THE ROYAL SOCIETY

doi 10.1098/rstb.2000.0762

Investigating protein conformation-based inheritance and disease in yeast

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Our work supports the hypothesis that a protein can serve as an element of genetic inheritance. This protein-only mechanism of inheritance is propagated in much the same way as hypothesized for the transmission of the protein-only infectious agent in the spongiform encephalopathies; hence these protein factors have been called yeast prions. Our work has focused on [*PSI*$^+$], a dominant cytoplasmically inherited factor that alters translational fidelity. This change in translation is produced by a self-perpetuating change in the conformation of the translation-termination factor, Sup35. Most recently, we have determined that new elements of genetic inheritance can be created by deliberate genetic engineering, opening prospects for new methods of manipulating heredity. We have also uncovered evidence that other previously unknown elements of protein-based inheritance are encoded in the yeast genome. Finally, we have begun to use yeast as a model system for studying human protein folding diseases, such as Huntington's disease. Proteins responsible for some of these diseases have properties uncannily similar to those that produce protein-based mechanisms of inheritance.

Keywords: amyloid; epigenetic; Huntington's disease; prion; PSI; Sup35

1. INTRODUCTION

Prions were first described as the pathogenic agents that cause a group of mammalian infectious neurodegenerative diseases, the transmissible spongiform encephalopathies (TSEs). Unlike conventional pathogens, prions are postulated to be composed entirely, or primarily, of protein. The key to the infectious character of the protein is that it replicates through protein derived from the organism itself. This protein, PrP, occasionally adopts an unusual pathogenic conformation that can convert other PrP molecules to the same conformation, simultaneously killing neurons and generating new infectious material. Mammalian prions are the subject of other papers (see Caughey, this issue; Weissmann *et al.*, this issue; Clarke *et al.*, this issue).

The prion concept of protein conformation-based infectivity was later invoked by Wickner to explain the dominant, cytoplasmic inheritance of two unusual genetic elements in yeast: [*PSI*$^+$] and [*URE3*] (Wickner 1994). This hypothesis, initially based on various unusual aspects of the genetic properties of these elements, is now substantiated by an overwhelming body of molecular data (Serio & Lindquist 2000; Tuite 2000). It is important to note that unlike the mammalian prion, these protein-based elements of heredity do not cause organisms to die, but simply change their metabolic states. [*URE3*] alters nitrogen metabolism and [*PSI*$^+$] increases the translational read-through of nonsense (stop) codons during protein synthesis (Lacroute 1971; Cox 1965). Like the mammalian prion, however, the protein determinants of these elements (Ure2 and Sup35, respectively) undergo self-perpetuating changes in conformation that alter the functions of the proteins. Because proteins in the altered conformation are passed from a mother cell to its progeny, the phenotypic change that is induced by the change in protein conformation is heritable. Remarkably, the altered protein conformation for both Sup35 and Ure2 is closely linked to the production of a protein amyloid, structurally related to the certain unusual protein conformations observed in many human diseases.

The realization that heritable changes in phenotype can be produced in yeast by a change in protein conformation, and the discovery that the type of change involved is related to those associated with human pathologies, raises many questions. Here, we briefly review work on one of the yeast prions [*PSI*$^+$] and then describe our recent progress on three outstanding questions. (i) Can we create new protein-based elements of inheritance, affecting novel functions, by genetic engineering? (ii) Do other protein-based elements of inheritance exist in yeast? (iii) Can we use yeast as a model system for studying related protein conformational changes involved in human diseases?

2. THE PRION [*PSI*$^+$] AND ITS PROTEIN DETERMINANT

Sup35, the protein determinant of the yeast genetic element known as [*PSI*$^+$], is a subunit of the translation termination complex. (The brackets indicate that it segregates in genetic crosses as a non-chromosomal element, as

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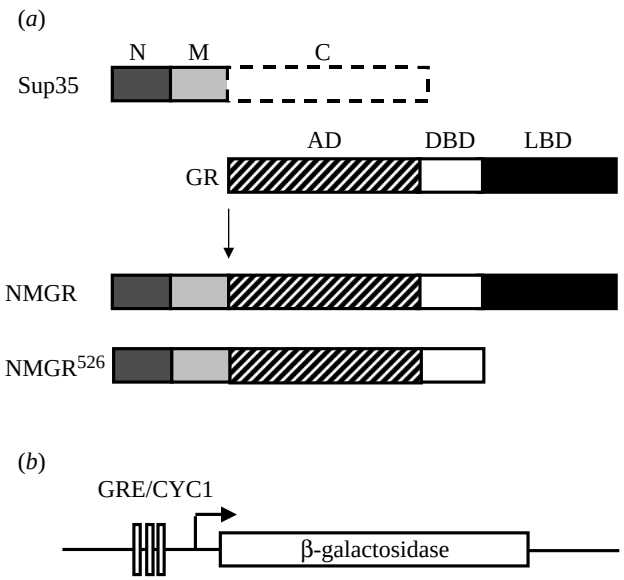

Figure 1. The glucocorticoid receptor (GR) reporter system. (*a*) A schematic structure of rat GR with the three distinct domains shown. AD, activation domain; DBD, DNA-binding domain; LBD, ligand-binding domain. (*b*) β-galactosidase reporter gene under the regulation of GRE and the yeast CYC1 promoter.

