Cell-to-cell propagation of infectious cytosolic protein aggregates

Julia P. Hofmann[a], Philip Denner[a], Carmen Nussbaum-Krammer[b], Peer-Hendrik Kuhn[c,d], Michael H. Suhre[e], Thomas Scheibel[e], Stefan F. Lichtenthaler[c,d,f], Hermann M. Schätzl[g], Daniele Bano[a], and Ina M. Vorberg[a,h,1]

[a]German Center for Neurodegenerative Diseases (DZNE), 53175 Bonn, Germany; [b]Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208-3500; [c]German Center for Neurodegenerative Diseases (DZNE), 81377 Munich, Germany; [d]Technische Universität München, 81377 Munich, Germany; [e]Lehrstuhl Biomaterialien, Fakultät für Angewandte Naturwissenschaften, Universität Bayreuth, 95447 Bayreuth, Germany; [f]Munich Cluster for Systems Neurology (SyNergy), 80336 Munich, Germany; [g]Departments of Veterinary Science and of Molecular Biology, University of Wyoming, Laramie, Wyoming 82071; and [h]Rheinische Friedrich-Wilhelms-Universität Bonn, 53127 Bonn, Germany

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Prions are self-templating protein conformers that replicate by recruitment and conversion of homotypic proteins into growing protein aggregates. Originally identified as causative agents of transmissible spongiform encephalopathies, increasing evidence now suggests that prion-like phenomena are more common in nature than previously anticipated. In contrast to fungal prions that replicate in the cytoplasm, propagation of mammalian prions derived from the precursor protein PrP is confined to the cell membrane or endocytic vesicles. Here we demonstrate that cytosolic protein aggregates can also behave as infectious entities in mammalian cells. When expressed in the mammalian cytosol, protein aggregates derived from the prion domain NM of yeast translation termination factor Sup35 persistently propagate and invade neighboring cells, thereby inducing a self-perpetuating aggregation state of NM. Cell contact is required for efficient infection. Aggregates can also be induced in primary astrocytes, neurons, and organotypic cultures, demonstrating that this phenomenon is not specific to immortalized cells. Our data have important implications for understanding prion-like phenomena of protein aggregates associated with human diseases and for the growing number of amyloidogenic proteins discovered in mammals.

Prions in mammals are unconventional infectious agents devoid of coding nucleic acid that cause transmissible spongiform encephalopathies (TSEs) by a protein-only mechanism (1). During the course of the disease, the cellular prion protein PrP^{C} misfolds into fibrillar aggregates termed PrP^{Sc}. Accumulating evidence supports the idea that PrP^{Sc} constitutes the major component of the TSE agent. Protein aggregates that replicate in a prion-like manner have also been identified in lower eukaryotes where they serve as epigenetic elements of inheritance (2). Fungal prions arise spontaneously by misfolding and assembly of cellular proteins, thereby conferring heritable phenotypes to the host (3, 4). By analogy to mammalian prions, yeast prions (2) propagate by a seeded polymerization process (5) in which a seed of abnormally folded protein catalyzes the conversion of the homologous soluble isoform. The prion conformation of the *Saccharomyces cerevisiae* translation termination factor Sup35 arises through conformational rearrangement into a less functional β-pleated polymer (3). The growing number of yeast proteins with prion properties (4) and the relative abundance of proteins with predicted prion-forming domains in lower and higher eukaryotes (6) support the idea that prion-like proteins are not rare in nature but have evolved as epigenetic elements even in higher eukaryotes (7).

The cellular prion protein is a membrane-anchored protein, confining prion formation to the cell surface or the endocytic pathway. Intriguingly, several systemic diseases and neurodegenerative disorders such as Alzheimer's disease, Parkinson disease, and Tauopathies are associated with aberrant intra- and extracellular deposition of highly ordered protein aggregates, so-called amyloid fibers. A prion-like mechanism of aggregate spreading has been suggested to underlie the stereotypical progression of pathology (8–10). The exact mechanism of prion replication is not well-understood. Mammalian prions have been extensively studied in vitro (11). In cell lines, PrP^{Sc} is faithfully propagated by progeny cells and spreads to neighboring cells, thereby inducing ongoing PrP^{Sc} formation. Aggregate uptake, induction, or intercellular transmission of a variety of disease-related protein aggregates has been observed in vitro (12–17); however, heritable aggregate phenotypes have rarely been reported (17). Importantly, intercellular induction of heritable self-perpetuating aggregates by cocultured donor cells has so far only been demonstrated for mammalian prions. It remains to be established if protein aggregates other than those derived from PrP can recapitulate the full prion life cycle in vitro.

