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Life cycle of cytosolic prions

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Prions are self-templating protein aggregates that were originally identified as the causative agent of prion diseases in mammals, but have since been discovered in other kingdoms. Mammalian prions represent a unique class of infectious agents that are composed of misfolded prion protein. Prion proteins usually exist as soluble proteins but can refold and assemble into highly ordered, self-propagating prion polymers. The prion concept is also applicable to a growing number of non-Mendelian elements of inheritance in lower eukaryotes. While prions identified in mammals are clearly pathogens, prions in lower eukaryotes can be either detrimental or beneficial to the host. Prion phenotypes in fungi are transmitted vertically from mother to daughter cells during cell division and horizontally during mating or abortive mating, but extracellular phases have not been reported. Recent findings now demonstrate that in a mammalian cell environment, protein aggregates derived from yeast prion domains exhibit a prion life cycle similar to mammalian prions propagated ex vivo. This life cycle includes a soluble state of the protein, an induction phase by exogenous prion fibrils, stable replication of prion entities, vertical transmission to progeny and natural horizontal transmission to neighboring cells. Our data reveal that mammalian cells contain all co-factors required for cytosolic prion propagation and dissemination. This has important implications for understanding prion-like properties of disease-related protein aggregates. In light of the growing number of identified functional amyloids, cell-to-cell propagation of cytosolic protein conformers might not only be relevant for the spreading of disease-associated proteins, but might also be of more general relevance under non-disease conditions.

Keywords: Prion, Sup35, amyloid, seeded polymerization

Abbreviations: GFP, green fluorescent protein; HA, hemagglutinin antibody epitope; NM, Prion domain of Sup35; PrP^{C}, cellular prion protein; PrP^{Sc}, abnormal prion protein; TNT, tunneling nanotube

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Prions—Self-Templating Protein Conformers with Infectious Properties

Prions were originally identified as the causative agent of the transmissible spongiform encephalopathies (TSE) in humans and other mammals.[1] Prion diseases include scrapie in sheep and goats, chronic wasting disease in deer, elk, and moose, bovine spongiform encephalopathies and human prion diseases such as Creutzfeldt-Jakob disease, Gerstmann-Sträussler-Scheinker disease, and fatal familial insomnia. Prion diseases typically occur sporadically or are of genetic origin. Importantly, prion diseases can also be acquired and can sometimes even be transmitted across species barriers. The term prion was first introduced by Stanley Prusiner to describe a novel class of "proteinaceous infectious particles" devoid of coding nucleic acids.[1] According to the protein-only hypothesis, mammalian prions consist of cellular prion protein PrP that gains infectious properties by a conformational transition into an aggregated, β-sheet rich prion isoform, termed PrP^{Sc}. The precursor of mammalian prions is the cellular prion protein, PrP^{C}. PrP^{C} is a highly glycosylated, gycosylphosphatidyl-inositol anchored cell-surface protein abundantly expressed in the central nervous system but also in

other tissues.[2,3] Mammalian prion formation is confined to the cell surface or cytosolic vesicles upon direct contact of the prion conformer PrP^{Sc} with PrP^{C} (for review, see ref. 4).

The self-perpetuating changes in protein conformation are also a hallmark of several proteins found in lower eukaryotes such as yeast and filamentous fungi. Fungal prion proteins share little sequence similarity with mammalian PrP. One exception is the translation termination factor Sup35 of *Saccharomyces cerevisiae* (*S. cerevisiae*), that harbors a peptide repeat region similar to the one in PrP.[5,6] Prion proteins of lower eukaryotes can also form insoluble, self-perpetuating amyloid-like polymers in vitro and in vivo.[7] Yeast prions constitute protein-based epigenetic elements that confer heritable phenotypic traits to their hosts.[8,9] At least nine different prion proteins of fungi have been identified that can be lethal or beneficial to the host.[10-12] Yeast prions are predominately cytoplasmic, with one exception that resides within the nucleus.[13] Thanks to the power of yeast genetics and the establishment of tractable systems to monitor prion states, yeast has proven to be a versatile model system to study mechanistic details of protein-based inheritance. Importantly, however, it must not be forgotten that mammalian and yeast prions differ in their cellular localization. Consequently, the exact factors required for prion induction and propagation for fungal and mammalian prions likely differ.

