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# The Three Faces of Sup35

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## Abstract

Sup35p is an essential protein in yeast that functions in complex with Sup45p for efficient translation termination. While some may argue that this function is the only important attribute of Sup35p, there are two additional known facets of Sup35p's biology that may provide equally important functions for yeast, both of which involve various strategies for coping with stress. Recently, the N-terminal and middle regions (NM) of Sup35p, which are not required for translation termination function, have been found to provide stress sensing abilities and facilitate the phase separation of Sup35p into biomolecular condensates in response to transient stress. Interestingly, the same NM domain is also required for Sup35p to misfold and enter into aggregates associated with the [*PSI*$^+$] prion. Here, we review these three different states or "faces" of Sup35p. For each, we compare the functionality and necessity of different Sup35p domains, including the role these domains play in facilitating interactions with important protein partners, and discuss the potential ramifications that each state affords yeast cells under varying environmental conditions.

## Abstract

Many proteins in yeast have been shown to change folding states in response to environmental stress. One protein, Sup35p, not only plays an essential role as a translation release factor but has been shown to undergo phase separation into biomolecular condensates as well as misfold to form the prion [*PSI*$^+$]. This budding topic describes the three states of Sup35p (translation termination, phase separation, and prion formation) and sheds light on how short and long-term stress impacts these three states.

## Introduction:

Sup35p is an essential protein that corresponds to the eukaryotic translation termination factor 3 (eRF3) in yeast(reviewed in Inge-Vechtomov et al., 2003).In addition toits critical role in translation, this simple protein also has some noteworthy attributes. Recently, it was found that Sup35p can respond to transient stress by undergoing phase transition. In the presence of short heat or pH stress, Sup35ptemporarily assembles into reversible condensates, which are localizedareas that contain high concentrations of protein(Franzmann et al., 2018).Another unique feature of Sup35p is that it can misfold and assemble into self-perpetuating, infectious amyloids that can be propagated for many generations(reviewed in Liebman and Chernoff, 2012). Here, we review the three different

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states or “faces” of Sup35p, including important interacting partners, the role of Sup35 domains in these protein interactions, and how Sup35p contributes to general cellular functions including the ability to respond to transient and long-term stress.

## Sup35p and translation

Two mutants, carrying mutations within *SUP35*or *SUP45*, were identified through screens for suppressors of nonsense mutations(Inge-Vechtomov, 1964; Smirnov et al., 1976). Both suppressors were later determined to encode eukaryotic release factors, eRF3 and eRF1, respectively, and are essential proteins required for translation termination(reviewed in Inge-Vechtomov et al., 2003; Nizhnikov et al., 2014). Sup45p plays a role in the recognition of stop codons. Similar to eRF1, Sup45p has a structure resembling a tRNA and initiates peptidyl tRNA hydrolysis (Bertram et al., 2000; Song et al., 2000; Stansfield et al., 1997). Sup35p supplies the GTPase activity, which together with Sup45p, leads to the termination of translation and release of the translational complex.

Sup35p is a modular protein that contains a C-terminal region that is both necessary and sufficient for translation termination, and is required for cell viability. This region, which contains a GTPase fold commonly found in G-proteins, has been shown to have GTPase activity (Salas-Marco and Bedwell, 2004; Stansfield et al., 1993) and interacts with Sup45p(Stansfield et al., 1995). For termination function, both Sup35p and Sup45p interact with the translating ribosome for stop codon recognition (Wada and Ito, 2014). GTP hydrolysis by Sup35p provides the energy for dissociation of the translation complex and release of the newly synthesized polypeptide (reviewed in Dever et al., 2016).

The C-terminus of Sup35p and other eRF3 proteins are important for translational termination function and are highly conserved (Chernoff et al., 1992; Kushnirov et al., 1988). However, regions outside of this C-terminal domain aredispensable for translational termination and are quite diverged (Chernoff et al., 1992; Ter-Avanesyan et al., 1993). In mammals, the non-essential N-terminal region of mammalian eRF3 has been shown to interact with PABP, the poly(A)-binding protein that is required for translation initiation and mRNA stabilization (Hoshino et al., 1999). *In vitro* translation experiments show that PABP directly affects translational termination activity by enhancing eRF3 binding to the ribosome(Ivanov et al., 2016). Authors have proposed that interaction between PABP and eRF3 plays an important role in positioning of the eRF3/eRF1 complex near stop codons for efficient translational termination (Ivanov et al., 2016).

