Integrated Structural Analysis of Nucleoprotein Complexes in Action

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1. INTRODUCTION

Large nucleoprotein complexes are challenging biological objects to study and require an integrated structure-function approach. The latter allows bridging various scales and resolution levels, from the atomic to the cellular level (Ménétret et al., 2013; Urzhumtseva et al., 2013, Orlov et al., 2015, Beinsteiner et al., Andronov et al.). In an effort to favour synergies across complementary disciplines we are combining biochemistry, bioinformatics, crystallography, single particle cryo electron microscopy (cryo-EM), cryo electron tomography (cryo-ET) and correlative fluorescence imaging in order to address the structure-function relationships of complexes involved in gene expression regulation, with a focus on ribosome complexes (Simonetti et al., 2013a & 2013b) and human DNA-bound nuclear receptors (Maletta et al., Nat. Com. 2014).

2. RESULTS

The presentation will focus on the integrated structural biology approach that we developed for the study of the human ribosome for which obtaining an atomic structure has remained a challenge to address. This included biochemistry, biophysical characterization with MALLS, AUC, MS and fluorescence anisotropy, single particle cryo-EM visualization for sample characterization and optimization of the sample preparation protocol and X-ray crystallography, which altogether lead to first crystals of the human 80S ribosome (Khatter et al., 2014). From these optimized samples, we determined the near-atomic structure of the human ribosome derived from high-resolution single particle cryo electron microscopy using the in-house Titan Krios electron microscope, advanced image processing and dedicated computing resources, and atomic model building using new crystallography refinement procedures. The structure reaches 2.9 Å resolution in the most stable regions and thus provides unprecedented insights into rRNA entities and amino acid side-chains of the human ribosome (Khatter et al., 2015). Furthermore, we will describe the 3D organization of ribosomes when bound to an mRNA strand and forming poly-ribosome assemblies such as left-handed supramolecular helices which we analysed by single and dual-axis cryo electron tomography (Myasnikov et al., 2013; Afonina et al., 2014; Afonina et al., 2015; Myasnikov et al., 2014).

3. CONCLUSION

Together, these results highlight the importance of advanced technologies in structural biology (high-resolution cryo electron microscopes, sensitive direct electron detectors, super-resolution imaging and X-ray crystallography) to allow a better multi-scale multi-resolution integration, notably for correlative microscopy at the light and electron microscopy interfaces. Such integrated tools are available through project-based access through the European and French distributed infrastructures http://frisbi.eu/ and http://www.structuralbiology.eu/.

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