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The Transcellular Spread of Cytosolic Amyloids, Prions, and Prionoids

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Recent reports indicate that a growing number of intracellular proteins are not only prone to pathological aggregation but can also be released and "infect" neighboring cells. Therefore, many complex diseases may obey a simple model of propagation where the penetration of seeds into hosts determines spatial spread and disease progression. We term these proteins *prionoids*, as they appear to infect their neighbors just like prions—but how can bulky protein aggregates be released from cells and how do they access other cells? The widespread existence of such prionoids raises unexpected issues that question our understanding of basic cell biology.

Imagine that you are a neuroscientist vacationing on Mars. One day you encounter a colony of Martians that, as it happens, look similar to water bottles. The Martians are highly distressed and seek your advice, as their community is plagued by an enigmatic transmissible disease. Intrigued, you agree to help. It turns out that the bodies of your exobiotic friends consist of bottles filled with a supersaturated salt solution. At some point crystals have started forming in one individual, and then crystallization has somehow been transferred to other community members. Lacking molecular insight, you would initially conclude that the Martians are affected by an infectious agent. Through ingenuity and technology, you may then discover that the infectious agent is exceedingly simple and homogeneous, that it lacks informational nucleic acids, and that it is generated both by ordered aggregation of an intrinsic precursor and by appositional growth of extrinsically added seeds. Your discovery will earn you the Intergalactic Nobel Prize, yet two crucial questions remain unanswered: how do the crystals transfer between individuals, and what can be done to prevent this from happening?

Middle-aged readers may feel reminded of the plot for *Andromeda Strain*, a stunningly prescient novel published in 1969 by the late Michael Crichton. But the sci-fi scenario described above is also the blueprint of Prusiner's hypothesis of prion propagation. Over time, we have learned that prions consist of PrP^{Sc}, higher-order aggregates of a physiological protein termed PrP^{C}. Accordingly, prions propagate through elongation and breakage of PrP^{Sc} aggregates (Aguzzi and Polymenidou, 2004)—not unlike the crystals vexing our extraterrestrial friends.

There is mounting evidence (Clavaguera et al., 2009; Frost et al., 2009; Ren et al., 2009; Desplats et al., 2009; Luk et al., 2009) suggesting that the events sketched above, far from being confined to science-fiction and prion diseases (whose incidence in humans is just $\approx 1/10^6$/year), may underlie highly prevalent human diseases of the brain and many other organs. The unifying characteristics of all these diseases is the aggregation of proteins into highly ordered stacks, henceforth termed "amyloids" irrespective of their size. Since PrP^{Sc} undoubtedly fulfills the latter definition of amyloid, one is led to wonder whether the prion principle may be much more pervasive than previously appreciated and whether many more diseases of unknown cause may eventually turn out to rely on prion-like propagation (Table 1, upper panel). Even more intriguingly, a number of proteins appear to exert normal functions when arranged in highly ordered stacks that are similar to amyloids and to prionoids (Table 1, lower panel).

Prions and Prionoids

There is one crucial difference between bona fide prion diseases and all other amyloids and prion-like phenomena hitherto described in uni- and pluricellular organisms (Table 1). Prions are infectious agents, transmissible between individuals, and tractable with microbiological techniques—including, e.g., titer determinations. Even if certain amyloids of yeast and mammals appear to infect neighboring molecules and sometimes neighboring cells, they do not propagate within communities, and none of them were found to cause macroepidemics such as Kuru and bovine spongiform encephalopathy. We have therefore termed these self-aggregating proteins "*prionoids*" (Aguzzi, 2009), since the lack of microbiological transmissibility precludes their classification as true prions.

