Published in final edited form as:

Protein Pept Lett. 2009 ; 16(6): 635–641.

Application of GFP-labeling to study prions in yeast

Lois E. Greene¹, Yang-Nim Park, Daniel Masison*, and Evan Eisenberg

Laboratory of Cell Biology, NHLBI, NIH, Bethesda, MD 20892-0301

* Laboratory of Biochemistry and Genetics, National Institute of Diabetes, Digestive, and Kidney Diseases

Abstract

Fluorescent live cell imaging has recently been used in numerous studies to examine prions in yeast. These fluorescence studies take advantage of the fact that unlike the normally folded form, the misfolded amyloid form of the prion protein is aggregated. The studies have used fluorescence to identify new prions, to study the transmission of prion from mother to daughter, and to understand the role of molecular chaperones in this transmission. The use of fluorescence imaging complements the more standard methods used to study prion propagation. This review discusses the various studies that have taken advantage of fluorescence imaging technique particularly in regard to understanding the transmission and curing of the $[PSI^+]$, the prion form of the translation termination factor Sup35p.

Introduction

Prions are infectious proteins that in normal form are harmless, but upon misfolding into an amyloid fiber rich in beta-sheet secondary structure, become pathogenic [1]. It then causes the conversion of the protein from the normal form to the misfolded form. Thus far, prions have been detected in yeast, other fungi and mammals [2]. In mammals, when conversion of prion protein to the amyloid form occurs on a large scale in the central nervous system, the amyloid form accumulates and is associated with neurodegeneration in fatal diseases such as scrapie in sheep, bovine spongiform encephalopathy in cows, and Cruetzfeld-Jacob disease in humans [3]. In yeast, prion infection does not occur in wild-type strains [4], suggesting that the misfolded conformation is deleterious to the cell. Yeast prions, which are transmitted by mating of prion-infected yeast to non-infected yeast, continue to propagate as the yeast divide, passing seeds or propagons from generation to generation.

Yeast has several prion proteins that can assume both a normal and misfolded amyloid conformation. The best-characterized yeast prion proteins are Sup35p, a translation termination factor, and Ure2p, which is involved in nitrogen metabolism [5,6]. More recently Rnq1p has been identified as a prion protein and New1p has been found to contain a domain that can substitute for the prion-forming domain of Sup35p [7–9]. All of these prion proteins have an Asn/Gln-rich prion-forming domain that can assume a beta-sheet rich, protease-resistant conformation in an autocatalytic manner.

To detect $[PSI^+]$ and $[Ure3]$ prions in yeast, a defined plate-based colony screening assay has been developed. A colorimetric assay is used to screen for $[PSI^+]$ prion, which takes advantage of the property of reduced translation termination activity in $[PSI^+]$ cells, causing read-through at nonsense stop-codons. When yeast with a nonsense stop-codon mutation in either *ADE1* or *ADE2* gene are grown on adenine supplemented media, the nonsense mutation is suppressed

¹Address correspondence to: Dr. Lois Greene, Laboratory of Cell Biology, NHLBI, 50 South Dr Rm 2537 MSC 8017, Bethesda, MD 20892-8017, Phone: 301-496-1228, Fax: 301-402-1519, E-mail: greenel@helix.nih.gov.

in [*PSI*⁺] yeast, but in [*psi*⁻] yeast there is termination of translation resulting in an accumulation of red adenine metabolic intermediate [10–12]. Therefore, red colonies indicate a [*psi*⁻] phenotype, whereas white, pink, or dark pink colonies indicate a [*PSI*⁺] phenotype, the color depending on the nonsense suppression strength of a particular [*PSI*⁺] strain [13]. To monitor [*Ure3*] yeast, which contains the amyloid form of Ure2p, there is an analogous colony screening assay using plates based on nitrogen metabolism [14]. This assay utilizes the ability of [*Ure3*] yeast, but not [*Ure0*] yeast containing the soluble form of Ure2p, to take up ureidosuccinate [14]. It should be pointed out since the colonies on the plate, which are derived from a single yeast cell, take several days to develop and during this time, the phenotype of the yeast daughter cells may change as the colony grows.

