Prions on the run: How extracellular vesicles serve as delivery vehicles for self-templating protein aggregates

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ABSTRACT. Extracellular vesicles (EVs) are actively secreted, membrane-bound communication vehicles that exchange biomolecules between cells. EVs also serve as dissemination vehicles for pathogens, including prions, proteinaceous infectious agents that cause transmissible spongiform encephalopathies (TSEs) in mammals. Increasing evidence accumulates that diverse protein aggregates associated with common neurodegenerative diseases are packaged into EVs as well. Vesicle-mediated intercellular transmission of protein aggregates can induce aggregation of homotypic proteins in acceptor cells and might thereby contribute to disease progression. Our knowledge of how protein aggregates are sorted into EVs and how these vesicles adhere to and fuse with target cells is limited. Here we review how TSE prions exploit EVs for intercellular transmission and compare this to the transmission behavior of self-templating cytosolic protein aggregates derived from the yeast prion domain Sup35 NM. Artificial NM prions are non-toxic to mammalian cell cultures and do not cause loss-of-function phenotypes. Importantly, NM particles are also secreted in association with exosomes that horizontally transmit the prion phenotype to naive bystander cells, a process that can be monitored with high accuracy by automated high throughput confocal microscopy. The high abundance of mammalian proteins with amino acid stretches compositionally similar to yeast prion domains makes the NM cell model an attractive model to study self-templating and dissemination properties of proteins with prion-like domains in the mammalian context.

KEYWORDS. exosome, extracellular vesicles, prion, spreading
Many, if not all cells, release a repertoire of vesicles in the extracellular milieu. Secreted vesicles shed from the plasma membrane or produced by the endosomal system are collectively termed extracellular vesicles (EVs). EVs are important mediators of intercellular communication and transfer proteins, RNAs and other cellular components between cells, thereby modulating diverse cellular processes in acceptor cells. As biomolecules incorporated into exosomes reflect the physiological state of their donor cells, they are also intensely surveyed as biomarker sources. Interestingly, pathogens such as viruses exploit exosomes for intercellular dissemination. EVs have received further attention for their proposed role as transfer vehicles for pathologic proteins in neurodegenerative diseases, including prions, SOD1, TDP-43, Aβ peptides, α-synuclein or Tau.

**Prions - Proteinaceous Infectious Particles**

The first pathogenic protein aggregates identified in exosomes were prions, self-templating protein particles that cause devastating neurodegenerative diseases in mammals. TSEs in mammals occur mostly sporadic, but can also be of genetic or iatrogenic origin and can be infectious. Scrapie in sheep and goats and chronic wasting disease in deer, elk and moose constitute prion diseases that naturally transmit horizontally. The extreme resistance to inactivation procedures that destroy nucleic acid and the discovery that the host-encoded prion protein PrP was the main component of the infectious particle led to the proposal that TSE agents are solely protein-based and devoid of coding nucleic acid. The cellular PrP (PrP\textsuperscript{C}) is a highly glycosylated, glycosylphosphatidylinositol (GPI)-anchored protein enriched in lipid raft microdomains on neuronal and non-neuronal cell membranes. In a seeded polymerization reaction, PrP\textsuperscript{Sc} serves as a template that induces the structural rearrangement of PrP\textsuperscript{C} monomers into β-sheet rich prion polymers. Accumulation of PrP\textsuperscript{Sc} in the central nervous system is associated with astrogliosis and spongiform degeneration. Remarkably, PrP\textsuperscript{C} cannot only take on one but a variety of self-templating conformations that are associated with different pathologies in their host. Substantial biophysical evidence supports the hypothesis that these prion strain properties are enciphered within the 3-dimensional fold of the prion polymer.