To gain insights into potential prion capacities of cytosolic protein aggregates, we developed a mammalian cell culture model based on the cytosolic expression of the yeast prion domain NM of Sup35 (18). The N-terminal and middle domain (NM) of Sup35 has no translation termination activity and shares no sequence homology with mammalian proteins, thus reducing the likelihood that its expression interferes with cellular function. Recombinant NM fibrils were capable of inducing self-perpetuating protein conformers in neuroblastoma cells that were stably passed on to daughter cells (19). Here we investigated if cytosolic NM aggregates have infectious properties and induce the NM prion state in neighboring cells. We demonstrate that aggregated NM exits the donor cell and gains entry into recipient cells, thereby triggering heritable conformational changes of endogenous NM. Cell-to-cell contact proved to be the most efficient route of transmission. Thus, cytosolic proteins can behave as infectious entities in mammalian cells, a finding that has important implications for understanding non–cell-autonomous protein aggregation in health and disease.

Results

Induction of NM Aggregates During Coculture. We have recently isolated several N2a cell clones that propagate morphologically and biochemically distinct NM-HA aggregates (NM-HA^{agg}) over multiple passages upon exposure to recombinant NM fibrils (Fig. S1) (19). N2a cells not exposed to NM fibrils express cytosolic soluble NM-HA (NM-HA^{sol}) (Fig. S1 *B* and *C*). In cell clones exposed to recombinant NM fibrils, a fraction of endogenous NM-HA becomes aggregated (Fig. S1*C*). Long fibrillar aggregates are

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[1]To whom correspondence should be addressed. E-mail: ina.vorberg@dzne.de.

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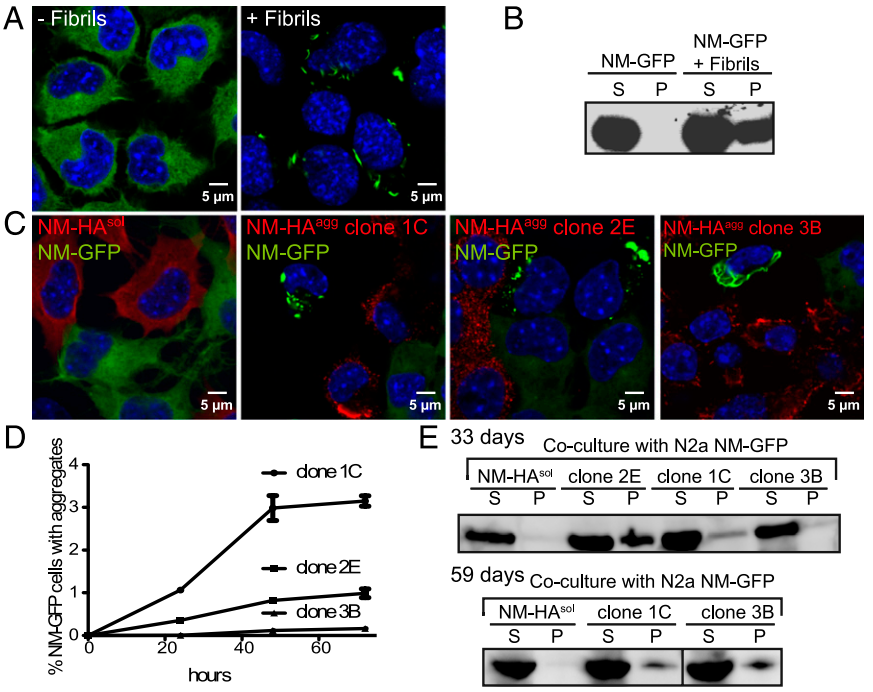

Fig. 1. Non–cell-autonomous aggregate induction by coculture. (*A*) N2a cells stably expressing cytosolic NM-GFP were exposed to 1 μM NM fibrils (monomer equivalent) for 24 h. Aggregate induction was monitored by confocal microscopy. (*B*) Sedimentation assay of lysates of N2a NM-GFP cells treated with recombinant NM fibrils. Antibody: anti-NM antibody 4A5. (*C*) Donor N2a cells expressing soluble NM-HA^{sol} or N2a cell clones 1C, 2E, or 3B bearing NM-HA^{agg} were cocultured with recipient N2a NM-GFP^{sol} cells for 48 h. Cells were stained for NM-HA using anti-HA antibody F7 (red) and counterstained with Hoechst. (*D*) Donor cell clones 1C, 2E, 3B, or N2a cells expressing NM-HA^{sol} were cocultured with N2a NM-GFP^{sol} cells for up to 72 h. The number of NM-GFP aggregate–bearing cells compared with N2a NM-GFP cells is shown. Experiments were performed in triplicate. (*E*) Sedimentation assay of NM-GFP in lysates of cocultures of donor cell clones 1C, 2E, 3B, or N2a cells expressing soluble NM-HA^{sol} with recipient N2a NM-GFP^{sol} cells. Additional lanes were excised for presentation purposes. Antibody: anti-GFP. P, pellet fraction; S, supernatant fraction. Errors represent SEM. (Scale bar, 5 μm.)

characteristic for N2a cell clone 3B, whereas more punctate NM-HA aggregates are propagated by clones 1C and 2E. In clone 1C, aggregates preferentially cluster in one area of the cell (Fig. S1*B*). Cell clones have been passaged for more than a year without obvious loss of aggregates. Cell viability was not impaired in any of the clones tested (Fig. S1 *D* and *E*).