Some prionoids may soon qualify for an upgrade to prion status. At least in select settings, amyloid A (AA) amyloidosis may exist as a truly infectious disease based on a self-propagating protein. AA amyloid consists of orderly aggregated fragments of SAA protein, whose deposition can damage many organs of the body. Somewhat bizarrely, AA aggregation is also present in the liver of force-fed geese, hence contributing to the pathophysiology of foie gras (Solomon et al., 2007). AA seeds can induce amyloidosis upon transfer of white blood cells (Sponarova et al., 2008). Furthermore, AA seeds are excreted with the feces, and AA amyloidosis is endemic in populations of cheetah (Zhang et al., 2008). It is therefore tantalizing to suspect that amyloid may entertain the complete life cycle of an infectious agent, including transmission by the orofecal and hematogenous route—similarly to enteroviruses and, perhaps,

Table 1. Potential Prionoids in Health and Disease (Adapted from Aguzzi, 2009)

Phenotype/Function	Protein	Molecular Transmissibility	Bona Fide Infectivity
Prion diseases	PrP^{Sc} (luminal)	yes	yes
Alzheimer's disease	Aβ (luminal)	yes	in APP-overexpressing mice
Tauopathies	Tau (cytosolic)	possibly	not shown
Parkinson's disease	α-synuclein (cytosolic)	host-to-graft	not shown
AA amyloidosis	SAA (luminal)	yes	probable
Huntington's disease	PolyQ (nuclear)	yes	not shown
Suppressed translational termination (yeast)	Sup35	yes	limited
Biofilm production (bacteria)	bacterial curlin	yes	questionable
Heterkaryon incompatibility (fungi)	Het-s	yes	limited
Pituitary secretory granules	peptide hormones	not shown	not shown
Mammalian skin pigmentation	Pmel17	not shown	not shown

scrapie prions. While there may be many other good reasons to avoid foie gras, including, e.g., animal welfare concerns, gourmets may not need to panic: under experimental conditions, AA amyloidosis is only transmitted to $AgNO_3$-pretreated mice that display elevated levels of the SAA precursor protein.

Alzheimer's disease (AD) has long been suspected to be a transmissible disease, but these suspicions have never materialized in epidemiological studies. On the other hand, Mathias Jucker and Lary Walker observed that injection of the Aβ peptide from human AD brains induced robust and convincing aggregation of Aβ in transgenic mice overexpressing the Aβ precursor protein, APP (Kane et al., 2000; Meyer-Luehmann et al., 2006). Jucker's finding raises an epistemologically significant question: if aggregation depends on the introduction of seeds and on the availability of the monomeric precursor, and if amyloid represents the primordial state of all proteins (Chiti and Dobson, 2006), wouldn't all proteins—under appropriate conditions—give rise to prionoids in the presence of sufficient precursor?

The issues sketched above go well beyond AD and prions. There are many other diseases—not necessarily involving the nervous system—whose pathogenesis involves ordered aggregation of proteins, but for which there is no evidence of transmission between individuals. The best-studied of these are the systemic amyloidoses, which come about through the nucleation of some aggregation-prone proteins such as transthyretin and immunoglobulin light chains. Yet ordered protein aggregation is by no means confined to the "classical" amyloidoses and extends to a number of conditions, some of which have been rather unexpected.

Type II diabetes is yet another disease whose pathogenesis may involve ordered protein aggregation. Evidence to support this idea was discovered over a century ago (Opie, 1901) but was largely forgotten until recently. It is now evident that aggregation of islet amyloid polypeptide (IAPP) is an exceedingly frequent feature of type II diabetes. IAPP amyloids damage the insulin-producing β cells within pancreatic islets and may crucially contribute to the pathogenesis of diabetes (Hull et al., 2004). It is unknown, however, whether IAPP deposition simply accrues linearly with IAPP production or whether it spreads prion-like from one pancreatic islet to the next.

A body of recent work supports the idea that many aggregation proteinopathies are, in one way or another, transmissible. A recent report showed that α-synuclein is released from neurons and is then taken up by the neighboring cells, thereby aiding in a progressive spread of the protein (Desplats et al., 2009; Lee et al., 2005). When exogenously added to cultured cells, fluorescently labeled, recombinant α-synuclein was internalized from the extracellular milieu into the cytosol. Furthermore, injection of GFP-labeled mouse cortical neuronal stem cells into the hippocampus of α-synuclein-transgenic mice led to the efficient uptake of the host α-synuclein into the grafted cells after just 4 weeks. These findings are reminiscent of the observation that healthy fetal tissue, grafted into the brains of Parkinson's disease patients, acquired intracellular Lewy bodies. The latter phenomenon is somewhat anecdotal and has been disputed (Mendez et al., 2008), yet it would be entirely compatible with the hypothesis that α-synuclein aggregates are prionoids (Li et al., 2008). A similar study conclusively demonstrated that exogenous α-synuclein fibrils induced the formation of Lewy body-like intracellular inclusions in vitro (Luk et al., 2009). This study also showed that the conversion of the host cell α-synuclein was accompanied by dramatic changes, including hyperphosphorylation and ubiquitination of α-synuclein aggregates—thus recapitulating some key features of the human pathology.

