INTRODUCTION: The formation of dynamic, membraneless compartments using intracellular phase transitions such as phase separation and gelation provides an efficient way for cells to respond to environmental changes. Recent work has identified a special class of intrinsically disordered domains enriched for polar amino acids such as glycine, glutamine, serine, or tyrosine as potential drivers of phase separation in cells. However, more traditional work has highlighted the ability of these domains to drive the formation of fibrillar aggregates. Such domains are also known as prion domains. They have first been identified in budding yeast proteins that form amyloid-like aggregates. Because these aggregates are heritable and change the activity of the prion-like aggregates, these domains are also known as prion domains. They have first been identified in budding yeast proteins that form amyloid-like aggregates. Because these aggregates are heritable and change the activity of the prion-like aggregates, they are thought to be a common mechanism for phenotypic inheritance in fungi and other organisms. However, the aggregation of prion domains has also been associated with neurodegenerative diseases in mammals. Therefore, the relationship between the role of these domains as drivers of phase separation and their ability to form prion-like aggregates is unknown.

RATIONALE: The budding yeast translation termination factor Sup35 is an archetypal prion-domain–containing protein. Sup35 forms irreversible heritable aggregates, and these aggregates have been proposed to be either a disease or an adaptation that generates reversible heritable aggregates, and these aggregates have been proposed to be either a disease or an adaptation that generates reversible heritable aggregates. Thus, an essential translation termination factor remains functional during the aggregation of Sup35 from irreversible aggregates to form biomolecular condensates. These condensates are liquid-like initially but subsequently solidify to form protective protein gels. Cryo-electron tomography demonstrates that these gel-like condensates consist of cross-linked Sup35 molecules forming a porous meshwork. A cluster of negatively charged amino acids functions as a pH sensor and regulates condensate formation. The ability to form biomolecular condensates is shared among distantly related budding yeast and fission yeast. This suggests that condensate formation is a conserved and ancestral function of the prion domain of Sup35. In agreement with an important physiological function of the prion domain, the catalytic guanosine triphosphatase (GTPase) domain of the translation termination factor Sup35 readily forms irreversible aggregates in the absence of the prion domain. Consequently, cells lacking the prion domain exhibit impaired translational activity and a growth defect when recovering from stress. These data demonstrate that the prion domain rescues the essential GTPase domain of Sup35 from irreversible aggregation, thus ensuring that the translation termination factor remains functional during harsh environmental conditions.

CONCLUSION: The prion domain of Sup35 is a highly regulated molecular device that has the ability to sense and respond to physiochemical changes within cells. The N-terminal prion domain provides the interactions that drive liquid phase separation. Phase separation is regulated by the adjacent stress sensor. The synergy of these two modules enables the essential translation termination factor to rapidly form protective condensates during stress. This suggests that prion domains are protein-specific stress sensors and modifiers of protein phase transitions that allow cells to respond to specific environmental conditions.
Phase separation of a yeast prion protein promotes cellular fitness

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Despite the important role of prion domains in neurodegenerative disease, their physiological function has remained enigmatic. Previous work with yeast prions has defined prion domains as sequences that form self-propagating aggregates. Here, we uncovered an unexpected function of the canonical yeast prion protein Sup35. In stressed conditions, Sup35 formed protective gels via pH-regulated liquid-like phase separation followed by gelation. Phase separation was mediated by the N-terminal prion domain and regulated by the adjacent pH sensor domain. Phase separation promoted yeast cell survival by rescuing the essential Sup35 translation factor from stress-induced damage. Thus, prion-like domains represent conserved environmental stress sensors that facilitate rapid adaptation in unstable environments by modifying protein phase behavior.

The formation of dynamic, membraneless compartments via intracellular phase transition provides an efficient way for cells to respond to metabolic changes (1, 2). This is because phase transitions are sensitive to small changes in physiochemical conditions, such as the cytosolic pH, which are a readout of metabolic state. Recent work has identified prion-like sequences as drivers of phase separation of protein compartments in cells (3–6). However, more traditional work has highlighted the ability of prion-like domains to form fibrillar assemblies that are thought to drive heritable phenotypic variation (7–9). Studies in humans and other mammals have implicated fibrillar assemblies of prion-like proteins in age-related neurodegeneration (10). Despite having been described almost 25 years ago (11), the physiological functions of prion-like sequences remain unclear. Uncovering this physiological function is an essential and important prerequisite for understanding whether the fibrillar assemblies are purely pathological or whether they have functional relevance.