While initially coined for TSE agents, the term “prion” was later adopted to describe proteinaceous particles that confer non-Mendelian traits in yeast. Prions in lower eukaryotes are insoluble, self-perpetuating amyloid-like polymers that act as epigenetic elements of inheritance. Unlike mammalian prions attached at the plasma membrane by a GPI-anchor, yeast prions are predominately cytoplasmic. Depending on the genetic makeup of the host and environmental factors, yeast prions can either be detrimental, benign or advantageous to their host. De novo yeast prion induction and replication involve rare spontaneous nucleation events followed by growth and fragmentation of highly ordered protein fibrils, a process similar to the proposed propagation mechanism of mammalian prions. The nucleation phase can be bypassed by exposure of yeast to in vitro formed prion aggregates or cytosolic “propagons” extracted from prion-containing strains. Yeast prion proteins share little sequence homology with PrP. Instead, prion activity is governed by so-called prion domains, disordered regions often enriched in uncharged residues such as glutamine, asparagine and glycine.

In 1982, Prusiner defined prions as “small proteinaceous infectious particles which are resistant to inactivation by most procedures that modify nucleic acid.” This original definition also holds true for protein aggregates in lower eukaryotes. We use the term “prion” to describe a biological process by which biologic information is enciphered, amplified and disseminated through protein conformation. To avoid any confusion in terminology, we will refer to prions causing TSEs as TSE prions, while we will term self-templating protein aggregates identified in yeast as “yeast prions.” Here, we specifically focus on the intercellular dissemination strategies of TSE prions and
compare these to the surprising self-propagating and dissemination properties of a yeast prion domain in mammalian cells. Remarkably, prion-like domains (PrLDs) compositionally similar to annotated yeast prion domains are present in 1% of mammalian proteins, including proteins forming pathogenic aggregates in Amyotrophic Lateral Sclerosis (ALS) or Frontotemporal Dementia (FTD). Prions derived from the yeast prion domain of Sup35 are not homologous to mammalian proteins and thus allow us to study protein aggregation and dissemination in the absence of a loss-of-function phenotype. As such, the yeast prion domain Sup35 constitutes an excellent tool to model general aggregation and dissemination propensities of proteins with related domains.

Extracellular Vesicles Are Involved in Intercellular Communication in Mammals

EVs are heterogeneous and differ in their biogenesis. Most vesicles that bud off the cell membrane (referred to as microvesicles) fall in the range of 200–500 nm, but smaller and larger membrane-bound particles have been described. Although EVs are discriminated by marker proteins, size and density, substantial overlap in all 3 parameters has been observed. Exosomes are EVs in the range of 40–100 nm, which arise through inward budding into specialized late endosomal structures, referred to as multivesicular bodies (MVBs). Fusion of MVBs with the plasma membrane liberates the intraluminal bodies (ILVs) as exosomes into the extracellular space. MVB are not only intermediates of exosome release but also subject to autophagosomal degradation. Although the selection mechanisms that define the fate of cargo proteins remain elusive, accumulating evidence suggests that cells secrete subpopulations of exosomes that differ in cargo composition, size, subcellular distribution and biogenesis. Recent research has highlighted some mechanisms that sort membrane associated proteins and cytosolic proteins into ILVs. These processes can act independently or collaboratively.

Protein sorting into exosomes involves “endosomal sorting complex required for transport” (ESCRT)-dependent and -independent processes. The ESCRT complex and additional regulatory proteins support sorting of ubiquitinated cargo into MVBs. Several other posttranslational cargo modifications have been reported, such as sumylation, phosphorylation or specific carbohydrate signatures. There is direct evidence showing that the number of N-linked glycans is a determinant for exosomal cargo sorting. Membrane microdomains enriched in ceramides were also shown to be involved in cargo sorting. Lipid components of raft-like domains, including cholesterol, ceramide, sphingomyelin, glycosphingolipids and phosphatidylcholine, are highly enriched in exosomes. The raft-like domain not only provides the platform for the ILVs budding, but is directly involved in cargo sorting. Specific lipids and integral membrane proteins such as tetraspanin interact with cargo. Furthermore, aggregation of proteins or lipids might serve as a general sorting signal for exosomes, as antibody-mediated aggregation of cell surface receptors induces their sorting into exosomes. Along these lines, higher-order oligomerization of plasma membrane associated retrovirus Gag protein is sufficient to target it to exosomes for hijacking exosome biogenesis for virus production. Key to the function of EVs is attachment and membrane fusion to deliver biologically active cargo to the target cell. Importantly, exosomes selectively adhere to specific cells, a tropism defined by ligand-receptor interactions. While some receptor and ligand pairs mediating this interaction have been identified, most have not been explored so far. Specific integrins and cell adhesion molecules abundant on EV surfaces can facilitate attachment onto target cells and mediate host cell tropism. Heparan sulfate proteoglycans, phosphatidylserin receptors and lectins can serve as EV receptors. EVs can fuse directly with the plasma membrane and release the vesicle content into the cytoplasm. Alternatively, EVs can be taken up by endocytosis or macropinocytosis.
been described for EV entry.\textsuperscript{36,37} The size limit of cargo that can be internalized by certain pathways might influence the preferred uptake route for EVs.\textsuperscript{38} It is possible that EVs use more than one entry route or use alternative pathways. One alternative route requires fusogenic proteins that mediate docking and direct fusion with host membranes, which has been shown for enveloped viruses and exosomes secreted by placenta.\textsuperscript{2} Moreover, certain exosomal tetraspanin compositions can also mediate EV-host cell adhesion and membrane fusion.\textsuperscript{39} How this fusion process is regulated for other EVs is so far unclear. Endocytosed EVs are either delivered to the lysosome or fuse with the limiting membrane of the late endosome to release their cargo into the cytosol.