Extracts generated from these cells induced aggregation of the homotypic soluble proteins in recipient cells (19). To assess if NM-HA aggregates can also naturally transfer between cells, a recipient N2a cell population stably expressing the Sup35NM domain fused to GFP was tested for its ability to form NM-GFP aggregates upon addition of recombinant NM fibrils. Confocal microscopy analysis (Fig. 1*A*) and sedimentation assays (Fig. 1*B*) confirmed that NM-GFP was soluble (NM-GFP^{sol}). Spontaneous NM-GFP aggregation was not observed, even under oxidative stress conditions (Fig. S2). By contrast, addition of recombinant NM fibrils successfully induced NM-GFP^{agg} (Fig. 1 *A* and *B*). Coculture of N2a NM-GFP^{sol} with N2a cell clones harboring induced NM-HA aggregates was sufficient to induce aggregation of NM-GFP independent of the donor cell clone tested (Fig. 1*C*). As observed previously, different aggregate phenotypes were induced in recipient cells (19). The efficiency of intercellular aggregate induction differed between cell clones (Fig. 1*D*), with aggregate induction being most effective with clone 1C as donor (induction 1% within 24 h). Very low induction rates were observed, with cell clone 3B producing long fibrillar aggregates (induction <0.002% within 24 h). Aggregate induction was detectable by sedimentation assay after 33 d in coculture with clones 1C and 2E and 59 d in coculture with clone 3B (Fig. 1*E*).

Propagation of Aggregates in Recipient Cells. Prion replication crucially depends on the autocatalytic formation of infectious entities, resulting in faithful inheritance of aggregates by daughter cells upon cell division (11). Exposure of cells to mammalian prions can induce a transient PrP^{Sc} formation that does not result in persistent infection (20). Mitotic stability of aggregates induced by coculture was assessed by live cell imaging. Analysis of cells expressing NM-HA^{sol} and cells expressing NM-GFP^{sol} revealed that coculture did not lead to spontaneous aggregation of NM during mitosis (Fig. 2*A*). By contrast, induced NM-GFP^{agg} were evenly distributed to both daughter cells during cytokinesis (Fig. 2 *B–D*; Movies S1, S2, S3, and S4). Thus, reminiscent of mammalian TSE agents in vitro, naturally transmitted NM-HA aggregates induce self-perpetuating heritable aggregates in recipient cells (11).

Efficient Intercellular Aggregate Induction Depends on Cell-to-Cell Contact. Aggregate induction in recipient cells was studied by live cell imaging of donor and recipient cells expressing

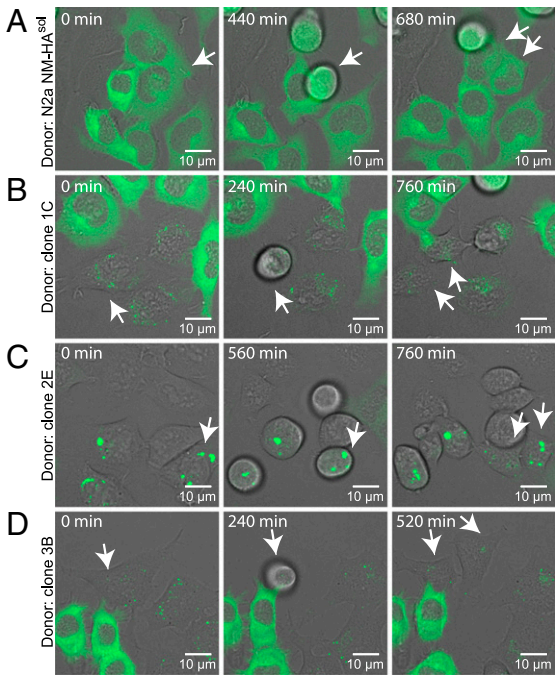

Fig. 2. Transmission of induced aggregates to progeny. Recipient N2a NM-GFP^{sol} cells cocultured with donor N2a cells expressing NM-HA^{sol} (*A*) or N2a NM-HA^{agg} clones 1C (*B*), 2E (*C*), or 3B (*D*) for 24 h were subjected to live cell imaging for 24 h. Arrows mark cells undergoing cell division. (Scale bar, 10 μm.)