Cells respond to stress by arresting the cell cycle, shutting down metabolism, and inducing stress-protective pathways. Upon cessation of stress, they must rapidly reprogram their metabolism and restart growth and division. When cells are stressed, they stop translation and release translation factors and mRNAs from poly-somes that are subsequently sequestered in granules (12, 13). After removal of stress, mRNAs reassociate with ribosomes, and translation factors ensure proper restart of protein synthesis.

Sup35 condensates form by pH-dependent phase separation and gelation

To provide a mechanistic understanding of pH-regulated condensation, we purified Sup35 and reconstituted the condensates in vitro. When 2 μM of purified Sup35 was incubated in physiological buffer, the protein remained diffuse (Fig. 2, A and B, and fig. S2A). However, when the pH was reduced from 7.5 to 6.0, condensates of Sup35 formed (Fig. 2, A and B, and fig. S2, B to D). Sup35 condensates adopted spherical shapes in solution and deformed when contacting the microscope slide (fig. S2B), suggesting that they are liquid-like. Supporting this idea, two Sup35 drops fused when brought together with an optical tweezier (Fig. 2C and movie S2), and photobleached regions within a Sup35 condensate quickly recovered fluorescence (Fig. 2D).

Using fluorescence recovery after photobleaching, we found that Sup35 was mobile in growing cells; it became immobile when sequestered into condensates, upon stress (Fig. 1D). We confirmed this behavior in vitro, where Sup35 initially phase-separated to form liquid droplets but then solidified into a gel-like state as suggested by fusion and photobleaching experiments (Fig. 2D and fig. S2, E to G). Cryo–electron tomography of Sup35 droplets revealed that gel-like droplets consisted of an amorphous, yet well-defined, meshwork with an average mesh size of ~10 nm (Fig. 2E; fig. S2, H to K; and movie S3). Such meshwork has not been seen in droplets formed by well-described stress and P granule proteins (3, 19). Gel-like condensates dissolved when the salt concentration or pH was raised or in the presence of small amounts of detergents, demonstrating reversibility in vitro (fig. S3, A to E). Thus, changes in pH regulate the formation of Sup35 into liquid droplets, which subsequently solidify.

The disordered M domain is a stress sensor that regulates phase separation of Sup35

The N-terminal region of Sup35 is intrinsically disordered and can be divided into two parts: an N-terminal prion domain (N) and a charged middle domain (M) (20, 21) (Fig. 2F). The conserved C-terminal guanosine triphosphatase (GTPase)
domain (C) is essential and catalyzes termination of protein synthesis. The N and M domains are dispensable, but conservation of the NM domain (22–25) indicates that they form a bipartite functional unit with an important function, which to date remains undefined.

A minimal module consisting only of the prion (N) and the M domain (NM) formed droplets in a reversible and pH-dependent manner in vitro (Fig. 2I, fig. S3F, and movie S4). The sequence of the M domain has a linear cluster of ionizable groups, specifically glutamic acid residues, located at the C-terminal end (Figs. 2F and 3A). Removing the charges within the negative cluster (Sup35M3 variant) yields a fully functional Sup35 variant (fig. S3, G and H) but with altered phase behavior, such that protein-rich droplets formed at pH 7.5 and the pH dependence of droplet formation was discernibly reduced in vitro (Fig. 2G) and in vivo (Fig. 2H and fig. S3I). Thus, pH sensing of Sup35 is facilitated by its charged M domain through protonation by its charged M domain through protonation.

In the presence of the M domain, the extent of irreversible aggregation was strongly reduced, in agreement with a solubilizing role of M (fig. S3L). The presence of M also partially restored condensate formation, but the amount of condensate formed was an order of magnitude lower than for the wild-type protein (Fig. 2I and fig. S3, L and M). Thus, the NM domain helps maintain the solubility of, and provides the pH sensitivity for, the C-terminal termination factor, in which the N domain provides the cohesiveness required for condensation. In other words, the disordered NM domain alters the phase behavior of the C domain by promoting the formation of reversible gels instead of irreversible aggregates. This phenomenon is consistent with the ideas of Semenov and Rubinstein, who predicted that gelation is driven by phase separation for so-called associative polymers (29). This phenomenon has been predicted to be relevant for linear multivalent proteins (30), and it appears to apply to Sup35 as well.

