Introduction

Scrapie in sheep, bovine spongiform encephalopathy (also called ‘mad cow’ disease) in cattle and Creutzfeldt-Jakob disease in humans are transmissible spongiform encephalopathies, which are also called prion diseases. A prion is a proteinaceous infectious particle that lacks nucleic acids, which means that it is an infectious protein [1]. In the prion, the altered conformers of a protein autocatalytically convert the normal structure to the altered form, which is an ordered aggregate called amyloid. Although this prion concept was developed for the mammalian neurodegenerative diseases in which the PrP protein participates, the concept has been extended to several non-Mendelian genetic elements in budding yeast, such as [PSI+] and [URE3] in Saccharomyces cerevisiae [2]. As yeast is quite a tractable model eukaryote, yeast prions can provide many important insights, which are usually difficult to achieve using mammalian prions, into prion biology [3–8]. In particular, the molecular mechanisms by which the prion proteins are propagated and transmitted have been unraveled in the yeast prion model. This review provides an overview on the transmissible entities of yeast prions, focusing mainly on the Sup35 protein in [PSI+].

Yeast prion [PSI+]

Although several dozen yeast proteins are known to behave as prions in vivo (for recent advances, see refs 9-12), the prion state of the Sup35 protein – the [PSI+] determinant – is the best-characterized prion.
Sup35 is an essential protein, which functions as a translation termination factor in cooperation with its partner, Sup45 [13]. The N-terminal portion of Sup35 is a glutamine/asparagine (Q/N)-rich domain, which has a high propensity to form amyloid fibrils in vitro [14–18] (Fig. 1). The C-terminal domain is sufficient to function as the termination factor (eRF3) and interacts with Sup45 (eRF1) [13]. The \([PSI^+]\) phenotype is a nonsense suppression caused by the amyloid-like aggregates of Sup35 [13]. The propagation of \([PSI^+]\) is strictly dependent on the presence of an appropriate amount of Hsp104 [19]. Impairment of Hsp104 function, by either the deletion of Hsp104 [19] or the addition of millimolar concentrations of guanidine hydrochloride (GuHCl), cures \([PSI^+]\) [20].

One of the central issues in prion biology is to identify the entity of the \([PSI^+]\) determinant. Several approaches, as described below, have been applied over the past decade.

### Biochemical approaches to investigate yeast prion aggregates

The proposal that non-Mendelian genetic elements (such as \([PSI^+]\)) in yeast are prions has opened the door to identify the molecular entity of prions [2]. The aggregates of Sup35 in the cells can be detected by the centrifugation of yeast lysates [21,22]. However, the centrifugation assay cannot address the detailed characteristics of the Sup35 aggregates, such as the structure and size of prion aggregates in vivo.

Kryndushkin et al. developed an ingenious method to characterize the prion aggregates in cells. They found that treatment of the \([PSI^+]\) lysate with 2% SDS disassembles the Sup35 prion aggregates into smaller, SDS-resistant particles (called polymers in their report), allowing analysis of their sizes on an agarose gel containing 0.1% SDS [23]. This method is referred to as semidenaturing detergent-agarose gel electrophoresis [23]. Using this method, they compared \([PSI^+]\) and \([psi^-]\) lysates and found that Sup35 was almost always in the oligomeric form in the \([PSI^+]\) lysate, while it was monomeric in the \([psi^-]\) lysate. They concluded that the SDS-resistant prion oligomers were heterogeneous in size, ranging from 700 to 4000 kDa, which should correspond to 8-50 Sup35 monomers [23].

The possibility of other interacting proteins being present in the Sup35 oligomers was tested by affinity isolation of Sup35–His6 in \([PSI^+]\) cells [24]. The affinity isolation revealed that the SDS-resistant Sup35 oligomers are associated with Sas1/2 proteins in a molar ratio of 0.5 Sas1/2 per Sup35, and with other minor components, including Hsp104, Ssb1/2, Sis1, Sse1, Ydj1 and Sla2 [24]. When the affinity-purified Sup35 oligomers were negatively stained and visualized by electron microscopy, the oligomers resembled short barrels and bundles, which seemed to be composed of barrels, rather than long fibrils [24].