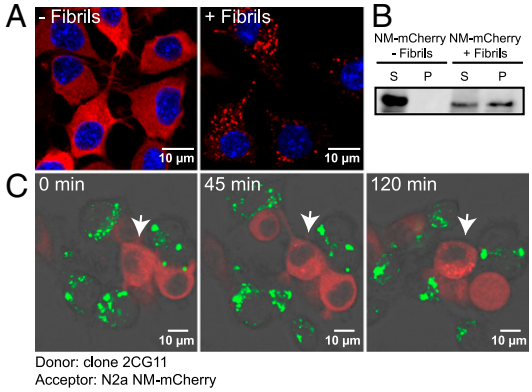

Fig. 3. Aggregate induction in neighboring cells. (*A*) NM-mCherry^{sol} cells were exposed to PBS (*Left*) or 1 μM recombinant NM fibrils (*Right*) and assayed for aggregate induction 48 h after fibrils application. Nuclei were counterstained with Hoechst. (*B*) Sedimentation assay of lysates of N2a NM-mCherry cells exposed to PBS or recombinant NM fibrils. P, pellet fraction; S, supernatant fraction. Antibody: anti-NM 4A5. (*C*) NM-mCherry^{sol} cells were cocultured with cell clone 2CG11 producing NM-GFP^{agg} for 57 h. (Scale bar, 10 μm.)

fluorescently tagged NM. Cytosolic NM-mCherry stably expressed by N2a cells is soluble but can be induced to aggregate upon exposure of cells to recombinant NM fibrils (Fig. 3 *A* and *B*). Time-lapse microscopy of N2a NM-mCherry^{sol} cells cocultured with N2a cell clone 2CG11 stably producing NM-GFP^{agg} demonstrated aggregate induction in some acceptor cells in direct proximity to donor cells, suggesting that aggregate induction could depend on close contact between donor and acceptor cells (Fig. 3*C*; Movies S5 and S6).

To test if infectious NM aggregates were secreted, recipient cells expressing NM-GFP^{sol} were either plated with normal WT N2a cells (to control for comparable cell numbers) or as a coculture with clone 2E producing NM-HA^{agg} (Fig. 4*A*). N2a NM-GFP^{sol}/WT N2a cells were subsequently cultured in the presence of conditioned medium of clone 2E or with conditioned medium of cells expressing NM-HA^{sol}. Medium from control cells or from clone 2E was ineffective or only partially effective in inducing NM-GFP^{agg} (Fig. 4*A*). Increasing overall cell density in N2a NM-GFP^{sol}/clone 2E cocultures increased induction efficiency (Fig. 4*B*). Long-term coculture experiments of cell clones 1C, 2E, and 3B producing NM-HA^{agg} with recipient N2a NM-GFP^{sol} cells confirmed that physical separation of donor and recipient cells by a 0.4-μm pore semipermeable transwell membrane drastically impaired induction of NM-GFP^{agg} (Fig. 4*C*).

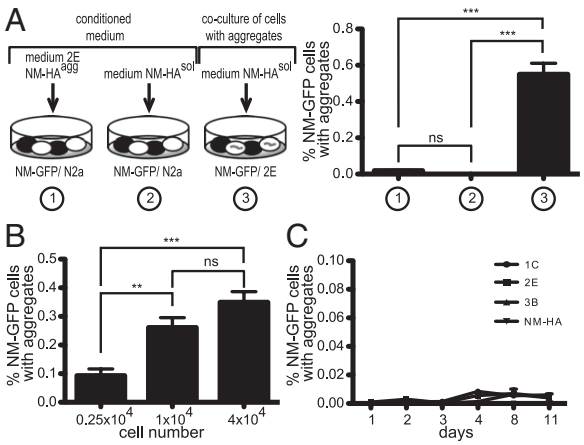

Fig. 4. Aggregate induction by cell-to-cell contact. (*A*) Intercellular aggregate induction by conditioned medium was assessed by incubating NM-GFP^{sol} cells with conditioned medium of either clone 2E NM-HA^{agg} (*Left*) or NM-HA^{sol} cells (*Center*) for 24 h. As a positive control, clone 2E NM-HA^{agg} and NM-GFP^{sol} cells were cocultured (*Right*). For comparison of equal cell numbers, NM-GFP^{sol} cells exposed to conditioned medium were cultured in the presence of WT N2a cells ($n = 6$). (*B*) Equal numbers of cells of clone 2E and N2a NM-GFP^{sol} were cocultured at different densities for 14 h and the number of cells harboring NM-GFP^{agg} was determined. $^{**}P \leq 0.01$. $^{***}P \leq 0.001$. ns, not significant. Error bars represent SEM. (*C*) Time course experiment of aggregate induction in N2a NM-GFP^{sol} cells cultured with N2a NM-HA^{sol} or N2a NM-HA^{agg} clones 1C, 2E, and 3B in transwells. Donor and recipient cells were subpassaged every 3 to 4 d and equal numbers of donor and recipient cells were plated.

