Neuron protection agency

Harry T. Orr

The results of an innovative way of tracing the life and death of neurons in culture favour one side of a debate about the protein accumulations associated with certain disorders of the nervous system.

On page 805 of this issue, Arrasate and colleagues[1] show that clumps of mutant protein, seen in certain neurons and characteristic of Huntington's disease, reduce the chance that the neurons will die — at least in culture. So, why is this worth noting? The answers lie in the ingenious manner in which the investigators made their observations, and because they address a point of fervent debate among those studying many human neurodegenerative diseases, including Huntington's disease.

Neuropathologists have long known that some disorders are characterized by an abnormal accumulation of macromolecules inside cells. These 'inclusion bodies' are found, for example, in the brains of patients suffering from Alzheimer's disease, prion diseases, amyotrophic lateral sclerosis (also known as Lou Gehrig's disease), Parkinson's disease, and a group of nine so-called polyglutamine diseases, of which Huntington's disease is the most widely known. The common factor in polyglutamine diseases is a genetic mutation that produces abnormal repeats of the amino-acid glutamine in the encoded protein, with more repeats being more pathogenic.

With the advent of studies by molecular geneticists and cell biologists, it became clear that, in the inherited forms of each of these diseases, inclusion bodies contain the protein encoded by the gene containing the disease-causing mutation. These observations reignited long-standing questions of whether and how the inclusions contribute directly to the disease process.

The debate has had especial intensity where polyglutamine diseases, and Huntington's disease in particular, are concerned[2,3]. For the polyglutamine diseases, inclusion bodies containing mutant protein sprang to public attention in a series of papers[2–5] that appeared in 1997. In the case of Huntington's disease, the critical protein is called huntingtin (htt), and inclusions containing mutant htt are found at the major site of neurodegeneration, the medium spiny neurons in a brain region called the striatum.

Depending on the system and the manipulations performed, over the years the experimental evidence has been interpreted as showing that the inclusion bodies cause disease, protect against disease, or are simply incidental[6]. The largest contingents are those who believe that inclusions are the major pathogenic species, owing to their ability to absorb other critical cellular proteins, and those who feel inclusions are protective, in that they sequester mutant protein out of harm's way. Resolution of this issue is necessary for many reasons, not least because many current studies aim to interfere with inclusion-body formation as a potential treatment for Huntington's disease and the other neurodegenerative disorders characterized by protein aggregates[7].

Arrasate and colleagues[1] have devised an elegant real-time technique for assessing the factors that affect the risk of neuron death in culture. They used a common model of Huntington's disease in which striatal neurons are transiently transfected with a pathogenic fragment of mutant htt (htt-exon1). They developed an automated microscopic system to simultaneously follow inclusion-body formation, the presence of diffuse htt and cell survival in the same neuron over a period of days. To visualize deposition of htt in transfected cells, they employed a construct of htt-exon1 fused with green fluorescent protein. As expected, inclusion bodies formed (Fig. 1), in the nucleus and in the cytoplasm, and neurons died.

To begin with, Arrasate *et al.* performed a mathematical analysis of the risk of death — a sort of actuarial assessment in tissue culture. From this analysis, they reached two conclusions. First, the risk of dying was low in neurons that had been transfected with a control (non-pathogenic) form of htt-exon1 and high in cells expressing htt-exon1 with an expanded polyglutamine tract; furthermore, the risk increased with increasing size of the mutant polyglutamine stretch. Second, they found that the risk of death in cells with mutant htt-exon1 was linear over time; that is, risk seemed to be largely time-independent.

So, what variables affected the risk of cell death? Not unexpectedly, inclusion bodies were one such variable. Importantly, the basic features of inclusion-body formation — size and growth with time — in this model system accurately replicated the picture seen in Huntington's disease. By following the same neuron over time, Arrasate *et al.* found that cells that failed to form inclusion bodies