### Genetic approaches to investigate the transmissible entity of yeast prions

As mentioned earlier, the addition of GuHCl leads to the elimination of \([PSI^+]\) through the inhibition of Hsp104 activity [20,25–29]. The kinetics of prion elimination exhibited a significant lag, corresponding to around four to five generations, before the gradual emergence of \([psi^-]\) [25]. Based on the kinetics, Tuite and co-workers proposed that GuHCl blocks a critical step in the replication of the prion conformers [25,30]. Assuming that the prion seeds are randomly segregated during cell division, they calculated that the number of prion seeds, which have been named ‘propagons’, in \([PSI^+]\) cells was approximately 60 [30]. Sophisticated yeast genetics revealed that \([PSI^+]\) cells contain propagons to transmit and maintain the prion phenotype.

### Green fluorescent protein-fused Sup35 aggregates as an indicator of \([PSI^+]\) cells

The expression of Sup35 fused with green fluorescent protein (GFP) in \([PSI^+]\) cells gives rise to the formation of visible spherical fluorescent aggregates, called
foci, in the cytosol [22] (Fig. 2A), although the size and the number of foci vary, depending on the expression level and the yeast strains used. Besides the spherical foci, ring/rod-shaped aggregates were also observed under some conditions, such as in [PSI\(^+\)] cells treated with GuHCl [31,32] (Fig. 2B). By contrast, Sup35–GFP does not form such visible foci, and is diffusely dispersed in the [psi\(^-\)] cells [22] (Fig. 2C). Most of the Sup35 in the [PSI\(^+\)] cell lysate exists as high-molecular-weight pellets, and is isolated by high-speed centrifugation [21,22]. In vitro, Sup35 fragments containing the N domain form β-sheet-rich amyloid aggregates [14–18]. Therefore, the spherical foci are considered to be an indicator of [PSI\(^+\)] because no such foci are seen in [psi\(^-\)] cells.

Regarding the relationship between the visible Sup35–GFP foci and [PSI\(^+\)], Zhou et al. [31] observed the formation of Sup35 aggregates in vivo during the de novo induction of [PSI\(^+\)] by the overexpression of Sup35 in [psi\(^-\)][PIN\(^+\)] cells, where [PIN\(^+\)] is a yeast prion phenotype that requires the induction of [PSI\(^+\)] [33]. Based on their detailed examination of the appearance of the visible aggregates in mother and daughter cells, as well as analyses of the timing of aggregate formation, they suggested that most of the heritable [PSI\(^+\)] seeds are too small to be visualized by conventional fluorescence microscopy [31]. Song et al. [34] also reported that fluorescent foci do not directly represent [PSI\(^+\)], based on the careful observation of visible foci in [GPSI\(^+\)] cells, in which GFP was inserted between the N and M domains of Sup35 in the chromosomally encoded SUP35 gene.

**Studying the dynamics of Sup35–GFP aggregates in living cells**

Prion phenomena are intrinsically dynamic processes, because prion aggregates propagate, remodel and transmit during the protein-based inheritance in yeast prions [3,8]. Simple static observations of Sup35–GFP aggregates by conventional fluorescence microscopy are insufficient to investigate the dynamic aspects of the prions in the cells. Recent advances using several techniques to investigate the dynamics of protein molecules in living cells have provided novel insights into the molecular mechanism by which the Sup35 prion aggregates are propagated and transmitted in [PSI\(^+\)] cells.