In experiments conceptually analogous to those discussed above, polyglutamine-containing protein aggregates similar to those present in Huntington's disease and in spinocerebellar ataxias exhibited prion-like propagation (Ren et al., 2009). There, aggregation of huntingtin progressed from the extracellular space to the cytosol and eventually to the nucleus. What is more, similar phenomena occurred upon exposure of cells to Sup35 aggregates, which consist of a yeast protein for which there are no known mammalian paralogs. This suggests that the prionoid properties are intrinsic to amyloids and are not tied to the origin or function of their monomeric precursor protein.

In another work, Tolnay and colleagues report a similar phenomenon in a mouse model of "tauopathy," a neurodegenerative disease due to intraneuronal aggregation of the microtubule-associated tau protein (Clavaguera et al., 2009). Aggregation-prone mutant tau, when extracted from the brain of transgenic mice, induced tauopathy in mice overexpressing wild-type tau. Assuming that tau pathology wasn't elicited by

some indirect pathway (tau-overexpressing mice develop tangles when exposed to Aβ aggregates [Götz et al., 2001]), these transgenic mice appear to behave like the Martian bottles, since tauopathy was not induced in mice expressing normal levels of tau. In yet another study, the microtubule binding part of the full-length tau was found to attack and penetrate cells when added exogenously, and this again induced host tau misfolding (Frost et al., 2009). This study also showed that aggregated intracellular Tau spontaneously transferred between two cocultured cell populations (Frost et al., 2009). In the case of both tau and polyglutamines, the protein aggregates appear to gain access to the cytosol and to cause further aggregation of their host counterparts—presumably by nucleation.

The unifying characteristics of all these diseases is the aggregation of proteins into highly ordered stacks, termed amyloids irrespective of their size; the growth of these structures also exhibits generic features (Knowles et al., 2009) shared with a wide class of self-assembly phenomena characterized by elongation and fragmentation, such as the formation of analogous aggregates in micro-organisms and in vitro. Two conclusions can be drawn from the recent studies: (1) an unexpected number of amyloidogenic proteins can be released from affected cells in the form of extracellular amyloid seeds, and (2) even more surprisingly, these seeds can then re-enter other cells and nucleate the aggregation of their intracellular counterparts—in the cytosol or even in the nucleus. The biological and practical implications are far-reaching. On the one hand, cell therapies of aggregation diseases may be more difficult than anticipated, as the transplanted cells may undergo infection. A possible remedy could consist in the removal of the genes encoding the precursor of the offending proteins from the cells utilized for therapy—e.g., using the zinc-finger nuclease strategy (Hockemeyer et al., 2009). On the other hand, a novel paradigm of amyloid pathogenesis is emerging from these data, whereby each prionoid behaves as a self-assembling and self-replicating nanomachine.

Conversely, these findings raise a number of enigmas for which we are lacking any satisfactory answer. Whereas PrP^{C} and the Aβ are luminally exposed, α-synuclein and tau are cytoplasmic—and huntingtin is even nuclear. Aggregates of both Aβ and PrP^{Sc}, as well as their monomeric precursors, are found in the extracellular space; it is hence intuitive that the nucleation process can propagate spatially across large distances. Instead, the propagation of cytoplasmic prionoids challenges our basic cell-biological understanding, since it posits that protein aggregates are released into the extracellular space and can subsequently reenter—and wreak havoc—in the cytosol of other cells. The release of cytosolic amyloids is supported by the amelioration of Lewy body pathology in α-synuclein transgenic mice immunized with human α-synuclein (Masliah et al., 2005). Similarly, anti-tau oligomer immunotherapy reduced brain pathology (Asuni et al., 2007), and immunization with mutant SOD1 led to clearance of SOD1 and delayed the onset of the disease in mice (Urushitani et al., 2007). All of these results indicate that cytosolic amyloids are somehow accessible to extracellular antibodies. This raises the question of how these proteins are released into the extracellular space ("cytosol to lumen") and how they subsequently re-enter cellular cytosol ("lumen to cytosol"). Both events require trespassing lipid bilayer barriers—by no means a trivial feat for proteins, let alone high-molecular-weight aggregates.