Lysates from yeast cells have also been examined to study the properties of prion aggregates. Biochemical techniques such as ultracentrifugation sedimentation and size exclusion column chromatography have been used to establish that the aggregated prion protein is much larger in size than the normally folded soluble form. Partial denaturation of yeast prions in sodium dodecyl sulfate followed by agarose gel electrophoresis has also been used to detect size differences between soluble and aggregated prion forms [15]. This technique has also been used to follow the size changes in the aggregated prion that occur as the yeast are cured of the prion form of the protein, i.e. as they are converted from [*PSI*⁺] to [*psi*⁻].

More recently, prion protein labeled with GFP has been used as a reporter to detect the state of prion protein in individual yeast cells on a real time basis. All of the yeast prion proteins, Sup35p, Ure2p, and Rnq1p, have been labeled with GFP [16–18]. When these proteins are aggregated GFP-fluorescent foci of various types form in the cytosol. In contrast, when these proteins are properly folded and soluble, the GFP fluorescence is diffusive. Since differences between the soluble and aggregated forms of these GFP-labeled proteins are readily detectable, fluorescence imaging is a valuable tool to study prion propagation and prion curing. The most extensive use of GFP to study prion propagation has been with Sup35p and therefore, for the most part, studies employing Sup35p labeled with GFP will be the focus of this review.

Labeling of Sup35p

When labeling a protein with GFP, the first point to consider is whether the fusion protein retains its normal physiological behavior. This is of particular importance with prion proteins, which occur in two very different conformational states. In this regard, several different GFP-labeled constructs of Sup35p have been made to study its aggregation. Sup35p can be subdivided into three domains: an amino-terminal domain (N) rich in polar amino acids that is necessary for prion formation of Sup35p, but is not required for its physiological activity, a highly charged middle domain (M) of unknown function that is also dispensable, and a C-terminal domain (C) that is necessary and sufficient for its physiological activity. Fusion proteins with GFP were first made by appending the fluorophore to the C-terminal end to the NM domains of Sup35p (referred here as NM-GFP) [16,19,20,18]. The expression of the truncated Sup35p-GFP constructs was regulated by an inducible promoter. When these fusion proteins were expressed in [*psi*⁻] yeast cells, the GFP fluorescence appeared diffusive, whereas GFP foci are present in [*PSI*⁺] cells [16]. The foci may be due to the truncated fusion proteins incorporating into existing endogenous Sup35p aggregates through N-domain interactions. Alternatively, they may be due to self-aggregation after the fusion protein is nucleated by endogenous Sup35p aggregates. With either scenario, these GFP-fusion proteins only indirectly report the state of the endogenous Sup35p. Unfortunately, although these truncated fusion proteins have been useful in distinguishing individual cells with either aggregated or soluble forms of Sup35p, they have the major drawback of actually causing the conversion of soluble Sup35p to aggregated Sup35p when expressed for a long time in [*psi*⁻] cells [16]; i.e.,

rather than simply acting as reporters, the overexpression of these truncated fusion proteins induced cells to change from $[psi^-]$ to $[PSI^+]$.

A second-generation of GFP labeled Sup35p has greatly improved the use of GFP fluorescence as a reporter for the conformational states of Sup35p [21,22]. To maintain the activity and conformational state of Sup35p, Masison's laboratory inserted GFP internally between the N- and M-domains of Sup35p, resulting in a full length GFP-labeled Sup35p, referred to as NGMC [22]. This construct was expressed on a centromeric plasmid under the control of its own promoter in a *sup35* deletion mutant strain. A similar full-length construct was made by Serio's laboratory, differing in that 3 glycine serine repeats flanked the *GFP* open reading frame (orf) [21]. The later construct used the *MFA-1* promoter to drive the expression of the fusion protein. This promoter, which is inactivated upon yeast mating, enabled them to follow GFP fluorescence temporally in yeast cells following mating. However, it is not clear how the expression level of the fusion protein driven by the *MFA-1* promoter compares to that of endogenous Sup35p protein. For the remainder of this review, both of these full-length constructs are referred to as Sup35p-GFP.