a) **Single-cell imaging system to monitor the fate of the Sup35–GFP foci**

Several genetic analyses, combined with fluorescent microscopic observations of visible Sup35–GFP foci, have suggested that the foci do not directly represent [PSI\(^+\)]. However, such an ensemble method does not provide direct evidence for the significance of the visible foci in the transmission of [PSI\(^+\)]. To gain insight into the dynamics of Sup35–GFP foci in [PSI\(^+\)] cells, an on-chip single-cell cultivation system was developed to investigate directly the dynamic properties of prion aggregates [35,36]. Specifically, the fate of the visible Sup35–GFP foci in single living [PSI\(^+\)] cells was directly monitored in a time-lapse manner. The on-chip cultivation system, in which the medium can be easily exchanged during cultivation, enabled us to...
continuously observe individual growing cells for long periods of time. The expression of Sup35–GFP was transiently induced to lead to the formation of visible foci in the cytoplasm of $[\text{PSI}^+]$ cells [35]. After stopping the further induction of Sup35–GFP, by exchanging the medium with one that lacks an inducer, the fate of the fluorescent foci was monitored in real time. Individual live-cell imaging showed that the diameter of the foci gradually decreased, and the foci eventually disappeared [36]. The disappearance of the foci was not caused by GFP photobleaching or degradation [36]. The disappearance of visible foci of Sup35–GFP was also reported by analyses of a microcolony assay system, in which preformed Sup35–GFP foci became undetectable as the cells grew [37].

The punctate foci reappeared when Sup35–GFP was re-induced, indicating that the seeds of the foci were not lost in the cells after the foci disappeared. The live-cell imaging showed the appearance of the foci in the daughter cell at almost same time as when the foci re-appeared in the mother cell, indicating that the seeds are transmitted from the mother cell to the daughter cell. In addition, several lines of evidence, including a nonsense suppression assay at a single-cell level, showed that the $[\text{PSI}^+]$ phenotype was maintained in the cells after the foci dispersed [36]. Taken together, the single-cell imaging of Sup35–GFP foci clearly revealed that the foci dynamically dispersed into a state that functions as the seeds of the foci and causes the nonsense suppression, which is sufficient to maintain the $[\text{PSI}^+]$ phenotype.

b) Fluorescence correlation spectroscopy

The single-cell imaging described in the previous section revealed that, after dispersion of the foci, the $[\text{PSI}^+]$ cells have an entity that behaves as prions. The next question is, what is the prion entity left in the cytoplasm after the foci have dispersed? Conventional fluorescence microscopic observation can barely distinguish the difference between $[\text{PSI}^+]$ cells without the foci and $[\text{psi}^-]$ cells in their appearance, as both cells have diffuse GFP fluorescence in the cytoplasm. To elucidate the physical properties of Sup35–GFP in living $[\text{PSI}^+]$ cells without the foci, fluorescence correlation spectroscopy (FCS) has been applied [36].

FCS is a technique used to analyze the diffusion properties of fluorescent molecules, by calculating the fluorescence autocorrelation function (FAF) in a microscopic detection volume at the femtoliter level [38,39]. FCS allows the determination of diffusion constants, which are directly correlated with the size of the molecules, of fluctuating fluorescent molecules under equilibrium conditions. The dynamic range of FCS is very wide: FCS can measure commonly accessed diffusion dynamics on a timescale from $\sim 1 \mu s$ to $\sim 1 \text{ s}$. Because FCS is usually combined with confocal laser-scanning microscopy, we can define the detection volume at any position of interest inside a living cell, in a non-invasive manner. As the dynamics of prions are basically dependent on the conversion from monomers to aggregates, and vice versa, FCS is ideally suited to estimate the size of Sup35–GFP in living yeast cells.

After confirming that FCS was applicable to living yeast cells, fluorescence fluctuations of Sup35–GFP in $[\text{psi}^-]$ and $[\text{PSI}^+]$ cells, with or without the foci, were measured using FCS [36]. The FAFs of Sup35–GFP in $[\text{psi}^-]$ cells were almost the same as those in cells expressing the GFP monomer alone, indicating that $[\text{psi}^-]$ cells contain mostly monomers of Sup35–GFP. By contrast, the FAF profiles in $[\text{PSI}^+]$ cells, irrespective of the presence of foci, were shifted to the right, compared with those in $[\text{psi}^-]$ cells, indicating that the Sup35–GFP species in $[\text{PSI}^+]$ cells were much slower, and thus larger, than those in $[\text{psi}^-]$ cells. These results indicate that the larger species, referred to here as diffuse oligomers, are dispersed in the cytoplasm of $[\text{PSI}^+]$ cells, regardless of the presence of foci [36].