Exosomes as Vehicles for Intercellular Dissemination of Transmissible Spongiform Encephalopathy Agents

TSE infection usually occurs through the intestinal route.\textsuperscript{6} The spreading of prions from the gut through the lymphoreticular system and peripheral nerves to the brain involves intercellular dissemination of infectious entities.\textsuperscript{40} How exactly TSE prions spread from cell to cell \textit{in vivo} is only poorly understood. Routes for prion transmission have been mainly studied in cell culture. The formation of the infectious PrP isoform occurs after PrPC has reached the plasma membrane, either directly on the cell surface or within recycling endosomes, endolysosomal vesicles and / or MVBs.\textsuperscript{6,41,42} Different dissemination strategies can be used by TSE prions, including direct cell contact,\textsuperscript{43,44} for example via tunneling nanotubes,\textsuperscript{45} or secretion of prions in EVs, such as microvesicles and exosomes.\textsuperscript{46-50} Cell culture derived exosomes containing PrP\textsuperscript{Sc} are infectious to permissive cell lines and produce clinical disease in mice.\textsuperscript{47,49} The observed differences in dissemination strategies might be related to prion strain differences or infected cell types.\textsuperscript{43-45,48,49,51,52} As EVs extracted from body fluids also contain prion activity, they are likely to contribute to prion dissemination \textit{in vivo}.\textsuperscript{53} Exosomal sorting is not restricted to PrP\textsuperscript{Sc}, as PrP\textsuperscript{C} is a normal constituent of intraluminal bodies in MVBs,\textsuperscript{47,54} and is found in exosomal preparations of immortalized cell lines and primary cells of diverse origins.\textsuperscript{42,48,55-56} Exosomes and microvesicles isolated from body fluids are also decorated with PrP\textsuperscript{C}, suggesting that PrP\textsuperscript{Sc} is a normal constituent of EVs.\textsuperscript{51,62} PrP\textsuperscript{C} expression has been shown to stimulate exosome secretion in primary astrocytes and fibroblasts.\textsuperscript{63} As both PrP\textsuperscript{C} and PrP\textsuperscript{Sc} are partitioned into intraluminal vesicles destined for secretion, protein polymerization is not a required trigger for secretion. Interestingly, the PrP\textsuperscript{Sc} glycosylation pattern often differs between cell extract and exosomes, arguing that specific subpopulations of PrP\textsuperscript{Sc} are selectively sorted into exosomes.\textsuperscript{64} The contribution of different sorting pathways is less clear and might be cell type or strain dependent.

