes, and we believe that comparison with the better studied eukaryotic SSU assembly pathway may provide useful insights into the possible roles of YbeY in the maturation of its bacterial-type counterpart (Fig. 7).

Consolidation of the platform in all organisms involves bringing together parts of the central and 3'-minor (helix 45) domains [75-78]. In eukaryotes, this critical step consists of several events (Fig. 7, upper side). The Eukarya/Archaea-specific ATPase Fap7 associates with uS11 and ensures its correct incorporation in the platform, probably with the help of the KH-domain assembly factor Krr1, with which they form a stable ternary complex [79-82]. The universally conserved rRNA methyltransferase Dim1 modifies two invariant adenines on the tip of h45 [83]. The tectonic movements shaping the platform involve a hierarchical action of two KH-domain assembly factors interacting with uS11: the already mentioned Krr1 and Pno1. The latter, being associated with h45, displaces Krr1 and takes its position next to uS11 [77, 78, 82, 84-87]. After these events, the endoribonuclease Nob1 processes the 3'-end of pre-18S rRNA [88, 89], permitting the incorporation in the platform of the last r-protein eS26 [86, 90].

Bacterial-type SSU assembly, although less well understood, likely follows similar logic and features related protein factors (Fig. 7, lower side). The methyltransferase KsgA/RsmA (TFB1M in mitochondria) modifies the same conserved adenines in h45 [91, 92]. Two KH-domain assembly factors are involved in the platform maturation: the already mentioned early-

binding GTPase Era and the late factor RbfA, which seem to interact with the SSU near the 3'-end of 16S rRNA in a mutually exclusive, possibly sequential fashion [25, 59, 62, 93-95]. The ultimate biogenesis step in bacteria is the 3'-processing of 16S rRNA, which likely licences the recruitment to the platform of the last r-protein bS21 (bS21m and mS37 in mitochondria), as suggested by structural, biochemical, and genetic data [3, 25, 48, 60, 75, 96-104]. In bacteria, deregulations of this assembly process compromise the final 3'-processing event [61, 105].

There are only two unassigned slots in this scheme which both benefit YbeY. It may act as a chaperone for uS11, a role supported by physical and genetic interactions between YbeY, Era and uS11 and the assembly phenotype observed in the mitochondria. It may also function as a 3'-processing endoribonuclease analogous to Nob1, which agrees with its *in vitro* RNase activity. These roles are not mutually exclusive: YbeY may first incorporate uS11 in the immature SSU and then, using its strategic position next to the 3'-end of 16S rRNA [34, 53], perform the processing reaction.

Both models must face certain challenges. For example, *E. coli* YbeY cannot process 16S rRNA precursors in the context of SSU intermediates *in vitro*, whereas four other exoribonucleases can do the job [48, 106]. In human mitochondria, rRNA processing is fully YBEY-independent [3, 4, 20, 21]. By contrast, in *B. subtilis* no other RNase seems to account for this cleavage, leaving YbeY the only plausible candidate [9]. So-far, all attempts to reconstitute precise 3'-processing by YbeY *in vitro* failed, suggesting that key ingredients of this reaction might be still missing [9, 16, 48]. On the other hand, the role of YbeY in uS11 assembly has only been demonstrated in mitochondria [3] and, although the YbeY-Era-uS11 interactions are conserved between the two systems, it is currently unknown whether YbeY has the same function in bacteria. Furthermore, this assembly model is so-far based on interactomic and phenotypic data; a more direct assay will be required to substantiate such a mechanism.

### **Future directions**

As for now, YbeY remains an *éminence grise* of ribosome biogenesis: its influence is vital, but its ways are still largely concealed. Their uncovering will require sorting out three key questions:

- (i) What is the genuine, biologically relevant activity of YbeY?
- (ii) What is the precise mechanism and the rationale of its interactions with its partners (especially Era and uS11)?
- (iii) How did the organisms which had lost YbeY learn to thrive without it?

Answering these questions will necessitate deeper mechanistic studies and will certainly benefit from looking in evolutionarily divergent genetic systems beyond traditional models, including the Candidate Phyla Radiation and the mitochondria of various eukaryotic clades (Fig. 1).

