THE ROLE OF RIBOSOME-ASSOCIATED PROTEIN QUALITY CONTROL IN A PRION-BASED EPIGENETIC STATE

by

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A THESIS

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Prions are heritable, alternatively-folded proteins that have been implicated in rare but fatal mammalian neurodegenerative diseases. However, they can also act as a beneficial epigenetic mechanism in budding yeast and other organisms by altering various cellular pathways such as gene expression and metabolism. A recently discovered yeast prion allows cells to adopt a 'live fast, die young' strategy, accelerating growth rate at the cost of a shortened lifespan. The [*BIG*$^{+}$] prion is a form of Pus4, a highly conserved RNA-modifying enzyme, and exhibits the following phenotypes: accelerated cell proliferation, increased cell size, shortened lifespan, and increased protein synthesis activity. The mechanism by which the [*BIG*$^{+}$] prion induces this change remains unknown, but increased translation activity may provide clues to its mechanism. Therefore, this thesis investigates the ribosome-associated protein quality control pathway (RQC), a cellular system involved in maintaining translation efficacy, as a potential contributor to the [*BIG*$^{+}$] protein synthesis phenotypes. A genetic approach was taken to knock out each of four genes coding for proteins involved in RQC. Luciferase reporter assays were used to examine the difference in translation phenotypes of these mutant strains in the context of the naïve and prion states. A

significant change in the [*BIG*$^{+}$] translation phenotype of mutants as compared to non-prion strains suggested the involvement of at least two genes in the RQC pathway, Hbs1 and Rli1. These results are some of the first mechanistic insights into how this prion affects translation, encouraging future investigations of the involvement of the RQC pathway in the [*BIG*$^{+}$] state.

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My family have my sincerest appreciation for their dedication to learning about my research topic, and I would not be where I am today without the support and influence of my incredible friends. I would also like to thank my cat, Linus Pawling, for enduring my countless practice talks, though he has refused to ever provide comments.

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This thesis is dedicated to the memory of Professor Jeff McKnight.

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Introduction

The term ‘prion’ emerged from Stanley Prusiner’s 1982 paper characterizing the *pro*teinaceous *in*fectious particle that gives rise to scrapie, which causes severe neurodegeneration in sheep. The particle was later named PrP for ‘prion protein’ and in its standard form (PrP^{C}) is an endogenous cellular protein, becoming a disease-causing agent only upon converting to an alternate form (PrP^{Sc}) (Shorter & Lindquist, 2005). This alternate form is a result of a conformational change from the standard (naïve) structure of a polypeptide to an alternate folding conformation, a process through which all prions arise (Figure 1). Because the function of proteins is highly dependent on their shape and structure, this shift in conformation is often accompanied by the development of a novel function. In the case of scrapie and other prion-based neurodegenerative diseases such as mad cow disease and Creutzfeldt-Jakob disease, this new function has detrimental and even deadly effects. With the alarming ability to propagate by self-templating, which converts copies of the naïve protein into the prion form, and to be inherited across generations of cells (Chakrabortee et al., 2016; Garcia & Jarosz, 2014), prions have long been regarded warily as a mysterious agent of disease.

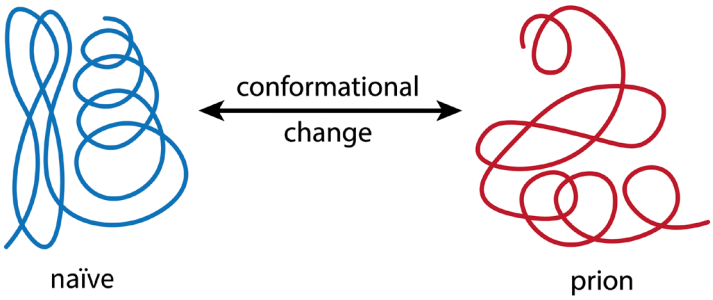

Figure 1. The structural change between naïve and prion forms of a protein.

A reversible conformational change results in an altered structure, often accompanied by a change in function or activity in the protein's prion form.