Release of Cytosolic Prionoids from Cells

The release of cytosolic proteins into the extracellular milieu is by no means an exclusive feature of amyloids. While most secreted proteins follow the conventional ER-Golgi biosynthetic pathway, several proteins have been reported to be secreted through a noncanonical pathway (Muesch et al., 1990; Nickel, 2003; Prudovsky et al., 2003). These proteins often lack a patent secretory signal sequence, and their release is not dependent on the intact ER/Golgi machinery. Prominent examples include the proangiogenic molecule, fibroblast growth factor-1 (Jackson et al., 1995; Mandinova et al., 2003; Prudovsky et al., 2002) and -2 (Engling et al., 2002; Mignatti et al., 1992), IL-1β (Andrei et al., 1999; Rubartelli et al., 1990), annexins, migration inhibitory factor1 (Flieger et al., 2003), galectins (Cleves et al., 1996; Cooper and Barondes, 1990; Lutomski et al., 1997), and caspase 1. While no single key mechanism has been documented for their exit from the cytoplasm, each of the following pathways discussed may potentially contribute to this phenomenon.

Direct Translocation at the Plasma Membrane

Transport of proteins across the plasma membrane via transporter complexes is uncommon, but not unheard of. Perhaps the only conclusive evidence for this type of translocation across the membrane has been shown for the growth factor FGF-2 (Schäfer et al., 2004). Reconstitution assays in inside-out vesicles (extracellular side inside and cytoplasm outside) indicate that cytosolic FGF-2 can directly translocate to the extracellular compartment in a temperature- and time-dependent manner. This translocation is also exhibited by galectin-1, but not by FGF-4 protein or MIFs, suggesting a certain degree of specificity. Polypeptides are transported across the endoplasmic reticulum bilayer by the Sec61 translocon and—in the opposite direction—by the retrotranslocon machinery (Lilley and Ploegh, 2004): one might therefore posit the existence of analogous transporters embedded in the plasma membrane.

Could cytoplasmic prionoids utilize such hypothetical transporters? Both α-synuclein and huntingtin can interact with acidic phospholipids enriched on the cytoplasmic leaflet (Kegel et al., 2005, 2009; van Rooijen et al., 2008) and have been proposed to form pores or conducting channels (Figure 1). However, the precise mechanics of this type of secretion remains mysterious. Although the interaction of aggregates with lipids has been documented to occur in protein-free liposomes, its importance for translocation across biological membranes remains unclear.

Apoptotic Blebs and Microvesicles

Apoptotic blebs are subcellular micelles that are released by dying cells and may encapsulate significant amounts of cytosol (Figure 1) (Cocucci et al., 2009). Whether there is a general incorporation of cytosolic proteins or there is specificity in the nature of proteins that undergo encapsulation is not yet clear. Rigorous proteomic analysis could address this issue (Alcazar et al., 2009). While similar to blebs in size and content, microvesicles are formed in healthy cells by outward budding of the plasma