Imaging Prions with Fluorescence Microscopy

Light microscopy imaging of full length and truncated Sup35p labeled with GFP has shown that the GFP fluorescence appears diffusive in $[psi^-]$ cells and aggregated in $[PSI^+]$ cells. The aggregates in $[PSI^+]$ cells have been described in various ways including bright points of coalescence, dots, foci, and rings [16,18]. In fact, we have found that there are many factors that contribute to the appearance of Sup35p-GFP in $[PSI^+]$ cells. One key factor is that the level of expression affects its appearance, as illustrated in Figure 1, which shows the fluorescence of Sup35p-GFP in $[PSI^+]$ cells expressed by having *NGMC* integrated into the *SUP35* genomic locus (panels a, c) and *NGMC* on a 2 micron multicopy plasmid using the *SUP35* promoter (panel b, d). The images in the top panels taken with the identical settings show that the fluorescence intensity is very uniform when *NGMC* is integrated into its genomic locus, whereas cells are much brighter and intensity more variable with the multicopy plasmid. These same cells were reimaged using optimal settings for each level of each expression in order to obtain the maximum fluorescence dynamic range. This reveals that the many fine foci apparent in yeast expressing Sup35p-GFP at endogenous levels (panel c) are less apparent in cells highly overexpressing Sup35p-GFP (panel d). On the other hand, regardless of the level of expression of the Sup35p-GFP, the GFP fluorescence appeared diffusive in $[psi^-]$ cells.

Aside from level of expression, growth medium can also affect appearance of the Sup35p-GFP foci in $[PSI^+]$ yeast. If cells are starved by either placing them into water or changing media from glucose to galactose containing medium, the Sup35p-GFP foci became more pronounced, as shown in Figure 2. Furthermore, this response to starvation may be a general stress response since the Sup35p-GFP foci also become larger when the cells are placed in hydrogen peroxide (Figure 2d). Arsenite and copper sulfate also cause the foci to become prominent about an hour after they are added to the medium (data not shown). In contrast, we could not detect any effect of these stress factors on the appearance of either Sup35p-GFP expressed in $[psi^-]$ cells or on the appearance of GFP alone in $[PSI^+]$ cells.

The growth phase of yeast also affects the appearance of the prion aggregate with the foci becoming more pronounced as yeast cells approach stationary phase [18,22]. Another factor is that the Sup35p-GFP aggregate can assume different conformations, which in turn affects the appearance of foci. For example, the appearance of NM-GFP is different depending on the conformation or "strain" of the $[PSI^+]$ prion [23]. Finally, it is important to point out that when yeast cultures are grown beyond mid-log phase, autofluorescent vacuoles become prominent

and these vacuoles can be mistaken for very large Sup35p-GFP foci. However, they can be distinguished from true GFP fluorophores by their broad fluorescence spectrum.

In addition to the many intrinsic factors that affect the appearance of the Sup35p-GFP foci, it is important to note that their appearance depends on the imaging method. Among the factors affecting the microscopic image are magnification, depth of field, and speed of image acquisition. If the foci are very small and rapidly moving, they may blur and be difficult to distinguish from each other unless high magnification and short acquisition times are used. This is in fact what happened when Sup35p-GFP was initially expressed in [*PSI*⁺] cells using a centromeric plasmid, foci were initially reported not to be present until the cells aged [22].

Quantitative Use of fluorescence to detect [*PSI*⁺] and [*psi*⁻]

Since the difference between [*PSI*⁺] and [*psi*⁻] yeast may be difficult to detect by fluorescence microscopy, two quantitative fluorescent techniques have also been used to detect differences in the mobility of fluorescence proteins in yeast. The first of these techniques is fluorescence recovery after photobleaching (FRAP). FRAP measures the relative mobility of GFP-labeled proteins by measuring the rate that fluorescence recovers after a limited region of the cell is photobleached [25]. When it was applied to Sup35p-GFP to distinguish [*PSI*⁺] from [*psi*⁻], the fluorescence recovery curves showed that the diffusion of Sup35p-GFP was very different in the [*PSI*⁺] and [*psi*⁻] cells [22]. Interestingly, FRAP curves show that GFP alone has very different diffusional mobility than Sup35p-GFP in [*psi*⁻] cells (Figure. 3) even though both proteins have a diffusive appearance (see below). Specifically, the fluorescence intensity of GFP, regardless of whether the cells were [*PSI*⁺] or [*psi*⁻], showed much less total recovery than that obtained with Sup35-GFP following photobleaching. Since these FRAP curves have not been corrected for the total bleaching of the fluorescent pool in the cytosol, more GFP molecules apparently enters into the bleached area than Sup35p-GFP molecules during the experiment. Therefore, even though the recovery curves do not show a significant difference in their rate of recovery between these proteins, the extent of the total fluorescence recovered indicate that GFP is diffusing much faster than Sup35p-GFP in [*psi*⁻] cells. FRAP has also been used to show that the diffusion of Sup35p-GFP in [*PSI*⁺] cells is very different depending on whether yeast is expressing wild-type Hsp70 or mutant Hsp70 (Ssa-21) [22]. However, one difficulty with the FRAP technique is that it requires that the yeast expresses enough fluorophore to obtain sufficient fluorescence signal for photobleaching. Specifically, we found that the fluorescence intensity of Sup35p-GFP is too low to do FRAP when this protein is expressed when *NGMC* is integrated in its genomic locus as opposed to having it on a centromeric or multicopy plasmid and choosing brighter cells.

