Structural characterization of human ribosome assembly by cryo-EM

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Ribosome assembly is a very complex process, that must be finely regulated to quickly adapt to cellular needs. It requires the production and the correct assembly of ~80 ribosomal proteins (RPs) with 4 ribosomal RNAs (rRNAs), and the transient intervention of more than 200 ribosomal biogenesis factors (RBFs) (Kressler et al., 2017). Defects in ribosome synthesis have been recently associated to an increasing list of human genetic diseases, called ribosomopathies, as well as inherited cancers. To tackle these diseases, it is now crucial to finely characterize each and every step of ribosome biogenesis. Molecular and functional studies have allowed to define a succession of cytoplasmic maturation steps for eukaryotic small ribosomal subunits (40S); however, little is known about the 3D structures of these precursors, and about the precise function of most of the RBFs required for these cytoplasmic steps. Using cryo-EM and single particle analysis coupled to functional analyses, our lab has solved the first 3D structure of a human pre-40S particle and showed the importance of the protein RACK1 in the last maturation steps of the human small ribosomal subunit (Larburu et al., 2016). With this PhD thesis, our aim is to understand the very final maturation steps of small ribosomal subunits, and this in normal and pathological conditions. To do so, the candidate will purify pre-ribosomes from human cells, both in control and mutant conditions blocking ribosome assembly and/or mimicking ribosomopathies. He/she will then use cryo-EM and single particle analysis to determine the 3D structure of the purified complexes at the highest possible resolution. These structures will be compared to one another and to known pre-40S subunit structures in order to highlight the structural remodeling events undergone by maturing small ribosomal subunits. The mechanisms suggested by this structural approach will be then tested using functional approaches in cellulo and in vitro. This powerful combination of cryo-EM and functional studies will shed light on the precise functions of RPs and RBFs involved in these maturation steps.

This work will be performed in a multidisciplinary group, which combines structural analysis of pre-ribosomal particles *in vivo* and *in vitro*, together with functional studies of the molecular mechanisms underlying ribosome synthesis (http://cbi-toulouse.fr/fr/equipe-pierre-emmanuel-gleizes).

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