To assess if NM-HA^{agg} were taken up by recipient cells, N2a NM-GFP^{sol} cells were tagged by transfection of a construct coding for histone H2B fused to monomeric red fluorescent protein (H2B-mRFP) (Fig. 5*A*). Coculture with clone 1C demonstrated the presence of both NM-HA^{agg} and NM-GFP^{agg} in recipient cells, revealing direct transfer of NM-HA aggregates between cells (Fig. 5*B*). No detectable NM-HA transfer was observed upon coculture of N2a NM-HA^{sol} cells with N2a NM-GFP^{sol} cells (Fig. S3). In conclusion, cytosolic NM-HA aggregates behave as infectious entities, capable of replicating and transmitting between mammalian cells.

Non–Cell-Autonomous Aggregate Induction in Primary Cells and Hippocampal Slices. To assess if NM exhibits prion characteristics in primary cells, murine astrocyte cultures, cerebellar granule neurons (CGNs), and cortical neurons stably expressing soluble NM-HA or NM-GFP (Fig. 6 and Figs. S4 and S5) were generated. Spontaneous aggregation of NM-GFP or NM-HA was not observed (Figs. S4*B* and S5). Recombinant NM fibrils induced aggregate formation in all primary cell types (Fig. 6 and Figs. S4 and S5). Similarly, direct coculture of astrocytes harboring NM-HA aggregates and CGNs expressing NM-GFP was sufficient to induce NM-GFP aggregation (Fig. 7 *A* and *B*), whereas coculture with astrocytes expressing soluble NM-HA was not (Fig. S6). Induction was not due to exogenous recombinant NM fibrils still attached to astrocytes, because NM fibrils are efficiently removed by trypsin treatment (Fig. S7). Astrocytes expressing NM-HA^{sol} or NM-HA^{agg} did not exhibit increased cell death as assessed by immunofluorescence staining for cleaved caspase 3 (Fig. 7*C* and Fig. S6*E*) and propidium iodide staining (Fig. S6*D*). Thus, release of aggregates resulting from cell toxicity unlikely contributes to aggregate transmission. Concomitantly, conditioned medium from astrocytes producing NM-HA^{agg} was significantly less efficient in inducing aggregates in recipient CGN NM-GFP cells compared with direct coculturing (Fig. 7*D*). Separation of donor and recipient cell populations by transwell membranes also drastically reduced aggregate induction (Fig. 7*D*), strongly suggesting that direct cell contact facilitated aggregate induction.

We next determined if NM aggregates could be induced in organotypic brain slices. Murine hippocampal slices ectopically expressing NM-GFP were overlaid with donor primary astrocyte cultures producing NM-HA aggregates (Fig. 8*A*) or astrocytes expressing soluble NM-HA (Fig. S8*A*). Immunocytochemical examination of fixed slices revealed induction of NM-GFP^{agg} in recipient cells (Fig. 8*B*). Often, aggregates were apparent in cells immediately adjacent to NM-HA–bearing astrocytes. Merged images demonstrated that a subset of NM-GFP and NM-HA aggregates colocalized, suggesting that aggregates were directly transferred between cells. No induction was observed when donor astrocytes expressed soluble NM-HA (Fig. S8*B*). In conclusion, cytosolic NM aggregates act as prions that faithfully replicate in progeny cells and spread to neighboring cells in permanent cell cultures, primary cells, and even in organotypic cultures.

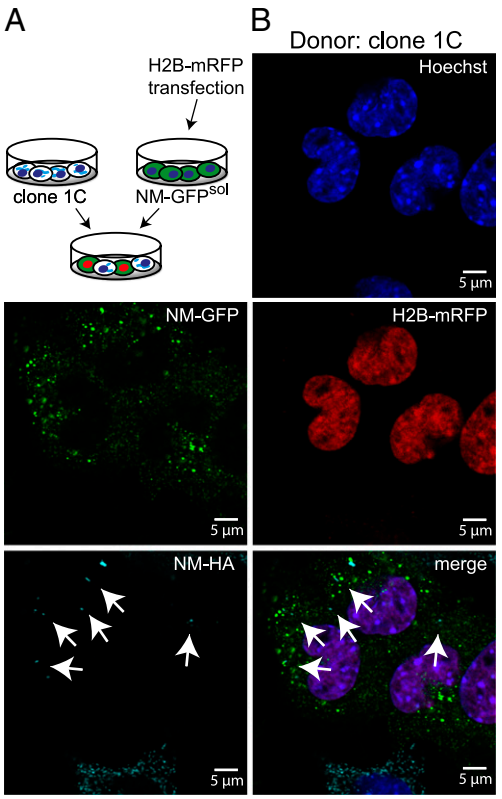

Fig. 5. Direct transfer of cytosolic aggregates to recipient cells. (*A*) N2a NM-GFP^{sol} cells transiently expressing nuclear H2B-mRFP were cocultured with clone 1C NM-HA^{agg} for 48 h. (*B*) Cells were stained using anti-HA antibody F7 (light blue) and nuclei were visualized by Hoechst staining. (Scale bar, 5 μm.)