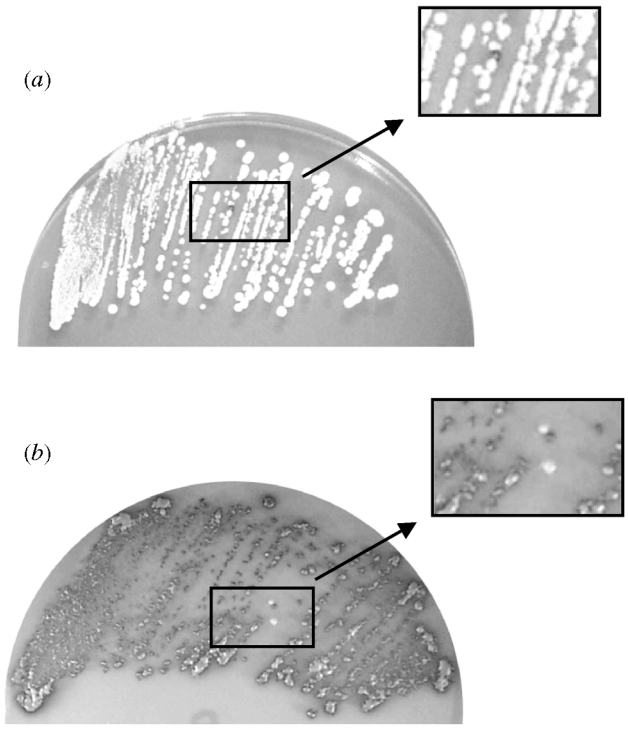

Figure 2. NMGR^{526} exists in two stable, distinct functional states. A single colony of (*a*) a white or (*b*) a blue isolate of yeast cells expressing NMGR^{526} and harbouring a GRE-lacZ reporter (strain 74D-694 Δ*NMSUP35*) was streaked onto selective medium for two days, then replica-plated onto X-GAL containing plate for colour visualization. A small area of each isolate streak is enlarged to show the metastability of the blue and white cells.

expected for a phenotype caused by a self-perpetuating change in protein conformation; the capital letters indicate that the change in phenotype is dominant.) In [*psi*$^-$] cells, Sup35 is soluble and promotes the recognition of nonsense codons in messenger RNAs (Stansfield *et al.* 1995; Patino *et al.* 1996). In contrast, in [*PSI*$^+$] cells, most Sup35 is insoluble—tied up in a self-perpetuating aggregate—and non-functional in translation termination (Patino *et al.* 1996). Ribosomes therefore read through nonsense codons at an appreciable frequency, and this condition is readily visualized by the suppression of nonsense codons in auxotrophic markers.

[*PSI*$^+$] propagates through the ability of Sup35 protein in the [*PSI*$^+$] state to influence newly synthesized Sup35 protein to adopt the same state. This process can be visualized in living cells with green fluorescent protein (GFP) fused to Sup35 (Patino *et al.* 1996). Newly synthesized protein remains soluble and broadly distributed in [*psi*$^-$] cells, but is captured into pre-existing aggregates of Sup35 in [*PSI*$^+$] cells and rapidly coalesces into discrete foci. The propagation of the [*PSI*$^+$] element can also be modelled *in vitro*. When the region of Sup35 that is responsible for the formation of coalescing protein particles (NM) is isolated, denatured and diluted into buffer, it assembles very slowly into amyloid fibres. However, if pre-existing fibres or lysates of [*PSI*$^+$] cells are added to the solution, fibres form very rapidly (Glover *et al.* 1997).

Seeded polymerization of Sup35 provides a molecular mechanism that explains the unusual capacity of the protein to produce a self-perpetuating change in conformation and a heritable change in phenotype. The polymerized (aggregated) state is difficult to achieve, but once it has formed, newly made protein readily adds on to it, perpetuating the altered state. This hypothesis is supported by a wide variety of biochemical and genetic experiments (Paushkin *et al.* 1997; DePace *et al.* 1998; Santoso *et al.* 2000).

3. CREATION OF A NEW PRION THROUGH GENETIC ENGINEERING

The yeast Sup35 protein contains three distinct regions (Kushnirov *et al.* 1988). The N-terminal region (N) is rich in glutamine, asparagine and other polar amino acids. It is critical for [*PSI*$^+$] propagation, but is dispensable for Sup35's normal cellular function (Kushnirov *et al.* 1988; Chernoff *et al.* 1993); hence it is termed the prion-determining domain. The middle region (M) is highly charged and provides a spacing or solubilizing role that affects the stability of [*PSI*$^+$] (J. J. Liu and S. Lindquist, unpublished data). The C-terminal region (C) possesses the translation termination activity that is essential for cell viability (Zhouravleva *et al.* 1995). Interestingly, like [*PSI*$^+$], the N-terminal region of Ure2 is also rich in polar amino acids, and dispensable for the protein's normal cellular function but essential for propagation of the [*URE3*] prion. The fact that the prion-determining domains and functional domains of both [*PSI*$^+$] and [*URE3*] have distinct biochemical properties suggests that the prion-determining domain might be modular. The question arises: can such prion-determining domains be transferred to other unrelated proteins to create novel prions, with new phenotypic traits? Engineered prions will not only shed light on basic aspects of prion