### Phase separation of Sup35 but not prion formation is conserved among distantly related yeast

The charge distribution, but not the sequence within the Sup35 M domain, is conserved across diverse fungi (Fig. 3A, fig. S4A, and table S1). Indeed, Sup35 from *Schizosaccharomyces pombe* exhibited similar behavior compared with that of *Saccharomyces cerevisiae*; in vivo, it formed stress-dependent intracellular condensates (Fig. 3, B and C, and fig. S4B), and in vitro, it formed reversible liquid droplets at low pH (Fig. 3, D to G; fig. S4, C to G; and movie S6) that crosslink into a meshwork that was indistinguishable from the one of Sup35 from *S. cerevisiae* (Fig. 3H; figs. S4, H to J; and movie S6). Importantly, and in contrast to *S. cerevisiae*, *S. pombe* is unable to induce and propagate the prion state of Sup35 (25). Thus, condensate formation, but not prion formation, is conserved among distantly related yeast that diverged more than

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**Fig. 1. Sup35 forms reversible condensates in *S. cerevisiae*.** (A) Fluorescence images of *S. cerevisiae* expressing green fluorescent protein (GFP)–labeled Sup35 during exponential growth (left), during energy depletion (middle), and after recovery from energy depletion (right). Energy depletion causes reversible condensation of Sup35 into intracellular puncta. White arrows point toward Sup35 condensates. The graph on the right shows a quantification of the cells with particles. About 150 to 200 cells per condition were used for quantification. (B) Fluorescence images taken from a time-lapse movie (see movie SI) of *S. cerevisiae* growing in a microfluidic device (CellASIC). Cells were grown in synthetic complete media for 2 hours. After 120 min, cells were energy-depleted to form Sup35 condensates. Condensates persisted during energy depletion and dissolved when the cells were recovered by being supplied with fresh synthetic medium. White arrows point toward Sup35 condensates. Cell growth was measured as the total increase in occupied area (cell area) as a function of time (t) (black). For particle signal, the maximum fluorescence signal was divided by the minimum fluorescence signal (magenta). Energy depletion coincides with growth arrest and Sup35 condensation. (C) Fluorescence images of *S. cerevisiae* expressing GFP-labeled Sup35 during recovery from stationary phase. Cells were grown to stationary phase for 2 days. White arrows point toward Sup35 particles. Supplying cells with growth medium (t0) caused dissolution of intracellular condensates and restart of cell growth. (D) The intracellular mobility of Sup35 was observed by fluorescence recovery after photobleaching. The recovery of fluorescence of GFP-labeled Sup35 was measured in exponentially growing (black) and energy-depleted (magenta) cells. (E) Fluorescence images of *S. cerevisiae* expressing GFP-labeled Sup35 in 100 mM phosphate, pH 5 (left), 100 mM phosphate buffer, 2 mM DNP, pH 5 (middle), and after 60 min of recovery with synthetic complete medium (right). About 150 to 200 cells were used for quantification of each condition. Box indicates intracellular pH as described by Munder *et al.* (16). Scale bar, 5 µm.
400 million years ago and suggests that condensate formation may be the ancestral function of the prion domain of Sup35.

Taken together, our data show that Sup35 forms condensates by pH-dependent phase separation and subsequent gelation into a porous polymer meshwork. The intrinsic disorder of the prion domain likely provides the necessary polymer meshwork. The intrinsic disorder of forms condensates by pH-dependent phase separation may be the ancestral function 400 million years ago and suggests that condensates dissolution could take several hours (Fig. 4, C and D, and fig. S5B). This suggests that the NM domain determines the material properties (reversible gel versus irreversible aggregate) of Sup35 in vivo. Concomitantly, Sup35C cells took longer to restart growth (Fig. 4, A and C, and movies S7 and S8) and exhibited reduced fitness when recovering from stationary phase (Fig. 4B).

