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## Review article

# Structure of the ribosome from highly developed organisms. Mini review

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**Abstract.** Ribosomes of highly evolved organisms are larger than their bacterial counterparts not only in terms of the number of proteins but also in terms of RNA length extensions. Some extensions do not fold into the three-dimensional structure of the ribosome in cryo-EM analysis and cannot be crystallized for X-ray examination. These expansion segments are not visible due to flexibility. In this mini-review, we propose an approach to study the chick embryo ribosome as an example of a highly evolved organism to visualize the expansion segments and study the mechanism of in situ tetramerization and crystallization of ribosomes.

**Keywords.** Cryo-EM, RNA extension segments, Structure, Chick embryo, Ribosome.

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## 1. Introduction

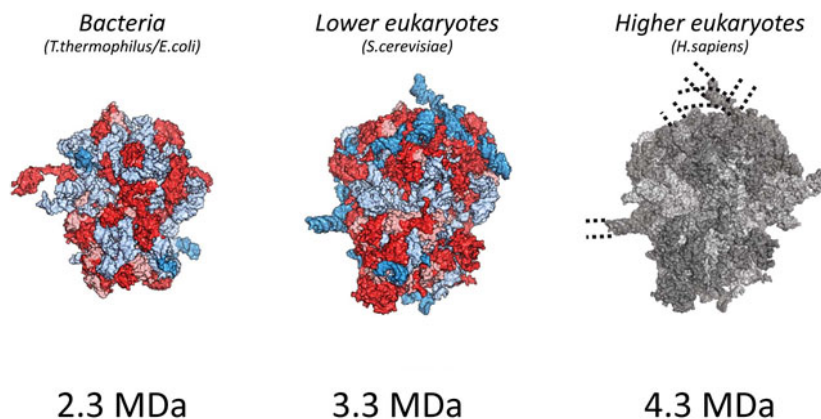
Ribosomes are multi-megadalton cellular machineries constituted of proteins and rRNA in charge of protein biosynthesis. Throughout evolution, ribosomes have increased in size. Expansions occur both at the protein level, with the enlargement of all large ribosomal proteins, and at the RNA level—with the increase in length of ribosomal RNA. The expansions within ribosomal RNA clusters in conserved regions facing the periphery of the ribosome. Recent biochemical and structural studies have shed light on how specific rRNA extension segments (ESs) interact with protein factors during translation. High-resolution crystal structures of bacterial and yeast ribosomes (Figure 1), in combination with more recent cryo-EM analyses, have been crucial in elucidating the fundamental mechanisms of protein synthesis in prokaryotic and eukaryotic systems (Mel-

nikov et al., 2012; Noller, 2024; Jackson et al., 2010). However, after 35 years of ribosome structural studies, there is little structural information about some ribosomal elements such as the L7/L12-stalk (P-stalk for eukaryote), the protein pentameric complex and several rRNA expansion segments (ES) of highly developed organisms (Melnikov et al., 2012; Yusupova and Yusupov, 2017; Nurullina et al., 2024).

A genome sequence comparison of ribosomal RNA from different kingdoms indicates an extension of the rRNA as the complexity of the organisms increases (Armache et al., 2010a; Armache et al., 2010b). Among the eukaryotes, the mass of the ribosome varies from 3.3 MDa in unicellular eukaryotes to 4.3 MDa in humans (Figure 1). This difference is mainly due to the enlargement of four specific expansion segments distributed across the rRNA (Hariharan et al., 2023). The expansion segments are generally located at the solvent surface and do not directly interact with ribosome functional sites (Armache et al., 2010a; Taylor et al., 2009; Ben-Shem et al., 2011). Even though the locations of the insertion sites of the

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**Figure 1.** Crystal structures of bacteria 70S and yeast 80S ribosomes with molecular weight according to 2.3 and 3.3 MDa. Cryo-EM structure of the human 80S ribosome, as an example of the ribosome from highly developed organisms with a molecular weight of 4.3 MDa.

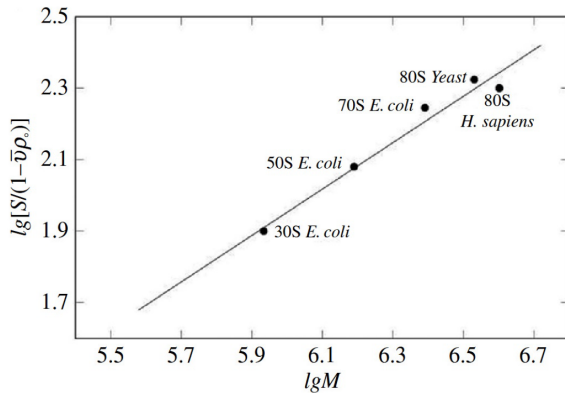
expansion segments coincide, their length and nucleotide composition differ among eukaryotes: ESs are generally more GC-rich in vertebrates than in invertebrates and unicellular eukaryotes (Armache *et al.*, 2010a; Armache *et al.*, 2010b).