Despite the divergence of non-essential sequences between the yeast Sup35 protein and the mammalian eRF3, the N-terminal region of Sup35p binds to the yeast homolog of the PABP protein, Pab1p(Roque et al., 2015). Yeast Two Hybrid and pull down assays show that a proline rich region in Pab1p (P domain; Fig. 1A)plays an important role in association with the N-terminus of Sup35p through a non-canonical Pab1p binding site (Cosson et al., 2002; Roque et al., 2015). Based on how mammalian PABP influences translational termination (Ivanov et al., 2016), we suspect that a similar mechanism occurs in yeast. It is possible that the interaction between yeast Pab1p and Sup35p positions the Sup35p/Sup45p complex near the stop codon. Such positioning could provide the translation machinery access to release factors in order to stop translation in a timely and efficient manner (Fig. 1B).

## Sup35p and phase separation

Translation is essential for cellular function. However, under times of transient stress, there may be a need to temporarily shut down processes like translation and shift the cellular energy load to other functions. Work in the last few years has shown that Sup35p, along with several other proteins, can change phase states and thus be conditionally pulled from the soluble protein pool into protein-rich biomolecular condensates in a process called phase separation. Phase separation provides the ability to locally organize proteins within the intracellular milieu without the need for distinct membrane organelles. These “membraneles sorganelles” allow proteins to be temporarily sequestered or stored into highly concentrated environments during stress. Upon removal of the stress, proteins compartmentalized through phase separation transition back into the aqueous cellular environment. Proteins containing low-complexity regions that are intrinsically disordered have been found to undergo phase transition (Franzmann and Alberti, 2018; Franzmann et al., 2018; Kato et al., 2012; Kroschwald et al., 2018; Molliex et al., 2015; Riback et al., 2017). It is thought that these low complexity or intrinsically disordered regions are sensitive to changes brought about by transient stress, and mediate the transition between a functional protein in a soluble environment and a protein that is sequestered into the protein dense environment of a membraneless organelle (reviewed in Alberti, 2017).

Stress granules are an example of membraneless organelles, and form in yeast in response to transient heat stress. Proteins move into stress granules through a process called demixing, where a protein population goes from a soluble state to localized regions of high protein concentration. Treatment of yeast cells at 46°C for 8 minutes leads to rapid demixing, yet return to normal temperatures leads to the disappearance of stress granules and release of proteins back to the soluble state (Wallace et al., 2015). 117 yeast proteins have been shown to undergo reversible phase separation in response to heat, many of which are associated with translation, RNA binding, and chaperone function (Wallace et al., 2015). Among the translation associated factors identified on this list were Sup35p, Sup45p, and Pab1p. The identification of these translation associated factors within stress granules is not surprising since there is a correlation between the formation of stress granules and decrease in translational efficiency (Buchan and Parker, 2009). As discussed above, interaction between Sup35p and Pab1p is mediated through the Sup35p N and M regions and the Pab1p hydrophobic P domain(Fig. 1; Cosson et al., 2002; Roque et al., 2015). The hydrophobic P domain of Pab1p has low complexity, and possibly works as a stress sensor (Fig. 2). Reducing the hydrophobicity of this proline-rich region reduces the ability of Pab1pto undergo phase separation. Therefore, this P domain may play an important role in keeping Pab1p soluble in non-stress conditions, but can also sense fluctuations in the environment to induce phase separation(Riback et al., 2017).

Data supporting the specific phase separation properties of Sup35p has been recently reported by Franzmann et al (2018). Using Sup35-GFP fusion proteins, Sup35p is able to transition into biomolecular condensates upon energy depletion, and return to the cytoplasm upon nutrient enrichment. In parallel, translation stops upon energy depletion, and is restored upon nutrient enrichment. Since pH levels decrease as a result of starvation, authors tested how mitochondrial uncouplers, which transiently lower cytosolic pH, influence

Sup35p phase separation. Similar to starvation, pH stress also causes Sup35p to phase separate into protein dense particles along with Pab1p(Fig. 2; Franzmann et al., 2018). The authors showed that acidic amino acids within the M region of Sup35p are important for phase separation in response to pH, since mutation of these acidic amino acids to polar residues reduces the ability to respond to stress. Similar to the proline-rich region of Pab1p, the ionizable nature of the Sup35p M domain may allow the protein to sense intracellular pH changes. While the N-terminal region does not have sensor ability, it appears to enhance the ability of Sup35p to enter into condensates(Franzmann et al., 2018).