Fluorescence correlation spectroscopy (FCS), another quantitative technique that has been used to measure the diffusion of Sup35p-GFP in yeast, does not have this technical problem arising from low concentration of fluorophore. Fluorescence correlation spectroscopy (FCS) examines the dynamics of fluorescent probes and molecules by measuring spontaneous fluorescence intensity fluctuations in a microscopic detection volume. An autocorrelation curve is then derived from the correlation between these fluctuations, which yields the number, diffusion time, and size of the oligomerized molecules [26,27]. In contrast to FRAP, which measures relative mobility, FCS enables the determination of size and concentration of the fluorescently-labeled molecule.

This method has been used in yeast to show quantitatively that the diffusion constant of GFP is the same in [*PSI*⁺] and [*psi*⁻] yeast cells and lysates [28]. Kawai-Noma, et al. [28] also showed that the diffusional mobility of NM-GFP was much slower in [*PSI*⁺] yeast cells than in [*psi*⁻] cells. Similarly, NM-GFP in [*PSI*⁺] yeast lysates was slower than in [*psi*⁻] lysates, with the latter having a diffusional mobility almost the same as GFP indicating that NM-GFP

is monomeric in $[psi^-]$ cells. Our laboratory has also used FCS to examine diffusion of the full-length Sup35p-GFP in $[PSI^+]$ and $[psi^-]$ yeast cells (Figure 3B). The results with the full-length construct were similar to those obtained with the NM-GFP construct, except that the diffusion constant of Sup35p-GFP in $[psi^-]$ was much slower than GFP, consistent with the FRAP data. It should be pointed out that the diffusion of GFP was the same in $[PSI^+]$ and $[psi^-]$ yeast cells. One interesting result is that the FCS data show Sup35p-GFP is not a monomer in yeast cytosol. If it were a monomer, its diffusion coefficient would have been 1.4 times larger than GFP since the diffusion coefficient is proportional to the cube-root of the molecular weight. However, the calculated diffusion coefficient is about 3 times larger than GFP, indicating that full-length Sup35p-GFP is in a complex with other molecules in $[psi^-]$ yeast cells. These results suggest that the C-terminal portion of Sup35p-GFP, which is not present in NM-GFP, is responsible for its complex formation.

Finally, FCS was used to study the properties of $[PSI^+]$ prion in dividing yeast cells expressing NM-GFP under the control of the *GALI*- inducible promoter [28]. With this technique, the mobility of small NM-GFP oligomers was found to be the same both in the mother and daughter cells regardless of whether the mother cell contained large foci that were not transmitted to the daughter cell. This showed that the transmission of $[PSI^+]$ phenotype from the mother cell to the daughter cell is caused by the small oligomers.

Application of fluorescence imaging to study curing of yeast prions

Yeast prions are cured by inactivation of the molecular chaperone, Hsp104, a hexameric protein with two triple-A ATPase domains per molecule. Its ATPase activity, which is essential for its biological activity, is inactivated either by adding guanidine or by expressing a dominant negative Hsp104 mutant in which both ATPase sites have been mutated with a double Lys-to-Thr substitution at residues 218 and 620 (Hsp104 KT) [29–31]. Our laboratory examined the change in fluorescence of Sup35p-GFP upon addition of guanidine to $[PSI^+]$ yeast cells [24]. Within one hour of guanidine addition, Sup35p-GFP formed large foci, which shows the incorporation of Sup35p-GFP into foci even in the absence of active Hsp104 [24]. However, these foci disappeared within six hours after addition of guanidine, resulting in Sup35p-GFP in $[PSI^+]$ yeast appearing as diffusive as it does in $[psi^-]$ yeast even though the yeast were still $[PSI^+]$. This diffusive fluorescence led us to suggest that cell division might not be necessary for curing. To test whether cell division was necessary to cure prion infected yeast, as previously proposed [32], cells were cured in alpha-factor to inhibit cell division [24]. Although we initially thought the curing was indeed independent of cell division, preliminary data indicates this is not the case due to the presence of a very small population of alpha-factor resistant cells that continued to divide in the presence of alpha factor.