Sup35 catalyzes an essential step during protein synthesis, namely translation termination. Indeed, translation was shut down upon energy depletion, as indicated by polysome disassembly (fig. S5, C and D). This coincided with Sup35 condensation. Conversely, dissolution of Sup35 condensates coincided with polysome reformation (fig. S5, C and D). Importantly, translation activity was specifically impaired after energy depletion in recovering Sup35C cells but not in control cells (Fig. 4E).

**The prion state interferes with Sup35 condensate formation and impairs recovery of yeast from stress**

How does the prion state of Sup35 affect the ability of cells to recover from stress? To investigate this, we compared prion-containing [PSI+] with prion-free [psi−] cells for their ability to regrow from the stationary phase (32). [PSI+] cells grew without a noticeable defect under normal conditions, but they showed a growth delay after recovery from stationary phase that was similar to that of Sup35C cells (Fig. 4F and fig. S5E). This suggests that the formation...

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**Fig. 2. The Sup35 prion domain synergizes with a pH sensor to drive phase separation into biomolecular condensates.** (A) Fluorescence images of 2 μM GFP-labeled wild-type (WT) Sup35 at indicated pH. Scale bar, 2 μm. (B) Phase diagram of 2 μM WT Sup35 with pH and salt concentration as order parameters. Phase separation was scored by the presence or absence of droplets in the samples. (C) Fusion of two Sup35 droplets at pH 6.0. Still images are shown on top. A force curve is shown at the bottom. Scale bar, 2 μm. (D) Internal rearrangement of Sup35 molecules was assayed by fluorescence recovery after photobleaching as a function of gelation time. A single pixel spot was bleached, and the change in the fluorescence was analyzed as a function of time. Still images are shown before and after bleaching. The drop size was ~3 μm. Analysis of the fluorescence recovery of 5-min-old (black; N = 19), 15-min-old (green; N = 9), and 60-min-old (magenta; N = 11) drops are shown. SD depicted as gray shadow. (E) Three-dimensional (3D)–rendered volume of WT Sup35 gel-like droplet imaged with cryo–electron tomography. Scale bar, 50 nm. (F) Disorder analysis (top) and schematic of the S. cerevisiae Sup35 domain structure. N (N) and middle (M) domain are disordered. The C-terminal domain is folded. Color gradient in M depicts the net charge along the sequence (blue, positive; magenta, negative; green, neutral). (G) Phase diagram of 2 μM Sup35M3 variant with pH and salt concentration as order parameters. Dashed line indicates the phase diagram of Sup35 WT as shown in (B). Representative images of 2 μM Sup35M3-GFP at indicated pH shown. Scale bar, 2 μm. (H) Fluorescence images and quantification of S. cerevisiae expressing Sup35M3 (top) and Sup35 WT (bottom) in 40 mM PIPES, pH 7.5, 2 mM DNP. Sup35M3 forms condensates at neutral pH, whereas WT stays diffuse. (I) Phase separation of Sup35 variants was probed at 2 μM final protein concentration (top). Dissolution was tested by increasing the salt concentration from 50 to 1000 mM NaCl (bottom). Scale bar, 2 μm.
of stress-protective Sup35 condensates is impaired in the presence of the prion and that [PSF+] cells have a reduced fitness when recovering from stress. This demonstrates that the ribosome critically depends on the availability of Sup35 after stress. Taken together, these experiments show that the Nm domain provides the catalytic C domain of Sup35 with the ability to recover rapidly from stress, and thus ensures a critical step in restarting translation (Fig. 5).

Discussion
Sup35 is a prominent member of a class of proteins with prion-like domains. These are low-complexity protein domains that consist primarily of polar and aromatic amino acids. They are called prion-like domains because they have been associated with the ability of proteins to spread through cellular populations in a prion-like manner (33). Indeed, numerous studies have highlighted the aggregation potential of prion-like domains when studied in isolation. Our data suggest that the prion domain, in the context of the full-length protein, adopts a benign role by increasing the solubility of the C-terminal catalytic domain at neutral pH and promoting Sup35 phase separation and gelation under stress. Thus, reversible gel formation, but not prion formation, is likely the ancestral function of the prion domain of Sup35. In agreement, many de novo formed variants of Sup35 prions cause cellular toxicity, suggesting that the prion state could be a sporadically occurring disease (34–37). Prion states may thus be a frequently occurring epiphenomenon of condensate-forming domains, which may or may not have adaptive value.