### **Perspectives**

- YbeY is an ultraconserved protein playing critical roles in the physiology of most bacteria and bacteria-derived organelles (mitochondria and chloroplasts).
- Current consensus implicates YbeY in key gene expression processes, such as ribosome biogenesis, quality control and turnover, RNA processing, and posttranscriptional regulation. Its role in the maturation of the small ribosomal subunit is deeply conserved and likely represents the main cause of the pleiotropic phenotypes associated with YbeY loss.
- Understanding the molecular mechanism of YbeY will require detailed studies of its enzymatic activities, the mode and the rationale of its interactions with partner proteins, such as GTPase Era and the ribosomal protein uS11. This will be facilitated by probing evolutionarily distant biological contexts, beyond standard model species.

### **AUTHOR CONTRIBUTIONS**

ZL. and CS. prepared the initial draft; CS and AS. prepared figures; AS. revised the draft and prepared the final version of the manuscript.

### **CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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## FIGURE LEGENDS

**Figure 1. Phylogenetic distribution of YbeY.** The phyla (capitalised) and supergroups (uppercase) which possess YbeY homologues are coloured with different colours, whereas those lacking YbeY are filled in grey (situation in January 2021, according to UniProt). In

certain clades (e.g., Mucoromycota and Peregrinibacteria), some species have YbeY whereas others have lost it), which is highlighted with partial grey filling. The species with YbeY homologues described in literature are shown. SAR – Stramenopiles-Alveolata-Rhizaria group, CPR – Candidate Phyla Radiation. Phylogenetic groups are given according to [109, 110].

**Figure 2. The likely hierarchy of phenotypes associated with YbeY deficiency in various species.** The primary driver of the pleiotropic phenotypes appears to be a severe small subunit (SSU) biogenesis defect, which manifests itself in the impairment of 16S rRNA 3'-processing (in bacteria and chloroplasts) or SSU assembly problems (in mitochondria). This results in SSU destabilisation and decreased translational activity, explaining the sensitivity of  $\Delta ybeY$  bacteria to ribosome-targeting antibiotics. Insufficient translation impacts protein synthesis directly and through destabilisation of mRNAs, resulting in growth inhibition. Through decreased production of positive (P) or negative (N) regulators, e.g., transcription factors, YbeY loss can cause pervasive downregulation or upregulation of their targets (T), respectively. This triggers multiple physiological and morphological phenotypes and impacts on ecological relationships (virulence, symbiosis) with other species. See Table 1 for a detailed overview of phenotypes in each species.

**Figure 3. YbeY is an ultraconserved protein.** (A) Sequence alignment of the YbeY homologues described in literature (see Fig. 1 for the phylogenetic positions of the species). Secondary structure elements are mapped based on the *E. coli* structure [111]. The multiple alignment is performed with COBALT [112]. The sequence logo is created with WebLogo [113]. Residues are coloured according to the physicochemical properties of their side chains (positively charged – blue, negatively charged – red, other hydrophilic – green, hydrophobic – black). YBEY homologues from *A. thaliana* and other plants often have an additional C-terminal Cof/HAD family hydrolase domain (IPR000150) of unknown functional significance. (B) Tertiary structures of four studied YbeY homologues. The three histidines of the HGxLHLxGYDH motif are shown in red, the coordinated  $Zn^{2+}$  observed in the *E. coli* structure is yellow. PDB codes: 1XM5, 1OZ9, 1TVI, 1XAX.

**Figure 4. Structural similarity between YbeY, matrix metalloproteinases and YfcM/EmpC.** The conserved metal-coordinating residues are shown in red,  $Zn^{2+}$  is yellow and  $Co^{2+}$  is pink. PDB codes: 2OW2, 1XM5, 3WTR.

**Figure 5. Overview of YbeY point mutations and their effects in diverse organisms.** (A) The corresponding residues are aligned between orthologues as shown in Fig. 3A, and their



effects on *in vitro* RNase activity, uS11 binding or stability, 16S rRNA processing, and other phenotypes are colour-coded, based on literature [3, 9, 15, 16, 18, 19, 41, 53]. For the *L. asiaticus* YbeY, only its ability to complement the *E. coli* *ybeY* deletion has been tested *in vivo* [41]. **(B)** Position of the corresponding residues in the 3D structure of the *E. coli* YbeY protein shown in ‘back’ (left) and ‘front’ (right) views. The colour code is the same between the two panels.