The presence of PrP\textsuperscript{C} and PrP\textsuperscript{Sc} in lipid raft microdomains suggests that PrP isoforms are sorted to exosomes in association with lipid rafts.\textsuperscript{26,65} Both ceramide dependent and Tsg101-ESCRT mediated pathways contribute to exosomal prion secretion in 2 cellular TSE models.\textsuperscript{42,52} While ESCRT Tsg101 subunit silencing directly affected exosome and PrP\textsuperscript{Sc} secretion, a compound inhibiting the ceramide-dependent exosome pathway only marginally affected exosome secretion but led to selective exclusion of PrP\textsuperscript{Sc} and infectivity from exosomes derived from a neuroglial cell line.\textsuperscript{52} This is in contrast to a study using a murine hypothalamic cell line where chemical impairment of the ceramide-dependent pathway reduced exosomes and exosome-associated PrP\textsuperscript{C} and PrP\textsuperscript{Sc} \textsuperscript{52}.

Little is known if TSE prion-containing exosomes derived from different cell types are equally infectious to different recipient cells. Generally, very few cell lines are permissive to TSE prions, and prion strains exhibit selective infectivity for specific cell lines and even subclones thereof.\textsuperscript{6} However, when tested in permissive cell cultures, exosomes isolated from different persistently infected cell lines proved infectious to recipient cells of different origin.\textsuperscript{42,49} TSE prion-containing exosomes might thus be taken up by recipient cells
unspecifically or via ligand-receptor pairs functional in the tested donor-recipient cell combinations. A problem in defining cellular pathways that mediate prion internalization and infection is that TSE prion infection takes days to weeks to be detectable in cell culture. The currently used assays rely on detection of newly formed PrPSc weeks post infection by cell colony blot or western blot. These assays do not measure single cell events and cannot discriminate between early events following internalization and subsequent secondary amplification and spreading events (Fig. 1A). Cellular uptake of PrPSc can also be visualized by confocal microscopy, but also non-permissive cells internalize PrPSc.67 Thus, these studies do not allow drawing conclusions on the internalization pathways that lead to productive infections.

A Yeast Prion Domain as a Model Protein to Study Dissemination Pathways of Cytosolic, Self-Templating Protein Aggregates in Mammalian Cells

Yeast prions have been studied extensively in the past to unravel basic principles of conformational templating. The translation termination factor Sup35 of S. cerevisiae is the best-studied yeast prion. Under rare circumstances, Sup35 adopts an inactive amyloid fold that induces heritable nonsense suppression in progeny and mating partners. Its prion propensity is governed by the prion domain N. The N domain together with a highly charged M domain are modular but otherwise dispensable for the termination function of the carboxyterminal C domain. Like most yeast prion domains, the N domain is enriched in uncharged amino acids, such as glutamine, asparagine, tyrosine, serine and glycine.15

Interestingly, the prionogenic properties of the Sup35 prion domain are conserved when it is expressed in bacteria68 and mammalian cell models.69 Investigating prion-like propagation and dissemination mechanism by using S. cerevisiae Sup35 can thus help to understand basic principles of cytosolic prion-like behavior in heterologous systems. Consistent with the finding that the Sup35 prion state can be induced in prion-free yeast cells by in vitro formed prion aggregates,15,70 we recently demonstrated that cytosolically expressed NM stays soluble in neuroblastoma cells but can be induced to aggregate upon addition of recombinant NM amyloid fibrils.69,71 Once induced, NM aggregates are faithfully propagated to daughter cells over multiple cell divisions. Furthermore, Sup35 NM protein aggregates in mammalian cells not only transmit vertically to progeny but also horizontally to naive cells in coculture. In analogy to the transmission pathways of TSE prions in mammalian cells, we found evidence for NM aggregate transmission to adjacent cells, potentially via actin-containing cytonemes,9,71 and via EVs.72 Although S. cerevisiae also secretes infectious prions in extracellular vesicles, so far it is unclear if these vesicles naturally transmit the prion state to bystander cells.14,73 Different N2a clones all produced NM-containing EVs that were taken up by recipient cells and induced aggregation of GFP-tagged NM in the cytosol. Induction efficiency was, however, low, compared with aggregate induction efficiency when cells were in close proximity, suggesting that direct cell contact is the most efficient way of NM aggregate dissemination in our model.71

As limiting dilution cloning had been successfully used in the past to isolate cell clones with increased susceptibility to TSE prions,74 we used the same strategy to isolate cell clones that secrete EVs capable of efficiently shuttling prion infectivity to recipient cells (Fig. 1B). Through sequential centrifugation and Optiprep gradients, prion activity could be traced to vesicle fractions that fall in the size and density range of exosomes. NM released via exosomes was protected from proteolysis, arguing that at least a fraction of NM was present in the exosomal lumen.72

