Prions tunnel between cells

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Prions are abnormal isoforms of host proteins that are the infectious agents in certain mammalian neurodegenerative diseases. How prions travel from their peripheral entry sites to the brain where they cause disease is poorly understood. A new study finds that tunnelling nanotubes are important for the intercellular transfer of prions during neuroinvasion.

Tunnelling nanotubes (TNTs) were discovered only a few years ago as conduits for a previously unrecognized form of cell-to-cell communication[1]. Since then, a growing number of cell types have been found to use TNTs to exchange diverse cargoes ranging from cytoplasmic signalling molecules such as calcium ions[2] to small vesicles. TNTs are membranous channels that connect cells over long distances. These tubes, with a diameter of 50–200 nm, form *de novo* between distant cells, which may be of the same or different types; they contain F-actin and have no contact with the substratum. The cytonemes observed in *Drosophila* imaginal disks[3], and the filopodial bridges induced by retroviruses such as between infected Cos-1 and XC sarcoma target cells[4], may be related to TNTs. In the past year, the human immunodeficiency virus (HIV) was found to spread between cells through these channels[5,6]. Now, among the molecules passing through TNTs, a significant cargo has been discovered: Gousset *et al.* report on page 328 of this issue that the prion protein PrP^{Sc} uses TNTs as an important intercellular route to invade the central nervous system (CNS)[7].

PrP^{Sc} is the infectious agent that causes spongiform encephalopathies such as scrapie in sheep, bovine spongiform encephalopathy in cattle and Creutzfeldt–Jakob disease in humans. According to the now widely accepted 'protein only' hypothesis[8], infectious PrP^{Sc} is simply a conformational isomer of the normal host cell protein PrP^{c}. Cellular PrP^{c} is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein that is normally expressed in neurons, various non-neuronal tissues and leukocytes. It is located predominantly on the cell surface and also appears in various compartments of the secretory pathway, including the endoplasmic reticulum and the Golgi apparatus, and the endosomal/lysosomal system. PrP^{c} is required for prion disease development, as direct interaction of endogenous PrP^{c} with the pathogenic PrP^{Sc} template is thought to drive conversion to the infectious prion isomer.

After entering the body through the gut, prions cross the intestinal epithelial cell layer and are then transported by bone marrow-derived dendritic cells (DCs) to lymphoid tissues such as the spleen. From these tissues, prions are thought to enter the peripheral nervous system and spread in a retrograde direction along the peripheral nerve fibres towards the CNS. How prions move from immune cells to nerve cells is largely unclear. One proposition is that prions transfer from one cell to another in small (30–100 nm diameter) vesicles called exosomes that are released from cells on fusion of multivesicular bodies with the plasma membrane[9]. Alternatively, the spread of prions into nerve cells may be mediated by conversion of PrP^{c} on the surface of one cell to PrP^{Sc} by contact with another cell bearing PrP^{Sc} on its surface[10].

Gousset *et al.* now present strong evidence that prions exploit TNTs as an important pathway to pass from immune cells to neurons, and between neurons. They show that fine tubular structures with the morphological characteristics typical of TNTs[11] connect DCs to primary hippocampal neurons, as well as to the cells of a CNS model cell line. Furthermore, these conduits permit the intercellular transfer not only of endocytic organelles, as previously reported for other cell lines[11], but also of endogenous and exogenous PrP^{Sc}. In the case of the CNS model cell line, PrP^{Sc} transfer between cells was evident only when a TNT connection was present. This indicates that TNTs may provide the exclusive, or at least the predominant, pathway for the crucial transfer of PrP^{Sc} from immune cells to neurons.

By using fluorescence video-microscopy with fluorescently labelled PrP^{Sc}, Gousset *et al.* have found discrete signals from PrP^{Sc} in TNTs; along with other observations this may indicate that at least a significant portion of PrP^{Sc} is conveyed in transport vesicles (Fig. 1). Given that a fraction of PrP^{c} cycles between the plasma membrane and the endosomal system[12], these vesicles may be of endocytic origin; this is in keeping with the idea that the endosome is an important compartment for the conversion of PrP^{c} to PrP^{Sc} (ref. 12). Discrete signals from fluorescently tagged PrP^{c} underwent directed movement with a speed in the range of myosin-dependent transport, consistent with the idea of an actomyosin-dependent transport system for PrP^{Sc} transfer suggested by earlier studies[11].

