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Prions and retroviruses: an endosomal rendezvous?

The past two decades have seen the emergence of endocytic pathways as highly regulated systems for the sorting, selective degradation and recycling of nearly all cell-surface membrane proteins. At the centre of these pathways are complex endosomal structures known as multivesicular bodies (MVBs) that house intraluminal vesicles (ILVs) formed by invagination and budding from the limiting membrane (Fig 1A). The ILVs contain membrane proteins from the Golgi complex and cell surface that are destined typically for degradation after the fusion of MVBs with lysosomes. This constitutive fate of ILVs is universal to eukaryotes and represents one of the main pathways for membrane protein degradation (Katzmann *et al*, 2002).

Complex multicellular organisms have elaborated on this basic MVB pathway, evolving additional regulatory mechanisms to retrieve and re-route MVB components to non-degradative fates (Fig 1B). Interest in the regulation of MVBs has exploded in recent years with the realization that many viruses, including human immunodeficiency virus 1 (HIV-1), exploit these transport pathways for their assembly and release from cells (Morita & Sundquist, 2004). This area of protein transport could attract another field of researchers—the prion protein aficionados. Intriguing new studies indicate a potentially key role for MVB pathways in prion transmission and metabolism, reinforcing the central importance of protein transport pathways as the molecular basis of various diseases.

Prion diseases are transmissible neurodegenerative disorders (Aguzzi & Polymenidou, 2004) in which the central player is the widely expressed, glycosylphosphatidylinositol (GPI)-linked cellular prion protein (PrP^{C}). The terminal stages of these diseases are characterized by the accumulation in the central nervous system of an abnormally folded and aggregated form of PrP^{C} known as PrP^{Sc}. Exogenously acquired PrP^{Sc} mediates the conversion of host-encoded PrP^{C} to PrP^{Sc}, leading to both PrP^{Sc} accumulation and the generation of more infectious units. Clearly, the mechanism of PrP^{C} to PrP^{Sc} conversion and the pathways of PrP^{Sc} spread within and between cells and organisms are of utmost importance to understanding prion disease transmission. However, surprisingly little is understood about how PrP^{Sc} is released from or transferred among cells.