Discussion

Here we demonstrate that Sup35NM prions fulfill all cellular replication criteria for mammalian prions: (*i*) the precursor protein resides in a stable nonprion state, (*ii*) prion formation can be initiated by exogenous seeds, (*iii*) induced prion aggregates persistently propagate over multiple cell divisions, and (*iv*) prions are capable of invading neighboring cells (*v*) in which they induce a self-perpetuating prion state of the substrate protein. Thus, in mammalian cells, NM can undergo the complete cellular life cycle of prions from uptake to propagation to egress. Importantly, NM prions also spread to postmitotic cells and to hippocampal brain slices, clearly demonstrating that cell division is not a prerequisite for cell-to-cell transmission of cytosolic aggregates. Three N2a clones propagating morphologically different NM aggregates all initiated the prion phenotype in acceptor cells, albeit with drastically differing efficiencies. Interestingly, lowest transmission efficiency was observed with a cell clone displaying long fibrillar aggregates. Possible explanations could be that clonal differences between donor cells account for the varying induction efficiencies or that the type of NM aggregate influences its transmission efficiency, just as small prion entities are more infectious in yeast (21, 22). Overt toxicity of NM prion propagation was not observed, which is in line with the in vitro propagation behavior of prions causing transmissible spongiform encephalopathies (11). The finding that N2a cells tolerate continuous formation of cytosolic aggregates is surprising and contrasts with previous studies demonstrating that intracellular misfolding and/or aggregation of disease-related and artificial β-sheet proteins can compromise cell viability (23, 24). The toxicity of aggregation-prone proteins is not well understood but has been attributed to disruption of membrane integrity, sequestration, and depletion of cellular proteins and interference with cellular quality control (23, 24). A direct comparison of the toxic potential of different aggregation-prone proteins in the same cellular model will help to better understand cellular processes underlying toxic gain of function phenotypes.

Cell-to-cell transmission is a characteristic of mammalian prions. Yeast prions propagate from mother to daughter, except during mating. Transmissibility of yeast prions is determined experimentally by cytoduction, a method by which a yeast population is mated to a yeast strain defective in nuclear fusion, resulting in transfer of organelles and virus-like particles (25). Alternatively, yeast prions can be transformed experimentally into yeast, where they induce the prion phenotype (26, 27). Our data demonstrate that the Sup35 prion domain behaves as a classical infectious entity in mammalian cells that naturally spreads horizontally and vertically. It has been hypothesized that tethering of amyloidogenic proteins to the cell membrane via a GPI anchor facilitates propagation and transmission (28, 29). Importantly, however, infectious prionosis can be induced in mice expressing anchorless secreted PrP (30). Although GPI-anchoring might facilitate CNS neuroinvasion of TSE agents (31), our data clearly show that membrane-anchoring is not a requirement for infectious properties of protein aggregates.

Induction of intracellular aggregation could involve direct NM seed transmission or indirect NM-independent stimuli. Cytokines unlikely contribute to aggregate induction because physical separation of donor and acceptor cells basically abolished this process. Whether coupling of donor and acceptor cells via electrical synapses (gap junctions) could influence aggregate induction is unknown. The existence of donor aggregates in acceptor cells strongly suggests that transmission of NM seeds triggers the NM prion phenotype. High cell densities necessary to achieve non–cell-autonomous aggregate induction and low induction efficiencies so far have prevented us from directly monitoring intercellular

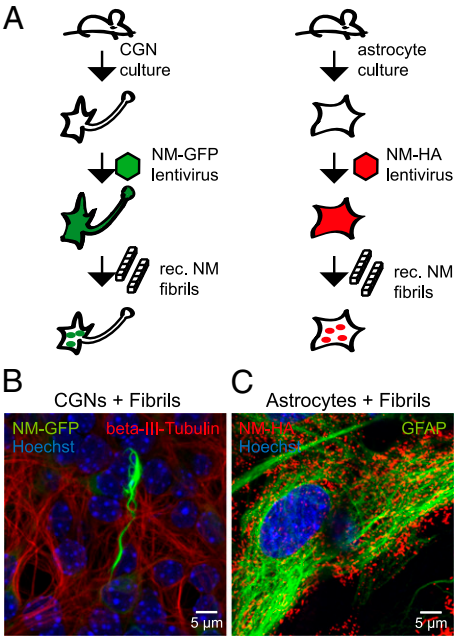

Fig. 6. Induction of NM aggregates in primary cells. (*A*) CGNs and primary astrocytes expressing NM-GFP (CGNs) or NM-HA (astrocytes) were exposed to 1 μM recombinant NM fibrils. (*B*) Neurons were stained 24 h after induction with antineuronal class III β-Tubulin antibodies (red). (*C*) Induced primary astrocytes were stained with anti-GFAP antibody (green); NM-HA was detected using anti-HA (red). Nuclei were stained with Hoechst (blue). Maximum intensity projections are shown. (Scale bar, 5 μm.)