Mammalian and yeast prions replicate by a seeded-polymerization mechanism in which misfolded prion protein catalyzes the conformational switch of the soluble protein and its incorporation into highly-ordered fibrillar aggregates, so-called amyloid. For multiplication of misfolded protein conformers, secondary nucleation events are required that generate daughter seeds.[14-17] Secondary nucleation is an essential step in the life cycle of prions. Results obtained with yeast prions highlight the essential role of chaperone-mediated aggregate shearing for prion maintenance.[18-20] During yeast budding, prion seeds transmit to the daughter cells where they recruit soluble prion proteins and grow into longer filaments. Severing of prion fibrils by cellular chaperones then generates fibril fragments that restore the mean prion population before cytokinesis is initiated again. In mammals, prion seed multiplication is required for efficient dissemination within and between organisms, but co-factors involved in this process have not been revealed so far. Under rare circumstances, prion seeds can form spontaneously, giving rise to sporadic prion diseases in humans or heritable phenotypic traits in yeast. The mechanism of spontaneous prion induction in mammals is not understood, but evidence from yeast prions demonstrates that other ordered protein polymers or even prions can theoretically act as heterologous seeds that cross-seed protein misfolding and thus induce heritable prion states.[21-23] As spontaneous formation of PrP-derived prions has so far not been observed in cellular models (for review see ref. 4), prions of lower eukaryotes serve as important models for studying cellular aspects of spontaneous prion induction.

Prions induce self-perpetuating prion states when transmitted to other cells. Cytoplasmic yeast prions are transmitted vertically to progeny or horizontally during mating.[24] Yeast prions can also be transmitted experimentally by cytoduction, a process of abortive mating, resulting in cytoplasmic mixing without nuclei fusion.[8] Extracellular phases in the yeast prion replication cycle have not been observed.[25] Prions in mammals follow a complex life cycle that enables horizontal transmission between individuals of the same species and even across species barriers. From the initial site of infection, prions spread within the host to eventually target the central nervous system, where they multiply to high levels.[26] The exact mechanism of intercellular spreading of mammalian prions is not fully understood, but cell culture models have shed some light onto the cellular mechanisms that might be involved also in vivo. Mammalian prions ex vivo transmit horizontally to bystander cells, in which they induce sustained infection (for a review, see ref. 4). In some models, direct contact between cells ex vivo is required.[27] Cytoplasmic bridges such as tunneling nanotubes (TNTs) appear to be involved in mammalian prion spreading ex vivo.[28] TNTs represent special types of intercellular bridges that allow intercellular exchange of cytoplasm and even organelles such as lysosomes and mitochondria.[29] Dissemination of mammalian prions ex vivo also includes extracellular phases. Mammalian prions have been found associated with exosomes, and those exosome fractions effectively induce prion infection in tissue culture and animal models.[30-33] It has been hypothesized that the efficient dissemination of mammalian prions is directly related to the membrane association of PrP, facilitating interaction with necessary co-factors for propagation and cell-to-cell spreading by membrane-bound vesicles or intercellular membrane interactions.[34] So is prion biogenesis in mammalian cells unique to PrP and its localization on the cell surface?