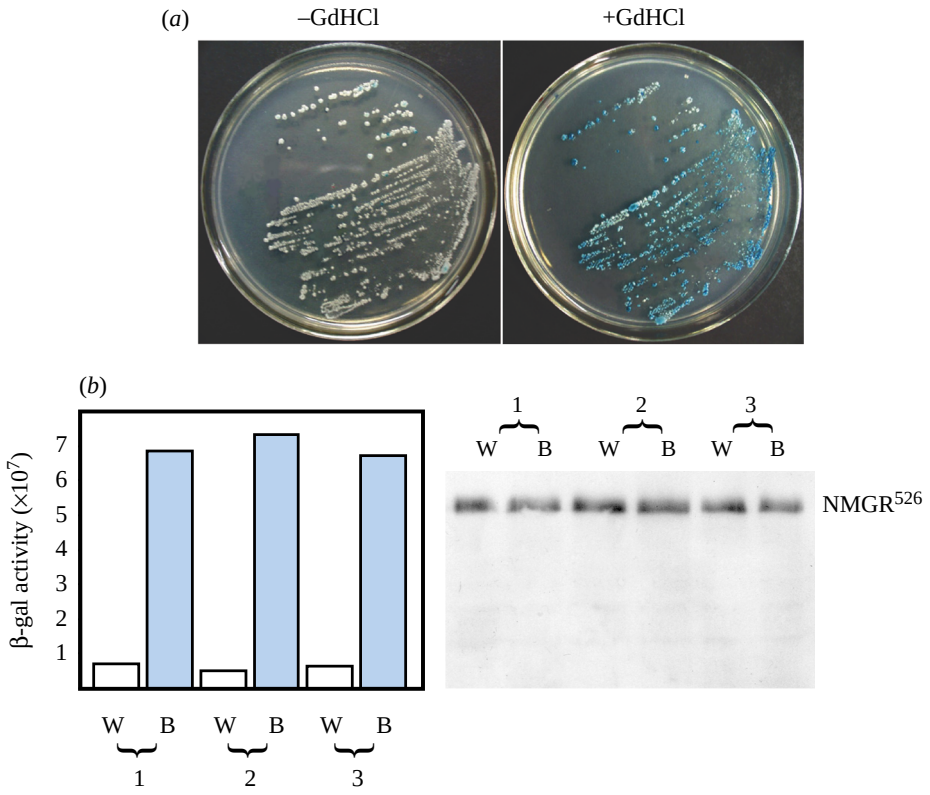

Figure 3. The reactivation effect of GdHCl on NMGR^{526}. (*a*) A white isolate of NMGR^{526} was streaked on a selective synthetic complete (SC) plate and replica-plated onto SC plates with or without 5 mM GdHCl. After two days' growth, cells were replica-plated onto X-GAL containing plates for colour visualization. (*b*) Three pairs of colonies were picked from the corresponding white (−GdHCl) and blue (+GdHCl) plates and inoculated in 3 ml SC liquid medium. After overnight growth at 30 °C, 1 ml culture was used to inoculate 10 ml fresh medium. Each of 3 ml culture was used for β-galactosidase assay and ethanol lysate preparation, respectively, after 4 h growth at 30 °C. The detection of β-galactosidase activity, SDS–PAGE, and immunoblotting analysis using GR-specific monoclonal antibody BuGR2, were performed as described in Li & Lindquist (2000).

propagation mechanisms, but also provide new systems for prion studies and new methods for manipulating biological phenotypes.

To test the idea that the prion-determining domain is transferable, we fused the N and M region of Sup35 to the rat glucocorticoid receptor (GR), an unrelated mammalian transcription factor (figure 1*a*), creating NMGR. We chose GR for several reasons.

(i) It is from an evolutionarily distant species and has a function completely unrelated to that of Sup35.
(ii) Its activity state does not influence the general metabolism of yeast.
(iii) GR activity can be easily assessed in yeast cells containing a reporter plasmid with a GR response element (GRE) fused to a β-galactosidase reporter construct (Schena & Yamamoto 1988) (figure 1*b*).
(iv) GR requires re-localization for its activity. This requirement is important because when the known yeast prion molecules switch to the prion conformational state, they form large complexes. In these complexes they may, or may not, lose biochemical function.

Previous attempts to create new prions by fusing the prion-determining domain of Ure2 to β-galactosidase or the NM domain of Sup35 to firefly luciferase failed to inactivate β-galactosidase and luciferase. One explanation for these results is that even if the prion domains of the fusion proteins were to switch states, the functional domains could remain active because their substrates and products are freely diffusible. Therefore, transcriptional factors or other proteins that require specific localization or assembly for their activities make better candidates for the creation of new prions. Since GR needs to be targeted to the nucleus for its function, the prion form of the NMGR fusion protein may cause aggregation and therefore eliminate GR function. In the presence of a GR-regulated β-galactosidase reporter gene (*GRE-lacZ*) and appropriate substrate (X-GAL), yeast cells containing NMGR with the normal conformational state should be blue, but cells with NMGR in the prion form should appear white.