This study further suggests that the transfer of PrP^{Sc} along TNTs may be accomplished in part by lateral diffusion along the plasma membrane (Fig. 1). This is on the basis of the observation that, in addition to discrete fluorescent signals of PrP^{c} in TNTs, continuous staining for PrP^{c} was also seen along the

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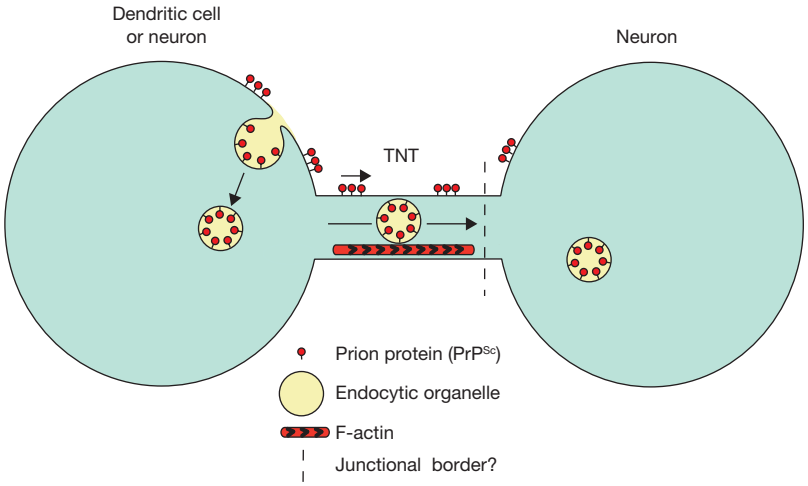

Figure 1 Intercellular transport of PrP^{Sc} mediated by TNTs. TNT bridges form between dendritic cells and neurons, as well as between neurons. PrP^{Sc} is conveyed along these bridges in endocytic vesicles inside TNTs by actin-dependent transport and/or by lateral diffusion within the TNT membrane. Whether the depicted membrane continuity between the TNT connections exists remains to be addressed experimentally.

TNT membrane. Similar lateral diffusion along the membrane of TNTs has also been reported for a model GPI-anchored protein[13]. However, for the protein to diffuse from one cell to another through the plasma membrane there must be continuity between the two cell membranes. Depending on the type, TNTs either provide this continuity or contain a junctional border that inhibits free diffusion of membrane components between connected cells[11,13]. We do not yet know which type of connection is present in the TNTs that transport PrP^{Sc}, so whether the mechanism of entry of vesicular or plasma membrane-bound PrP^{Sc} into connected cells involves a transient or long-lasting membrane continuity, or exo/endocytic events remains an open question. Whatever the mechanism, Gousset *et al.* highlight the role of TNTs and TNT-like structures as efficient and selective conduits for the intercellular transfer of various cargo molecules, in accordance with other reports[11,13].

Although the authors did not include a model for peripheral neurons (such as dorsal root ganglia) to analyse the transfer of PrP^{Sc} from immune cells to the initial neuronal entry sites, it is likely that peripheral neurons also generate TNTs, since they have shown that neurons of the CNS do. A more important consideration, however, is whether the *in vitro* data reflect the situation in living tissues. This could be addressed by *in vivo* imaging of prion trafficking in a lymphoid tissue such as the spleen; a technically challenging task owing to the constraints in optical resolution under these conditions. Nevertheless, a recent study succeeded in providing the first evidence that TNTs exist *in vivo*: long TNT-like bridges were observed between putative DCs in whole-mount corneas from chimaeric mice expressing green fluorescent protein[14]. In other words, there is good reason to believe that immune cells gathering in the spleen could generate a TNT network that would promote neuroinvasion by PrP^{Sc}.

In addition to PrP^{Sc}, other pathogens also use TNT-like structures to promote their spreading between cells. These include bacteria[13] and retroviruses[4] which are transported on the surface of TNT-like bridges, and the HIV virus which is translocated within TNT-like structures, to infect connected cells[5,6]. Unfortunately, our current knowledge of the cellular functions of these structures is very poor. The exchange of PrP^{Sc} between cells may provide a useful tool to probe the normal cellular functions of TNTs. For example, it could be used as an assay to search for molecules involved in the formation of TNTs and the transport of cargo through them. It could also be used to screen for reagents that selectively inhibit intercellular PrP^{Sc} transport. Such an approach may identify potential therapeutic molecules for prion-based neurodegenerative diseases and viral infections such as HIV. Particularly in the case of prion diseases the very long latency period between the time of infection and the clinical manifestation of the disease provides a wide window for intervention after infection has occurred but before brain damage is initiated.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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