More generally, organisms must adapt to sudden changes in the environment, independent of transcriptional and translational regulation. In agreement with previous conjectures (38), we suggest that prion domains are protein-specific stress sensors and modifiers of phase transitions that allow cells to respond to specific environmental conditions. In the case of Sup35, this condition is the lowering of the cytosolic pH under energy stress. However, yeast contains more than 200 proteins with predicted prion-like domains, and Dictyostelium contains more than 1000 such proteins (39, 40). It seems likely that organisms deploy prion-like domains to generate protein-specific environmental responses. In agreement, another prion-like domain has recently been shown to tune the phase behavior of the yeast stress granule protein Pab1 (5). Prion domains are therefore crucial stress-adaptive regions that allow organisms to explore and persist in stressful and unstable environments.

Materials and methods
Strains and culture conditions
S. cerevisiae was grown at 30°C in yeast extract peptone dextrose (YPD), synthetic complete (SC) or synthetic dropout (SD) medium. Sc. pombe

Fig. 3. Phase separation of Sup35 is conserved in the evolutionarily distant fission yeast Sc. pombe. (A) Comparative bioinformatic analysis of Sup35 from S. cerevisiae (left) and Sc. pombe (right). Sup35 has three domains. The N (green) and M (blue-magenta) domains are predicted to be disordered, and the C domain (gray) is a folded and conserved GTPase domain (degree of predicted disorder: IUPred). FCR, fraction of charged residues; NCPR, net charge per residue; Hydro, hydrophobicity. M is disordered and contains a high density of charged residues with a blocky architecture. It carries an overall net negative charge with a net positive charge at the N-terminal half and a strong net negative charge at the end of M. Arrows point toward the M3 charge cluster. (B) Fluorescence images of Sc. pombe expressing mCherry-labeled Sup35 grown in medium (left) and after 30 min of energy depletion (right). Scale bar, 5 μm. (C) Fluorescence images of Sc. pombe expressing mCherry-labeled Sup35 exposed to 100 mM phosphate buffer of stress-protective Sup35 condensates is impaired in the presence of the prion and that [PSF+] cells have a reduced fitness when recovering from stress. This demonstrates that the ribosome critically depends on the availability of Sup35 after stress. Taken together, these experiments show that the Nm domain provides the catalytic C domain of Sup35 with the ability to recover rapidly from stress, and thus ensures a critical step in restarting translation (Fig. 5).

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was grown at 30°C in YE5 or EMM5 medium. A list of yeast strains is in table S2.

**Plasmids and cloning**

A list of plasmids used here can be found in table S2. Gateway cloning (Invitrogen) was carried out as described previously (43). *Sc. pombe* Sup35-mCherry was generated according to (42) using standard primers for amplification of the linear tagging cassette carrying the fluorescence protein tag. *Sc. pombe* was transformed with purified PCR product (43) and selected clones were verified using standard primers (42).

**Energy depletion of cells**

*S. cerevisiae* and *Sc. pombe* cells were energy depleted as described previously (16, 17). In short: Exponentially growing yeast were transferred to and incubated in liquid SC medium or EMM medium, respectively, without glucose containing 20 mM 2-deoxyglucose (2-DG, inhibition of glycolysis) and 10 μM antimycin A (inhibition of mitochondrial ATP production). Treatment causes about 95% reduction in cellular ATP (44). Recovery of cells from energy depletion was by replacing energy depletion media with media containing 2% glucose. In a microfluidic setup (CellAsic), cells were grown for 2-3 hours with medium prior to treatment. Medium was pumped with 2 PSI. Exchange of medium was carried out with 4 PSI for 2 min.

**pH stress**

Exponentially growing *S. cerevisiae* and *Sc. pombe* cells were transferred to 100 mM phosphate buffer of different pH containing 2 mM 2,4-dinitrophenol (DNP). Control samples were treated equally, but DNP was omitted. Cell recovery was by replacing buffer with medium containing 2% glucose. In a microfluidic setup (CellAsic), cells were grown for 2-3 hours prior to treatment. Medium was pumped with 2 PSI. Exchange of medium was carried out with 4 PSI for 2 min.