The combination of FCS with the on-chip single cultivation system (a time-lapse FCS system) allows measurement of the size of Sup35–GFP in the daughter cells immediately after the transmission from the mother $[\text{PSI}^+]$ cells [36]. Autocorrelation functions of both the mother and daughter cells were measured as the $[\text{PSI}^+]$ cell with the foci was budding. Strikingly, the autocorrelation function of Sup35–GFP in the daughter cell in an early budding step was almost the same as that in the mother cell, indicating that the diffuse oligomers are transmissible to daughter cells [36]. These time-lapse FCS experiments, combined with the retention of the seeds of the foci in the daughter cells, demonstrated that the oligomeric species dispersed in the mother cells are directly transmitted to their daughter cells [36].

The single mother–daughter pair analysis using FCS was extended to investigate the effect of Hsp104 on the transmission of Sup35–GFP [32]. An FCS analysis of GuHCl-treated $[\text{PSI}^+]$ cells revealed that Sup35–GFP diffusion in the daughter cells was faster; that is, the Sup35–GFP particle was smaller than that in the mother cells under the Hsp104-inactivated conditions [32] (see below for details).
c) Fluorescence recovery after photobleaching

As an alternative approach to analyze the protein dynamics in living cells, the diffusion of a fluorescent protein can be measured using a photobleaching technique known as fluorescence recovery after photobleaching (FRAP) [38]. In this technique, fluorescent molecules in a small region of the cell are irreversibly photobleached by transient exposure to a laser beam, and the subsequent recovery of fluorescence in the photobleached region is recorded [38]. The fluorescence intensity recovers when the fluorescent molecules diffuse, as the bleached molecules diffuse away and the unbleached molecules diffuse into the irradiated region. The kinetics of the recovery provides information about the diffusion property of the molecules: faster recovery means faster motion, indicating the greater diffusion constant of the molecules. As the exposure to the laser beam for the bleaching is only transient, this method is usually not harmful to living cells. The technique usually involves the production of a specific protein of interest fused to GFP or other fluorescent proteins, and has been applied to the Sup35–GFP fusion proteins in living yeast cells [32,34,37,40–42].

A modified [PSI+] strain, in which a functional Sup35–GFP fusion protein (referred to as NGMC) was created by introducing GFP between the N-terminal and middle domains of endogenous Sup35, was used for the FRAP analysis to measure the diffusion of Sup35–GFP proteins [34]. The FRAP analysis showed that the fluorescence recovery was slower in [PSI+] cells than in [psi−] cells, indicating that the NGMC proteins were in an aggregated form in [PSI+] cells [34]. In a subsequent study, FRAP was used to monitor the NGMC states in GuHCl-treated [PSI+] cells [40]. The cytoplasm in the cells showed a slower rate of recovery after 1 hour of incubation in GuHCl, but the rate of FRAP increased after 5 hours of incubation in the GuHCl-containing medium, and became identical to the rate observed in [psi−] cells [40]. In an independent study, FRAP was also used to measure the physical state of Sup35–GFP in the Hsp104-inactivated [PSI+] cells, by either the Hsp104 mutant or the GuHCl treatment [37]. The measurements indicated that Sup35–GFP became largely immobile, with no recovery of fluorescence in the Hsp104-inactivated cells [37]. This immobility was interpreted as a cause of the segregation bias of Sup35 aggregates in the Hsp104-inactivated cells, eventually leading to the loss of [PSI+] [37].

Transmission of Sup35 from mother cells to daughter cells is critical in the prion phenomena. Neither conventional FRAP nor FCS can be used to investigate the flux of Sup35 between mother cells and daughter cells; however, a technique based on FRAP has been developed to investigate the flux [32]. In the conventional FRAP technique, fluorescent proteins in a small region of the cell are photobleached. In the modified FRAP technique, the GFP fluorescence in the whole daughter cell is photobleached to assess the flux rate from the mother cell to the daughter cell. When the modified FRAP, called MD-FRAP (mother to daughter), was conducted with the [PSI+] cells, the flux of Sup35NM–GFP in the [psi−] cells was faster than that in the [PSI+] cells, reflecting the existence of diffuse oligomers of Sup35–GFP in the [PSI+] cells. The MD-FRAP in the GuHCl-treated [PSI+] cells yielded two distinct distributions of the flux rates. About half of the cells transmitted the Sup35NM–GFP with a flux rate that was almost identical to that in the [psi−] cells, suggesting that the cells were already cured. By contrast, either no, or extremely slow, flux was observed in the other half of the cells, reflecting the severe impairment of the mother–daughter transmission in the cells [32].