**Figure 6. Genomic context of *ybeY* genes in model bacterial species.** Recurrently co-occurring genes and the corresponding protein families are expanded on the right. Additional genes and protein families found next to *ybeY* only in some clades are encoded as follows: “*cydA*” - cytochrome oxidase d subunit I (PF01654: Cyt\_bd\_oxida\_I); “*cydB*” - cytochrome oxidase d subunit II (PF02322, Cyt\_bd\_oxida\_II); “*EF-G*” - elongation factor G, *fusA* (PF00679: EFG\_C, PF14492: EFG\_III, PF03764: EFG\_IV, PF00009: GTP\_EFTU, PF03144: GTP\_EFTU\_D2); “*lysS*” - lysyl-tRNA synthetase (PF00152: tRNA-synt\_2, PF01336: tRNA\_anti-codon); “*MMCoA*” - methylmalonyl-CoA mutase,  $\alpha$ -subunit, chain A (PF01642: MM\_CoA\_mutase); “*murC*” - UDP-N-acetylmuramate-*L*-alanine ligase (PF01225: Mur\_ligase, PF02875: Mur\_ligase\_C, PF08245: Mur\_ligase\_M); “*nfeD*” - PF01957: NfeD, PF01972: SDH\_sah; “*rsmB*” - 16S rRNA m<sup>5</sup>C967 methyltransferase (PF01189: Methyltr\_RsmB-F, PF01029: NusB); “*speE*” - polyamine aminopropyltransferase (IPR030374: PABS, IPR029063: SAM-dependent\_Mtases, IPR001045: Spermi\_synthase); “*tmcAL*” - tRNA<sup>Met</sup> cytidine acetate ligase (PF05636: HIGH\_NTase1); “*ydfA*” - UPF0365 protein (PF12127: YdfA\_immunity); “*1015*” - PF06245: DUF1015; “*H*” - heat-inducible transcription repressor, *hrcA* (PF01628: HrcA); “*J*” - chaperone, *dnaJ* (PF00226: DnaJ, PF01556: DnaJ\_C, PF00684: DnaJ\_CXXCXGXG); “*K*” - PF07295: DUF1451; “*L*” - *yqzL* (PF14006: YqzL); “*M*” - cytidine deaminase, *ccd* (PF00383: dCMP\_cyt\_deam\_1); “*N*” - PF02698: DUF218; “*P*” - UPF0719 transmembrane protein (PF03994: DUF350); “*Q*” - glycyl-tRNA synthetase  $\alpha$ -subunit, *glyQ* (PF02091: tRNA-synt\_2e); “*S*” - glycyl-tRNA synthetase  $\beta$ -subunit, *glyS* (PF02092: tRNA\_synt\_2f); “*T*” - cation efflux system membrane protein (PF01545: Cation\_efflux, PF16916: ZT\_dimer); “*U*” - nitrogen fixation protein, *nifU* (PF08712: Nfu\_N, PF01106: NifU); “*V*” - PF13785: DUF4178; “*W*” - heme chaperone, *hemW* (PF06969: HemN\_C, PF04055: Radical\_SAM); “*a*” - shikimate dehydrogenase (NADP<sup>+</sup>), *aroE* (PF18317: SDH\_C, PF01488: Shikimate\_DH, PF08501: Shikimate\_dh\_N); “*b*” - ribosomal protein uS7, *rpsG* (PF00177, Ribosomal\_S7); “*c*” - PTS system, glucose-specific IIA component, *crr* (PF00358: PTS\_EIIA\_1); “*d*” - ribosomal protein uS12, *rpsL*