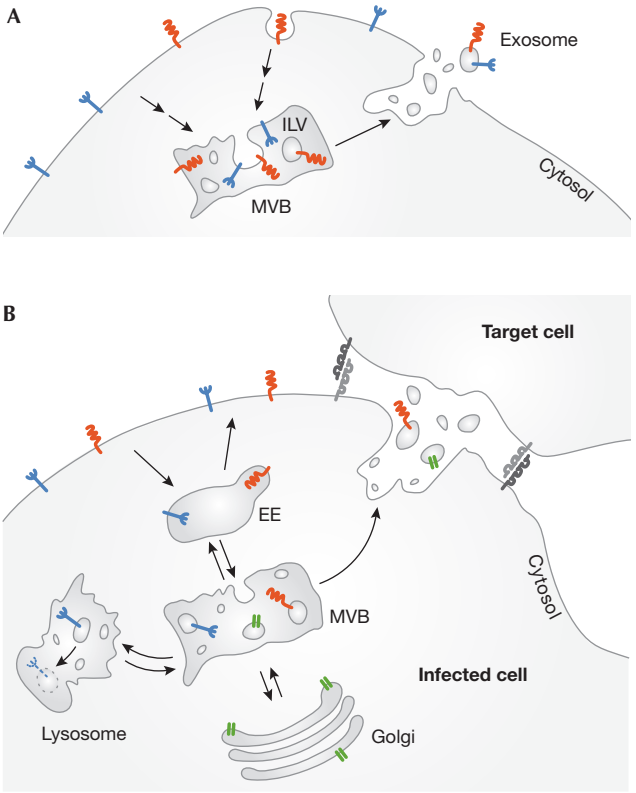

Fig 1 | Protein transport in the endo-lysosomal pathways. (**A**) Topological relationships of the multivesicular body (MVB). Different types of cell-surface proteins (indicated by blue and red molecules) can be internalized and transported by various routes (arrows) to endosomal compartments. The endosomal compartment is known as an MVB when its lumen contains intraluminal vesicles (ILVs) that are formed by budding from the limiting membrane. If the MVB fuses with the plasma membrane, ILVs are released into the extracellular space and become known as exosomes. (**B**) Transport into and out of the multivesicular body (MVB). Pathways linking the MVB with other compartments, such as the early endosome (EE), Golgi, lysosome and plasma membrane, are all regulated in a substrate-specific and cell-type-specific manner to control the localization and fate of various membrane proteins (blue, red and green molecules). Alterations of these transport pathways is emerging as a key facet of viral pathogenesis and might also influence the intracellular accumulation and cell-to-cell transmission of prions. For example, adjacent cells, through interactions among cell surface signalling or adhesion molecules, might stimulate localized MVB fusion with the plasma membrane to facilitate exosome release and intercellular transfer of their cargo.

Fevrier *et al* (2004) demonstrated that PrP^{Sc}-infected cells in culture discharge both PrP^{C} and PrP^{Sc} into the extracellular medium in association with exosomes, the name given to ILVs released after the fusion of MVBs with the plasma membrane (Fig 1A).

PrP^{Sc}-containing exosomes were enriched in prion infectivity, raising the provocative, albeit speculative, idea that exosomes are important carriers of PrP^{Sc} between cells and tissues of an infected organism. Implicit in this hypothesis is the prediction that manipulation of the pathways leading to exosome generation should markedly influence both PrP^{Sc} release and its infectious spread. As some of the most active research in MVB transport is being carried out by virologists, studies in this field might offer insight into the cellular release of PrP^{Sc}.

Retroviruses including murine leukaemia virus (MuLV) and HIV-1 have been found to bud into endosomal membranes by hijacking the same machinery used for the generation of ILVs (Morita & Sundquist, 2004). On delivery of MVBs to the plasma membrane, viral particles, similar to ILVs, are released from the cell. The obvious advantages of this mode of viral assembly are the minimal exposure of viral proteins to extracellular immune surveillance during budding and the opportunity to acquire host endocytic proteins that might contribute to immune evasion by viral particles. It has been suggested that these viruses have even evolved mechanisms to avoid degradation by inhibiting the transport of MVBs to lysosomes while temporally and spatially regulating their release at the plasma membrane (Fig 1B). Thus, retroviruses seem to subvert the endocytic transport pathways markedly to stimulate MVB formation and release at the cell surface. Could this influence the cellular release of PrP^{C} and/or PrP^{Sc}?

The recent findings of Leblanc *et al* (2006), published in the 21 June issue of *The EMBO Journal*, suggest that the pathways of viral release and PrP release might indeed intersect at the MVB. These authors used a combination of immunoelectron microscopy, immunoisolation and co-fractionation methods to show that both MuLV and HIV-1 infection of cultured cells markedly stimulates the release of PrP^{C}. MuLV infection also stimulates PrP^{Sc} release, and this was inhibited by budding-incompetent MuLV. The released PrP^{C} and PrP^{Sc} were found largely in association with viral particles and cellular exosomes. By using a transwell co-culture assay, the authors could further show that virus-stimulated release of PrP^{Sc} led to increased infection of target cells. These results indicate that retrovirus infection stimulates the release and spread of PrP^{Sc}, potentially by modulating MVB transport pathways.

The authors speculate that retroviruses, perhaps endemic in certain flocks of sheep, might act as (presumably non-obligatory) co-factors in the infectious spread of prions. Validation of this provocative suggestion must await future studies. Nonetheless, the combined work of Fevrier, Leblanc and colleagues provides the field of prion transmission with a new cell biological focus: the highly regulated endocytic pathways of intracellular transport. The rapidly emerging intricacies of MVB formation, transport and exosome release might help to reconcile otherwise disparate observations on prion infectivity. In the discussion below, we consider a few of the more intriguing recent findings to exemplify how the framework of endocytic transport might point towards testable hypotheses for future study.