How is NM prion activity packaged into exosomes? We found the neutral sphingomyelinase inhibitor Spiroepoxide significantly reduced exosome and NM release, suggesting that ceramide-mediated exosome biogenesis is involved in NM secretion. Both soluble and insoluble protein was packaged into exosomes, and no correlation existed between NM
FIGURE 1. Cell culture assays to study prion infection by exosomes. (A) Cell culture assays to study exosome-mediated TSE prion infection. Published exosome-mediated TSE infection assays are time consuming and rely on the detection of newly formed, proteinase K (PK) resistant PrPSc. Naive cells permissive to infection with the respective TSE prion strain are exposed to exosomal preparations isolated from prion-infected cells for 4–5 days, followed by several weeks of culture. Read-out is PK-resistant PrPSc detected by cell blot or western blot.42,47-49,51 (B) Quantitative imaging of exosome-mediated NM aggregate induction. Recipient NM-GFPsol cells are seeded on a 384 well plate for 1 hour. Exosomes isolated from conditioned medium of donor cells are added to the wells. Life or fixed cells are subjected to automated high throughput confocal microscopy. Read-out is induction of NM-GFP aggregates in recipient cells. Life imaging analysis demonstrates the appearance of NM-GFP aggregates as soon as 3 hours post exosome addition. The arrowhead marks cells with exosome-induced NM-GFP aggregates. The assay can also reveal bidirectional inheritance of NM aggregates by daughter cells, a characteristic of TSE prions replicating in cellular models.6
aggregation state and exosome numbers. Donor cells expressing soluble NM secreted even more vesicle-associated NM than donor clones containing NM prions, arguing that aggregation per se was not a required trigger for incorporation into exosomes. NM shares no sequence homology with mammalian proteins, so it is unlikely that specific recognition signals mediated selective recruitment.

The finding that different cell clones secreting exosomes with distinct infectivity can be isolated correlates with findings for TSE infected cells. NM prion producing cell clones had been originally derived from a bulk population of N2a cells transduced with lentivirus coding for NM that were subsequently exposed to recombinant NM fibrils. Cell clones differ in NM expression levels and show phenotypic variation of NM aggregates. The morphological phenotype of NM prions is remarkably persistent and does not change even over prolonged culture. We compared 2 cell clones for their secretion of NM aggregates via EVs. Interestingly, cell clone 1C expressed more total NM and also secreted more total NM in association with exosomes than cell clone s2E. Cell clone s2E selected for its production of highly infectious conditioned medium secreted approximately 6 x more exosomes than 1C clone, but exhibited a seeding activity which was approximately 280 x higher than that of exosomes derived from clone 1C. Secreted EVs from clones 1C and s2E did not differ in size. Filter trap assay and SDD AGE demonstrated that a considerable amount of aggregated NM was present in exosomal preparations of both cell clones. However, comparison of the aggregation states of exosome-packaged NM revealed that lower-order NM oligomers were preferentially sorted into exosomes by the clone s2E. The finding that the aggregation state of NM within exosomes was distinct from that seen in whole cell extracts suggests that NM aggregate sorting into exosomes is a selective process. Our data are in line with the hypothesis that lower-order oligomers constitute highly active templates for seeded polymerization. Notably, rupture of exosomal membranes by sonication left NM oligomers relatively unaffected but drastically reduced the infectivity of the preparation, strongly arguing that only intact exosomes efficiently deliver NM aggregates to target cells.