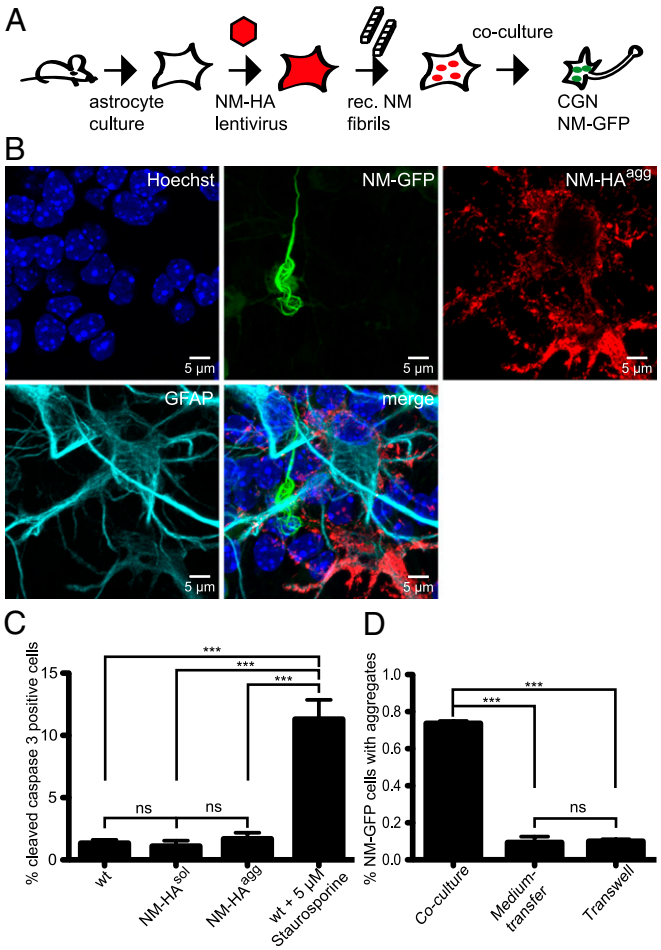

Fig. 7. Astrocytes bearing NM-HA^{agg} induced NM-GFP^{agg} in CGNs in co-culture. (*A*) Astrocyte NM-HA cultures were exposed to recombinant NM fibrils and passaged two times before coculture with CGNs expressing NM-GFP. (*B*) Maximum intensity projection of NM-GFP^{agg} induction imaged 48 h after plating. CGNs express NM-GFP (green); astrocyte cultures harbor NM-HA^{agg} (anti-HA F7 antibody; red) and express GFAP (light blue). Nuclei were stained with Hoechst. (*C*) Astrocyte cultures were stained for cleaved caspase 3. Cells treated with 5 µM staurosporine for 4 h served as a positive control (n = 6). (*D*) CGNs NM-GFP^{sol} were cocultured with astrocytic cultures with NM-HA^{agg} for 48 h either directly or in transwells. CGN NM-GFP^{sol} cells were exposed to conditioned medium of astrocytes harboring NM-HA^{agg} for 48 h (n = 3). ***$P \leq 0.001$. ns, not significant. Error bars represent SEM. (Scale bar, 5 µm.)

NM prion transfer. Only few acceptor cells harbored visible NM-HA donor aggregates, suggesting that either uptake of a small number of NM-HA seeds is sufficient to induce NM-GFP aggregation or that transmitted NM-HA aggregates are usually too small to be visualized. Size determination of transmitted NM-HA aggregates by confocal microscopy analysis revealed that particles of up to 0.5 × 1 µm in diameter could be identified in acceptor cells; however, the possibility that smaller entities reassembled into larger aggregates after intercellular transfer cannot be excluded.

In vitro, mammalian prions use exosomes (32), direct cell contact, or tunneling nanotubes (33, 34) for entry and egress. That conditioned medium of NM^{agg}-producing cells was less potent in inducing aggregates in acceptor cells argues that secretion of NM^{agg} was not the main route of prion dissemination. Our data point to an infection process that involves transmission at cell–cell interfaces. NM seeds could be shuttled to recipient cells via filopodial bridges or nanotube-like structures, as observed for certain viruses (35) and prions (34). Of note, time-lapse studies demonstrated cytoplasmic bridges between cells (Movies S5 and S6), but induction by other adjacent donor cells cannot be excluded. NM aggregates could also be released into the intercellular cleft to be taken up by the adjacent cell. A specialized mechanism of particle shedding and subsequent uptake has recently been described for intercellular melanosome transfer (36). Secretion and transmission of infectious particles through so-called "virological synapses" between cells of the immune system have been described for retroviruses (37).