An apparent requirement of cell-to-cell contact for the efficient intercellular spread of PrP^{Sc} (Kanu *et al*, 2002; Liu *et al*, 2002) seems at odds with exosome-mediated transfer. However, the sites for exosome release are unlikely to be random and might be stimulated by extracellular cues that are still poorly understood. In the case of viral release, local fusion of MVBs at sites of close cell-to-cell apposition (virological synapses) is thought to mediate efficient infection of nearby cells (Morita & Sundquist, 2004). Thus, an analogous mechanism of stimulated, focal release of PrP^{Sc}-laden exosomes might explain why in some cell types or under certain culture conditions, close juxtaposition of cells is necessary for the spread of PrP^{Sc} (Fig 1B). We speculate that such a directed intercellular transfer of PrP^{Sc}-enriched exosomes might be pertinent early in the course of prion infection, when PrP^{Sc} is robustly replicated and spread throughout lymphoreticular tissues and lymphoid organs (Aguzzi & Polymenidou, 2004). Consistent with this idea, most examples of regulated viral and exosome release involve cells of the immune system (Morita & Sundquist, 2004), making them attractive model systems for studying the regulation of exosome dynamics and perhaps PrP^{Sc} spread.

If PrP^{Sc} is recruited into ILVs and can be stored in MVBs (or constitutively degraded through lysosomes) until stimulated to be released, an appropriate stimulus could release bursts of infectivity. Two studies have shown that inflammation, induced by either lymphofollicular mastitis in sheep (Ligios *et al*, 2005) or nephritis in mice (Seeger *et al*, 2005), can induce PrP^{Sc} release in milk and urine, respectively. It is tempting to hypothesize that inflammatory signals can stimulate exosome release and hence promote local PrP^{Sc} spread. This is a particularly appealing idea because immune cells have already been shown to regulate MVB formation and exosome release on stimulation by extracellular stimuli. Although the mechanisms involved in stimulation of exosome release and the role of inflammation in this process remain to be clarified, the predictions are both specific and testable.

Finally, the endo-lysosomal system has long been implicated not only in the normal turnover of PrP^{C}, but also in the (albeit rather slow) degradation of PrP^{Sc}. Because both PrP^{C} and PrP^{Sc} transport intersect in endosomes and lysosomes, it is widely thought that the conversion of PrP^{C} to PrP^{Sc} occurs in these intracellular compartments. Examination of this idea requires a mechanistic understanding of how PrP is sorted in MVBs under normal circumstances. This step is not only presumably a decisive event in the constitutive degradation of PrP in lysosomes, but also a point of probable divergence in PrP transport during the course of disease.

An intriguing aspect of sorting in the endocytic system is that the oligomerization state of proteins has been shown to alter their transport itineraries markedly (Marsh *et al*, 1995; Vidal *et al*, 1997; Wolins *et al*, 1997). For example, both the oligomerization and aggregation of cell-surface molecules have been shown to cause their prolonged retention in endosomal structures through the inhibition of recycling mechanisms. It is thus plausible that PrP^{Sc}, by virtue of its oligomeric structure, could modify the cellular transport of PrP^{C} in such a way as to evade degradation and perhaps facilitate replication in a sequestered, partly denaturing and immune-privileged environment. In such a scenario, lysosomes are 'bioreactors' for the replication of PrP^{Sc}, an idea suggested many years ago for the scrapie agent by Laszlo and colleagues on the basis of morphologic analysis of infected brain (Laszlo *et al*, 1992). Clearly, testing these ideas will require not only a quantitative and mechanistic understanding of PrP^{C} biosynthesis, transport and metabolism, but also the tools to manipulate individual steps experimentally. The illumination of MVBs as a potentially key branch point in the transport pathways of PrP^{C} and PrP^{Sc} opens up a range of possibilities for future work on prion disease transmission and spread.

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