Prion Propensity of a Yeast Prion Domain in the Mammalian Cytosol

Recent findings now argue that the cytosol of mammalian cells also supports aggregate propagation and that membrane anchorage is not a prerequisite for prion replication.[35,36] Evidence for this comes from experiments with mammalian cell cultures ectopically expressing the prion domain of the *S. cerevisiae* Sup35 protein, a subunit of the translation termination factor. In yeast, prions composed of aggregated Sup35 cause a nonsense suppression phenotype due to occasional read-through of stop codons.[9] Sup35 is a 686 amino acid residue protein comprising domains N, M, and C domain. Prion propensity of Sup35 is governed by a transposable N-terminal (N) and middle domain (M). The N domain is enriched in glutamine and asparagine residues and harbors a peptide repeat domain. N is required to form the self-propagating amyloid in yeast.[37-39] The highly charged middle domain (M, aa 125–253) enhances the protein solubility and is required for sustained mitotic and meiotic stability of the prion phenotype.[40] The C-terminal domain (C, aa 254–686) has translation termination activity in yeast and is dispensable for prion formation.[40]

To elucidate if the mammalian cytosol can also support prion-like propagation of protein aggregates, the Sup35 NM prion domain was stably expressed

in murine cells and its prion propensity was assessed upon induction with exogenous NM aggregate seeds. As the NM domain by itself does not normally exist in mammalian cells, it allows monitoring cytosolic prion propagation without loss-of-function phenotype. Cytosolic prion propagation was initially studied in the mitotically active mouse neuroblastoma cell line N2a and subsequently also in primary neurons and astrocytes (**Fig. 1**) and organotypic hippocampal slices.[35,41] In all models, ectopically expressed cytosolic NM remained soluble and did not significantly affect cell viability. Remarkably, experimentally induced raise in reactive oxygen species which trigger aggregation of other amyloidogenic proteins, did not affect solubility of the NM domain in neuroblastoma cells.[41] Even upon continuous culture, spontaneous induction of NM prion aggregates was not observed, demonstrating that Sup35 NM is not prone to spontaneously misfold when cytosolically expressed. To induce aggregation of Sup35 NM in mammalian cells, we employed previously established prion induction protocols using recombinant yeast prion protein fibrils.[42-45] In yeast, the prion phenotype can be induced by transformation of recombinant Sup35 NM fibrils via liposomes or polyethylene glycol (PEG).[42,43,45] Also in mammalian cells, exogenously added recombinant NM fibrils were efficiently taken up by cells and induced aggregation of the ectopically expressed soluble NM.[35] The mechanism of NM internalization is unclear, but pathways involved in the uptake of PrP-derived prions or other amyloidogenic protein aggregates, such as endocytosis or macropinocytosis might play a role,[46-51] potentially after binding to specialized membrane regions enriched in cholesterol.[52]

Once induced, Sup35 NM prion aggregates were remarkably stable.[35] Even under prolonged cell culture over months, the prion phenotype was faithfully maintained. Self-templating aggregates were also induced in a PrP-deficient cell line stably expressing NM, demonstrating that the cellular prion protein is not involved in NM prion induction and propagation.[36] NM aggregates differed in morphology, ranging from small punctate aggregates to longer, worm-like aggregates that were maintained even upon cell division. Subsequent isolation of N2a clones by limiting dilution cloning confirmed that aggregate phenotypes were inherited by daughter cells, arguing that aggregates had self-propagating properties. Surprisingly, N2a cells appeared to be unaffected by the continuous presence of NM aggregates, as significant changes in cell viability were not observed.[41] Again, continuous culture of several clones did not lead to a loss of the prion phenotype, and even years after the first induction, more than 90% of the cells in closed populations contain NM prions. This is in line with mammalian prion propagation in mitotically active tissue culture cells.[4] Ex vivo propagation of mammalian prions is generally not cytotoxic, and sustained propagation ex vivo over years has been achieved for several mouse-adapted prion strains.[53-55]

