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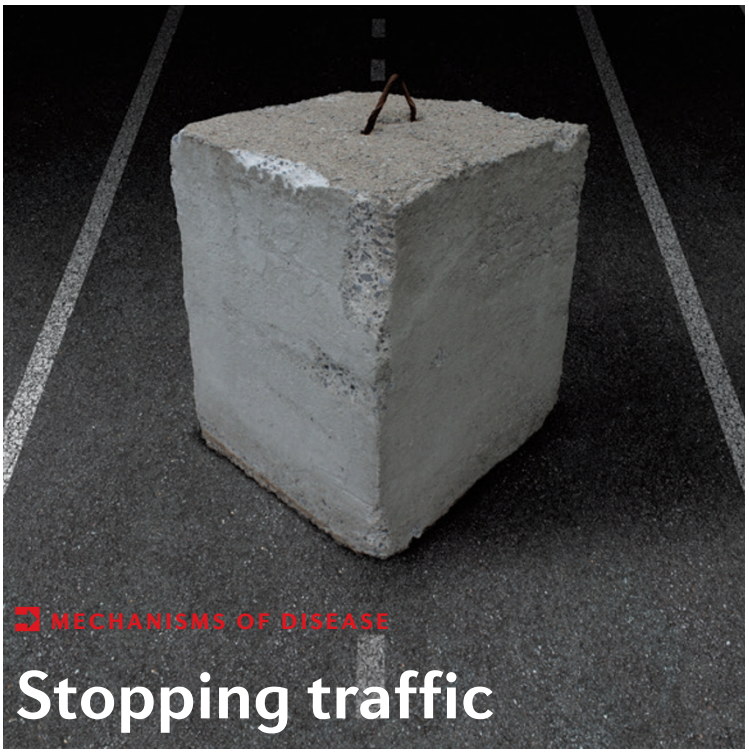

Brain light/Alamy

MECHANISMS OF DISEASE

Stopping traffic

> in addition to impairing mRNA export, β-protein aggregates might perturb mRNA processing

Numerous neurodegenerative diseases are associated with the aggregation of aberrant proteins in the cytoplasm and nucleus, but the mechanism by which these toxic aggregates cause pathology has been unclear. New research published in *Science* indicates a particular role for cytoplasmic, but not nuclear, aggregates in stopping nucleo-cytoplasmic traffic.

Two artificial proteins from a combinatorial library designed to form β-strands — β17 and β23 — were confirmed to form aggregates in the cytoplasm and nucleus of HEK293T cells, causing cell toxicity. To test whether this toxicity is compartment specific, a nuclear export sequence (NES) or nuclear localization sequence (NLS) was added to the proteins. NLS-β17 and NLS-β23, which form nuclear inclusions, were significantly less toxic than NES-β17 and NES-β23, which form cytoplasmic inclusions.

The expression of NES-β17 and NES-β23 resulted in the partial dislocation of nuclear pore complex (NPC) proteins from the nuclear envelope to the cytoplasmic inclusions, whereas NLS-β17 and NLS-β23 did not seem to have any effect on NPC integrity. In keeping with this, NES-β17 inhibited both the nuclear import and the nuclear export of a GFP reporter, indicating impairment of nucleo-cytoplasmic protein transport. NES-β17 also prevented nuclear translocation of the nuclear factor-κB (NF-κB) subunit p65 after stimulation of the cells with tumour necrosis factor, despite normal initiation of the signalling pathway. This effect on protein traffic through nuclear pores was also observed in cells containing cytoplasmic inclusions of polyQ-expanded Huntingtin exon 1 (Htt96Q), which is associated with Huntington disease.

Furthermore, the authors showed that cytoplasmic, but not nuclear, aggregates also affect mRNA transport, as a marked nuclear accumulation of mRNAs was observed in cells expressing NES-β17, NES-β23, Htt96Q or a C-terminal fragment of TAR DNA-binding protein 43 (TDP43; which forms cytoplasmic aggregates in neuronal cells of patients with amyotrophic lateral sclerosis). This impaired nuclear export of mRNAs significantly reduced protein biosynthesis. Quantitative interactome analysis identified the seven-subunit THO complex (THOC), which is involved in mRNA export, as being highly enriched for interaction with β17. Expression of NES-β17, Htt96Q or the carboxy-terminal fragment of TDP43 caused mislocalization of THOC2 from the nucleus to the cytoplasm, whereas NLS-β17 did not co-aggregate with nuclear THOC2. Finally, β17 was also shown to interact with splicing factors and other RNA-binding proteins, raising the possibility that, in addition to impairing mRNA export, β-protein aggregates might perturb mRNA processing.

In summary, these studies of cytoplasmic aggregates formed by artificial β-sheet proteins and disease-causing proteins indicate that their formation impairs nucleo-cytoplasmic transport of mRNA and protein, which may contribute to the pathology associated with aggregate formation. It remains to be determined why nuclear aggregates do not have the same effect and whether nuclear sequestration of toxic aggregates might be a protective mechanism.

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ORIGINAL ARTICLE Woerner, A. C. *et al.* Cytoplasmic protein aggregates interfere with nucleo-cytoplasmic transport of protein and RNA. *Science* http://dx.doi.org/10.1126/science.aad2033 (2015)