Dynamic properties of the diffuse oligomers in the [PSI+] cells

After the extension of the prion concept into yeast non-Mendelian genetic elements, a variety of techniques, as described above, have unraveled the molecular entity of yeast prions, such as the [PSI+] determinant, over the past decade. Together, the data from centrifugation assays, biochemical isolation, yeast genetics, GFP fusion methods and several single-cell approaches have revealed that the transmissible entities of [PSI+] are the oligomeric states of Sup35 within the cytoplasm. Importantly, the nature of the diffuse oligomers is not static, but highly dynamic. Although the role of protein dynamics in prion propagation was well summarized in a recent review [8], we will further discuss the details of the diffuse oligomers in the prion transmission, based mainly on our recent findings.

a) Sup35–GFP foci are in equilibrium with diffuse oligomers in [PSI+] cells

Analyses using the on-chip single-cell imaging and FCS revealed the dynamics of visible foci derived from Sup35–GFP. The visible fluorescent foci, which are one of the indicators of the [PSI+] phenotype, are dispersed throughout the cytoplasm as diffuse oligomers, which are sufficient to maintain the [PSI+] phenotype [36]. In addition, the diffuse oligomers are transmitted...
to their daughter cells, where the foci can reappear [32,36]. Taken together, the foci are not dead-end aggregates, but are highly dynamic species that are in equilibrium with the diffuse oligomers.

b) What is the molecular structure of the diffuse oligomers?

What is the molecular structure of the diffuse oligomers? The in vitro properties of recombinant Sup35 proteins, which form amyloid fibrils with cross β-sheet structures, led us to hypothesize that the amyloid structures of Sup35 are critical for the propagation and transmission of [PSI+] in vivo. However, there is no direct connection that links the in vitro amyloid fibrils with the in vivo transmissible entities of the prions. Nevertheless, several lines of evidence support the proposal that [PSI+] cells contain amyloid structures of Sup35. First, in vivo, Sup35 aggregates in [PSI+] cells are stained by an amyloid-staining dye, thioflavin S, indicating the presence of cross β-sheet structures in [PSI+] cells [43]. Second, amyloid fibrils prepared from recombinant Sup35 in vitro can efficiently convert cells from [psi−] to [PSI+] after incorporation of the in vitro fibrils into the [psi−] cells [44,45], indirectly showing that amyloid fibrils are propagated in the [PSI+]–converted cells. Third, an electron micrographic analysis of Sup35 oligomers isolated from [PSI+] cells revealed barrels ~20 nm wide and larger structures (bundles) [24]. Although the appearance of these structures does not resemble the typical amyloid fibrils formed in vitro, it has been pointed out that these structures look similar to the prion oligomers made of recombinant Sup35, when prepared in the presence of Hsp104 plus ATP [46]. Fourth, a simulation of the FCS data on Sup35–GFP oligomers in the [PSI+] lysate, based on a semidenaturing detergent–agarose gel electrophoresis analysis, clearly showed that the diffuse oligomers are not spherical, but adopt a rod shape (C. G. Pack, S. Kawai-Noma, et al., manuscript in preparation), suggesting that the diffuse oligomers are in amyloid-like structures. Finally, electron micrographic observations of GuHCl-treated [PSI+] cells, in which large rod-like or ring-like aggregates of Sup35–GFP were visible, showed that the visible rod-shaped aggregates are composed of bundled fibrils (S. Kawai-Noma, A. Hirata et al., manuscript in preparation). The diameters of the fibrils in cells closely resemble those of amyloid fibrils formed in vitro. The bundled structure is considered to be a consequence of the impaired Hsp104 function, which results in the formation of longer fibrils that bundle together to form the rod-shaped structure. Taken together, the diffuse oligomers in [PSI+] cells are most likely to be fragmented amyloid fibrils.

c) Balance between growth and division of the diffuse oligomers

Prion propagation involves two distinct steps: a growth phase in which the existing amyloid particles elongate in a self-catalyzed manner; and a division (or fragmentation) phase in which the amyloid particles are divided for multiplication. [3] Stable maintenance of the prion phenomena relies on a delicate balance between the growth and division phases, resulting in the dynamic properties of prion particles (Fig. 3).