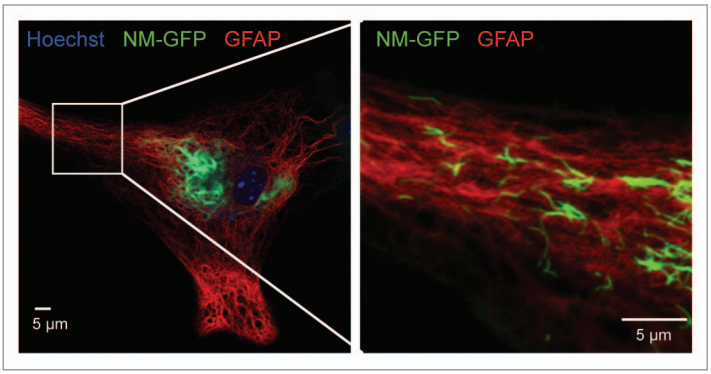

Figure 1. Confocal image of an astrocyte transduced with NM-GFP encoding lentivirus. The cells were treated with 1 µM recombinant NM fibrils (monomer equivalent) for 24 h and fixed 48 h post fibril addition. A net-like NM-GFP aggregate structure with very high fluorescence intensity was visible close to the nucleus (left). Higher magnification of the cell periphery showed further spindle-shaped NM-GFP (green) aggregates present throughout the cytoplasm (right). Nuclei were stained with Hoechst (blue) and GFAP with anti-GFAP antibody (red). (Scale bar = 5 µm).

But how exactly can protein aggregates propagate in mammalian cells? Clearly, the stable propagation of mammalian prions in dividing cells argues that aggregate propagation is quite efficient, because the prion multiplication effectively outcompetes prion loss due to cell division or clearance. Secondary nucleation events that produce more seeds from a preformed seed and bidirectional seed distribution to both daughter cells are key characteristics of yeast and mammalian prions in mitotically active cells.[24,36,56] Cells are equipped with sophisticated proteostasis mechanisms that sense, refold or ultimately degrade aberrantly folded proteins. As misfolded proteins can be cytotoxic and thus pose a potential risk to cells, damaged and misfolded proteins are often retained by the mother cell during cytokinesis to increase fitness of progeny.[57,58] Proteins that can function as prions appear to have evolved mechanisms that allow them to sustain complete clearance by cellular degradation machineries. Still, interaction of prions with the cellular protein folding and clearance machinery can also aid in prion propagation. Yeast prions crucially depend on the cellular chaperone system for seed multiplication. A major player in yeast prion propagation is the AAA+ ATPase Hsp104 capable of resolubilizing proteins and rapidly dissociating protein aggregates.[59,60] Hsp104 shears high molecular weight prion aggregates into smaller seeds,[18] a process that is tightly regulated by the interplay of Hsp104 with chaperones Hsp70 and Hsp40.[20,61,62] Experimental evidence suggests that the rate of aggregate fragmentation is dictated by fibril properties and host cell environment and is a key determinant for transmissibility.[16] The effective shearing of growing prion aggregates thus enables sustained prion propagation in rapidly dividing populations.[18,63-65] Importantly, aggregate shearing results in multiplication of mammalian prions in a test tube in vitro, suggesting that aggregate fragmentation by host factors might also contribute to mammalian prion replication in vivo.[66,67] While processes that support secondary

seed formation in mammalian prion replication are unknown, the propagation mechanism of cytosolic NM prions likely differs from that of mammalian prions that replicate along the endocytic pathway. As the prion disaggregase Hsp104 necessary for prion maintenance in yeast lacks mammalian homologs,[68] other cellular factors or processes must trigger cytosolic aggregate shearing in mammalian cells. First evidence for a protein depolymerizing system in mammals came from studies with mammalian cell extracts revealing an ATP-dependent disaggregating activity capable of dissolving preformed protein aggregates and even amyloid fibrils.[69,70] Hsp70 and Hsp40 are also key chaperones in mammals that prevent protein misfolding and promote refolding into functional proteins. Recent studies now demonstrate that cooperation with specific co-chaperones confers disaggregation activity to the Hsp70-Hsp40 chaperone system.[70,71] Hsp110 proteins represent a subgroup of the Hsp70 superfamily that function as nucleotide exchange factors, stimulating ADP release and substrate release by chaperone Hsp70.[72,73] Hsp110[74,75] synergizes with Hsp70 and Hsp40 to slowly disaggregate protein aggregates.[70,71] Chaperones Hsp70, Hsp40, and Hsp110 also stimulate NM fibril disassembly in vitro,[76] and could thus also be involved in NM protein polymer fragmentation in mammalian cells. Alternatively, the autophagy machinery could contribute to prion seed formation, as has been suggested for mammalian prions.[77,78] Of note, Sup35 NM aggregates can associate with autophagic vesicles in *Caenorhabditis elegans*, suggesting that this pathway is involved in NM aggregate clearance in higher eukaryotes.[79] The fact that NM prions propagate efficiently in mammalian cells argues that a substantial number of prion seeds are either not recognized or escape the cellular clearance machinery.