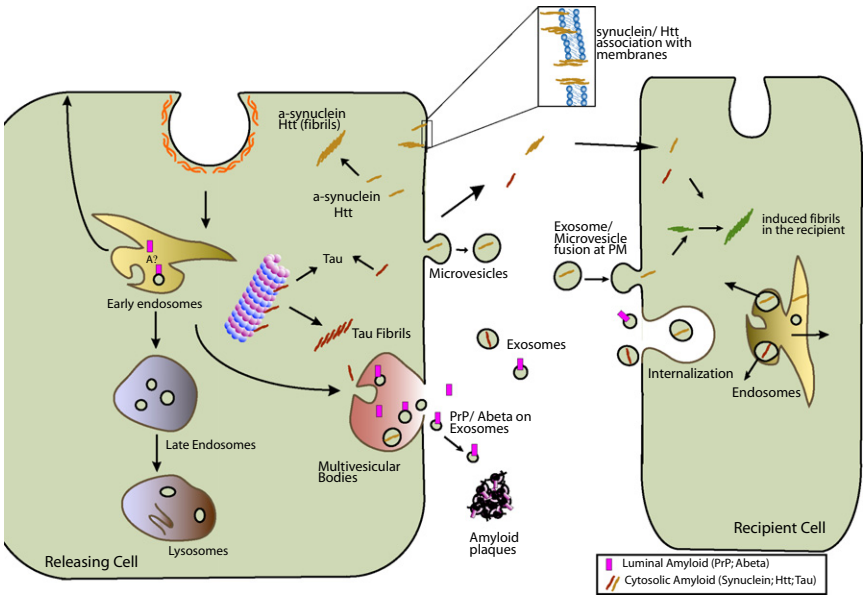

Figure 1. Cellular Routes for the Release and Uptake of Cytosolic and Luminal Amyloids

membrane—a process analogous to the release of enveloped viruses. FGF-2 was also found in microvesicles (Taverna et al., 2003), as was capsase-1, another protein that is secreted via a non ER-Golgi pathway. Interestingly, caspase-1 itself is involved in the secretion of several cytosolic proteins by a mechanism that is poorly understood (Keller et al., 2008; Pétrilli et al., 2007). Caspase-1 silencing by RNAi or its pharmacological inhibition inhibits the secretion of several cytosolic proteins, including galectins, fgf-1 and -2, MIfs, and IL1-α and -β (Keller et al., 2008). Experiments with small-molecule inhibitors suggest that the proapoptotic function of caspase-1 or its proteolytic activity is essential for the secretion. The critical factor that enables secretion upon cleavage and activation by caspase is still unknown: its identification will definitely shed light on the mechanism by which cytosolic proteins are secreted.

Release through the Exosome Shuttle

Several cytosolic proteins, including alix, enolase, heat shock proteins, caspase-1, and galectin, are released via exosomes (Mathivanan et al., 2009; Olver and Vidal, 2007; Wubbolts et al., 2003). Although exosomes are mostly implicated in the sorting and release of membrane proteins, they also carry a substantial amount of cytosol. During endocytosis, the plasma membrane invagination (outside-in) gives rise to early endosomes, the limiting membrane of which undergoes another round of invagination (inside-out) to form the intraluminal vesicles, which give the endosome a multivesicular appearance. Upon invagination, these intraluminal vesicles encapsulate cytosolic material. Multivesicular bodies harboring the intraluminal vesicles can now fuse with the plasma membrane to release these ILVs as exosomes. This also explains the topology of exosomes being identical to that of the plasma membrane (outside-out; inside-in) with the cytosol encapsulated within them (Figure 1). By taking the exosome shuttle—but also by hijacking blebs and microvesicles—protein aggregates may depart from their cells of origin without the need to cross any membrane.

Both microvesicles and exosomes are found in plasma and other body fluids, suggesting that these fluids may act as vectors for prionoids.

Exosomes deserve special consideration because of their relevance to neurodegeneration. Luminally exposed amyloid-forming proteins, such as Aβ and PrP, are both secreted on exosomal vesicles, and this association has been implicated in disease progression and pathogenesis. Amyloidogenic processing of amyloid precursor protein by β- and γ-secretase generates Aβ peptide in early endosomes (Rajendran et al., 2006), trafficked to multivesicular bodies (Rajendran, et al., 2007), and is then released from the cells via exosomes. Both PrP^{C} and the infectious PrP^{Sc} are also trafficked in cells via multivesicular bodies and are associated with exosomes (Fevrier et al., 2004; Veith et al., 2009). Since amyloid β peptide and prion release have pathological consequences, it is possible that exosomes function as Trojan horses facilitating the release of these pathogenic peptides and playing a role in disease progression (Rajendran, et al., 2007). Perhaps aggregates of α-synuclein, tau, and huntingtin could be transported inside exosomal vesicles for long-range signaling or deposition. Moreover, exosomal vesicles contain lipids that drive fibrillation and mediate accelerated amyloid formation (Yuyama et al., 2008).