Non–cell-autonomous propagation of protein misfolding has been suggested for many disease-linked proteins (38). In the CNS, transmission of protein aggregates might involve secretion into the synaptic cleft. Small oligomers or fibrils of disease-associated proteins such as tau, polyglutamine-rich proteins, or α-synuclein have been shown to gain entry to the cytosol, where they induce aggregation of the cellular soluble isoforms (12–16). Further, secreted forms of tau, α-synuclein, or superoxide dismutase 1 (SOD1) (14, 17, 39–41) have been reported. Defining possible routes of intercellular protein aggregate transfer and their potential involvement in cell-to-cell transport of specific protein aggregates will be key to understanding progressive spreading of pathology.

Are neurodegenerative disease-linked amyloidogenic proteins true prions? We found that NM aggregates in N2a cells were mitotically stable, a characteristic of prions in lower eukaryotes (3) and mammalian prions in permissive cell lines (11, 42). Prion maintenance depends on the continuous propagation of prion particles and their bidirectional segregation to daughter cells, processes that involve fragmentation of polymers into seeds. In yeast, the Sup35 prion state relies on Sup35 polymer fragmentation by heat shock protein Hsp104, a chaperone that has no homolog in mammalian cells (43). Which cellular factors contribute to Sup35NM aggregate fragmentation in mammalian cells is currently unknown. Strong inheritance of the aggregated state has so far only been demonstrated for SOD1 (17), and,

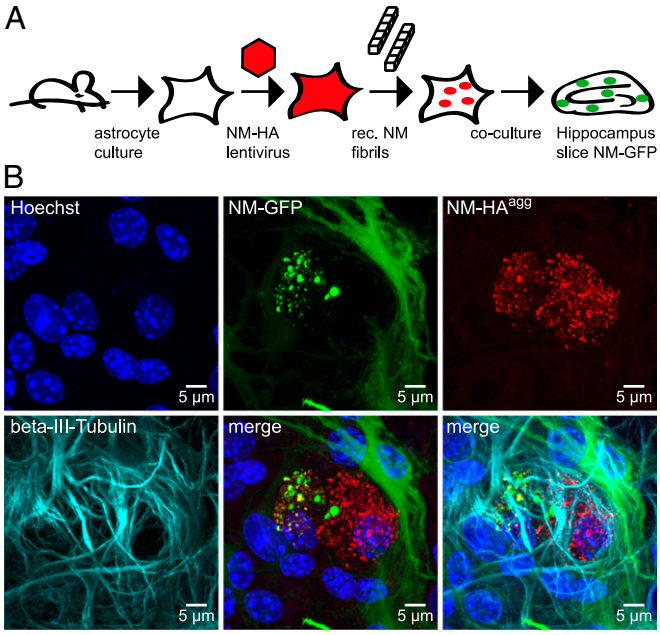

Fig. 8. NM-GFP aggregate induction in organotypic hippocampal slices. (*A*) Hippocampal slices were transduced with lentivirus coding for NM-GFP and slices were subsequently cocultured with primary astrocyte cultures producing NM-HA^{agg}. (*B*) Twelve days after coculture, nuclei were stained using Hoechst and neurons were stained using anti-beta-III-Tubulin antibodies (light blue). NM-HA was detected using anti-HA antibodies F7 (red). Maximum intensity projections are shown. (Scale bar, 5 µm.)

to a very low degree, polyglutamine aggregates (15). In fact, asymmetric inheritance of damaged and misfolded proteins constitutes an evolutionary conserved mechanism that enables survival of progeny cells (44). Thus, the degree to which disease-linked proteins fulfill the cellular replication criteria for prions likely differs. Efficient aggregate partitioning and egress routes combined with escape from the cellular quality control mechanisms might be the survival strategy of infectious protein aggregates. In light of compelling evidence for protein-based inheritance in fungi and the discovery of proteins that form functional amyloid even in mammals (45–48), one can speculate that such strategies could also be used for normal intercellular communication. The mammalian genome encodes a diverse array of proteins with prion-like domains comparable to the one in NM, some of which are known to aggregate (49). Transmission of aggregates between cells could thus add another dimension to non–cell-autonomous regulation of cellular function. Future experiments will show if the reported exchange of cytosolic proteins with prion characteristics is of functional significance in mammals.

Materials and Methods

Construction of the NM-HA plasmid was described previously (18). For external induction of NM-HA, NM-GFP, and NM-mCherry aggregates, 1 μM recombinant Sup35NM fibrils (monomer equivalent) were added to the cell culture medium for 24 h. A detailed description of procedures is provided in *SI Materials and Methods*.

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