However, recent studies have identified prions as capable of acting as epigenetic mechanisms, allowing cells to respond to the environment with a heritable, reversible change in the structure of a protein. This can, contrary to the initial impression that prions are only disease-causing agents, create phenotypes that are advantageous to cell growth and survival. The prion [*GAR*$^{+}$] demonstrates an ability to change the metabolic program of yeast in response to cross-kingdom chemical signaling from bacteria, allowing it to generalize its metabolic pathways and adapt to different environments (Jarosz et al., 2014). [*PSI*$^{+}$] was found to be induced by environmental stressors, allowing yeast to respond to changing conditions by increasing translational readthrough and altering gene expression (Byers & Jarosz, 2014; Tyedmers et al., 2008). Because of these novel functions, scientists are beginning to look past the negative connotations of prions and view them as a potentially strategic method of altering a cell's biological program.

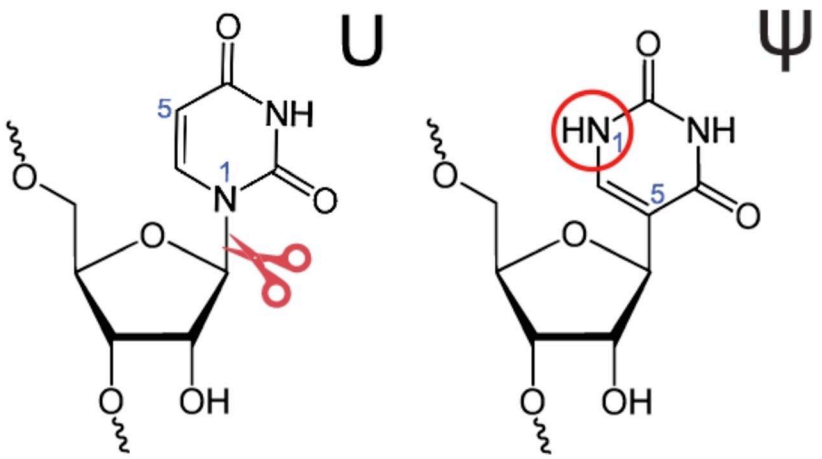

Figure 2. The conversion of the RNA base uridine (U) to pseudouridine (Ψ).

The enzyme Pus4 cleaves a bond in uridine to create its isomer pseudouridine, creating another available hydrogen bond with various effects on translation and RNA stability.

Another beneficial epigenetic state has been induced by transiently overexpressing the protein Pus4 in *Saccharomyces cerevisiae* (budding yeast), forming a prion named [*BIG*$^{+}$] for better in growth. Pus4 is a pseudouridine synthase, an enzyme that converts the base uridine in RNA to pseudouridine (Figure 2). Pus4 primarily pseudouridylates tRNAs, serving to stabilize their tertiary structure (Arnez & Steitz, 1994), though it has also been found to affect translational capacity, degradation, and expression of some mRNAs (Franco & Koutmou, 2022; Karikó et al., 2008). Though the [*BIG*$^{+}$] prion retains pseudouridylation activity (Shaw & Aladin, unpublished), it is unclear how this function relates to the phenotype produced by the prion. By a mechanism yet to be determined, yeast cells harboring [*BIG*$^{+}$] exhibit a significant increase in growth rate and cell size (Figures 3A and 3B), though at the cost of a shortened lifespan. This prion is the primary target of study in the Garcia lab and shows striking similarities to previously characterized prions, such as the heritability of the altered epigenetic state it

produces, its dependence on chaperone protein activity, and the potential of this phenotype to be used for adaptation to different environments (Garcia et al., 2021).

