50 Years Ago

The British Home Office seems to be modifying slightly its attitude to the tests by which motorists in Britain can now be convicted of driving under the influence of alcohol. A recent paper … by Professor J. B. Payne … suggests that the methods used … are far from accurate … So far the Home Office has not been forced to act, because no motorist accused of driving under the influence of drink has quoted Professor Payne's work in his defence … Originally … police surgeons were advised to take small samples of capillary blood for use in the test, although it was also open to them to take venous blood if they preferred. The work at the Royal College of Surgeons suggests that the latter is likely to give more accurate results … The Home Office has now sent a circular to police authorities pointing out that it is within their discretion to take venous rather than capillary blood … The circular also points out that the motorist has the right to keep a sample of his own blood for independent analysis.
From *Nature* 23 March 1968

100 Years Ago

Now that our rations of food, particularly of meat and wheaten bread, have been so appreciably reduced the necessity of arranging our diet so as to ensure a sufficient supply of those elusive substances, the so-called "vitamines", is more important than ever. It is known that these substances exist in certain foods, and that an adequate supply of them is necessary to health, but they have not yet been isolated in a pure condition, although several workers claim to have done so successfully. As a result of some recent work, McCollum and Davis concluded that two distinct types of vitamine exist, the "fat-soluble A" and the "water-soluble B".
From *Nature* 21 March 1918

branching and are less densely packed than poly(GA) ribbons[7].

Second, when the authors used powerful computational approaches to search for known macromolecular complexes in each aggregate, they found many proteasomes incorporated in poly(GA) aggregates (Fig. 1). Indeed, biochemical data suggested that as many as 50% of the proteasome complexes in the neuron become highly entangled within poly(GA) ribbons. Removal of proteasomes from their normal location in cells through this sequestration mechanism might explain the reduced proteasomal activity in cells harbouring these aggregates[4,8]. Complexes called ribosomes, which mediate protein production and are comparable in size to proteasomes, were largely excluded from poly(GA) ribbons, suggesting that poly(GA) aggregates are actively recruited or retained by proteasomes. By contrast, poly(Q) fibrils did not contain proteasomes, but formed close contacts with membranes from multiple types of organelle. This interaction leads to deformation of the membranes around organelles, such as the endoplasmic reticulum. Such deformation might alter pathways involved in protein translation, trafficking and degradation[7].

The proteasome consists of a barrel-shaped core particle in which substrate cleavage takes place, and one or two regulatory particles that cap the ends of the barrel, restricting access to the core so that only proteins tagged with the pro-degradation molecule ubiquitin can enter. Regulatory particles have been observed in multiple conformations[9,10], indicating that proteasomes progress through a reaction cycle that involves ground, committed and substrate-engaged states. Guo *et al.* used computational particle averaging to quantify the proteasomal states (technique reviewed in ref. 11), and found both ground and substrate-engaged states within poly(GA) aggregates. They also found a large increase in the proportion of doubly capped proteasomes (indicating engagement with substrate) compared with control neurons that did not contain poly(GA) products. Almost one-quarter of the proteasomes within aggregates adopted a conformation recently described[9] as substrate-engaged yet stalled, in which the substrate becomes trapped in the barrel. And that proportion rose to 36% for those proteasomes closest to poly(GA) ribbons.

Why might this stalling occur? The authors' tomographic reconstructions revealed numerous regions of electron density located between a poly(GA) ribbon and the site where the protein RAD23 binds to the proteasome. RAD23 is involved in recruiting ubiquitin-tagged proteins to the proteasome and is known to be enriched in poly(GA) aggregates[8]. Thus, this electron density could indicate RAD23-associated ubiquitin that is attached to proteins within the aggregate. Which protein or proteins the proteasome is choking on is currently unclear, although possibilities include the poly(GA) peptides themselves, which probably inhibit proteasome activity directly. Regardless of the mechanism, it seems likely that depletion of proteasomal activity in the cell proper would be detrimental to protein-degradation pathways, thereby contributing to cellular toxicity.

These results raise several important questions. First, pathogenesis in cases of ALS driven by *C9orf72* expansion has been linked both to changes mediated by poly(GA) formation and to changes caused by reduced production of C9orf72 protein — but what are the relative contributions of each mechanism? C9orf72 is part of a complex involved in autophagy[12], a process by which cellular material, including proteins, is degraded and recycled. It is therefore possible that reduced C9orf72 levels conspire with poly(GA)-dependent proteasome inhibition to increase neuronal toxicity. Second, is toxicity promoted by the capture of other proteins within poly(GA) aggregates? One candidate is the autophagy cargo receptor p62, which is known to accumulate in poly(GA) aggregates[8]. Third, several molecular machines involved in disassembling aggregates do not accumulate in poly(GA) structures, but the reasons for this are unclear.

Finally, although poly(GA) is the most abundant repetitive protein produced by *C9orf72* expansion, it is not the only one — mutation can also produce tracts of glycine–arginine (poly(GR)) and proline–arginine (poly(PR)). How do the structures of these other aggregates compare to that of poly(GA) proteins? Most data on poly(GR) and poly(PR) aggregates indicate that they do not accumulate proteasomes, suggesting alternative toxicity mechanisms[13,14]. Further analysis by 3D cryo-ET, and analysis of natural products of *C9orf72* expansion rather than the engineered product used in the current study, might clarify the similarities and differences between the aggregates that occur in patients and the model aggregate structures studied by Guo and colleagues.

In sum, the current work highlights the unprecedented resolution of 3D cryo-ET for visualizing fundamental processes within cells[11]. Moreover, it sets the stage for a more comprehensive mechanistic understanding of aggregate-associated neurodegenerative diseases. ■

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1. DeJesus-Hernandez, M. *et al. Neuron* **72,** 245–256 (2011).
2. Taylor, J. P., Brown, R. H. Jr & Cleveland, D. W. *Nature* **539,** 197–206 (2016).
3. Yamakawa, M. *et al. Hum. Mol. Genet.* **24,** 1630–1645 (2015).
4. Zhang, Y.-J. *et al. Acta Neuropathol.* **128,** 505–524 (2014).