Huntington's disease: The ultrastructure of huntingtin inclusions revealed

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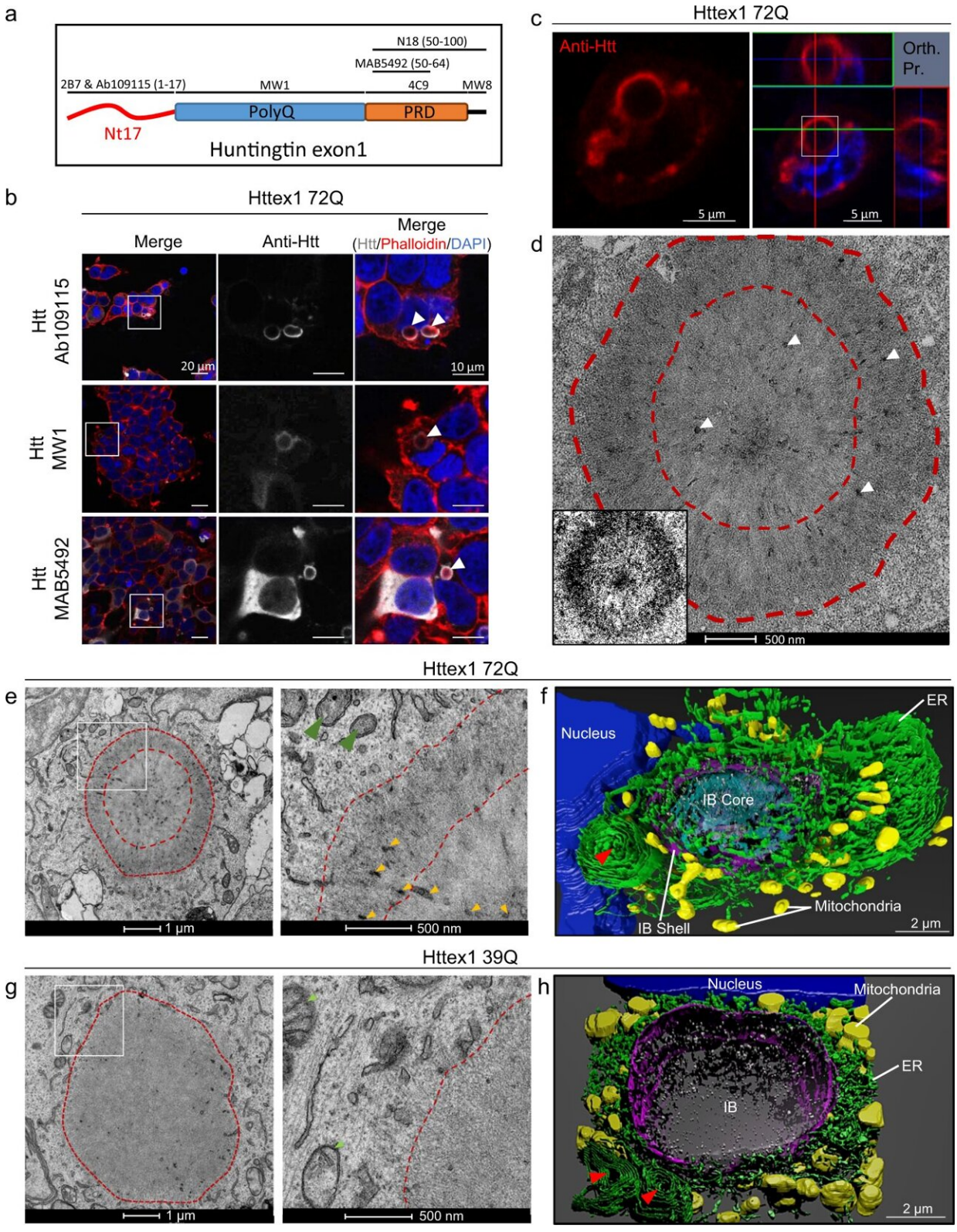