Recently, Serio's laboratory also examined the fluorescence of Sup35p-GFP in $[PSI^+]$ yeast during curing caused by either expression of the dominant negative Hsp104 mutant or guanidine treatment [33]. They first examined whether aggregated prion protein can remodel the soluble form of the Sup35p protein by examining whether diffusive Sup35p-GFP in $[psi^-]$ would aggregate upon mating to non- fluorescent $[PSI^+]$ cells both in the presence and absence of active Hsp104. Yeast expressed the Sup35p- GFP under the control of the *MFA-1* promoter, which is readily repressed upon mating. Using this construct, they found that when $[psi^-]$ cells containing the soluble Sup35p-GFP were mated to non-fluorescent $[PSI^+]$ cells, Sup35p-GFP appeared aggregated in the zygotes within six minutes of cytoplasmic mixing whether or not the Hsp104 was active [21]. This agrees with our observation that Sup35p-GFP can be added to aggregates in the absence of active Hsp104. However, in contrast to our experiments, Serio's laboratory did not observe in guanidine a subsequent decrease in size of the aggregates or an increase in mobility of aggregates when measured by FRAP. These data

indicate that Sup35p can add on to preexisting aggregates, but it takes active Hsp104 to split the aggregates into smaller more mobile fragments.

Hsp104 was also found to be necessary for long-term remodeling of preexisting Sup35p complexes [33]. This was determined by following the fluorescence foci in microcolonies formed from zygotes in which the Sup35p-GFP was again expressed under the *MFA-1* promoter. As expected, when Hsp104 was active the Sup35p-GFP aggregates were no longer visible either in the original zygote or in the progeny since the Sup35p oligomers are constantly being severed and the smaller fragments are incorporating into newly made non-fluorescent Sup35p. On the other hand, foci were still apparent in the original zygotes when Hsp104 was inactivated either by guanidine or by overexpression of Hsp104 KT. This again shows that Hsp104 activity is necessary for the aggregates to be severed into smaller mobile fragments, which, in turn, is necessary for continued propagation of the aggregates into daughter cells during cell division.

Finally, by examining the aggregates during cell division when Hsp104 activity was inhibited, Serio's laboratory found that the aggregates segregated asymmetrically between the daughter cells, perhaps because they were large and immobile [33]. Their results also suggested that, although soluble Sup35p can be incorporated into the aggregates in the absence of Hsp104 activity, there is a limit to how much incorporation can occur. As the cells were cured in guanidine, a soluble pool of Sup35p-GFP developed that was not incorporated into the large foci, perhaps because these large foci did not provide enough ends for unlimited incorporation of the soluble protein.

Limitations in the use of fluorescence to examine curing

The foci of Sup35p-GFP observed by fluorescence microscopy have been reported to be functionally equivalent to the seeds or propagons present in $[PSI^+]$. However, this point has yet to be proven and it is important to note that, as described above, during curing of $[PSI^+]$ by guanidine, Sup35p-GFP can appear completely diffusive even though the yeast are still $[PSI^+]$ [24]. Therefore, the absence of visible foci does not always correlate with the absence of $[PSI^+]$. Moreover, it has been reported that during the curing of $[PSI^+]$, the Sup35p can form dead-end aggregates [32]. Since the relationship between aggregates and foci is not yet clearly defined, in future experiments it is necessary to count foci in $[PSI^+]$ yeast during curing to determine the exact correlation between Sup35p-GFP foci and propagons or seeds.