The details of the growth phase in vivo are poorly understood. By contrast, the division phase has been extensively investigated because trans-acting Hsp104 and other chaperones play a critical role in this process. Hsp104 is a member of the AAA+ superfamily of ATPases (ATPases associated with various cellular activities), which is not required under normal growth conditions but is critical for surviving extreme stress, such as temperatures of 50 °C [47]. Hsp104, with the aid of the Hsp70/40 system, breaks protein aggregates in an ATP-dependent manner [47]. Perturbation of cellular Hsp104 levels dramatically affects the maintenance of [PSI+]: overexpression, inactivation and deletion of Hsp104 cure [PSI+] [19]. Although the molecular basis by which Hsp104 overexpression cures [PSI+] remains to be solved, accumulating evidence

Fig. 3. Dynamics of the yeast prion Sup35 in the cell. Prion propagation involves two distinct steps. In the growth phase, pre-existing amyloid particles elongate in a self-catalyzed manner. After translation at the ribosomes, Sup35 monomers (green circles) are incorporated into the pre-existing amyloid fibrils (red arrowheads). In the division (or fragmentation) phase, the amyloid particles are divided for multiplication. Stable maintenance of the prion phenomena relies on the delicate balance between the growth and division phases, resulting in the dynamic properties of prion particles.
has revealed a mechanism by which the inactivation of 
Hsp104 cures $[PSI^+]$. 

First, microcolony observations and FRAP revealed 
that the Sup35 aggregates became immobile as a result 
of their increased size upon Hsp104 inactivation [37]. 
Second, the rod-shaped Sup35–GFP aggregates were 
accumulated in GuHCl-treated $[PSI^+]$ cells [31,32], 

probably because of an insufficient fragmentation of 
Sup35 amyloid fibrils, which was caused by impaired 
Hsp104 function. Third, a single mother–daughter pair 
analysis using FCS in GuHCl-treated $[PSI^+]$ cells 
showed that Sup35–GFP diffusion in the daughter cells 
was faster, that is, the Sup35–GFP was smaller, than 
that in the mother $[PSI^+]$ cells, and it eventually 
reached the diffusion profiles found in $[psi^-]$ cells [32]. 
Finally, MD-FRAP revealed that the flux of the dif-
fuse oligomers in the GuHCl-treated $[PSI^+]$ cells 
was completely inhibited [32]. 

Taken together, these studies indicate that the 
Hsp104 inactivation causes the severe transmission bias 
between mother cells and daughter cells [32,37]. In 
other words, inactivation of Hsp104 alters the dynam-
ics of the diffuse oligomers of Sup35 by disrupting the 
delicate balance to maintain $[PSI^+]$, eventually curing 
$[PSI^+]$. So far, the mechanism by which Sup35 
proteins are transmitted to daughter cells is unclear. 
Is there an energy-dependent transmission system, such 
as the actin cytoskeleton? Alternatively, simple diffu-
sion might suffice for the transmission to the daughter 
cells. We can assume a diffusion barrier at the bud 
neck, in which the transmission of large Sup35 oligo-
mers or long amyloid fibrils is more or less restricted 
by an unknown mechanism.

d) Size-dependent transmission of the diffuse 
amyloid oligomers 

Recent data on the protein dynamics under the 
Hsp104-perturbed conditions prompted us to propose 
that the propensity of prion entity transmission to the 
daughter cell partly depends on the size of the diffuse 
oligomers. In this model, larger aggregates are less 
transmissible to the next generations, whereas smaller 
oligomers are more transmissible. Considering the 
observation that Sup35 forms amyloid fibrils even in 
cells, we depicted the size-dependency of the transmis-
sible propensity in Fig. 4A, where the short amyloid 
fibrils are represented as diffuse oligomers. 