Structurally, rRNA expansion segments can be divided into two clusters: the first type is known to interact with ribosomal proteins or rRNA, such as ES31L and ES39L (Figure 4); the second type of rRNA ES includes long rRNA helices anchored to the ribosome via their bases. ESs belonging to this group can adopt different conformations, as observed for the flexible ES6S and ES27L, although their functional role is not yet clear (Melnikov *et al.*, 2012). Studies have been carried out to investigate the involvement of ESs in translational fidelity, ribosome biogenesis, and protein folding (Hariharan *et al.*, 2023). ES6S, located in the vicinity of the mRNA entry and exit sites, and ES27L, localized close to the polypeptide exit tunnel, were shown to help translation factors access the ribosome (Shankar *et al.*, 2020; Beckmann *et al.*, 2001). Using systematic deletion experiments of specific ESs, it was shown that almost all ESs in *Saccharomyces cerevisiae* are required and vital for ribosome biogenesis (Ramesh and Woolford, Jr., 2016). Also, it was demonstrated that ES27L and ES7L orchestrate the binding and anchoring of the acetyltransferase NatA complex, which is required for acetylation of the nascent peptide chain and facilitates binding of the conserved enzyme methionine aminopeptidase (MetAP) responsible for cleaving

off the N-terminal methionine residue (Knorr *et al.*, 2019; Fujii *et al.*, 2018). Generally, ESs are believed to be involved in many different processes that maintain ribosome efficiency (Hariharan *et al.*, 2023).

An interesting observation was made while comparing the sedimentation coefficients of bacterial, yeast and human ribosomes. Comparison of the sedimentation coefficients of the bacterial ribosomal 50S and 30S subunits, the bacterial 70S ribosome, the yeast 80S ribosome and the human ribosome showed a partially unfolded structure of the human ribosome. The experimental sedimentation coefficients of the human ribosome and the yeast ribosome were 78S and 80S, respectively (Figure 2). However, the molecular weight of the human ribosome is approximately 1,000,000 Da more than the yeast ribosome owing to the longer expansion segments of the 28S RNA. A partially unfolded structure of the human ribosome expansion segments made the crystallization of this ribosome difficult and made other structure functional studies challenging to accomplish.

Most studies regarding the function of expansion segments have been carried out in yeast, whose expansion segments are shorter than in mammals (Shankar *et al.*, 2020). The main challenges in answering these enduring questions lie in the different intrinsic limitations of structural techniques, such as the need for crystal growth in crystallography and the limited resolution caused by flexibility and averaging in cryo-EM and cryo-ET. The rRNA expansion



**Figure 2.** Dependence of the sedimentation coefficient of the ribosome particle on molecular weight. Graphic kindly provided by S. Agalarov.

segments and protein elements mentioned above are highly flexible and often unstructured, which hinders both proper crystallization—crucial for achieving high-resolution data—and accurate image alignment in cryo-EM and cryo-ET, essential for resolving fine structural details (Armache et al., 2010a; Armache et al., 2010b; Holvec et al., 2024). A successful approach to visualize expansion segments is cryo-ET in situ, which has been used efficiently to resolve ES of *Dicystostelium discoideum* ribosomes. Despite the low resolution achieved, Hoffmann and collaborators depicted the three-dimensional features of rRNA ES in a near-physiological environment, capturing them in complex with several translation factors. Their structural work supported the long-standing hypothesis behind the role of ES in translation fidelity and its fine-tuning, thus suggesting their transversal role in the evolution of higher eukaryotes (Hoffmann et al., 2022). An alternative way to investigate the structure of these flexible regions involves a more biological rather than technical approach. Starting from functional data, researchers made the hypothesis that some protein factors could lock such regions in a certain conformation, allowing their visualization. It is the case of the maturation protein Arx1, which interacts with ES27L in the pre-60S particles of *S. cerevisiae* ribosomes. The interaction with Arx1 stabilizes the region, making the ES27L flexible helix a more stable hub for further protein interactions that would lead to the export of the pre-60S ribosomes to the cytosol (Greber et al., 2012).