Even though this review has focused primarily on Sup35p-GFP, GFP fluorescence has also been used to monitor the $[URE3]$ prion; and here too several problems occurred. One unexpected difficulty is that overexpression of GFP fusion proteins of full-length or truncated Ure2p cures the $[URE3]$ prion [17]. This was rather unexpected because overexpression of proteins susceptible to prion conversion typically causes an increase in spontaneous prion formation [34]. For example, spontaneous induction of $[PSI^+]$ was observed upon overexpression of NM-GFP [16]. It has also been reported that overexpression of Ure2p-GFP causes non-specific aggregation that is not always associated with $[URE3]$ formation [35]. Moreover, non-specific aggregation of Ure2p-GFP was present even after $[URE3]$ was cured by overexpression of Ure2p-GFP in an Ure2/Ure2-GFP heterozygote. These complications have limited the use of Ure2-GFP in studying the $[Ure3]$ prion [36].

Application of fluorescence to screen for prions

Since prions form amyloid aggregates, while the normally folded protein is diffusive, appending GFP to putative prions has been useful in identify new prions. This was used to show that Rnq1p exists in both a soluble and insoluble form in yeast, with the latter form being a prion in that it passes to the daughter cell through the cytoplasm [9]. In Liebman's laboratory,

a protein screen for the $[PIN^+]$ prion, which causes spontaneous induction of $[PSI^+]$, was conducted by examining proteins that cause aggregation of NM-GFP [37]. This screen uncovered nine novel candidate prion genes all carrying prion domains. Moreover, they identified that aggregates of Rnq1p were responsible for the $[PIN^+]$ prion. Similarly, Weissman's laboratory found that two proteins, New1p and Rnq1p, cause induction of $[PSI^+]$ [38]. In their study, GFP labeling of either New1p or fragments of New1p was used to study its aggregation properties and its ability to induce $[PSI^+]$. This helped them to identify that the Asn/Gln -rich domain of New1p was essential for inducing the $[PSI^+]$ phenotype.

The study of yeast prions has also taken advantage of the fact that many different fluorophores can be appended to proteins to do multicolor imaging. CFP and YFP fluorophores were used to show that the prion-domains of *S. cerevisiae* and *Candida albicans* do not colocalize [8]. Two color fluorescence microscopy was also used to show that New1p aggregates do not colocalize with aggregates of the NM-domain of Sup35p [38]. In a recent study by the Craig laboratory, two color fluorescence microscopy was used to follow newly synthesized Rnq1p as cells are cured of $[PIN^+]$ [39]. This established that the newly synthesized protein partitioned to both soluble and aggregated pools of Rnq1p.

Measuring the $[PSI^+]$ phenotype by fluorescent reporters

Although GFP fusion proteins have proven useful in studying prions in yeast, this technique is limited in that it does not provide a direct measure of the prion phenotype. Two methods have recently been developed to detect the $[PSI^+]$ phenotype by taking advantage of the property that in $[PSI^+]$ yeast, but not in $[psi^-]$ yeast, there is stop-codon read through of mRNA [28,33]. The strategy used to detect $[PSI^+]$ by fluorescence in yeast employed a stop signal upstream of a red fluorescent protein. Cells that are $[PSI^+]$ would have red fluorescence, but not the $[psi^-]$ cells. At the same time by expressing GFP-labeled Sup35p, GFP fluorescence can be imaged along with the red fluorescence.

In one such experiment, a stop codon was inserted between orfs of glutathione-S- transferase and DsRed protein [33]. $[PSI^+]$ yeast then showed red fluorescence, but $[psi^-]$ yeast did not. However, this method did not always detect the $[PSI^+]$ phenotype. $[PSI^+]$ yeast cells grown several generations in guanidine showed no red fluorescence even though the yeast were still $[PSI^+]$, as shown by the appearance of red fluorescence upon washing out the guanidine. It is possible that before the guanidine washout, the cells contain both properly and aggregated Sup35p and therefore, red fluorescence is not produced even though the cells are $[PSI^+]$. Therefore, the absence of red color does not prove the cells are $[psi^-]$.

Another construct to report the $[PSI^+]$ phenotype had a stop codon inserted between a nuclear localization sequence and a red fluorescent protein orf [28]. A red fluorescent signal localized in the nucleus was obtained in $[PSI^+]$ cells, but not $[psi^-]$ yeast. However, here too all of the $[PSI^+]$ cells were not red; only 10% of the $[PSI^+]$ cells showed red fluorescence in the nucleus with this construct. Therefore, although reporter proteins can detect $[PSI^+]$ on a single cell basis in real time, it is important to note that cells can often be $[PSI^+]$ without triggering the reporter proteins.