To avoid the need to add hormone, we employed a constitutive version of GR called GR^{526}. To avoid complications from endogenous NM present on Sup35, we employed a yeast strain in which this region was deleted from the chromosome (Δ*NMSUP35* 74D-694). To determine if NMGR could function as a prion on its own, this strain was transformed with GR^{526} and NMGR^{526} expression plasmids. Indeed, both blue and white colonies were identified from cells expressing NMGR^{526}, but only blue colonies were observed for GR^{526}. Upon re-streaking, the NMGR^{526} cells maintained their colours (figure 2). Like the [*PSI*$^{+}$] element, which can spontaneously switch

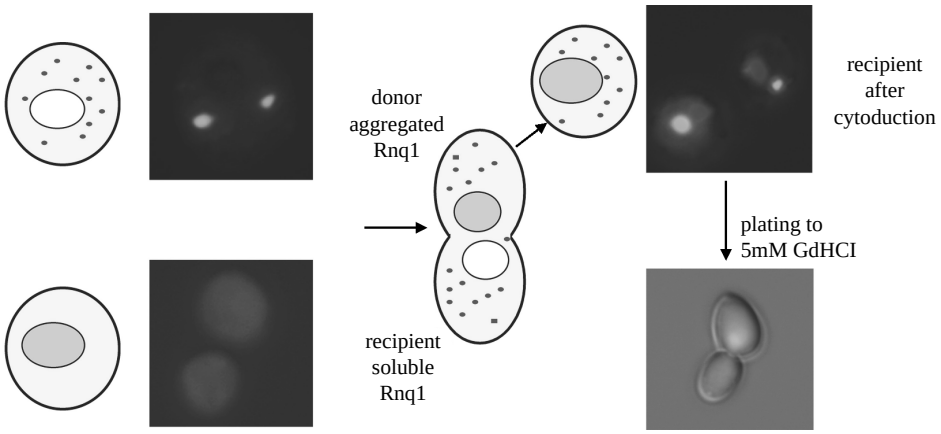

Figure 4. Cytoduction reveals cytoplasmic inheritance of a Rnq1-based prion. The presence of a *kar1-1* allele in one of two mating partners causes cytoduction, cytoplasmic fusion between two mating partners without nuclear fusion or exchange. Therefore it is ideal for demonstrating cytoplasmic inheritance of traits. In this experiment a Rnq1 aggregating donor strain (Trp−, containing mitochondrial DNA) is mated to a Rnq1 soluble recipient (Trp+, lacking mitochondrial DNA). The correct haploid progeny can be selected on media lacking tryptophan and containing glycerol as a carbon source. Expression of Rnq1-GFP demonstrates the aggregation state of the Rnq1 protein. The ability to cure the yeast of the aggregated state of Rnq1 is demonstrated by plating to 5 mM GdHCl.

between [*psi*$^-$] and [*PSI*$^+$] states at a low frequency (Cox 1965), blue cells gave rise to white cells and white cells gave rise to blue cells at a low rate (figure 2). In the case of [*PSI*$^+$] and another yeast prion [*URE3*], treatments with a low concentration of the protein denaturant, guanidine hydrochloride (GdHCl), can induce a high rate of switching from the non-functional prion conformational state to the soluble, functional conformational state (Tuite *et al.* 1981). This reactivating process is termed 'curing'. To test whether NMGR prions are subject to curing, the white cells of NMGR^{526} were replica-plated onto media with or without 5 mM GdHCl before colour visualization on X-GAL plates. Although cells without GdHCl treatment remained white, cells that had passaged through GdHCl became blue (figure 3*a*). Three pairs of colonies were picked from plates with or without GdHCl to quantify the reporter activity. The difference in the activity of GR in white and blue colonies was estimated to be *ca.* 12-fold (figure 3*b*, left). Immunoblotting analysis using a GR-specific antibody indicated that both white and blue cells produced a similar amount of NMGR^{526} fusion protein (figure 3*b*, right). Thus, GdHCl treatment of the yeast cells did not change the expression level of the fusion transcriptional factor. Instead, it induced a heritable change in its activity, probably due to a change in NMGR^{526} conformation. Another test of the protein conformation switch in GR activity employs the protein modelling factor Hsp104, which plays a critical role in [*PSI*$^+$] propagation. Either overexpression or deletion of Hsp104 abolishes [*PSI*$^+$] (Chernoff *et al.* 1995; Patino *et al.* 1996) Similarly, when the white NMGR^{526} cells were transformed with an Hsp104 overexpression plasmid, they changed to blue (data not shown). Even if the Hsp104 expression plasmid was removed, they remained blue, indicating that a transient expression of Hsp104 is enough to cause in a heritable switch a change of NMGR^{526} from the inactive prion conformation to an active conformation.