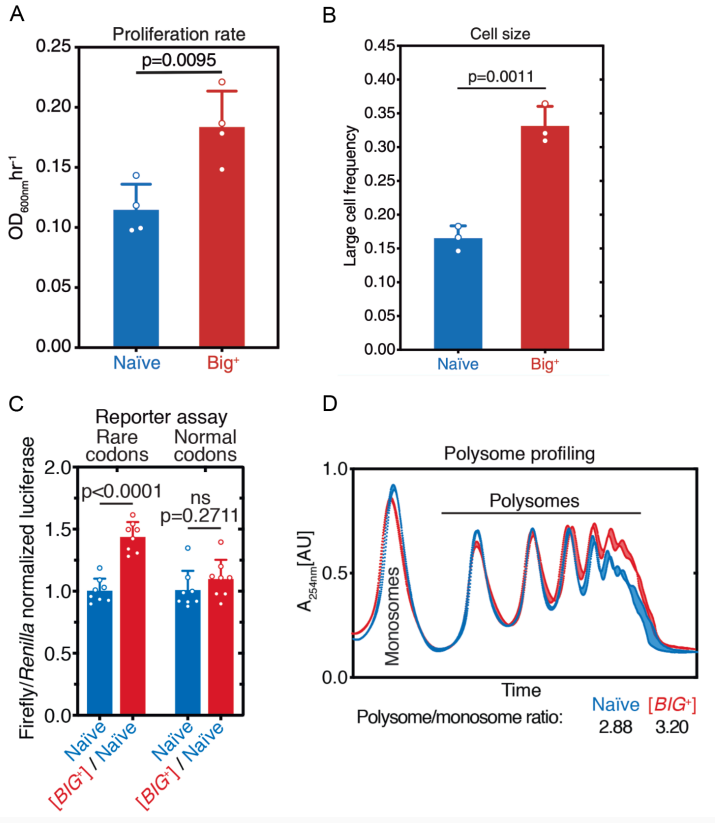

Figure 3. [*BIG*$^+$] phenotype characterization.

A) The proliferation rate of [*BIG*$^+$] yeast is significantly higher than that of naïve strains.

B) The large cell frequency (along with average cell size, data not shown) of [*BIG*$^+$] yeast is significantly higher than that of naïve.

C) [*BIG*$^+$] yeast demonstrates a significant increase in translation as compared to naïve strains when using 'rare' codons to translate Firefly luciferase.

D) [*BIG*$^+$] yeast exhibits a higher frequency of polysomes as compared to naïve yeast. Adapted from Garcia et al. (2021).

[*BIG*$^+$] has also been shown to affect the process of translation, i.e., the synthesis of proteins according to RNA sequence, suggesting a potential alteration in translation regulation or efficacy in this epigenetic state (Figure 3C). This is consistent with

findings that [BIG+] cells harbor more polysomes, which are strings of multiple ribosomes translating the same piece of mRNA (Figures 3D and 4A). This may be related to the increased translation activity found in [*BIG*$^{+}$] cells, as more ribosomes actively translating proteins can increase the output of protein products. However, a stalled ribosome in a polysome can cause trailing ribosomes to collide with it and halt their translation activity as well (Figure 4B). If [*BIG*$^{+}$] yeast rely on polysomes for increased translational capacity, ribosome stalling would affect many more ribosomes than it ordinarily would in a cell with fewer polysomes—yet they continue to exhibit beneficial growth phenotypes. Based on these characterizations of [*BIG*$^{+}$], it is possible that a protein quality control pathway is involved in the prion's mechanism and ability to endow the cell with the observed alterations in translation phenotype.

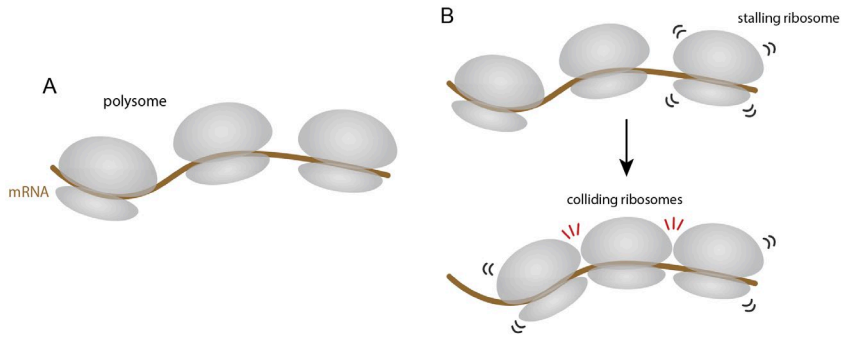

Figure 4. Polysomes and ribosome collisions.

A) Polysomes consist of an mRNA transcript with multiple ribosomes actively translating.

B) A stalling ribosome in a polysome will cause the trailing ribosomes to collide with it, creating a translation 'traffic jam' in which all ribosomes are stalled.

The importance of translation for cell growth and survival renders it a heavily regulated process. Ribosomes stalling, pausing mid-protein synthesis, may interrupt the normal translation process (Figure 5). This not only prevents a partially completed protein from

reaching completion which wastes cellular energy and resources, but also traps the ribosome on the mRNA, unable to be recycled onto other transcripts and reducing the ribosomes available for protein synthesis. Protein quality control pathways such as the ribosome-associated protein quality control (RQC) pathway monitor the status of ribosomes during translation to mitigate these problems. The RQC specifically rescues stalled ribosomes through a series of proteins acting on the stalled complex, disassembling the large and small ribosomal subunits so they can be recycled to assemble on another mRNA transcript, marking the partial nascent protein for degradation because it likely will not function as it should, and degrading the mRNA that may have caused the ribosome to stall originally (Brandman et al., 2012).