Further evidence that distinct exosomes are released from different donor cell populations comes from new experiments with human HEK cells engineered to express NM. Similar to our N2a model, exposure of engineered HEK cells to recombinant NM fibrils turned soluble cytoplasmic NM into morphologically heterogeneous, self-templating protein aggregates that were stably propagated by individual cell clones (Fig. 2A, B). Also HEK cells released soluble and aggregated NM in association with exosomes (Fig. 2C). Consistent with our previous results, we did not observe increased exosome release in cells with aggregated NM-HA (Fig. 2D). HEK donor cells secreted significantly less exosomes compared with N2a donor clones 1C and s2E (Fig. 2E). While we expected to achieve lower induction rates due to lower exosome numbers, exosomes derived from HEK NM-HAagg cells were basically non-infectious to HEK NM-GFPsol recipient cells (data not shown). Comparison of the NM aggregation states in donor cell populations revealed that the oligomerization state of NM in the cell lysates of all donor cell populations was remarkably similar (Fig. 2F). Exosome-associated NM from HEK NM-HAagg cells was also enriched for lower-order oligomers comparable to the exosomes produced by highly efficient donor clone s2E (Fig. 2F). The lack of aggregate induction by NM oligomer-bearing exosomes in the HEK system argues that there is considerable difference in composition and activity of EVs isolated from different cell lines and even cell clones. Possible differences in the seeding activities of exosome populations are likely related to the relative number of secreted exosomes, the relative expression level of amyloidogenic protein, and the relative amount and oligomerization state of incorporated aggregated protein (Fig. 3). Another intriguing possibility is that subsequent exosome-target cell interactions could influence the biologic activity of the NM cargo. This possibility, however, needs further elucidation.
Evidence for Secretion of Human Proteins with PrLDs in Association with Exosomes

Algorithms devised to identify novel prion proteins predict that approximately 1% of mammalian proteins contain PrLDs.15,16,76 The majority of mammalian proteins with PrLDs are nucleic acid binding proteins. The PrLDs play critical roles in protein function by mediating protein-protein interactions or phase transition required for the formation of physiologically relevant membrane-less organelles, such as stress granules.16 A prominent protein known to contain a PrLD is the RNA-binding protein TIA-1, an essential component of stress granules. The finding that replacement of TIA-1 PrLD with the Sup35 prion domain restores its normal function argues that yeast prion domains and predicted PrLDs are indeed functionally related.77

FIGURE 2. Human HEK cells secrete both soluble and aggregated Sup35 NM in association with exosomes. HEK cells expressing HA-epitope tagged NM (NM-HA) before (A) and after (B) NM aggregate induction by recombinant NM fibrils. NM-HA<sup>sol</sup>, soluble NM-HA. NM-HA<sup>agg</sup>, aggregated NM-HA. NM-HA was stained with anti-HA antibody (red) and nuclei were counterstained with Hoechst (blue). Maximum intensity projections were generated from Z-stacks. (C) Western blot analysis of exosomes from HEK NM-HA<sup>sol</sup>, NM-HA<sup>agg</sup> and N2a NM-HA<sup>agg</sup> s2E cell clones for exosomal marker Alix and NM-HA. Exosomes were isolated according to a previously described method.72 (D) Exosome numbers released from HEK NM-HA<sup>sol</sup> and NM-HA<sup>agg</sup> were determined using ZetaView PMX 110-SZ-488 Nano Particle Tracking Analyzer with the same measurement setting. Results shown are means ± SD (n = 3; *** p < 0.001; unpaired student t test). (E) Exosome numbers released from HEK NM-HA<sup>agg</sup>, N2a NM-HA<sup>agg</sup> clone s2E (selected for high aggregate inducing activity in recipient cells) and N2a NM-HA<sup>agg</sup> clone 1C. Results shown are means ± SD (n = 3; *** p<0.001; one-way ANOVA). (F) Glutaraldehyde cross-linking of proteins in cell lysates or exosomes from HEK NM-HA<sup>sol</sup>, HEK NM-HA<sup>agg</sup> and N2a NM-HA<sup>agg</sup> clones s2E or 1C to determine the oligomerization state of NM-HA. Cross-linking was done as described previously.72
Importantly, aberrant aggregation of proteins with PrLDs might be the underlying cause of degeneration in several neurodegenerative diseases and myopathies. ALS is a fatal motor neuron disease that is mostly sporadic. Ten percent of cases are genetic and have been linked to mutations in a variety of proteins, such as SOD1, VCP, OPTN, TDP-43, hnRNPA1, hnRNPA2 and FUS, many of which form insoluble pathological inclusions. FUS, TDP-43, hnRNPA1 and hnRNPA2 contain putative PrLDs similar to annotated yeast prion domains. Systematic screens in yeast recently identified TAF15 and EWSR1 as further aggregation-prone PrLD-bearing proteins linked to neurodegenerative diseases. Several other proteins listed as PrLD-like proteins await further characterization. Deregulated PrLD-mediated protein assembly has been proposed to promote the formation of protein aggregates with self-templating and dissemination properties. Indeed, a recent study showed that replacement of the Sup35 prion domain with the human hnRNPA2B1 PrLD generates a protein with definite prion activity in yeast, arguing that PrLDs of human proteins can drive prion assembly at least in lower eukaryotes.