The above studies suggest that both Pab1p and Sup35p individually have regions that sense cellular stress and mediate phase transition. These same two sensor regions also mediate the interaction between Sup35p and Pab1p during translation (Fig. 1A and Fig. 2A). The existence of sensor regions that also mediate the interaction of these proteins may not be coincidental. It is possible that while these regions are important for translation efficiency, conformational changes due to transient stress allow these proteins to be temporarily sequestered possibly resulting in translational issues. Yet upon the release of stress, these proteins quickly solubilize and are available for translation (Fig. 2B). It is foreseeable that phase separation can affect other important processes involving proteins with low complexity regions. For example, several proteins associated with transcription and chromatin remodeling contain low complexity regions. One protein, Snf5p, is glutamine and asparagine rich, and also appears to phase separate in response to pH stress (Gutierrez et al., 2018). It is intriguing to speculate that inherent sensing abilities via low complexity regions could provide a wide array of proteins, particularly those involved in global functions such as transcription and translation, with a rapid mechanism for responding to transient stress.

## Sup35p as a prion

At the time when Sup35p mutants were being pulled out of the original nonsense suppressor screens in the 1960s, a cytoplasmic element named *ψ* was also shown to suppress nonsense mutations (Cox, 1965). Years later, this cytoplasmic element was shown to be the misfolded, self-perpetuating, infectious form of the Sup35 protein, called the [*PSI*$^+$] prion (reviewed in Liebman and Chernoff, 2012). In contrast to transient phase separation of Sup35p into reversible biomolecular condensates, the [*PSI*$^+$] prionenables the Sup35p protein to assemble into stable amyloid aggregates that can be propagated for many generations. Cell viability is not severely impacted in [*PSI*$^+$] strains, suggesting that there must be a sufficient amount of functional Sup35p to ensure some basal level of translation termination (Pezza et al., 2014; Zhou et al., 1999). However, toxicity is observed when Sup35p is overexpressed in the presence of [*PSI*$^+$] (Derkatch et al., 1996). This toxicity appears to be caused by overexpression driving the excess Sup35p into aggregates. Likewise, the excess Sup35p pulled into aggregates sequesters Sup45p away from essential translational functions (Vishveshwara et al., 2009), suggesting that there is a delicate balance between maintaining the prion and ensuring that Sup35p and Sup45p are available for essential translation termination functions.

The ability of Sup35p to become a prion is dependent upon the region that is dispensable for translation termination but contributes to phase separation, the N-terminal region (Fig. 3A;

Ter-Avanesyan et al., 1994). The N-terminus of Sup35p contains a region rich in glutamine and asparagine, followed by a repeat region that contains several degenerate tandem repeats required for stabilizing the intermolecular interactions between Sup35p molecules within the aggregate during prion formation (Liu and Lindquist, 1999; Parham et al., 2001). While the M region and the C-terminus are not required for prion formation, the M region has been shown to foster the formation of specific Sup35p conformations called prion strains or variants(Bradley and Liebman, 2004), and shown to be important for [*PSI*$^+$] stabilization (Liu and Lindquist, 1999). [*PSI*$^+$] variants have different size and biochemical characteristics, as well as stabilities and translational termination efficiencies. For example,without M sequences, strong [*PSI*$^+$] variants are able to stably propagate for many generations,whereas weaker [*PSI*$^+$] variants are quickly lost from the population (Bradley and Liebman, 2004).

The propagation of [*PSI*$^+$] over many generations requires protein quality control factors such as chaperones. A complex of molecular chaperones, such as Hsp104p, Sis1p, and Ssa1p, work together to recognize the prion particles and ultimately shear the prion into smaller particles. The transmission of these smaller prion particles to daughter cells ensures that the prion is propagated to the next generation(reviewed in Liebman and Chernoff, 2012).It has been shown that the M region of Sup35p plays a role in the interaction with chaperone machinery to enhance the propagation of the prion (Helsen and Glover, 2012). The ability to propagate [*PSI*$^+$] over many generations may have some adaptive value to cells, potentially due to the phenotypic variation facilitated by the readthrough of nonsense mutations or subtle variation in translation termination efficiency (True and Lindquist, 2000). However, [*PSI*$^+$] does not cause dramatic global proteomic changes (Chan et al., 2017). Instead, ribosomal profiling studies show that approximately 100 genes are susceptible to stop codon readthrough in the presence of [*PSI*$^+$], and [*PSI*$^+$] appears to impact reading frame selection for a subset of genes (Baudin-Baillieu et al., 2014).