Sup35 NM Prions Exhibit an Infectious Life Cycle in Mammalian Cells

The first evidence for the infectious properties of mammalian cell-derived NM came from experiments using N2a cell extracts containing NM aggregates.[35] Addition of this cell extract to N2a cells expressing soluble NM triggered persistent NM aggregation in a subset of cells. Transmission of cytosolic NM aggregates induced the Sup35 prion phenotype also in yeast, arguing that the protein aggregates represent true prions with infectious properties. Remarkably, NM prions in neuroblastoma cells also horizontally infected neighboring cells, in which they induced ongoing prion propagation.[41] When N2a cells with NM prions were co-cultured with N2a cells expressing soluble NM, NM aggregation was induced in recipient cells (**Fig. 2**). The presence of donor NM aggregates in recipient cells strongly argues that direct seed transmission induced the prion phenotype in acceptor cells. The induction efficiency in the recipient cell population ranged between 0.1–0.5% within 24 h. Donor clones producing long fibrillar NM aggregates exhibited lower induction efficiencies compared with clones producing small punctate aggregates. If this was due to the type of aggregate or clonal differences in transmission efficiencies remains to be determined. Cell-to-cell transmission of NM aggregates and subsequent seeding was not only confined to proliferating tumor cell lines, as aggregate induction was also apparent upon coculture of aggregate bearing astrocytes with primary neurons and organotypic slice cultures.[41] Thus, NM aggregates gained additional prion characteristics in mammalian cells compared with NM prions present in *S. cerevisiae*. This spreading behavior of NM prions in tissue culture is reminiscent of mammalian prion dissemination ex vivo.[27,80] The horizontal spreading of NM aggregates clearly demonstrates that prion characteristics in mammalian cells are not confined to PrP and that GPI-anchoring is not generally required for prion replication and transmission at least in cell culture models.[41]

But how do cytosolic NM prions transmit to bystander cells? Possible mechanisms of intercellular aggregate spreading could include an extracellular phase, by transmission via prion secretion or directly by cellular interactions. Aggregates in recipient cells are probably not induced by aggregates released from dying donor cells, since aggregate-bearing cells did not show signs of overt toxicity.[41] Indeed, culture medium transferred from