Since the experiments described were performed in a yeast strain whose endogenous NM region of Sup35 was deleted from the genome, we concluded that NMGR^{526} is capable of undergoing self-perpetuating changes in conformation that result in heritable changes in phenotype, i.e. the NMGR^{526} fusion protein can act as a prion on its own. The observation that the white NMGR^{526} cells are capable of converting to blue upon GdHCl treatment and Hsp104 overexpression also indicates that the critical determinant for GdHCl and Hsp104 interaction is within the NM domain of Sup35. Taken together, the above results demonstrate that the NMGR^{526} fusion protein can exist in at least two different conformational states, which are interchangeable upon experimental manipulations. Thus, the prion determination domain of Sup35 is both modular and transferable.

4. IDENTIFICATION OF NEW PRIONS

Prion-like genetic elements have been found in both *Saccharomyces cerevisiae* and *Podospora anserina*. Interestingly, the [Het-s] prion of *Podospora* may underlie an important adaptive mechanism that prevents the spread of viruses (Coustou *et al.* 1997). It is possible, therefore, that prions provide an important and evolving mechanism for cellular control in fungi and other organisms. It may well be, however, that they represent highly unusual products of accidental sequence alterations. The only way to understand this question is to identify and study the activities and evolution of prions in these systems.

To initiate a search for other prions in the yeast genome, we first looked for similarities between the prions [*PSI*$^+$] and [*URE3*] that might provide signature features. Although the physiological consequences of the two known prions are quite distinct—read-through of stop codons or derepression of nitrogen uptake—there is an obvious similarity between the two proteins that underlie these prions. Both Sup35 and Ure2 contain a region called the prion domain that is extremely rich in polar amino acids. In both cases, the prion domain is essential for the formation of the prion, but is not required for the 'normal' function of Sup35 or Ure2.

We exploited this common feature in a search for new prion proteins. A BLAST search of the yeast genome

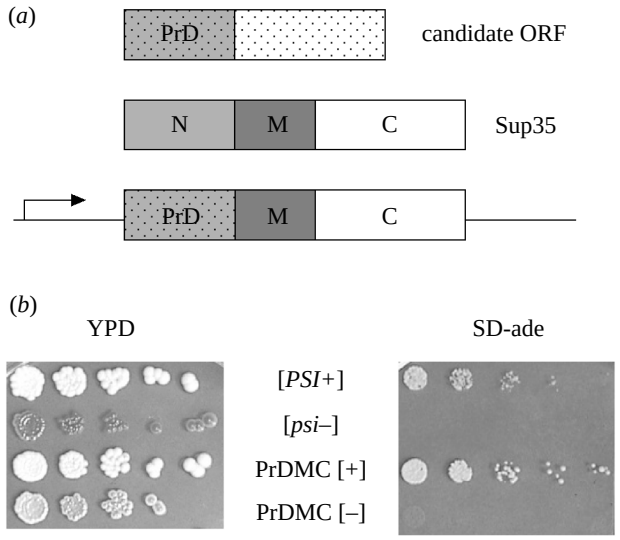

Figure 5. A domain-swapping method for examining the prion-forming capacities of putative prion domains (PrD). The PrD of a candidate open reading frame is inserted into the genome in place of the N-terminal PrD of Sup35 (*a*). The capacity of this fusion protein is analysed in several ways. First, it must complement the function of Sup35. Second it must allow the existence of two prion states, one viable on SC-ade (see [PSI^+] and PrDMC [+] in (*b*)), and one inviable on SC-ade (see [psi^-] and PrDMC [−] in (*b*)). The experiment shown in figure 4*b* uses Rnq1(153–405) as the substituted PrD.

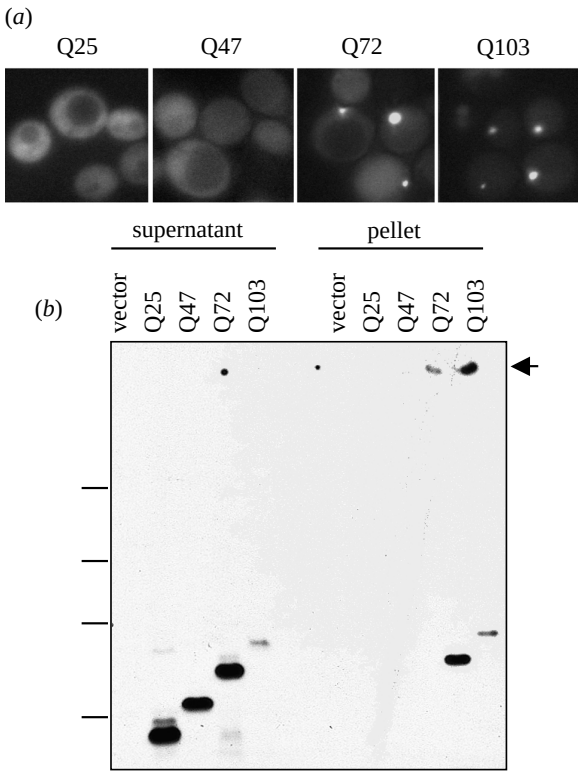

Figure 6. Coalescence of huntingtin depends on the length of its polyglutamine repeat. (*a*) Visualization of huntingtin coalescence in living yeast cells by fluorescence. Cells were allowed to adhere onto polylysine-treated slides and microscopy was performed with an Axioplan 2 microscope (Zeiss, Germany). (*b*) Solubility assay. The cell walls of yeast wild-type cells expressing different huntingtin–GFP fusion proteins were removed, cells were lysed and Sarkosyl was added (final concentration 2%). After centrifugation, supernatant and pellet fractions were subjected to 8% SDS–PAGE and immunoblotting using antibodies directed against GFP. Marker positions are indicated on the left from the top 212, 122, 83, 51.8 (kDa). The arrow marks the top of the gel.