(PF00164: Ribosom\_S12\_S23); “e” - transcription inhibitor protein, *gfhI* (PF01272: GreA\_GreB, PF03449: GreA\_GreB\_N); “g” - *grcA* (PF07750: GcrA); “i” - glutamate/aspartate import solute-binding protein, *gltI* (PF00497: SBP\_bac\_3); “j” - glutamate/aspartate import permease protein, *gltJ* (PF00528: BPD\_transp\_1); “k” - DUF1893 domain-containing protein (PF08973: TM1506); “l” - lipoyl synthase, *lipA* (PF04055: Radical\_SAM); “p” - isoprenyl transferase, *uppS* (PF01255, Prenyltransf); “q” - *yqfC* (PF07873: YabP); “s” - PF08238: Sel1; “u” - PF08850: DUF1820; “v” - PF01988: VIT1; “y” - *yqfD* (PF06898: YqfD); “z” - putative metal-dependent peptidase (PF04298, Zn\_peptidase\_2). The locus between *lnt* and *gltI* in enterobacteria is highly variable even within one species: for example, it contains a transposase gene (PF01609: DDE\_Tnp\_1, PF05598: DUF772) in *E. coli* K-12 strains, a phosphohydrolase gene (PF01243: Putative\_PNPOx) in *Y. enterocolitica*, but is empty in most other Enterobacterales.

**Figure 7. Possible molecular mechanisms of YbeY.** Key SSU biogenesis processes accompanying the maturation of the platform in eukaryotes are summed up in the upper side of the scheme. Their hypothetical counterparts in the biogenesis of bacterial-type SSUs and two possible roles for YbeY are shown in the lower side. Transient or short events are marked with vertical black lines. The physical interactions between Krr1, Fap7 and uS11 and an analogous association between Era, YbeY and uS11 are indicated with blue dotted lines. ‘*KH-switch*’ refers to the main tectonic movement that brings h45 bound to Pno1 to its mature position in the platform, provoking the displacement of Krr1.

**Table1. Phenotypic consequences of YbeY deficiency in various species.**

Organism	Physiological phenotypes of YbeY deficiency	Molecular phenotypes of YbeY deficiency	Source
<b><math>\alpha</math>-proteobacteria</b>			
<i>Agrobacterium tumefaciens</i>	Not essential. Moderate growth defect in complex medium; reduced swimming motility; sensitivity to translational inhibitors and hydroxyurea; impaired virulence.	Multiple gene expression changes; mild processing defect for 16S rRNA (17S rRNA accumulation).	[33]
<i>Sinorhizobium meliloti</i>	Not essential. Severe growth delay; strong symbiotic defect on alfalfa; impaired swimming and swarming motility; sensitivity to oxidative, envelope, and heat stresses, DNA damaging agents, cell wall-targeting and translational antibiotics.	Pervasive gene expression changes; 70S ribosome assembly defect (paralleled by increased free subunits); 3'- and 5'-processing defect; accumulation of a 16S rRNA precursor; mild decrease of mature 16S rRNA; 23S rRNA is not visibly affected.	[18, 31, 36, 107]
<i>Brucella abortus</i>	Not essential. Small colonies; moderate growth defect; morphological abnormalities; impaired virulence in murine macrophages; sensitivity to oxidative and envelope stresses; multiple metabolic deregulations (e.g., inability to grow on butyric and caproic acids).	Multiple gene expression changes; accumulation of a 16S rRNA precursor, but no visible effect on the levels of mature 16S and 23S rRNAs.	[18, 32]
<b><math>\gamma</math>-proteobacteria</b>			
<i>Escherichia coli</i>	Not essential. No growth defect in minimal medium but slower growth in LB medium at 37°C; severe thermosensitivity (growth arrest at 42°C, heat shock intolerance); sensitivity to cell wall-targeting antibiotics, DNA damaging agents, translational inhibitors, envelope and oxidative stresses; resistance to hydroxyurea.	Pervasive gene expression changes; moderate translation defect at 37°C; complete loss of translation at 42°C; decreased polysomes and increased free subunits already at 37°C; higher frame-shift and stop codon read-through rates; the SSU is defective: low activity in translation, severe loss of IF2 binding, 16S rRNA processing and stability defects (decreased 3'- and 5'-processing, accumulation of 17S rRNA and a truncated 16S* rRNA species), decrease of mature 16S rRNA (completely disappears at 45°C); LSU is not visibly affected but 23S and 5S rRNA precursors do accumulate, too.	[14-17, 22, 23, 29, 53, 108]
<i>Enterohemorrhagic E. coli (EHEC)</i>	Not essential. Slow growth at 30°C, 37°C, and 42°C; severe swimming motility defect.	Decreased expression of type 3 secretion system genes; translation defect; accumulation of 17S rRNA and a decrease of mature 16S rRNA upon shifting the culture to 45°C.	[34]
<i>Yersinia enterocolitica</i>	Not essential. Severe growth defect at 4°C, 22°C, and 37°C; no growth at 42°C; enhanced aggregation after overnight growth at 37°C; intolerance to heat shock and low pH; decreased arabinose and galactose utilization; inability to grow on <i>myo</i> -inositol; reduced adhesion to HeLa cells but no effect on cell invasion.	Pervasive gene expression changes; global upregulation of virulence plasmid-encoded genes; 'cold-shock-like' response; 16S rRNA is strongly reduced; LSU rRNAs are not affected.	[30]

