doi:10.1017/S1431927616001252
Microsc. Microanal. 22 (Suppl 3), 2016
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Structural Characterization of Mutant Huntingtin Inclusion Bodies by Cryo-Electron Tomography

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Huntington's disease (HD) is a devastating neurodegenerative disorder caused by an abnormal expansion of the poly-glutamine (polyQ) repeat of huntingtin. Expanded polyQ repeats cause protein aggregation and the consecutive formation of large inclusion bodies (IBs) in neurons of patients with HD. The lack of information on the architecture and cellular interactions of mutant huntingtin (mHtt) IBs has so far precluded a detailed understanding of the roles of these structures in mHtt toxicity. Here we employ advanced techniques in cryo-electron tomography to analyze the structure of mHtt IBs within human cells in close-to-native state and at molecular resolution.

We used fluorescence light microscopy to target the small fraction of living cells containing mHtt IBs, highlighting the importance of the correlative approach in cryo electron microscopy (cryo-EM). IBs had a typical diameter of 5-8 µm and were typically located close to the nucleus, making them not accessible for cryo-EM without further thinning. Therefore, we employed cryo-FIB [1,2] to expose 25-30 µm wide lamellas containing the IB and their cellular environment. Lamellas were imaged in a FEG Titan Krios (FEI) at 300 kV equipped with a post column energy filter (968 Quantum, Gatan) and a direct detection camera (K2 Summit, Gatan) operated in counting mode. Essential for visualization and further post-processing of the IBs architecture and the interaction of the aggregated mHtt was the imaging close-to-focus with a Volta phase plate [3], as the molecular details of the aggregates were not visible with classical defocus acquisition. Lamellas were sputtered with a thin metallic platinum layer after FIB preparation to prevent charging effects that are visible with the Volta phase plate.

In conclusion, to investigate the architecture and the cellular interactions of mHtt IBs of HD in human cells, the following pipeline of advanced techniques in Cryo-EM is necessary: correlative light microscopy, lamella preparation by cryo-FIB, EFTEM with direct electron detectors with the essential combination of a Volta phase plate and close-to-focus acquisition. [4]

References:

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[4] This work was supported by the ERC Synergy Grant ERC-2012-SyG, European Research Council.

https://doi.org/10.1017/S1431927616001252 Published online by Cambridge University Press

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