using the prion domains of Sup35 and Ure2 (amino acids 1–124 and 1–65, respectively) gave us an initial list of candidates. We pared down this list by selecting proteins that had the glutamine/asparagine-rich region at one end of the protein sequence that contained another domain with normal amino-acid composition, and by selecting proteins that were expressed at a constant level during the cell cycle. We then fused the candidate prion domains to GFP to determine whether expression of the hybrid open reading frame caused the formation of aggregates. Several of the candidate open reading frames formed these aggregates; we decided to focus on Rnq1 because its aggregation was intense and was eliminated in the absence of Hsp104. This elimination was a relevant finding because studies have shown that [PSI^+] is dependent upon the presence of Hsp104 (Chernoff *et al.* 1995). This criterion is not a necessary feature of a prion, but it provides a convenient tool for further analysis.

Using a variety of techniques we demonstrated that Rnq1 was a genuine prion element in *S. cerevisiae* (Sondheimer & Lindquist 2000). The protein existed in two different states, aggregated and soluble, and could be changed from the soluble to the aggregated state using cytoduction (see figure 4). In this assay two strains with the protein in different states were fused—allowing cytoplasmic mixing, but no nuclear transfer. When the initially non-aggregating strains were re-isolated, it was observed that they had all gone into the aggregating state, indicating that the protein state had been transmitted by cytoplasmic contact alone. The two different states were stable and heritable, and the protein could be switched between the two states using genetic and biochemical techniques (such as *Hsp104* deletion and GdHCl treatment) (Sondheimer & Lindquist 2000; figure 4).

The function of Rnq1 is unknown, and the prion state of Rnq1 does not produce an observable phenotype. To determine if Rnq1 was capable of inducing a prion-like switch in protein function, we created a domain-swapping assay to test directly for epigenetic control. The prion domain of Rnq1 was inserted in place of the prion domain of genomic Sup35 (figure 5*a*) and the ability of the Rnq1 prion domain to produce two different translation activity states (similar to the [PSI^+] and [psi^-] states) was analysed. The fusion of Rnq1(253–405) with Sup35(125–653) created a fully functional protein with prion behaviours that were indistinguishable from that of Sup35 (figure 5*b*).

Given the many promising candidates found in our database search, it seems likely that further studies will increase the number of known prion proteins in *S. cerevisiae* and other fungi. The open reading frame New1 contains a region that is capable of modifying the activity of the Sup35 C-terminal domain in an assay similar to our domain-swap with Rnq1 (Santoso *et al.* 2000). These studies will help to answer the critical questions of the

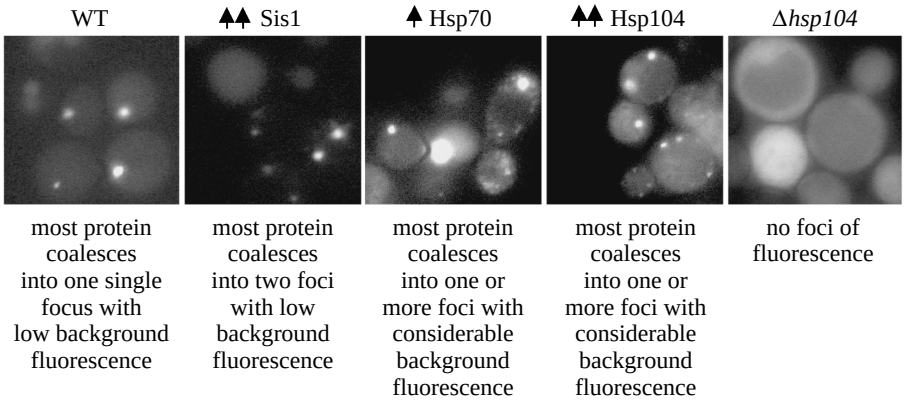

Figure 7. Chaperone proteins affect the formation of huntingtin aggregates. Microscopy was performed with yeast wild-type or Hsp104-deficient cells expressing the N-terminal region of huntingtin with 103 glutamines. Additionally, wild-type cells producing huntingtin with 103 glutamines were transformed with plasmid-DNA to overexpress Sis1, Hsp70 or Hsp104.

fungal prion field: How common are prions in nature? How do prion domains evolve? How are they of benefit to their hosts?

5. YEAST AS A MODEL SYSTEM FOR HUNTINGTON'S DISEASE

The aforementioned studies indicate that the yeast system is a powerful tool for investigating self-perpetuating conformational changes of proteins. A basic mechanism underlying many human neurodegenerative disorders involves altered conformation of proteins. Although several model systems were created to investigate which factors play a role in the aggregation process, none is as accessible to genetic analysis as yeast. We hope to take advantage of the specific tools we developed for the analysis of conformational changes in our yeast prion studies, together with the general power of genetic analysis in yeast, to investigate these disorders. Our first focus of study is Huntington's disease (HD).