Concluding remarks

Using GFP and fluorescence imaging to study yeast prions has become a valuable tool in understanding the mechanism of prion propagation in yeast and the role of chaperones in curing the prion phenotype. Although the presence of fluorescent foci may not always correlate with the prion phenotype, fluorescence imaging complements the more standard methods used to study prion propagation. Future studies will be necessary to determine under what conditions the fluorescent foci are equivalent to the Sup35p-GFP propagons that transmit the prion

phenotype. Until the relationship between foci and propagons is completely understood, however, it is important not to over-interpret the fluorescence patterns of GFP-labeled prion proteins in yeast cells.

References

1. Prusiner SB, Scott MR, DeArmond SJ, Cohen FE. *Cell* 1998;93(3):337. [PubMed: 9590169]
2. Wickner RB, Edskes HK, Maddelein ML, Taylor KL, Moriyama H. *J Biol Chem* 1999;274(2):555–558. [PubMed: 9872986]
3. Collinge J, Clarke AR. *Science* 2007;318(5852):930–936. [PubMed: 17991853]
4. Nakayashiki T, Kurtzman CP, Edskes HK, Wickner RB. *Proceedings of the National Academy of Sciences* 2005;102(30):10575.
5. Wickner RB. *Science* 1994;264(5158):566–569. [PubMed: 7909170]
6. Tuite MF, Cox BS. *Nat Rev Mol Cell Biol* 2003;4(11):878. [PubMed: 14625537]
7. Derkatch IL, Bradley ME, Zhou P, Chernoff YO, Liebman SW. *Genetics* 1997;147(2):507–519. [PubMed: 9335589]
8. Santoso A, Chien P, Osherovich LZ, Weissman JS. *Cell* 2000;100(2):277. [PubMed: 10660050]
9. Sondheimer N, Lindquist S. *Mol Cell* 2000;5(1):163–172. [PubMed: 10678178]
10. Parry JM, Cox BS. *J Gen Microbiol* 1965;40:235–241. [PubMed: 5867756]
11. Silver JM, Eaton NR. *Biochemical and Biophysical Research Communications* 1969;34(3):301. [PubMed: 5767024]
12. Chernoff YO, Lindquist SL, Ono B, Inge-Vechtormov SG, Liebman SW. *Science* 1995;268(5212):880–884. [PubMed: 7754373]
13. Tanaka M, Chien P, Naber N, Cooke R, Weissman JS. *Nature* 2004;428(6980):323. [PubMed: 15029196]
14. Wickner RB, Edskes HK, Shewmaker F. *Methods* 2006;39(1):3. [PubMed: 16793280]
15. Kryndushkin DS, Alexandrov IM, Ter-Avanesyan MD, Kushnirov VV. *J Biol Chem* 2003;278(49):49636–49643. [PubMed: 14507919]
16. Patino MM, Liu JJ, Glover JR, Lindquist S. *Science* 1996;273(5275):622–626. [PubMed: 8662547]
17. Edskes HK, Gray VT, Wickner RB. *Proceedings of the National Academy of Sciences* 1999;96(4):1498–1503.
18. Zhou P, Derkatch IL, Liebman SW. *Molecular Microbiology* 2001;39(1):37–46. [PubMed: 11123686]
19. DePace AH, Santoso A, Hillner P, Weissman JS. *Cell* 1998;93(7):1241. [PubMed: 9657156]
20. Borchsenius AS, Wegrzyn RD, Newnam GP, Inge-Vechtormov SG, Chernoff YO. *Embo J* 2001;20(23):6683–6691. [PubMed: 11726504]
21. Satpute-Krishnan P, Serio TR. *Nature* 2005;437(7056):262–265. [PubMed: 16148935]
22. Song Y, Wu YX, Jung G, Tutar Y, Eisenberg E, Greene LE, Masison DC. *Eukaryot Cell* 2005;4(2):289–297. [PubMed: 15701791]
23. Diaz-Avalos R, King CY, Wall J, Simon M, Caspar DLD. *Proceedings of the National Academy of Sciences* 2005;102(29):10165–10170.
24. Wu YX, Greene LE, Masison DC, Eisenberg E. *Proc Natl Acad Sci U S A* 2005;102(36):12789–12794. [PubMed: 16123122]
25. Wu YX, Masison DC, Eisenberg E, Greene LE. *Methods* 2006;39(1):43–49. [PubMed: 16793282]
26. Hess ST, Huang S, Heikal AA, Webb WW. *Biochemistry* 2002;41(3):697–705. [PubMed: 11790090]
27. Hausteil E, Schwillle P. *Annual Review of Biophysics and Biomolecular Structure* 2007;36(1):151–169.
28. Kawai-Noma S, Ayano S, Pack CG, Kinjo M, Yoshida M, Yasuda K, Taguchi H. *Genes to Cells* 2006;11(9):1085–1096. [PubMed: 16923127]
29. Eaglestone SS, Ruddock LW, Cox BS, Tuite MF. *Proceedings of the National Academy of Sciences* 2000;97(1):240–244.
30. Jung G, Masison DC. *Curr Microbiol* 2001;43(1):7–10. [PubMed: 11375656]

