Names (Presenter in bold font)	**Sean S. Keating**, Adekunle T. Bademosi, Rebecca San Gil, Adam K. Walker
Affiliations	Queensland Brain Institute, The University of Queensland
Title	CRISPR-tagging for live-cell analyses of endogenous TDP-43 protein aggregation dynamics in ALS and FTD models
Abstract (max 300w)	Dysfunction and aggregation of the RNA-binding protein, TDP-43, within neurons is the unifying hallmark of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). TDP-43-related neurodegeneration encompasses multiple changes to normal physiological TDP-43, which undergoes nuclear depletion, mislocalisation, post-translational modification, aberrant liquid-liquid phase separation, and self-assembly, preceding protein inclusion deposition. While toxic cytoplasmic aggregation of TDP-43 has been thoroughly studied and is a major therapeutic focus, concurrent depletion and dysfunction of functional, normal nuclear TDP-43 in cells with pathology is a relatively unexplored but likely key factor potentiating neurodegeneration. However, previous difficulties in simultaneously but separately modelling, visualising, and modulating normal and pathological forms of TDP-43 has left the driving mechanisms and consequences of these concurrent processes unresolved. To define processes driving TDP-43 dysfunction, we used CRISPR/Cas9-mediated fluorescent tagging to investigate how disease-associated stressors and pathological TDP-43 alter abundance, localisation, self-assembly, aggregation, solubility, and mobility dynamics of normal nuclear TDP-43 over time in live cells. Oxidative stress stimulated TDP-43 liquid-liquid phase separation into droplets or spherical shell-like anisosomes, which were not formed by over-expressed wild-type TDP-43. Further, nuclear RNA-binding-ablated or acetylation-mimicking TDP-43 rapidly formed anisosomes and inclusions that readily sequestered and depleted free normal nuclear TDP-43. Endogenous TDP-43 sequestered into anisosomes retained high protein mobility and solubility. However, cytoplasmic RNA-deficient TDP-43 formed large, phosphorylated inclusions that occasionally sequestered endogenous TDP-43, rendering it insoluble and immobile, indicating irreversible pathological transition. These findings suggest that RNA-binding deficiency and post-translational modification exacerbate TDP-43 aggregation and dysfunction by driving sequestration, mislocalisation, and depletion of normal nuclear TDP-43 in neurodegenerative diseases.

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