As extreme cases, visible foci or rod/ring-shaped 
aggregates are barely transmissible. By contrast, the 
monomeric form of Sup35 is the easiest to transmit. 
Therefore, diffuse oligomeric states, which have prop-
erties distinct from those of the monomer in the 

protein function, would be the appropriate size for the 
prion phenomena of protein-based inheritance. 

The behavior of diffuse oligomers of Sup35 in the 
GuHCl-treated $[PSI^+]$ cells can be explained by an 
impairment of the division phase by the inactivation of 
Hsp104 (depicted as the absence of the division phase in 
Fig. 4B). Moreover, this scenario can be extrapolated to 
the size-dependent transmission of the prion aggregates, 
even in the presence of functional Hsp104 (Fig. 4B).
The proposal that there is a transmission bias, even in the presence of functional Hsp104, might extend to yeast prion ‘strains’ and other amyloid-forming protein phenomena. \([\text{PSI}^+]\) has multiple phenotypic strains, including strong and weak \([\text{PSI}^+]\) [48–52]. The fibrils that cause the strong \([\text{PSI}^+]\) are known to be fragile, resulting in the smaller size [51]. Size-dependent transmission explains why the strong phenotype is induced by the smaller fibrils that tend to be transmitted to the daughter. In addition, a polyglutamine sequence fused with the C-terminal domain of Sup35 forms amyloid aggregates, but is not inherited as a prion [53,54]. If the polyglutamine aggregates were generally large, then the preferential retention of the large aggregates in the mother cells might result in the impaired inheritance.

Regarding the transmission from mother cells to daughter cells, the mechanism by which Sup35 proteins are transmitted to daughter cells is unclear. Is there an energy-dependent transmission system, such as the actin cytoskeleton? Alternatively, simple diffusion might suffice for the transmission to the daughter cells. We can assume that a diffusion barrier exists at the bud neck, where the transmission of large Sup35 oligomers is more or less restricted by an unknown mechanism. In fact, because the bud neck has diffusion barriers, such as septin rings [55,56], this assumption might be feasible.

**Implication to other prions**

The importance of the diffuse oligomers in \([\text{PSI}^+]\) yeast cells could be extended to other yeast prions as well as to mammalian prions. In fact, the dynamic nature of the visible foci is not restricted to Sup35 aggregates in \([\text{PSI}^+]\) cells. Single-cell imaging revealed that foci derived from Rnq1–GFP in \([\text{RNQ1}\]), a yeast prion, also disappeared during cell growth [36], suggesting that foci derived from other prions besides those formed by the Sup35 and Rnq1 proteins are dynamic during their propagation. In another yeast prion \([\text{URE3}\]) soluble forms of the \([\text{URE3}\]) determinant Ure2 protein were linked to the \([\text{URE3}\]) phenotype [57], suggesting that the diffuse oligomers are critical for maintaining the \([\text{URE3}\]) prion. Regarding mammalian prions, the physiological relevance of PrP oligomers remains to be identified. However, we note that recent studies have shown that PrP can also form soluble oligomeric states [58–60].

**Concluding remarks**

Yeast prions are not toxic amyloids. Instead, amyloid forms are utilized to switch the functional state of a protein. Typically, the monomeric form is active, whereas the aggregated (amyloid) form is inactive, which is the molecular basis of the prion phenotypes. To maintain the phenotype from generation to generation, that is, the protein-based inheritance, the amyloid structures must propagate. For the propagation, the amyloid fibril, an ordered aggregate, has adopted a growth-and-division strategy for the protein switch, leading to the dynamic remodeling of the diffuse oligomers. In this context, the amyloids that are used in the prion should be fragile. Understanding, in greater detail, the intrinsic fragility of the amyloids and their susceptibility to trans-acting factors, such as Hsp104, will provide important insights into prion biology as well as into other amyloid-forming proteins in the cell.

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H. Taguchi and S. Kawai-Noma

Oligomer-based transmission of yeast prions