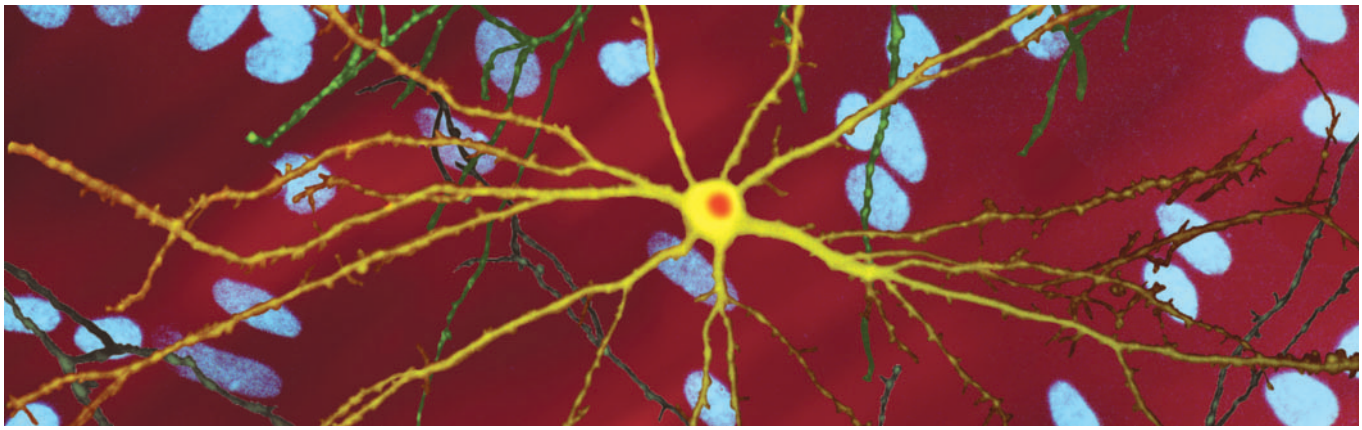

Figure 1 **Picture of health? This image, produced by Arrasate *et al.*[1], shows a striatal neuron (yellow) that has been transfected with the disease-associated version of huntingtin, the protein that causes Huntington's disease. The orange–red structure is an inclusion body. Arrasate *et al.* show that transfected neurons that form such bodies live longer than those that do not. Nuclei of untransfected neurons (blue) are seen in the background, along with projections from other neurons (green).**

had an increased risk of death, indicating that the inclusion body is not required for polyglutamine-induced neuronal death. Remarkably, when neurons expressing equal levels of mutant htt-exon1 were followed individually, those neurons in which inclusion bodies formed had a significantly reduced risk of dying compared with neurons in which mutant htt-exon1 remained diffuse. Moreover, inclusion-body formation reduced the amount of diffuse mutant htt elsewhere in the neuron.

So inclusion-body formation actually prolonged survival and protected neurons, seemingly by reducing the amount of a toxic, diffusely distributed form of mutant htt. But although the results provide compelling evidence that readily visible (1 μm^2), mutant polyglutamine inclusion bodies are protective, and not pathogenic, they do not rule out the possibility that the major toxic species are the early precursors to inclusion bodies — typically referred to as microaggregates.

The long-term strength of this study[1] lies in the approach itself: the ability to find out whether a cellular feature of a disease is pathogenic, beneficial or merely incidental will help greatly in understanding disease mechanisms. It will also be interesting to see whether the results end the debate on the pathogenic role of inclusion bodies in polyglutamine diseases. If they don't, one wonders what would. ■

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Nuclear physics

Neutron halo slips

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In neutron-rich nuclei, weakly bound neutrons form a halo surrounding a compact core. Unexpectedly, it seems that this halo does not improve the chances of the nucleus fusing with another nucleus.

Accelerator facilities that can supply beams of highly unstable, radioactive isotopes are making it possible to study nuclear reactions that occur naturally only in violent cosmic events such as supernovae, but which determine many of the elemental and isotopic abundances found on Earth. Some of the most neutron-rich of these nuclei have a diffuse neutron cloud that extends to large distances beyond the compact nuclear core. Based on the lessons learned from the fusion of stable nuclei, this halo might be expected to enhance, many times over, the probability of nuclear fusion at low energies. But, on page 823 of this issue, Raabe and colleagues[1] describe an ingenious measurement that shows no such enhancement, indicating that the behaviour of the neutron halo is more unusual than expected.