**Microscopy of yeast**

Samples were prepared as described above. Imaging was with a DeltaVision (Applied Precision) microscope (Olympus IX70 stand, Osram Mercury short arc HBO light source, Olympus UPlanSApo objective, CoolSnap HQ2 camera). Z stacks with 6 planes were collected. Imaging settings were: 5% excitation intensity, 0.15 s exposure time, 512x512 pixels, 2x2 binning.

**FRAP measurements**

In vivo and in vitro fluorescence recovery after photobleaching (FRAP) experiments were carried out with an Andor spinning disc microscope (Nikon TIE inverted stand, Nikon Apo 100x, NA 1.49 Oil objective, Andor Ixon+ camera, EM gain 200, imaging laser intensity of 0.3% for reconstituted protein droplets and 5% for cells) equipped with a FRAPPA unit (Andor). A single pixel was bleached with a 405-nm laser pulse (1 repeat, 10% intensity, dwell time 10 ms). Recovery from photobleaching was recorded in a single focal plane. Image analysis was carried out in Fiji.

**Ratiometric pH measurements**

Cytosolic pH measurements were carried out as described in (16, 17). In short: pHluorin2 (45) was expressed in W303 ADE+ under control of a GPD promoter. pH calibration was obtained as described previously (16, 46). Imaging was carried out using DAPI/FITC (Excitation: DAPI; Emission: FITC) and FITC/FITC (Excitation and emission: FITC) filter sets on a DeltaVision (Applied Precision) microscope (Olympus IX70 stand, Osram Mercury short arc HBO light source, 100x Olympus UPlanSApo objective, CoolSnap HQ2).

**Fig. 4. The prion domain and a pH sensor work in synergy to protect the C domain from stress-induced damage.** (A) Growth analysis of *S. cerevisiae* expressing WT Sup35 (black) or Sup35C (magenta) growing in synthetic complete medium in a microfluidic setup for 3 hours (left) and upon exposure to and recovery from pH 5.0/DNP for one hour (indicated in gray). SEM is shown in gray, N = 9 fields of view. (B) Spot-titer growth assay of *S. cerevisiae* expressing WT Sup35 (WT) and Sup35C (C) (Ctrl: exponentially growing cells were spotted) (left) and after recovery from stationary phase. (C) Fluorescence images of *S. cerevisiae* expressing WT Sup35-GFP (top) and Sup35C-GFP (bottom) before (Before), during pH 5.0/DNP (before), during and after recovery (Recovery). WT Sup35-GFP particles dissolve rapidly during recovery, and cells grow and divide. Yellow arrows point toward newly formed yeast buds. Particles formed by Sup35C-GFP persist, and cells remain in an arrested state for a long time. Scale bars, 5 μm. (D) Fluorescence images of *S. cerevisiae* expressing WT Sup35-GFP (left) and Sup35C-GFP (right) after exposure to pH 5.0/DNP for 60 min (top) and after 60 min of recovery (bottom). Arrows point toward Sup35 condensates. (E) Translational activity was determined for cells expressing WT Sup35 and Sup35C during exponential growth (first data point), during 60 min of energy depletion (ED; highlighted in gray) and during recovery (Recovery). SD is shown: N = 500 to 700 cells per data point. (F) Spot-titer growth assay of the exponentially grown [psi−] and [psi+] yeast (Ctrl) and cells that were grown to stationary phase cells for 6 days (Day 6).
subjected to ultracentrifugation for 2.5 hours onto the sucrose gradient. The samples were OD260 nm of 20 of the supernatant were layered by centrifugation for 5 min at 8000 × g, the beater for 5 min at 30 Hz. The lysate was cleared by centrifugation (20,000 rpm, JA-25.50) and was briefly cleared by centrifugation (20,000 rpm, JA-25.50). Lysates was resuspended in 1% (v/v) Triton X-100, 30 mM NEM, 1 x Complete Protease Inhibitor (Roche). The cells were then lysed using glass beads (TissueLyser II from Qiagen, settings: 15 min, 25/sec) and were briefly spun at 2,000 rpm to sediment debris. 90 μl of supernatant were mixed with 4 × sample buffer (2 x TAE, 20% (v/v) glycerol, 4% (w/v) SDS, bromophenol blue). Samples were incubated at room temperature for 10 min and 50 μl were loaded onto a 1.5% agarose gel containing 1 x TAE and 0.1% SDS. The gel was run in 1 x TAE, 0.1% SDS at 100 V, followed by blotting onto a nitrocellulose membrane (GE Healthcare Life Sciences), as described in (47). Detection was with ECL plus solution (GE Healthcare Life Sciences) and a primary antibody against the C-terminal domain of Sup35 (Sup35C antibody kindly provided by R. Halfmann) and a secondary anti-mouse antibody.