A hallmark of HD is the presence of nuclear and neuritic aggregates formed by N-terminally truncated huntingtin protein with an expansion of polyglutamine in brain tissue of HD patients and transgenic mice (Reddy *et al.* 1999). The N-terminal region of huntingtin, containing 25, 47, 72 or 103 glutamines (Q25, Q47, Q72, Q103), was fused to GFP and expressed in wild-type yeast. Fluorescence of Q25 was diffusely distributed in all cells (figure 6*a*). Q47 was generally diffusely distributed, with less than 2% of the cells exhibiting a single focus of fluorescence. More than half of the cells expressing Q72 contained a single focus with considerable diffuse background fluorescence. In most cells expressing Q103 the protein coalesced into a single focus and the background fluorescence was low (figure 6*a*). Thus, the coalescence of N-terminal huntingtin fragments depends on the length of the polyglutamine repeat.

To investigate whether these coalescent foci reflect the formation of higher-order complexes, yeast cells were spheroplasted and lysed in the presence of the detergent Sarkosyl. After centrifugation, the supernatant and pellet fractions were boiled in loading buffer with 5% SDS for 10 min, separated by 8% SDS–PAGE and analysed by immunoblotting (figure 6*b*). Q25 or Q47 was detected

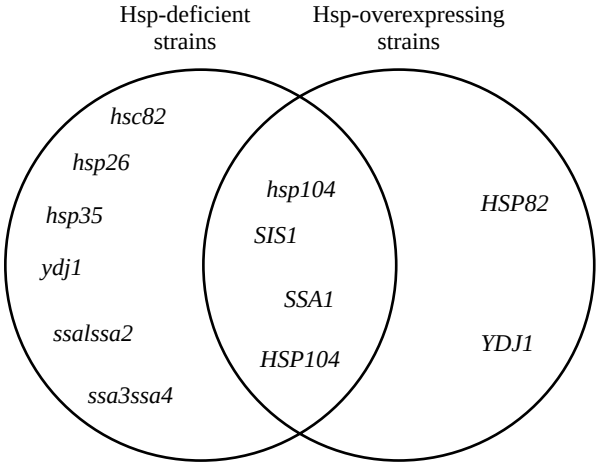

Figure 8. Huntingtin aggregation depends on the balance of chaperone activities in the cell. The illustration summarizes the *hsp* deletion or Hsp overexpressing strains used. In strains listed in the middle of the circles, changes in the fluorescence pattern were observed in comparison to the fluorescence pattern of wild-type cells.

only in the supernatant fraction, whereas Q72 was found at equal concentrations in both fractions. In contrast, most Q103 was detected in the pellet fraction, and a major fraction of this remained on the top of the gel. We conclude that the coalescence is due to the formation of higher-order complexes.

Chaperone proteins and the ubiquitin/proteasome pathway are thought to be involved in the misfolding and aggregation of polyglutamine-expanded mutant proteins (Chai *et al.* 1999*a*,*b*; Cummings *et al.* 1998; Stenoien *et al.* 1999; Wyttenbach *et al.* 2000). To address the question of whether chaperone proteins and the ubiquitin pathway are involved in the misfolding of huntingtin we examined fluorescence from the Q103–GFP fusion in strains with variant expression of several Hsps (deletion mutants and overexpression plasmids) (figure 7) and with mutations in the ubiquitin/proteasome pathway.

(a) *Hsp deletion strains*

The deletion of the genes for most chaperone proteins had no significant effect on the formation of aggregates

(figure 8). A dramatic effect, however, was seen in a strain deficient for Hsp104. The fluorescence of Q103 was distributed diffusely throughout the cell, whereas in wild-type cells a single fluorescent focus was observed (figure 7).

(b) *Hsp overexpression*

The overexpression of three chaperone proteins had a significant effect on the aggregation of Q103. In contrast to wild-type cells, which had a single intense focus, cells overexpressing Sis1 (the yeast Hdj1 homologue) exhibited two intense foci (figure 7). In cells overexpressing Hsp70 or Hsp104, one or more coalescent foci were observed and the background fluorescence was considerably higher than in wild-type cells (figure 7).

(c) *Proteasome-deficient strains (partial loss of function)*

No obvious effect on Q103 aggregation was observed in any of the three strains we examined with partial loss-of-function mutations in the proteasome pathway (data not shown). In these strains the ubiquitin-activating enzyme (ubal), a catalytic subunit of the 20S proteasome (doa3-1), or a subunit of the 19S proteasome regulatory complex (sen3-1) are affected.

These observations establish yeast as a model system for investigating proteins that affect the misfolding of huntingtin proteins with expanded glutamine tracts. Although huntingtin is broadly distributed in neuronal and non-neuronal tissue, the reason why it is toxic in only a subset of neurons is still a mystery. In our yeast system the expression of N-terminally truncated huntingtin fragments with glutamine expansion is non-toxic (either in the soluble or aggregated state), and therefore advantageous for a search for factors that influence toxicity of glutamine-expansion mutants. Additionally, yeast screens with different polyglutamine-expanded proteins can be used to identify potential therapeutic agents and cell type-specific factors that affect misfolding, aggregation and degradation.

