A Native Molecular View of Cells by Cryo-ET - Structural Biology Done In-Situ

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Abstract

In situ cryo-electron tomography - i.e. cryo-focused ion beam (FIB) milling in conjunction with cryo-electron tomography (cryo-ET) - has changed the way biological systems can be analyzed and modeled. Using this approach, intact cells can now be imaged in a native state and at high resolution, allowing both the quantitative analysis of cellular constituents (visual proteomics) as well as modeling of dynamic cellular processes. Some of these processes might be rare (1-2 events per cell), or localized to specific sites within a cell (e.g. endoplasmatic reticulum, cell membrane, etc.) and therefore need to be specifically targeted, to not be missed during the milling process. We therefore use a 3D-correlative approach, combining confocal cryo-fluorescence microscopy (FLM) and FIB milling to study such rare biological events.

In our research, we address how ribosomes are formed in a variety of organisms. For eukaryotic cells - including the unicellular green alga Chlamydomonas reinhardtii - ribosome precursors are produced in the nucleolus, where essential processing steps take place, before the individual precursors are exported to the cytoplasm where they are assembled and mature into a fully functioning ribosome. Using cryo-FIB milling together with template matching and subtomogram averaging, we were able to dissect the organization of this non-membrane-bounded organelle, and shed light on the molecular details of ribosome biogenesis in C. reinhardtii. We here present the first in situ averages of its small subunit (SSU) as well of its large subunit (LSU) precursor, the pre90S and pre60S particles respectively. We use advanced classification techniques to ensure we only keep true positives during subtomogram averaging. This has allowed us to clearly discriminate between distinct states of ribosome precursor maturation as well as their native spatial organization within the nucleolus.

In addition to ribosome biogenesis, we also apply triad of cryo-FLM, cryo-FIB and cryo-ET to address open questions in the field of autophagy, namely clearing of aberrant endocytic events. Autophagy (or more specifically macro-autophagy) is an essential process for protein homeostasis inside cells. Structures that are damaged, or no longer needed can be directed toward degradation via sequestration into a double-membrane structure, the autophagosome, which later fuses with the lysosome or the vacuole to submit its payload for degradation. Cargo specificity can be realized though means of receptors, which bind to particular cellular structures and thus target them to the autophagosomes. We have identified a new type of receptor, an 'intrinsic receptor', which on one hand is involved in a specific cellular process (endocytosis), but which in itself can also act as a receptor for autophagy if said cellular

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process becomes abnormal. We apply 3D-correlated cryo-light and electron microscopy to study this receptor and its effect on the autophagic machinery to reveal the early stages of clearing aberrant endocytic events on a molecular resolution level.