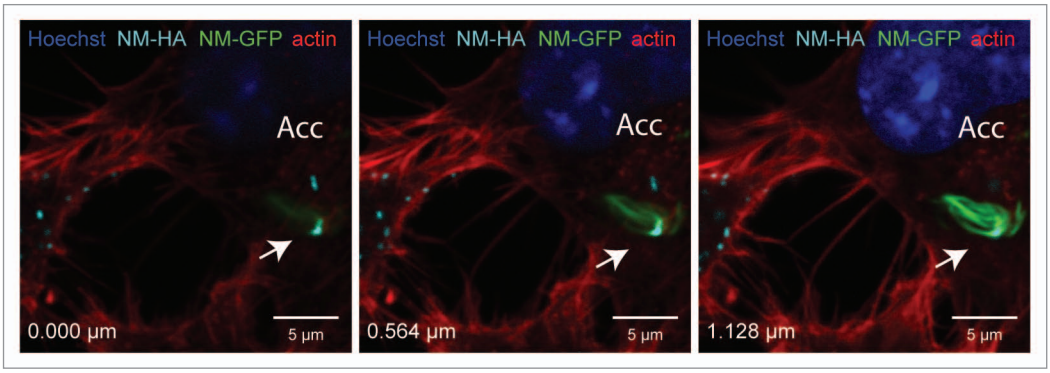

Figure 2. NM-HA aggregates transmitted from N2a donor to acceptor cells (Acc) co-localize with induced NM-GFP aggregates. Confocal microscopy revealed the presence of NM-HA and NM-GFP aggregates in the same N2a cell. Donor cells with induced NM-HA aggregates were co-cultured with acceptor cells expressing soluble NM-GFP. Different layers of a Z-stack are shown (Z distance = 0.564 µm). NM-HA aggregates were stained with anti-HA antibody (light blue) and F-actin was stained with iFluor-546-Phalloidin (red). Nuclei were stained with Hoechst (blue) and NM-GFP is displayed in green. (Scale bar = 5 µm).

aggregate bearing donor cells to recipient cells was significantly less efficient at inducing intracellular NM aggregation than cocultures. NM aggregates spread most efficiently between cells that are in direct contact, suggesting that physical contact between donor and acceptor cells was the prominent route of transmission. Intercellular filopodia-like bridges might be involved in transfer of aggregates, as NM aggregates were sometimes found in actin-rich protrusions connecting donor and acceptor cells (**Fig. 3**). Interestingly, TNTs also assist in transmission of polyglutamine aggregates between cells, suggesting that intercellular bridges might generally serve as conduits for the exchange of protein aggregates between cells.[81] It is also possible that adjacent cells secrete NM aggregates into the intercellular cleft as known for neuronal synapses. In this case, NM prions could be released in the extracellular space, either as naked aggregates or packaged into vesicles, and immediately internalized by the recipient cells. Therefore multiple mechanisms can underlie the aggregate transmission of interconnected cells.

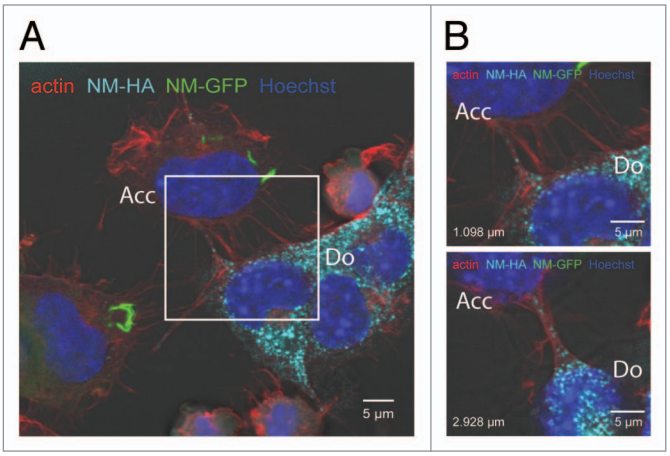

Figure 3. NM-HA aggregates in actin-rich protrusions. (**A**) Confocal image of a coculture of donor clone 2E (Do) and acceptor cells (Acc) expressing soluble NM-GFP. Cells were fixed after 48 h for immunofluorescence staining. The image shows one layer of a Z-stack. NM-HA aggregates of clone 2E were stained with anti-HA antibody (light blue) and F-actin was stained with iFluor-546-Phalloidin (red). The image shows transmitted light (gray) and NM-GFP in green. Nuclei were stained with Hoechst (blue). NM-GFP in the acceptor cell localizes in inclusions. The white square indicates the inset shown in B. (**B**) Excerpts of two layers of the Z-stack described above. The distance between the two layers is 1.830 µm. The distances to the bottom are indicated. The images show NM-HA aggregates in actin-rich protrusions between donor and acceptor cell. (Scale bar = 5 µm).