The spontaneous formation of [*PSI*$^+$] in yeast cells occurs at a very low rate of approximately $10^{-7}$ per generation (Allen et al., 2007; Lancaster et al., 2010; Lund and Cox, 1981). Overexpression of Sup35p or its N- and M- regions (Sup35NM) can marginally increase prion formation, while prion formation can be dramatically enhanced by overexpression in the presence of a second prion. For example, the presence of the prion form of the Rnq1p protein called [*PIN*$^+$], also known as [*RNQ*$^+$], can enhance the conversion of Sup35p to the prion state(Fig. 3B; Derkatch et al., 2001; Osherovich and Weissman, 2001; Sondheimer and Lindquist, 2000). Two models have been suggested to mediate the process by which [*PIN*$^+$] induces [*PSI*$^+$] formation. The first is the inhibitor titration model, which suggests that the [*PIN*$^+$] prion sequesters or titrates a factor such as a chaperone that normally inhibits the aggregation of Sup35p(Derkatch et al., 2001; Osherovich and Weissman, 2001). However, there is little evidence to support or negate this model. The second is the cross seeding model in which a pre-existing aggregated protein is used as a template to enhance the misfolding and aggregation of a second heterologous protein (Derkatch et al., 2001; Osherovich and Weissman, 2001). It has been shown from *in vitro* studies that Rnq1p fibers can cross seed the formation of Sup35p fibers (Keefer et al., 2017; Sharma and Liebman, 2013; Vitrenko et al., 2007), and *in vivo* studies have provided both co-localization and genetic support for the cross seeding model (Derkatch et al., 2004;

Keefer et al., 2017). Within the sequence of Rnq1p, the long glutamine/asparagine (Q/N) rich region near the C-terminus is not only essential for the propagation of [*PIN*$^+$], but even a Q-R substitution in this region can dramatically reduce [*PSI*$^+$] induction (Fig. 3B; Derkatch et al., 2001; Keefer et al., 2017).It has been suggested that the heterotypic interactions between [*PIN*$^+$] and the high concentration of Sup35NM through overexpression fosters amyloid nucleation and bypasses the formation of condensates. When [*PIN*$^+$] is absent, it is well established that prion formation is diminished and the overexpression of Sup35NM leads to condensate formation rather than the prion state (Khan et al., 2018).

The process of [*PIN*$^+$] dependent prion formation can be monitored both biochemically and visually using fluorescently tagged Sup35NM fusion proteins. During formation, Sup35p is initially converted from a monomericform to small SDS-resistant oligomeric complexes(Salnikova et al., 2005; Sharma et al., 2017). At the time of this initial SDS-resistant oligomer detection, Sup35NM-GFP visibly exhibits diffuse cytoplasmic fluorescence indicating that Sup35p is associated with extremely small aggregates. As the oligomers assemble into larger SDS-stable aggregates over time, small highly mobile fluorescent foci are visually detected. These foci quickly coalesce into a single fluorescent dot before being sequestered near the cell periphery (Lyke and Manogaran, 2017; Sharma et al., 2017). Once residing at the periphery, the majority of the diffuse Sup35NM quickly converges into the aggregate within 20 minutes. This quick sequestration coincides with the growth of the aggregate into dots, or ring and line-like structures(Sharma et al., 2017; Zhou et al., 2001). Cells containing these structures are considered to be hallmarks of [*PSI*$^+$] since cells that contain aggregates give rise to [*PSI*$^+$] cells but diffuse cells do not (Ganusova et al., 2006; Sharma et al., 2017).