Fig. 1: Confocal microscopy and CLEM revealed the ring-like structure of the

Httex1 72Q inclusions in HEK cells. a Epitope mapping of the Httex1 antibodies used in this study. b Confocal imaging of Httex1 72Q inclusions formed 48 h after transfection in HEK cells. All the Htt antibodies showed strong immunoreactivity to the periphery of the inclusions. The nucleus was counterstained with DAPI and the F-actin with phalloidin. White arrows indicate the colocalization of the F-actin with the ring-like structure of Httex1 inclusions. Scale bar = 20 μm and 10 μm. c, d CLEM of Httex1 72Q inclusions. c Confocal imaging of Httex1 72Q inclusions formed 48 h after transfection in HEK cells. The selected area (white square) was examined by EM (d). Orthogonal projection (Orth. Pr.), Scale bars = 5 μm. d The presence of membrane fragments and vesicles are indicated by the white arrowheads. The binary image (inset) shows the core and the shell ultrastructure of the Httex1 72Q inclusion. Scale bar = 500 nm. e Representative EM images of Httex1 72Q inclusion. Higher magnification (white square) are shown in the right panel. Dashed lines delimit the aggregate and the core of the inclusion. Internalized membranous structures and the mitochondria are indicated by the orange and the green arrowheads, respectively. Scale bar = 1 μm and 500 nm. f 3D model of Httex1 72Q cellular inclusion and surrounding organelles (top view). The Httex1 inclusion body (IB) shell is represented in purple, the core in cyan. ER membranes are shown in green, intra-inclusion membranous structures in white, the nucleus in blue, mitochondria in yellow, and the stacked ER cisternae are indicated by a red arrowhead. Scale bar = 2 μm. g Representative EM images of Httex1 39Q inclusion formed 48 h after transfection in HEK cells. The white square indicates the area shown in the right panel at higher magnification. Dashed lines delimit the inclusion. Scale bar = 1 μm and 500 nm. h 3D model of the Httex1 39Q IB is shown in purple, surrounded by mitochondria (yellow), ER structures (green), stacked ER cisternae (red arrowheads), intra-inclusion membranous structures are shown in white and the nucleus in blue. Scale bar = 2 μm. Credit: DOI: 10.1038/s41467-021-26684-z

Huntington's disease is a progressively debilitating brain disease that causes uncontrolled movements, psychological problems, and loss of cognition. Huntington's is caused by a mutation in the gene that encodes huntingtin, a protein that normally plays important roles in keeping brain

cells healthy and active. But the mutation gives huntingtin an abnormally long tail of glutamine amino acids, which cause huntingtin to aggregate inside neurons and eventually kill them.

These aggregates, or inclusion bodies, of huntingtin have been the subject of a lot of research efforts in the attempt to find a way to understand and treat Huntington's. What has been missing, though, is a deep analysis of the inclusions' ultrastructure—a term that describes the level of structure that lies beyond the capacity of a conventional microscope to observe.

Looking deep

Using advanced microscopy and proteomics, scientists led by Hilal Lashuel at EPFL, in collaboration with the Biological Electron Microscopy Facility, have now successfully carried out the first study that interrogates both the ultrastructure of huntingtin inclusion bodies and their composition in cells that model the pathological formation of Huntington's disease. The work is published in *Nature Communications*.

The researchers used an advanced technique known as correlative light-electron microscopy (CLEM) that can identify inclusions in different cell compartments (cytoplasm or nucleus) and study their structural and organizational features of inside cultured neurons. CLEM essentially combines an electron microscope with a fluorescence microscope. The first provides high-resolution information down to the nano-scale, while the fluorescence microscope can (literally) highlight regions of interest.

The researchers also used electron tomography, which allowed them to visualize individual aggregates within these inclusions. "We wanted to go beyond just painting a detailed picture of how these inclusions form," says Nathan Riguet, the study's first author. "This is why we extended our studies to understand how these inclusions form, how they impact

their subcellular environment, the function of organelles in their vicinity, and ultimately the viability of neurons."

Different mechanisms of aggregation

The study found that the aggregation of huntingtin and the formation of inclusions in the cytoplasm and nucleus of neurons occurs through different mechanisms, lead to distinct biochemical and ultrastructural properties. What this means is that these two types of inclusions may also be toxic through different ways, meaning that treatments must employ different strategies to address their formation, maturation, and toxicity.

The study also suggests that finding ways to modify the growth of inclusions and their interactions with other proteins and organelles is a valid, alternative or complementary strategy for slowing the progression of Huntington's, especially after disease onset. This is because this process leads to the sequestration of functional proteins and disrupts the function of important organelles in the cell such as the mitochondria. The authors state: "…[W]e believe that targeting inclusion growth and maturation represents a viable therapeutic strategy."

Fluorescent tags

"Our findings also caution against the use of large fluorescent protein tags to develop disease models of HD and screen for drugs that modify Huntingtin aggregation and inclusion formation," says Lashuel. "This has significant implications for Huntington's disease and other neurodegenerative diseases, where fusion of fluorescent proteins is commonly used to investigate mechanisms of protein aggregation and in drug discovery."

"Our work opens up new insights into the composition and ultrastructure of huntingtin inclusions," he concludes. "They advance our understanding of the mechanisms of huntingtin aggregation but also point to new directions for therapeutic interventions, which we plan to pursue."

More information: Nathan Riguet et al, Nuclear and cytoplasmic huntingtin inclusions exhibit distinct biochemical composition, interactome and ultrastructural properties, *Nature Communications* (2021). DOI: 10.1038/s41467-021-26684-z

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