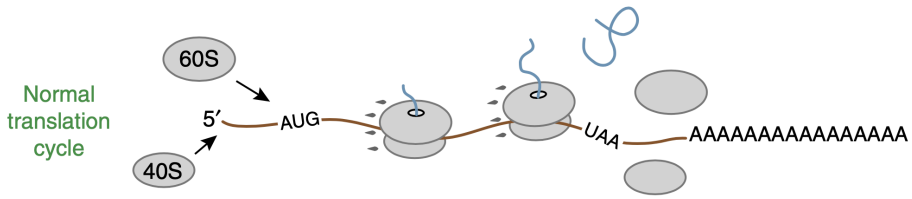

Figure 5. The normal translation cycle.

Ribosomal subunits assemble at the start codon on an mRNA transcript. The protein polypeptide is created as tRNAs escort the appropriate amino acids to the ribosome by matching codons in the mRNA. The nascent protein is formed by the linkage of these amino acids and released upon reaching a STOP codon. Adapted from Brandman & Hegde (2016).

Ribosome stalling during translation can be caused by damaged mRNA, strings of certain unusual or difficult-to-translate codons in the mRNA, tertiary structures within the mRNA, and other similar impediments (Figure 6) (Shoemaker & Green, 2012). Genetic constructs have been developed to use these triggers in a deliberate manner when attempting to induce RQC activity (Brandman et al., 2012; Brandman & Hegde,

2016). Upstream RQC protein interactions with the ribosome identify and mark ribosomes stalled during translation (Brandman & Hegde, 2016), while the rest of the pathway facilitates dissociation of the ribosomal subunits and targets the nascent protein for degradation (Shao et al., 2015). Together, the proteins in the pathway mitigate the effects of translation obstructions to increase the efficiency of protein synthesis.

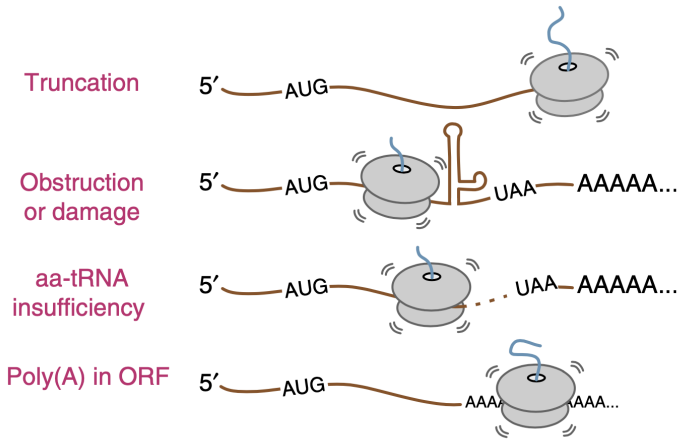

Figure 6. Several triggers for ribosome stalling during translation.

Factors such as mRNA truncation, physical obstruction or damage, or insufficiency of available tRNAs carrying the appropriate amino acid can contribute to failure of the ribosome to complete the normal translation cycle. Adapted from Brandman & Hegde (2016).

The proteins involved in this pathway are numerous in yeast and include Asc1, Hel2, Dom34, Hbs1, Rli1, Rqc2, Ltn1, Rqc1, and a complex of Cdc48-Ufd1-Npl4 (Figure 7), all of which have homologs in humans (Brandman & Hegde, 2016). For the purposes of this investigation, four of the first proteins in the pathway (Asc1, Hel2, Hbs1, and Rli1) are the first to be studied in relation to the [*BIG*$^{+}$] prion.

Asc1 and Hel2 have been identified to function by targeting stalled ribosomes, facilitating the downstream steps of the RQC pathway (Sitron et al., 2017; Winz et al., 2019). Hel2 has also been found to facilitate nascent protein degradation, as well as to

exhibit some RNA cleaving activity (Ikeuchi et al., 2019), while Asc1 has been recently implicated in ribosome disassembly (Juszkiewicz et al., 2020). Dom34 and Hbs1 are closely associated with one another in the process of ribosomal subunit dissociation (Doma & Parker, 2006; Shoemaker et al., 2010), along with Rli1 as an essential component of ribosomal release (Van den Elzen et al., 2014). There are several subtle differences in the type of ribosome stalling mediated by each protein, such as apparent preference of the Dom34-Hbs1-Rli1 pathway for ribosomes stalled specifically at the 3' end of a truncated mRNA (Brandman & Hegde, 2016). This allows the RQC to respond to a variety of translation obstructions.