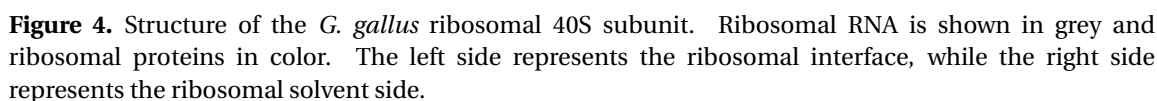
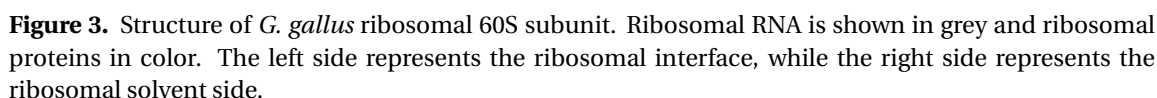
## 2. Natural crystals of ribosomes of highly developed organisms

An important observation, made several decades ago, was the identification of ribosome hibernation in *Gallus gallus*. During study of the hypothermic effects on chicken microtubules, highly ordered bidimensional layers of ribosomes were discovered (Barbieri, 1979a; Barbieri, 1979b; Barbieri, 1979c). Bidimensional layers were studied using electron diffraction techniques, thus demonstrating their crystalline nature, with each unit cell containing four ribosomes—tetramers (Milligan and Unwin, 1986). Tetramers were found and isolated from all tissues of the embryo, demonstrating that under cold exposure chicken ribosomes can exist in various forms: monomeric, tetrameric, and in crystalline layers (Barbieri, 1979b; Milligan and Unwin, 1982). Several questions regarding the formation, the structure and the role of these tetramers and larger aggregates remain unanswered. Due to the compact form of both layers and tetramers, it is easy to think that expansion segments and protein factors might play a role in the formation of ribosomal sheets, by stabilizing ribosomes via rRNA–rRNA and/or rRNA–protein interactions. The study of this phenomenon began in the early 70s, but due to a lack of technical capabilities, no certain results have been obtained.

Ribosome crystalline layers have been found in sand lizard (*Lacerta agilis*) and common chicken eggs (*Gallus gallus*) exposed to cold stress (Porte and Zahnd, 1961; Ghiara and Taddei, 1966). We can conclude that this is a physiological response occurring under conditions of stress-induced hypothermia. The ability of the ribosomes from chick embryos to form tetramers and 2-D crystals in situ under stress conditions allows its purification and recrystallization (Milligan and Unwin, 1982). Isolated *G. gallus* ribosomes could be used for the stabilization and structural study of expansion segments.

## 3. *Gallus gallus* ribosomes for structural study. Possible stabilization of expansion segments

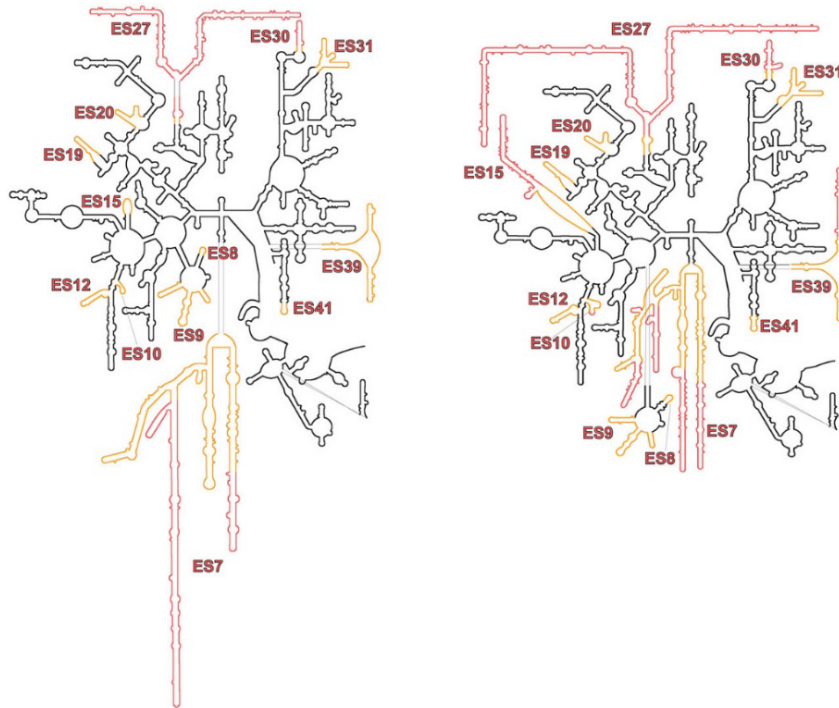
As a starting point of our research, the recently structure of isolated *G. gallus* 80S ribosomes (Figures 3 and 4) has been determined by a cryo-EM study (Nurullina et al., 2024).