Polysome profiling
Polysome profiling was adapted as described in (48). In short: 250 mL yeast cultures were grown to OD600 nm = 0.5, 100 mL were treated as untreated control sample, 150 mL were energy depleted as described above. After energy depletions, cycloheximide was added at a final concentration of 0.1 mg/ml to arrest polysomes and the samples were incubated on ice for 5 min. Cells were centrifuged 5 min at 3000 rpm and washed with 5 mL iced-cold polysome extraction buffer (PEB: 20 mM Tris-HCl pH 7.4, 140 mM KCl, 5 mM MgCl2, 0.1 mg/ml cycloheximide, 0.5 mM DTT, DEPC treated H2O). Cells were resuspended in 800 μL PEB supplemented with 500 μL glass beads and lysed using a bead beater for 5 min at 30 Hz. The lysate was cleared by centrifugation for 5 min at 8000 × g, the absorbance at 260 nm was determined and an OD260 nm of 20 of the supernatant were layered onto the sucrose gradient. The samples were subjected to ultracentrifugation for 2.5 hours at 35 k rpm in an SW40 Ti rotor (Beckman). The gradients were subjected to UV 260 nm readings using a peristaltic pump.

Translation activity assay
Cells were grown to mid-log phase in SC medium. Samples were taken before, during, and after energy depletions at indicated time points. Newly synthesized proteins were labeled by resuspending the cells in SD medium depleted for methionine and supplemented with the methionine analog HPG (Invitrogen) for a 10-min pulse. Samples were fixed with 3.7% formaldehyde, washed twice with PBS and the cell wall was digested with Zymolyase (ZymoResearch). Cells were washed twice with PBS, 3% v/v BSA and permeabilized with 0.5% Triton X-100. Samples were mounted in 4-well dishes treated with polylysine. Click chemistry for HPG labeling was carried out according to the manufacturer protocol (Click-iT, Invitrogen). Samples were imaged with a DeltaVision Elite as described above. Image analysis was carried out with Fiji.

Protein purification
Recombinant Sup35 and variants were expressed as N-terminal MBP-fusion proteins with a C-terminal His-Tag. Expression was by baculovirus expression in SF9 insect cells (3). Cells were lysed in buffer A (50 mM Tris-HCL, 1 M KCl, 2 mM EDTA, 1 mM DTT, pH 7.5) supplemented with cOmplete Protease Inhibitor Cocktail (Roche) using an Emulsiflex C5 (Avastin). Lysates was cleared by centrifugation (20,000 rpm, JA-25.50 rotor (Beckman Coulter), 60 min, 4°C). Supernatant was applied to MBP resin and washed with 20 column volumes buffer A. Elution was with 20 mM Maltose in buffer A. Samples were pooled and GST-tagged precision protease was added to cleave off the MBP- and His-tag and dialyzed against buffer A overnight at 4°C. The sample was cleared by centrifugation and subjected to size exclusion chromatography using a Superdex-200 26/60 column (GE Healthcare Life Sciences) equilibrated with buffer A running on a BioCad 60 (Applied Biosystems) at RT. Pooled samples were concentrated and frozen in liquid nitrogen.