<i>Vibrio cholerae</i>	Essential. Depletion causes severe growth defect; small colonies; loss of pigmentation; impaired biofilm formation; sensitivity to bile salts, heat and cold; virulence defect in an infant mouse model; sensitivity to translational, transcriptional, and cell wall-targeting antibiotics, UV, and oxidative stress.	Deregulation of several sRNAs; impaired cholera toxin production; 70S ribosome assembly defect (paralleled by increased free subunits); 3'- and 5'-processing defect for 16S rRNA and accumulation of a 16S rRNA precursor; decrease of mature 16S rRNA; LSU rRNAs are not affected.	[17]
<i>Pseudomonas aeruginosa</i>	Not essential. Moderate growth defect; severe virulence defect in a murine acute pneumonia model; enhanced cytotoxicity; sensitivity to oxidative stress; reduced biofilm formation.	Pervasive gene expression changes; decreased catalase activity; low $\sigma^S$ transcription and translation; deregulated Rsm system; upregulated type 3 secretion system genes; downregulated type 6 secretion system genes; 70S ribosome assembly defect (paralleled by increased free subunits); accumulation of a 16S rRNA precursor; decrease of mature 16S rRNA.	[19, 35]
<b>Firmicutes</b>			
<i>Bacillus subtilis</i>	Essential. Depletion causes growth arrest, which can be rescued by deletion of RNase R.	70S ribosomes are nearly undetectable (paralleled by increased free subunits); slight accumulation of pre-16S rRNA; impaired 3'- but normal 5'-processing of 16S rRNA; LSU rRNAs are not affected.	[9]
<b>Actinobacteria</b>			
<i>Corynebacterium glutamicum</i>	Not essential. Impaired growth.	4.5S RNA 3'-processing defect.	[37]
<b>Thermus-Deinococcus group</b>			
<i>Thermus thermophilus</i>	Not essential.	Pervasive gene expression changes.	[65]
<b>Streptophyta</b>			
<i>Arabidopsis thaliana</i> (chloroplast)	Essential. Disruption causes pale-green leaves and cotyledons; slow growth and developmental defects up to seedling lethality (if expression is completely abrogated); diminished photosynthesis; lower amount of chloroplasts; abnormal thylakoid system.	Reduced chlorophyll content; decreased PSII supercomplexes, PSI monomers/PSII dimers; moderately increased MHCII trimers; strong decrease of some plastid-encoded proteins; no anomalies at the level of mRNAs and tRNAs; processing defect of 16S, 23S and 4.5S rRNAs (accumulation of precursors with both ends unprocessed); mature 16S and 4.5S rRNAs are moderately decreased; 5S rRNA is not visibly affected.	[2]
<b>Chordata</b>			
<i>Homo sapiens</i> (mitochondrion)	Mitochondrially essential (knockout cells do not grow on non-fermentable carbon sources). Slow growth on glucose; enlarged and misshaped mitochondria with few cristae and other morphological defects; impaired cellular respiration; increased glycolysis.	Severe complex I and IV deficiencies; levels of multiple mitochondrial transcripts are affected; mitochondrial translation is nearly fully abolished; the mitochondrial SSU is defective: 12S rRNA is decreased and unstable, several r-proteins in the head and the platform (uS11m, bS21m, mS37) are depleted; mild LSU assembly defect; significant misprocessing of tRNA <sup>Ser</sup> <sub>AGY</sub> .	[3, 4]