The *de novo* formation of [*PSI*$^+$] has been shown to be influenced by both the actin cytoskeleton and protein quality control systems. Loss of genes that influence the formation of endocytic cortical actin patches leads to decreased dot, ring, and line formation (Ganusova et al., 2006; Manogaran et al., 2011). It has been proposed that the glutamine-rich proteins of the endocytic actin patch may provide a location near the periphery of the cell that allows the cross seeding of Sup35p into dot, ring, and line aggregates (Ganusova et al., 2006). Protein quality control also influences prion formation. Mutations that disrupt autophagy, oxidative stress response, or the ubiquitin proteasome system results in increased prion formation (Allen et al., 2007; Chernova et al., 2011; Doronina et al., 2015; Speldewinde et al., 2015), suggesting that under normal conditions, protein quality control mechanisms actively limit prion formation. Conversely, long-termstress can also enhance prion formation. Exposure to high salt or hydrogen peroxide stress for 12–24 hours leads to high levels of cell death. However, of the small surviving population, prion formation was shown to be enhanced by approximately 60 fold (Tyedmers et al., 2008). The enhancement of prion formation in response to long-term stress may provide a means to generate phenotypic variation through the readthrough of nonsense mutations or alteration of translation termination efficiency. Therefore, this variation may lead to a small subset of the population that is well suited for the extreme environment.

### The three faces of Sup35

Here, we have discussed three separate states for Sup35p: a functional translation termination state, a phase separation state, and a prion state.The role of Sup35p in translation termination is widely accepted, however the purpose of Sup35p's ability to be sequestered into biomolecular condensates and the ability to form a prion is highly debated. It has been proposed that the ancestral function of the N- and M- regions of Sup35p is to sense acute stress and foster reversible phase separation into condensates rather than encourage prion formation (Franzmann and Alberti, 2018; Franzmann et al., 2018).This argument is to some extent supported by the fact that [*PSI*$^{+}$] can be detrimental, as only those variants with translation termination function are able to survive (Wickner, 2011). However, the ancestral function of the N and M regions of Sup35p is likely much more complicated than simply preferring phase separation over prion formation. [*PSI*$^{+}$] formation is enhanced by cross seeding and through chronic stress. While [*PSI*$^{+}$] is not abundant in nature, other prions such as [*PIN*$^{+}$] are found in both wild and commercial strains(Halfmann et al., 2012; Nakayashiki et al., 2005). It is possible the existence of [*PIN*$^{+}$] in the wild provides a means to efficiently induce [*PSI*$^{+}$] in response to chronic stress. This formation of [*PSI*$^{+}$] could be advantageous, since it has been shown that [*PSI*$^{+}$] provides growth benefits under distinct stress conditions and genetic backgrounds (True and Lindquist, 2000). It is possible that cells need both phase transition and prion formation to serve specific purposes. Under acute stress, phase transition allows for the short-term sequestration of Sup35p in order to transiently shut down translation termination. Under chronic conditions, prion formation could allow for phenotypic variation in populations. Those cells that endure the stress while retaining the prion would be able to divide and propagate the prion form for many generations (Fig. 4).

The three separate states of Sup35p are reminiscent of the 1957 film, *The Three Faces of Eve*. A story of a woman with three separate personalities: Eve White, a modest unassuming woman who takes care of the home, Eve Black, a tempestuous woman who quickly appears and disappears during conflict, and Jane, who emerges after many years as a stable and consistent individual. Similarly Sup35p could be viewed as having multiple personalities: the first involved in translational termination, the second who seamlessly phase separates into and out of biomolecular condensates in response to acute stress, and third, the stable prion that can be propagated for many generations.Given that intriguing facets of Sup35p have continued to be uncovered over the 50+ years of study, experiments over the next decade will further define these Sup35p states, and may reveal new and unexpected Sup35p states in the future.

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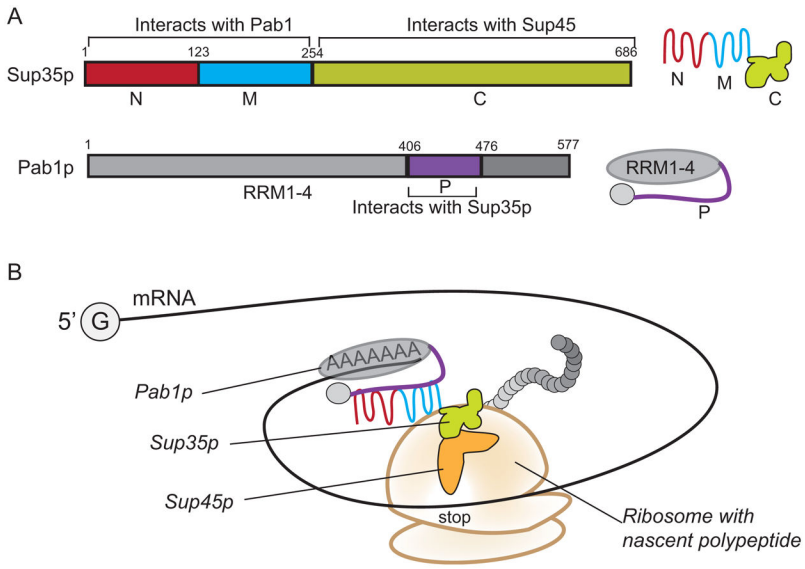