Autophagy, by which certain cytosolic proteins are engulfed, may also influence exosome secretion (Fader et al., 2008), yet whether autophagy regulates the release of cytosolic proteins remains to be seen. The uptake of exosomes and exosome-capsulated proteins could shuttle proteins between cells, as recently observed for the transfer of Wnt protein between synapses via exosome-like vesicles (Korkut et al., 2009).

Discharge of Aggregates through Pores

Biophysical studies of membrane lipid-Aβ interactions suggest that Aβ peptides can form pores on the membrane (Kayed et al., 2004, 2009; Lashuel et al., 2002). These pores can mediate the leakage of ions and small molecules. α-synuclein and huntingtin were also found to interact with membrane lipids and form pores at the membrane akin to those mediating the entry of toxins into the cytosol (Georgieva et al., 2008; Zhu et al., 2003). Lipid association or membrane anchoring of these peptides may drive their oligomerization and, ultimately, pore formation (van Rooijen et al., 2009). However, these pores are thought to be similar to ion channels, and it is not trivial to envisage how they could eject such large protein aggregates.

On a more practical note, the findings that cytosolic amyloids may be released from cells raise the intriguing possibility that the

aggregates may be found in body fluids such as blood and cerebrospinal fluid (CSF). If so, they could serve as biomarkers of disease progression. In fact, CSF tau is a relatively sensitive though unspecific biomarker of AD (Blennow and Hampel, 2003), probably because it is released by dying neurons. In the light of these new findings, one could imagine that *aggregated* tau and other cytosolic amyloids could also be released from cells in a regulated way without neuronal death. Further work is required to assess whether other cytosolic amyloid proteins such as SOD1 (involved in amyotrophic lateral sclerosis or ALS) and TDP-43 (in frontotemporal dementias and ALS) are also released and nucleate their siblings in foreign cells. Indeed, antibodies against intracellular proteins are detected in Alzheimer's disease. Regulated exocytic release of these proteins could explain their presence in the CSF and, in some cases, in the plasma of the affected individuals.

Internalization: The Arduous Path of External Aggregates into the Cytosol

If the externalization of protein aggregates presents an interesting quiz, their internalization and the subsequent nucleation of cytosolic proteins in target cells is even more puzzling. If prionoids are internalized through endosomes, the limiting membrane of the endosomes poses a barrier for these proteins to diffuse across the bilayer into the cytosol. In the following, we review few examples of extracellular proteins ending up in the cytosol.

Direct Penetration of the Plasma Membrane

Peptides derived from viral proteins (HIV-tat, HSV-VP22) and Antennapedia proteins have been documented to have cell-penetrating activity (Elliott and O'Hare, 1997; Frankel and Pabo, 1988), and this has been utilized to target drugs to cytosolic compartments (Rajendran et al., 2009). While the mechanism of cell penetration is still poorly understood, interactions of peptides with positively charged phospholipids are thought to induce a conformational change that allows the passage of these peptides through the bilayer (Wender et al., 2000). It is theoretically possible that prionoids gain access to the cytoplasm via such a mechanism, but it is difficult to imagine that high-molecular-weight aggregates would avail themselves of this pathway.

Release from the Endosomes by Endo-Osmolysis or Endosomal Fusion

Certain bacterial toxins such as diphtheria and cholera toxins bind to their receptors at membrane from the extracellular side, traffic to the endosomes or Golgi, and enter the cytosol (Johannes and Decaudin, 2005). It is within this compartment that they gain access to their targets—a property that can be exploited by conjugating drugs to the ligands/toxins (Rajendran et al., 2009). Enveloped viruses, on the other hand, deliver their genome to the cytosol (and in some cases to the nucleus) by either fusing their envelopes with the plasmalemmal or endosomal membrane or by releasing their contents by pore formation in endosomes (endosomal fusion) (Leopold and Crystal, 2007). The influenza virus carries a fusogenic peptide sequence at the N terminus of viral hemagglutinin, which allows the virus to fuse with the endosomal membrane. Other pathogen-associated proteins, such as diphtheria toxin, colonize the cytosol by disrupting endosomal membranes (endo-osmolysis) (Huang et al., 2003).