Biological Implications

Our studies on NM prion domain replication have demonstrated that artificial protein aggregates can behave as prions in mammalian cells. So far, the only mammalian protein with clear-cut prion characteristics is PrP. So is the prion propensity unique to PrP or could other mammalian proteins also function as prions? Prions derived from PrP induce uniformly fatal diseases, but prions in lower eukaryotes sometimes fulfill beneficial functions for their hosts. One such example is the (Het-s) prion of the filamentous fungus *Podospora anserina* required for heterokaryon incompatibility between illegitime mating partners.[82] The increasing number of proteins that exhibit prion-like characteristics in lower eukaryotes suggests a functional relevance of prion conformers. It has been proposed that the prion phenotype is an evolutionary conserved mechanism in response to environmental changes.[10] Bioinformatics algorithms that analyze compositional similarity with known and synthetic yeast prions have identified several novel prions in yeast[83-88] and have predicted candidate proteins in mammals.[89] Strikingly, key candidates include proteins known to aggregate in devastating neurodegenerative diseases.[89,90] Prion algorithms so far are based on the sequence determinants of yeast prions rich in glutamine (Q) and asparagine (N) and thus will not identify prions that are not enriched in Q and N. Q/N-rich stretches are not a general requirement for prion propensity, and the mammalian PrP,[5] the yeast prion Mod5[91] and the Het-s prion[92] of *Podospora anserina* are not enriched in Q and N. Refinement of algorithms to include compositional information of novel prions and those without Q/N-rich stretches will likely generate lists of new candidates. A thorough investigation into their prion properties will reveal if those proteins fulfill all prion criteria. In light of the growing number of known prions in lower eukaryotes and the identification of amyloid with biological function also in humans,[93,94] it is tempting to speculate that aggregate transfer and conformational templating might be of general biological relevance.

Our study has also important implications for protein misfolding diseases. Increasing evidence argues that intracellular aggregate induction by extracellular proteinaceous seeds is not unique to mammalian and fungal prions, but also a prominent feature of several protein aggregates linked to neurodegenerative diseases in humans.[95] In these diseases, unrelated proteins such as Huntingtin, Tau, or α-synuclein also template their amyloid states in a seeded-polymerization reaction. Intriguingly, extracellular protein seeds of misfolded, disease-related protein can be taken up by cells and induce an aggregation state of the homologous endogenous protein.[49,96-98] Those disease-related cytosolic proteins can even spread within tissues, thereby contributing to pathology progression in mouse models of several neurodegenerative diseases.[99-102] The similarities in the seeding and spreading capacities of true prions and other amyloidogenic proteins have blurred boundaries between infectious and non-infectious amyloid. Mammalian prions are true infectious agents, because they (at least several of them) can naturally spread from individual to individual. Epidemiological data do not support an infectious potential of other neurodegenerative diseases. Clearly, there is a lack of fundamental understanding of essential properties of true infectious proteins on

all levels, ranging from organismal to cellular to molecular levels.

Simple cell culture models such as those discussed here can help to dissect similarities and differences between prions and prion-like proteins on a cellular level. Mitotically active cells are invaluable for studying aggregate multiplication, because induced protein aggregates with low propagation efficiency will be ultimately lost during cell division. The ex vivo life cycles of membrane-bound, PrP-derived prions and cytosolic NM-derived prions are remarkably similar and include six steps: (1) The endogenous protein is in a soluble state, (2) exogenous homotypic seeds can be internalized by cells, (3) the exposure to exogenous seeds causes intracellular aggregate formation, (4) the prion entities are stably inherited during mitosis, (5) aggregates naturally spread and invade neighboring cells, and (6) transmitted aggregates induce the self-perpetuating prion state in the infected cells (**Fig. 4**). A wealth of data argues that steps 1–3 (invasion of bystander cells and induction of protein aggregation) are shared by many protein aggregates. Oligomers or fibrils formed in vitro from recombinant polyglutamine proteins,[97] Tau fragments,[50,98,103] mutant superoxide dismutase 1 (SOD1),[49] α-synuclein,[96,104,105] or patient-derived insoluble TDP-43[106] have all been shown to be taken up by cells ex vivo and gain access to the cytosol, where they induce the aggregation of endogenous, homotypic proteins. In some instances, protein aggregates were introduced by transfection, so it is unclear if cells take up those aggregates under more physiological conditions.[106]