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Discussion

H. Peto (*Department of Biochemistry, University of Cambridge, UK*). Chris Dobson mentioned earlier in the meeting that he felt that the original form of proteins was amyloid, and that they evolved subsequently to be globular. If this was the case early in evolution, would you not expect that the transition from globular to amyloid to be a common feature of regulatory processes involving proteins? Might Sup35 be the thin end of the wedge?

S. Lindquist. Most proteins have evolved away from the situation where they form amyloid fibrils. However, the ability to form inactive aggregates may be a valuable trait in some circumstances. This effect gives yeast cells the opportunity to have two stable but different heritable phenotypes with the same genome. Since there may be 25–30 such proteins in the yeast cell, we have a world of genomic plasticity that is encoded not just in the nucleic acids but in the proteins.

Anonymous. Can you use the yeast Prp domain to see if you can make the yeast prion infectious for mammals?

S. Lindquist. No. We have put only the yeast prion domains into the Sup35 protein of yeast, replacing the prion domain of Sup35. We have not yet tried putting the Sup35 protein into mammals.

Anonymous. Is hspl04 designed specifically for this role?

S. Lindquist. This is an interesting question. We thought initially that hspl04 existed to provide thermotolerance, but its role in the prion phenomenon seems important too. We have recently taken a hspl04 homologue from *Arabidopsis* and found that by altering levels of hspl04 in this plant, we can either increase or decrease the thermotolerance of whole plants. So the role of hspl04 in thermotolerance is highly conserved and thus likely to be selected in evolution. We can now look at its influence on epigenetic mechanisms in plants.

Anonymous. Your prion-forming domains look very similar, but nevertheless the phenotypes are not interrelated. Why is this?

S. Lindquist. The phenotype is entirely dependent upon the other protein domain that the prion domain is attached to. The whole process depends on the fact that the prion domain goes into an altered state that attracts other proteins to that same state, and it takes the attached domain out of circulation. So the phenotype is due to the fact that the attached protein is no longer functional. The prion domain is only the facilitator, it is not determining the phenotype.

C. M. Dobson (*Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, UK*). The similarity with the amyloid diseases might be even greater than you imply because you can introduce mutations that increase the amyloidicity of a globular protein, not just due to destabilization of the fold, but also by increasing the rate of aggregation in the denatured state.

Anonymous. Did I understand you to say that the human prion protein that you used has a leader sequence?

S. Lindquist. No. The variant we used has no leader sequence and the protein folds in the cytosol.

Anonymous. Is hspl04 specific for dissolving particular aggregates?

S. Lindquist. That is an important question: it is not specific—it will disaggregate a wide range of heat-denatured proteins in yeast. With polyglutamine proteins the situations are more complex. Hspl04 overexpression will cause polyglutamine protein aggregates to dissolve, but hspl04 deletion also causes these aggregates to dissolve. We think that with the latter proteins the unfolding activity of hspl04 is required to get the protein chains into a susceptible state. What then happens to chains in a susceptible state depends on whether there is a pre-existing prion there or not. Perhaps Hspl04 will unfold many polyglutamine-rich proteins, but it does not unfold other proteins unless they are already in an aggregate.

M. F. Perutz (*Medical Research Council Laboratory of Molecular Biology, Cambridge, UK*). I notice in your repeat sequence that not only do you have glutamine side-chains with H-bonds to neighbouring chains, but also asparagine and tyrosine side-chains. Have you tried mutagenesis to see whether if, say, you replace the tyrosine by phenylalanine, this reduces prion formation?

S. Lindquist. Jonathan Weissmann looked for mutations that interfere dominantly with the propagation of the prion, and most of these are in the glutamine residues. We are just starting to do similar mutagenesis studies.

M. Pepys (*Department of Medicine, Royal Free and University College Medical School, London, UK*). Another possible analogy with amyloid diseases is an obscure phenomenon called amyloid enhancing factor in the mouse model of reactive AA amyloidosis that is induced by chronic inflammation. The onset of histochemically detectable amyloid deposition can be accelerated dramatically by giving before the inflammatory stimulus a single injection of very small amounts of extracts of amyloidotic organs, or even *ex vivo* amyloid fibrils. This reduces the onset from six weeks down to 24 hours in the mice. This looks like a seeding phenomenon, similar to your findings with yeast prions.

Anonymous. You talked about plasticity of genetic elements. In the ageing field we now have plasticity of life span, because demographers tell us that genes contribute only *ca.* 25% to longevity. So researchers in ageing are now very interested in epigenetic elements.

M. E. Goldberg (*Pasteur Institute, Paris, France*). Your experiments on fibre formation *in vitro* with the [PSI^+] protein were done during the folding of the protein. If you mix the two native states of the protein, I understand that they do not interchange under non-folding conditions, but what happens if you add hspl04?

S. Lindquist. We are doing such experiments now. We do not know what the 'normal' state of the NM domain is. It might be normally partly unfolded, and bound to hsp70 in the cell. The C-terminal domain is known to be folded but the state of the NM domain is unclear.