TDP-43 is a nuclear RNA-binding protein involved in transcription and splicing and is associated with cytoplasmic inclusions in ALS and FTD. The predicted PrLD of TDP-43 mediates its aggregation in vitro and in vivo. Recombinant TDP-43 fibrils and TDP-43 aggregates extracted from ALS and FTD patients have seeding activity and cause mislocalization and aggregation of TDP-43 in cell culture. TDP-43 oligomers or aggregates also transmit from donor to recipient cells in culture, either through tunneling nanotubes or exosomes. Cell culture experiments suggest that the ceramide-dependent exosomal pathway is involved in exosomal TDP-43 release. As TDP-43 is also present in exosomal fractions from brains and CSF of healthy controls, its assembly into disease-associated aggregates might not cause the sorting into EVs. Still, exosome-associated TDP-43 was reported to be increased in ALS

FIGURE 3. Factors influencing seeding activity of protein aggregates incorporated into exosomes. Exosome-mediated secretion of NM-HA by donor cells and subsequent uptake and seeding of NM-GFP prions in recipient cells. Infectivity of NM-HA bearing exosomes is likely determined by the following parameters: (1) Enhanced secretion of exosomes, (2, 3) Selective sorting of low-order oligomers, (4, 5) Specific exosome-target cell interaction (ligand-receptor recognition). This could include cell specific ligand-receptor interactions or differences in the intracellular fate of endocytosed exosomes. After internalization, the NM-HA aggregates contained in exosomes are released and induce new aggregate formation in N2a NM-GFP cells. The mechanism of NM-HA release into the cytosol is so far unknown.
brains compared with controls.88 Interestingly, mammalian proteins that harbor intrinsically disordered domains with amino acid compositions similar to yeast prion domains15,76 appear to be frequent constituents of exosomes. Of the human RNA-binding proteins with PrLDs,80 71% have been previously reported in exosomal fractions (http://www.exocarta.org/). PrLD-containing proteins can even be actively involved in the selective sorting of specific microRNAs into EVs for secretion. A sumoylated form of hnRNPA2B1 controls the sorting of a subpopulation of microRNAs into exosomes.90 The presence of PrLD containing proteins in exosomes could thus reflect the physiological function of the respective protein. Whether aberrantly folded proteins with PrLDs are generally sorted into exosomes and how this might contribute to intercellular aggregate spreading remains to be established.

CONCLUSION

Research over the last years has demonstrated that not only TSE prions are sorted into exosomes, but also pathogenic protein aggregates associated with more common neurodegenerative diseases. Among them, proteins with domains compositionally similar to yeast prion domains have been found associated with EVs, suggesting that EVs might contribute to their intercellular dissemination. Our knowledge on mechanisms that drive cargo sorting into EVs and uptake by recipient cells is limited. There is an urgent need for assays that monitor cargo delivery to target cells that are amenable to high throughput screening. Here we showed that the non-mammalian prion domain of Sup35 can serve as a versatile tool to study exosome-mediated induction of self-templating protein aggregates. The NM prion cell assay has been successfully adapted to automated high throughput confocal microscopy. The fast and accurate detection of aggregate induction in recipient cells will help to characterize general cellular pathways involved in aggregation and dissemination of protein aggregates.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

No potential conflicts of interest were disclosed.

ACKNOWLEDGMENTS

We thank Sybille Krauss and Philip Denner for critical comments on this manuscript. We are grateful to Philip Denner and Birgit Kurkowsky for advice on automated imaging.

FUNDING

This work was supported by the Helmholtz Portfolio Wirkstoffforschung.

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