**Figure 1. Sup35p,Pab1p, and the model of protein interaction during translation.**

A. Sup35p is comprised of three regions: The N-terminal (1-123; red) and middle domains (124-254; blue) and the C-terminal region (255-686; light green). A symbol for the folded Sup35 protein and its domains is shown to the right. The C-terminal region of Sup35p interacts with Sup45p and has a GTPase fold necessary for GTPase activity. The N and M regions of Sup35p interact with the poly(A)-binding protein, Pab1p. The Pab1 protein is also modular, containing four RNA recognition motifs (RRM1-4), and a proline rich region (P domain, shown in purple) that interacts with Sup35p.A symbol for Pab1p and its domains are shown to the right. B. During translation, protein interactions between Sup35p NM regions and Pab1pcould position theSup35p/Sup45pcomplex near stop codons. As the translating ribosomes encounter stop codons, the positioned Sup45p is able to recognize the stop codon and initiate tRNA hydrolysis, while Sup35p provides the GTPase activity for the release of the translational complex.

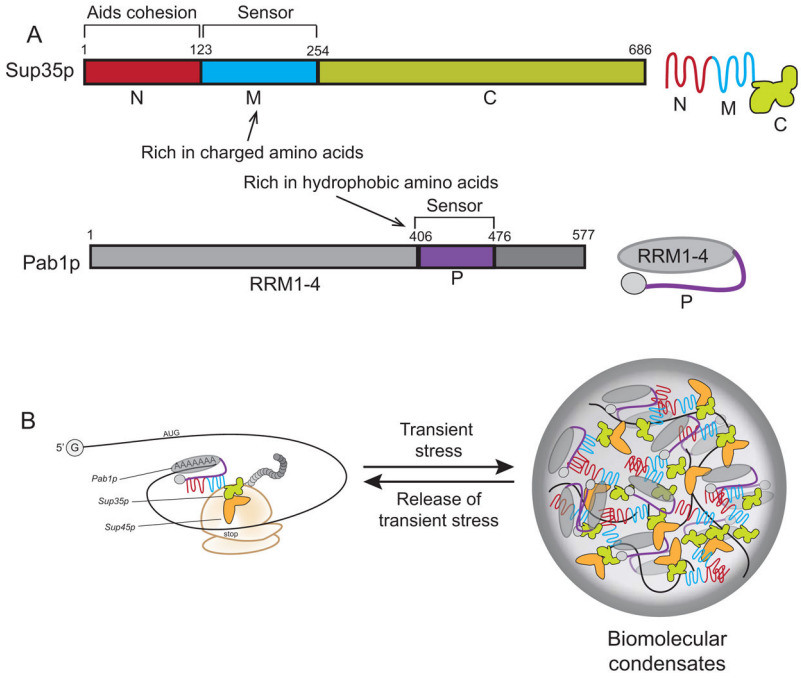


**Figure 2. Sup35p, Pab1p, and the model of phase separation.**

A. Sup35p and Pab1p are described in figure 1, but the same domains provide different functionalities in phase separation. The charged amino acids within the M region of Sup35pare responsive to changes in pH, allowing Sup35p to enter into biomolecular condensates at low pH. The N-terminal region of Sup35p appears to enhance condensation. The P domain (purple) of Pab1p is hydrophobic, is temperature responsive and can also phase separate. B. During transient stress, the sensor regions of both Sup35p and Pab1p signal the proteins to undergo phase separation into biomolecular condensates. It should be noted that the sensor regions of both proteins also participate in protein interactions between Sup35p and Pab1p as shown in figure 1.