Atomic nuclei, which are made up of protons and neutrons, exist because of the attractive nuclear force between their constituents. This force depends on many variables, but in particular favours equal numbers of protons and neutrons. Thus, for example, the most common isotopes of the elements helium and nitrogen, having two and seven protons respectively, are ^{4}He and ^{14}N. If more neutrons were added to a nucleus, either one by one, or in pairs, the nucleus would become less strongly bound. Eventually, at the point where the energy of the new nucleus is greater than that of its 'ingredients', the nucleus would become unbound. The transition from bound to unbound neutron-rich nuclei occurs at the so-called neutron drip-line, which marks the boundary of the existence of nuclei (Fig. 1).

For the examples of helium and nitrogen, the drip-line isotopes are ^{8}He and ^{23}N. These and other neutron-rich nuclei are not found on Earth because they undergo β-decay, in which a neutron is transformed into a proton, improving the balance between neutrons and protons.

Nuclei close to the neutron drip-line can have one or two extremely weakly bound neutrons. The energy required to separate the two weakly bound neutrons from ^{6}He is only 0.973 MeV (megaelectronvolts), compared with typically 8 MeV to remove a single neutron from a stable nucleus. The low binding energy of these neutrons results in quantum-mechanical tunnelling to large distances, allowing their wavefunctions (or probability distributions) to extend well beyond the tightly bound core, forming a diffuse neutron cloud or halo (Fig. 1).

The effect that this halo might have on nuclear fusion has been the subject of some controversy. Because the nuclear force has a short range, the attractive force between two nuclei is closely related to the degree of overlap of their matter distributions. The potential barrier (fusion barrier), which must be overcome in fusion, occurs at the radial separation where this force balances the repulsive Coulomb force between the positively charged protons in the two colliding nuclei. According to this picture, the neutron halo might be expected to contribute a longer-range attractive force, which would reduce the energy of the fusion barrier. Furthermore, in reactions between stable nuclei, the likelihood of fusion occurring is known to be enhanced at energies well below the fusion barrier if the transfer of one or more

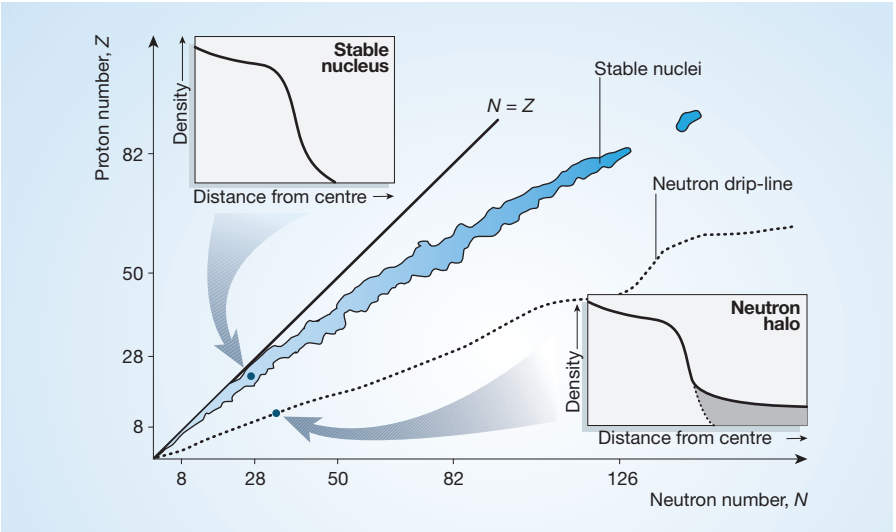

Figure 1 **Nuclear stability and neutron halos. In stable nuclei, the number of neutrons tends to exceed the number of protons. Nuclei with too many neutrons, however, are unstable; beyond the 'neutron drip-line', nuclei become unbound. Isotopes close to this line may have one or two neutrons that are weakly bound to the core nucleus. Through quantum-mechanical tunnelling, and because their binding energy is low, these neutrons form a nuclear halo: the neutron density extends to greater distances (inset, right) than is the case in a well-bound, stable nucleus (inset, left). According to Raabe *et al.*[1], the existence of this neutron halo does not make it any more likely that the nucleus will undergo fusion.**