31. Wegrzyn RD, Bapat K, Newnam GP, Zink AD, Chernoff YO. *Mol Cell Biol* 2001;21(14):4656–4669. [PubMed: 11416143]
32. Ness F, Ferreira P, Cox BS, Tuite MF. *Mol Cell Biol* 2002;22(15):5593–5605. [PubMed: 12101251]
33. Satpute-Krishnan P, Langseth SX, Serio TR. *PLoS Biol* 2007;5(2):e24. [PubMed: 17253904]
34. Masison DC, Wickner RB. *Science* 1995;270(5233):93–95. [PubMed: 7569955]
35. Fernandez-Bellot E, Guillemet E, Ness F, Baudin-Baillieu A, Ripaud L, Tuite M, Cullin C. *EMBO Rep* 2002;3(1):76–81. [PubMed: 11751580]
36. Ripaud L, Maillet L, Cullin C. *Embo J* 2003;22(19):5251–5259. [PubMed: 14517262]
37. Derkatch IL, Bradley ME, Hong JY, Liebman SW. *Cell* 2001;106(2):171. [PubMed: 11511345]
38. Osherovich LZ, Weissman JS. *Cell* 2001;106(2):183. [PubMed: 11511346]
39. Aron R, Higurashi T, Sahi C, Craig EA. *EMBO J* 2007;26(16):2794–2803.

Fig. 1. Effect of expression of Sup35p-GFP (NGMC) on the appearance of aggregates in $[PSI^+]$ yeast. NGMC was expressed by being integrated into the genomic locus of *SUP35* (a,c) and on a 2 micron plasmid with the *SUP35* promoter (b,d) in a *sup35* deletion mutant strain. In images (a,b), the identical microscopic settings (e.g. laser power, laser gain, zoom) were used to enable direct comparison of the fluorescence intensity of Sup35p-GFP. In images (c,d), the settings were adjusted for the level of expression to obtain the maximal dynamic range of fluorescence. Specifically, laser power and gain were changed, but zoom and data acquisition times were kept constant. All images were acquired on the Zeiss Live Fast Scanning confocal Microscope. Scale bar, 5 μ m.

Fig. 2. Effect of conditions on the aggregation of Sup35-GFP in [*PSI*⁺] cells. Yeast strain with *NGMC* integrated into its genomic locus was grown to mid-log phase in synthetic defined (SD) medium (a). These cells were incubated 1 h in H₂O (b), 1h in galactose inducing medium (c), 1h in SD-medium with 100 μM H₂O₂ (d). Cells were imaged using the identical settings on the Zeiss Live Fast Scanning confocal Microscope. Scale bar, 5 μm.

Fig. 3.

Measurement of diffusional mobility of GFP constructs in yeast. In (A), FRAP was used to measure the rate of fluorescence recovery of Sup35p-GFP in [PSI⁺] cells (open squares), Sup35p-GFP in [psi⁻] cells (closed triangles), and GFP alone in [PSI⁺] cells (closed squares). These FRAP curves were not corrected for the total loss of fluorescence intensity of the GFP pool from bleaching the small region of interest, which accounts for partial recovery of the fluorescence intensity. In (B), FCS was used to obtain autocorrelation curves of Sup35p-GFP in [PSI⁺] cells, Sup35p-GFP in [psi⁻] cells and GFP alone in [PSI⁺] cells. The FRAP and FCS data for GFP alone were the same in [PSI⁺] and [psi⁻] cells