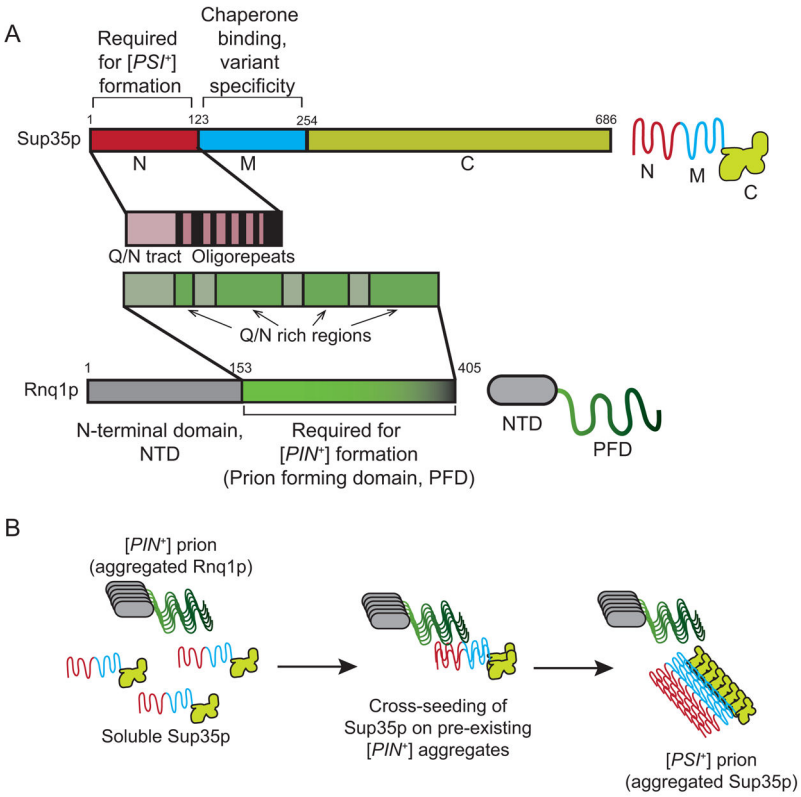


**Figure 3. Sup35p, Rnq1p, and the model of prion formation through cross seeding.**

A. The N-terminal region of Sup35p contains both aglutamine and asparagine (Q/N) rich region and oligopeptide repeats that are required for prion formation. The M region interacts with chaperone machinery and fosters the formation of certain [*PSI*$^{+}$] variants. The Rnq1 protein has a dispensable N-terminal region and a C-terminal domain that contains 4 Q/N rich regions involved [*PIN*$^{+}$] prion maintenance. A symbol for the Rnq1 protein and its domains is shown to the right. B. [*PSI*$^{+}$] formation is proposed to be enhanced through a cross seeding mechanism in which the[*RNQ*$^{+}$] prion is able to nucleate Sup35p assembly into the [*PSI*$^{+}$] prion. It is thought that interactions between Q/N rich regions mediate cross seeding.

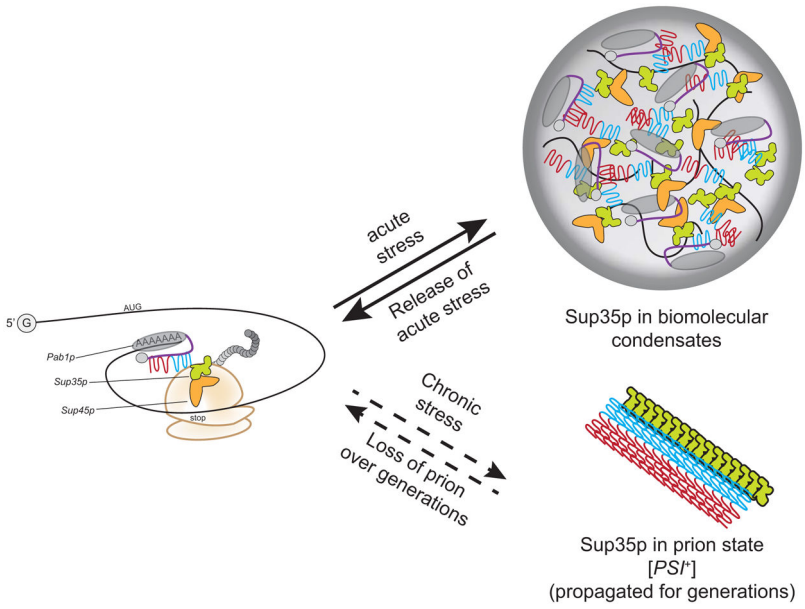


**Figure 4. Sup35p response to different stresses.**

The ability of Sup35p to enter phase transition could be dependent upon the type of stress. Transient (acute) stress could foster Sup35p to condense into biomolecular condensates, whereas extreme, long-term (chronic) stress could foster Sup35p into the prion form.