It is not inconceivable that prionoids possess the properties demonstrated by other pathogens, as outlined above, but it is also possible that binding to cationic phospholipids at endosomal pH triggers their entry into the cytosol. Lee and colleagues showed that α-synuclein is internalized in endosomes via a dynamin-1-dependent pathway (Desplats et al., 2009), but how α-synuclein leaves the endosome is shrouded in mystery. Once in the cytosol, these nuclei may elongate, fracture, and produce further nuclei—ultimately promoting the formation of inclusion body-like structures in the cytosol of acceptor cells. This process relies on the integrity of lysosomes, as lysosomal pH disruption using v-ATPase inhibitors aggravates the formation of inclusion bodies. How lysosomal function is coupled to a process that occurs in the cytosol is unclear, but lysosomal degradation of these cytosolic structures might be essential for homeostasis.

Exosome Fusion at the Plasma Membrane or in Endosomes

If prionoids travel via exosomes, the cytosolic contents of exosomes might be released into the cytosol of the target cell by direct fusion with the plasma membrane, or by fusion with the limiting membrane of early endosomes after internalization. In view of their virus-like properties, which include their budding (Morita and Sundquist, 2004) and their contents of genetic material (miRNA and mRNA) (Valadi et al., 2007), one wonders whether exosomes may behave like primitive viral-like particles that ferry molecules from one cell to the other. In fact, exosomes contain CD9, a fusogenic protein involved in gamete fusion and in viral fusion (Théry et al., 1999). For each of these reasons, exosomes are prime candidates for the release, the extracellular transit, and the eventual cytoplasmic delivery of prionoids. Maybe exosomes carry amyloids on the membrane but sequester the cytosolic amyloids inside the vesicle. If so, the topology after fusion would be maintained. This would ensure proper localization of the amyloids to compartments where further conversion may occur.

Propagation of Prionoids in the Brain

In α-synucleinopathies, the pathology spreads progressively from the canonical sites of Parkinson's disease to remote areas of the brain (Brundin et al., 2008). In tau pathologies, a similar spreading occurs from the transentorhinal cortex to the hippocampus. In addition to the mechanisms discussed above, prionoids may utilize nanotubes as an extremely direct means of propagating from cell to cell. Indeed, prions appear to hijack tunneling nanotubes for their intercellular spreading (Gousset et al., 2009). Even if naked α-synuclein and polyQ could enter cells from the extracellular space, nanotubes may play a role in the spatial spreading of pathology. At least in the case of prions, there is strong evidence that transfer of pathology from one neuron to another occurs transsynaptically (Glatzel et al., 2001; Prinz et al., 2003), and a series of legendary experiments has demonstrated progressive colonization of the entire visual

system, including the superior colliculus, the lateral geniculate, and the optical cortex after retinal prion inoculation (Fraser, 1982). The released amyloids could also be taken up by the surrounding astrocytes and microglia network and could aid in the expansion of the pathology. On the other hand, tau and α-synuclein pathology occur not only in neurons but also in glia (Park et al., 2009), suggesting that the astrocyte-microglia network might also internalize the cytosolic amyloids. It remains an open question whether the latter is a good or a bad thing for the brain: microglia may be the brain's most important defense against prions and prionoids (Falsig et al., 2008), but conversely could also assist in their spread (Beringue et al., 2000) (Figure 1).

Conclusion

The wave of these recent reports on the prion-like behavior of disparate pathogenic proteins raises many more questions than it answers. Here we have highlighted a number of open issues related to mechanisms of cell-to-cell spread of prionoids. The resolution of such issues may constitute the first step toward the development of rational strategies aimed at blocking transcellular propagation. There is justified hope that the latter may decelerate the progression of pathology and, consequently, help toward fighting the devastating outcome of aggregation proteinopathies.

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