The interface between ribosomal subunits, as well as the area surrounding the mRNA entrance and the polypeptide exit tunnel, is highly conserved and contains very few expansion segments and eukaryote-specific proteins. The ribosomal RNA expansion elements are located predominantly on the periphery of the solvent-exposed sides of both subunits of the eukaryotic ribosome.

The insertions of expansion segments in the sequence of the rRNA of *G. gallus* is conserved among other eukaryotic organisms. However, the length of these inserted ES differs between species, and some features of the expansion segments are specific to avians (Figure 5). Our map revealed differences in the structural arrangement of expansion segments in the 28S rRNA of *Gallus gallus*. The main differences, when compared to its human counterpart, were observed in the highly variable expansion segment ES7L and between the interaction patterns of the expansion segments ES15L, ES10L, ES9L and ES7L. These differences suggest that the packaging of expansion segments may vary from species to species. The ES7L tentacle was found to be longer than in other species,





**Figure 5.** Secondary structure of 28S rRNA: on the left, the secondary structure from *Gallus gallus*; and on the right, the one from *Homo sapiens*. In black are the rRNA conserved between the two species. Expansion segments are represented in yellow/red color. Red indicate the parts of expansion segments which are flexible and not visible in cryo-EM three-dimensional structure.

and one of our assumptions was that this tentacle could participate in the formation of tetramers and aggregates (Figure 5). The solved structure of the 80S *G. gallus* ribosome can be further utilized while working on the structure of the ribosomal tetramers and aggregates.

#### 4. Conclusion

We suggest that the stabilization of chick embryo ribosomes is possible in an environment similar to stress cold conditions in vivo. It is impossible to imagine the crystallization of ribosomes containing unfolded expansion segments into 3-dimensional crystals, as was shown earlier (Milligan and Unwin, 1982). We conclude that the behavior of human ribosome during sedimentation experiments is the result of partial unfolding of ribosomal RNA during purification and sample preparation. Similar results of unfolded expansion segments were obtained in all cryo-EM studies of ribosomes from highly developed or-

ganisms including human and chick embryos (Nurullina et al., 2024; Armache et al., 2010a; Armache et al., 2010b). Therefore, it is becoming clear that some sort of stabilization is needed to decipher the ES spatial disposition. Finding the right model to address these structural questions about the role of ES is of paramount importance to dive deeper in the understanding of translation regulation at the molecular level. For this reason, we suggest using in situ cryo-ET to study ribosomal tetramers and aggregates that are stabilized under cold stress conditions. This could help resolve their three-dimensional structure, elucidate the mechanism of aggregate formation, and visualize expansion segments during ribosomal function or intermolecular contact formation.

#### Declaration of interests

The authors do not work for, advise, own shares in, or receive funds from any organization that could benefit from this article, and have declared no affiliations other than their research organizations.