Reconstitution and microscopy of protein-rich droplets
Protein-rich droplets of Sup35 were formed by dilution of the protein from a stock solution into 20 mM PIPES, 2% Polyethyleneglycol 20K. pH was adjusted with NaOH and the respective pH of the buffer is denoted in the figures and figure legends. Sup35 phase separation was tested at concentrations ranging from 0.1-20 μM. Phase diagrams were obtained at 2 μM Sup35. Samples were mixed in low-binding PCR vials and imaged on PEG-silane pacified microscopy slides and/or in 384 low-binding multi-well microscopy plates (Greiner Bio-One). Phase separation was scored yes or no, depending on the presence or absence of protein droplets. Samples for phase diagrams were imaged with a DeltaVision Elite (GE Healthcare Life Sciences), equipped with a multi-well plate holder using an Olympus UPlan SAPochromat 100x, NA 1.4 Oil objective. Excitation of GFP labeled samples was at 488 nm and emission was at 520 nm. 20 μm x-stacks with 1 μm spacing were taken. For statistical representation and analysis 16 fields of views with a 50 μm spacing were recorded per sample. Enrichment measurements were carried out on an Andor spinning disc confocal microscope using a Nikon Apo 100x, NA 1.49 Oil objective on a Nikon TIE, inverted stand. Excitation was with a 488 nm DPSS laser.
Cryo–electron microscopy and tomography

Copper Quantifoil grids (R2/1, Cu 200 mesh grid, Quantifoil Micro Tools) were glow discharged for 45 s and incubated with BSA-coated 15 nm gold nanoparticles. The solution was allowed to dry to adhere gold particles to the grid support and serve as fiducials for the alignment of tilt series. Phase separation was carried out immediately prior to the application of samples to the grids. 4 μl from each sample were deposited on grids and allowed to settle 30 s. Grids were plunged-frozen into liquid ethane/propane mixture at -180°C, and subsequently frozen into liquid nitrogen to a pixel size 0.342 nm with target defocus of 4 μm. Individual frames acquired by K2 camera were aligned using an in-house implementation following procedures developed by Li et al. Tilt-series were collected using SerialEM software (5b). Tilt-series projection images were performed with gold nanoparticles as fiducials with IMOD software. Final alignment of the tilt-series images was performed using the linear interpolation option in IMOD and a low pass filter (cut off 0.35; sigma 0.05). CTF correction was performed. Filtered volumes were generated in Matlab (Mathworks 2015) using TOM Toolbox and a low pass filter (cut off, 0.35; sigma, 0.05). No CTF correction was performed. In IMOD and a low pass filter (cut off, 0.35; sigma, 0.05). No CTF correction was performed.

Bioinformatic analysis

Sequence analysis of Sup35 from S. cerevisiae and Sc. pombe was performed using localCIDER (52), IUPred (59), and the Superfamily database (54). For each protein, six analysis tracks were generated. SupFam defines the functionally annotated domains, identifying the three well-characterized folded regions in the C-terminal domain. IUPred describes the degree of predicted disorder; the N and M domains are predicted to be disordered while the C domain is predicted to be folded. The remaining four tracks use a 20-residue sliding window to compute local sequence properties of relevance to disordered regions. FCR describes the fraction of charged residues and shows the N domain has relatively few charged residues while the M domain is substantially enriched. NCPR describes the net charge per residue. Despite substantial sequence divergence, the M domains have a characteristic charge distribution of a positively charged N-terminal region and a negatively charged C-terminal region. Hydro describes the local hydrophobicity using the Kyte-Doolittle hydrophobicity scale. Comparative charge distribution of various yeast species was also carried out using the emboss explorer charge module with a sliding window of 20-residues. Sequence identity analysis was carried out with ClustalOmega.

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Phase separation of a yeast prion protein promotes cellular fitness

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Science 359 (6371), eaao5654.
DOI: 10.1126/science.aao5654

Biophysical responses of proteins to stress

Much recent work has focused on liquid-liquid phase separation as a cellular response to changing physicochemical conditions. Because phase separation responds critically to small changes in conditions such as pH, temperature, or salt, it is in principle an ideal way for a cell to measure and respond to changes in the environment. Small pH changes could, for instance, induce phase separation of compartments that store, protect, or inactivate proteins. Franzmann et al. used the yeast translation termination factor Sup35 as a model for a phase separation-induced stress response. Lowering the pH induced liquid-liquid phase separation of Sup35. The resulting liquid compartments subsequently hardened into gels, which sequestered the termination factor. Raising the pH triggered dissolution of the gels, concomitant with translation restart. Protecting Sup35 in gels could provide a fitness advantage to recovering yeast cells that must restart the translation machinery after stress.

Science, this issue p. eaao5654