However, efficient secondary nucleation events, natural horizontal transmission, and the induction of self-perpetuating protein aggregation in recipient cells (life cycle steps 4–6) might be efficiently accomplished only by few protein aggregates. Stable inheritance of induced protein aggregates over multiple cell divisions (life cycle step 4) has so far only been reported for mutant SOD1,[49] while exogenously induced polyglutamine aggregates were only inefficiently maintained by mitotically active cells.[97] The observed differences in aggregate maintenance could be due to different efficiencies in secondary nucleation, aggregate clearance or aggregate toxicity. So far it is unclear if protein aggregates induced by endogenous seeds of Tau fragments, TDP-43 or α-synuclein exhibit mitotic stability. In cellular models, polyglutamine-rich proteins,[81] Tau fragments,[98,103] α-synuclein,[101,107] and recombinant SOD1[49] have been demonstrated to transmit horizontally from one cell to the next (step 5). In some instances, transmission of those proteins initiated endogenous homotypic protein interaction[103] or aggregation.[98,101,107] The finding that disease-related, aggregation-prone proteins transmit from one cell to the next (life cycle step 5) has sparked tremendous interest in the intercellular transmission of those disease-related proteins, but it remains to be demonstrated that the transmitted proteins induce a sustained, self-perpetuating aggregation phenotype (life cycle step 6) just as PrP-derived prions (as reviewed in ref. 4) or NM-derived prions[41] do. In terms of an infectious entity, the transient induction of a protein aggregate by an exogenous seed would be somewhat reminiscent of an abortive infection by a virus that enters the cell but cannot initiate a full replication cycle that generates infectious progeny. The efficiency of secondary seed generation within the cell is thus a major determinant of prion infectivity. Surprisingly, the necessity for secondary nucleation events that generate secondary nuclei from pre-formed aggregates has so far often been overlooked. The mammalian Sup35 NM model represents a versatile model to study faithful propagation of cytosolic prions on a cellular level.

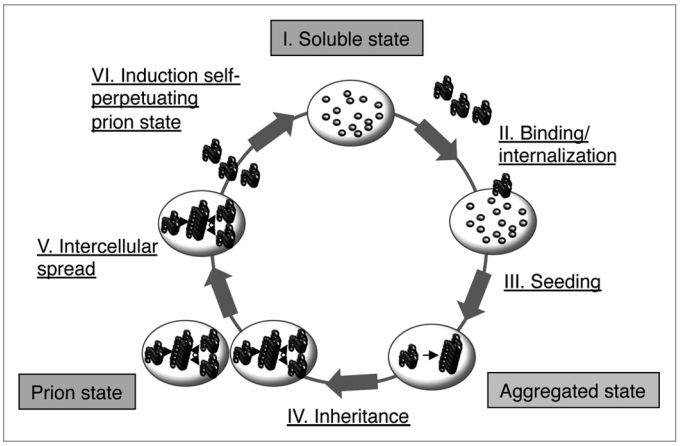

Figure 4. Life cycle of both PrP-derived and NM-derived prions in mammalian cells cultured ex vivo. Note that PrP^C and NM differ in their cellular localization. PrP^C is a cell surface bound glycoprotein, whereas NM is ectopically expressed in the cytosol. Both proteins are soluble and spontaneous conversion to a prion conformation has not been observed in mammalian cells ex vivo. The prion conformation can be induced by addition of infectious aggregates derived from the respective proteins. For PrP-derived prions, the inoculum is prepared from prion infected tissue or cell culture (for review, see ref. 4) or produced in vitro.[108] The prion phenotype is faithfully inherited by daughter cells during cell division. For PrP-derived prions, so far unknown susceptibility factors restrict persistent propagation to few cell lines (for review, see ref. 4). PrP-derived and NM-derived prions are transmitted horizontally to neighboring cells, in which they induce persistent aggregation of the prion proteins PrP or NM, respectively.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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