## References

- Armache, J. P., A. Jarasch, A. M. Anger, et al., "Cryo-EM structure and rRNA model of a translating eukaryotic 80S ribosome at 5.5-A resolution", *Proc. Natl. Acad. Sci. USA* **107** (2010), pp. 19748–19753.
- Armache, J. P., A. Jarasch, A. M. Anger, et al., "Localization of eukaryote-specific ribosomal proteins in a 5.5-A cryo-EM map of the 80S eukaryotic ribosome", *Proc. Natl. Acad. Sci. USA* **107** (2010), pp. 19754–19759.
- Barbieri, M., "Ribosome crystallization in homogenates and cell extracts of chick embryos", *J. Supramol. Struct.* **10** (1979), pp. 349–357.
- Barbieri, M., "Ribosome crystallization in nuclei of chick embryos", *J. Supramol. Struct.* **10** (1979), pp. 365–375.
- Barbieri, M., "The role of temperature in the crystallization of ribosomes in chick embryos", *J. Supramol. Struct.* **10** (1979), pp. 359–364.
- Beckmann, R., C. M. Spahn, N. Eswar, et al., "Architecture of the protein-conducting channel associated with the translating 80S ribosome", *Cell* **107** (2001), pp. 361–372.
- Ben-Shem, A., N. Garreau de Loubresse, S. Melnikov, L. Jenner, G. Yusupova and M. Yusupov, "The structure of the eukaryotic ribosome at 3.0 Å resolution", *Science* **334** (2011), pp. 1524–1529.
- Fujii, K., T. T. Susanto, S. Saurabh and M. Barna, "Decoding the Function of Expansion Segments in Ribosomes", *Mol. Cell.* **72** (2018), 1013–1020.e6.
- Ghiara, G. and C. Taddei, "Cytological and ultrastructural data on a special type of basophil constituents of the cytoplasm of follicular cells and ovarian oocytes of reptiles", *Boll. Soc. Ital. Biol. Sper.* **42** (1966), pp. 784–788.
- Greber, B. J., D. Boehringer, C. Montellese and N. Ban, "Cryo-EM structures of Arx1 and maturation factors Rei1 and Jjj1 bound to the 60S ribosomal subunit", *Nat. Struct. Mol. Biol.* **19** (2012), pp. 1228–1233.
- Hariharan, N., S. Ghosh and D. Palakodeti, "The story of rRNA expansion segments: Finding functionality amidst diversity", *Wiley Interdiscip. Rev. RNA* **14** (2023), article no. e1732.
- Hoffmann, P. C., J. P. Kreysing, I. Khusainov, M. W. Tuijtel, S. Welsch and M. Beck, "Structures of the eukaryotic ribosome and its translational states in situ", *Nat. Commun.* **13** (2022), article no. 7435.
- Holvec, S., C. Barchet, A. Lechner, et al., "The structure of the human 80S ribosome at 1.9 Å resolution reveals the molecular role of chemical modifications and ions in RNA", *Nat. Struct. Mol. Biol.* **31** (2024), pp. 1251–1264.
- Jackson, R. J., C. U. Hellen and T. V. Pestova, "The mechanism of eukaryotic translation initiation and principles of its regulation", *Nat. Rev. Mol. Cell Biol.* **11** (2010), pp. 113–127.
- Knorr, A. G., C. Schmidt, P. Tesina, et al., "Ribosome-NatA architecture reveals that rRNA expansion segments coordinate N-terminal acetylation", *Nat. Struct. Mol. Biol.* **26** (2019), pp. 35–39.
- Melnikov, S., A. Ben-Shem, N. Garreau de Loubresse, L. Jenner, G. Yusupova and M. Yusupov, "One core, two shells: bacterial and eukaryotic ribosomes", *Nat. Struct. Mol. Biol.* **19** (2012), pp. 560–567.
- Milicevic, N., L. Jenner, A. Myasnikov, M. Yusupov and G. Yusupova, "mRNA reading frame maintenance during eukaryotic ribosome translocation", *Nature* **625** (2024), pp. 393–400.
- Milligan, R. A. and P. N. Unwin, "In vitro crystallization of ribosomes from chick embryos", *J. Cell. Biol.* **95** (1982), pp. 648–653.
- Milligan, R. A. and P. N. Unwin, "Location of exit channel for nascent protein in 80S ribosome", *Nature* **319** (1986), pp. 693–695.
- Noller, H. E., "The ribosome comes to life", *Cell* **187** (2024), pp. 6486–6500.
- Nurullina, L., S. Terrosu, A. G. Myasnikov, L. B. Jenner and M. Yusupov, "Cryo-EM structure of the inactive ribosome complex accumulated in chick embryo cells in cold-stress conditions", *FEBS Lett.* **598** (2024), pp. 537–547.
- Porte, A. and J. P. Zahnd, "Structure of the ovarian follicle in *Lacerta stirpium*", *CR Seances Soc. Biol. Fil.* **155** (1961), pp. 1058–1061.
- Ramesh, M. and J. L. Woolford, Jr., "Eukaryote-specific rRNA expansion segments function in ribosome biogenesis", *RNA* **22** (2016), pp. 1153–1162.
- Shankar, V., R. Rauscher, J. Reuther, W. H. Gharib, M. Koch and N. Polacek, "rRNA expansion segment 27Lb modulates the factor recruitment capacity of the yeast ribosome and shapes the proteome", *Nucleic Acids Res.* **48** (2020), pp. 3244–3256.
- Taylor, D. J., B. Devkota, A. D. Huang, et al., "Comprehensive molecular structure of the eukaryotic ribosome", *Structure* **17** (2009), pp. 1591–1604.
- Yusupova, G. and M. Yusupov, "Crystal structure of eukaryotic ribosome and its complexes with inhibitors", *Phil. Trans. R. Soc. Lond. B Biol. Sci.* **372** (2017), article no. 20160184.