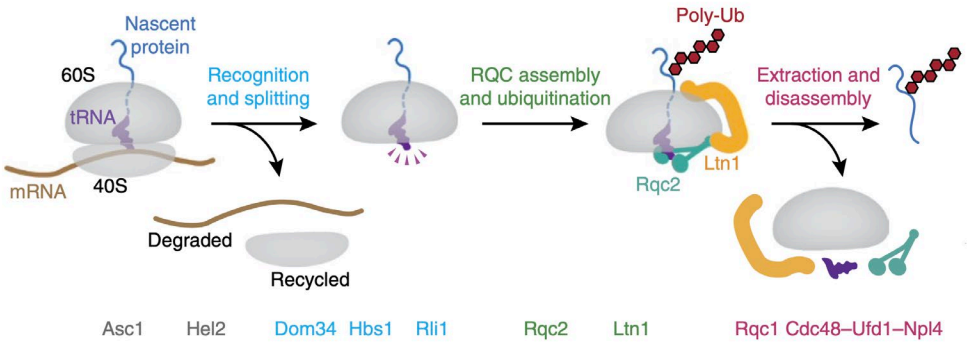

Figure 7. The ribosome-associated protein quality control (RQC) pathway.

The RQC involves a sequence of events to split the stalled ribosome, extract and recycle translation components, and degrade the nascent protein. Adapted from Brandman & Hegde (2016).

As a major component of translation regulation, the RQC pathway could potentially be implicated in the mechanism of the [*BIG*$^{+}$] prion causing the observed translation phenotype. This project investigates the effects of this pathway on the [*BIG*$^{+}$] phenotype, aiming to elucidate part of the prion's epigenetic mechanism and add to the overall understanding of prions as beneficial cellular agents. This is achieved by the creation of biological models in which the pathway is disrupted and the use of these

models in reporter assays that measure relative amounts of translation activity in cell cultures.

The mechanisms of cell growth, inheritance, and gene expression are crucial for understanding biology, particularly that of human disease. Protein quality control pathways and prion proteins acting as epigenetic mechanisms constitute two significant components of these processes, and thus two intriguing areas of study. This thesis aims to combine both areas in an effort to uncover how the RQC pathway may be impacted by the epigenetic influence of prions.

Methods

Creation of deletion strains

Strains of yeast with single gene deletions for each of the RQC genes in question (*ASC1*, *HEL2*, *HBS1*, and *RLI1*) were created to investigate the effects of removing these genes on the [*BIG*$^{+}$] translation phenotype (Figure 8).

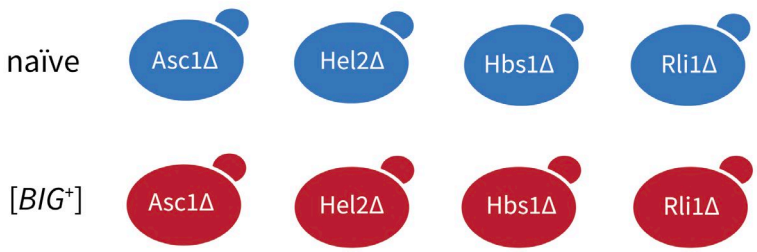

Figure 8. Sets of deletion mutants created for assays.

Single deletions of each gene were crossed into either a naïve or [*BIG*$^{+}$] background.

Yeast strains from pre-made stocks containing each of these deletions were mated with wild-type naïve or [*BIG*$^{+}$] phenotype strains of the opposite mating type by patching them together on a YPD agar plate to produce diploid offspring with a genome copy from each parent. This was done to create yeast containing both the specific gene deletion and the prion. The next step, sporulation, resulted in distribution of the genes from each parent cell to create haploid offspring. The non-Mendelian inheritance pattern of prions as cytoplasmic elements passed down to all daughter cells promoted retention of the prion phenotype in [*BIG*$^{+}$] crosses and therefore the presence of a daughter cell with the prion phenotype in addition to the desired deletion (or wild-type allele in the other half of progeny) (Figure 9).

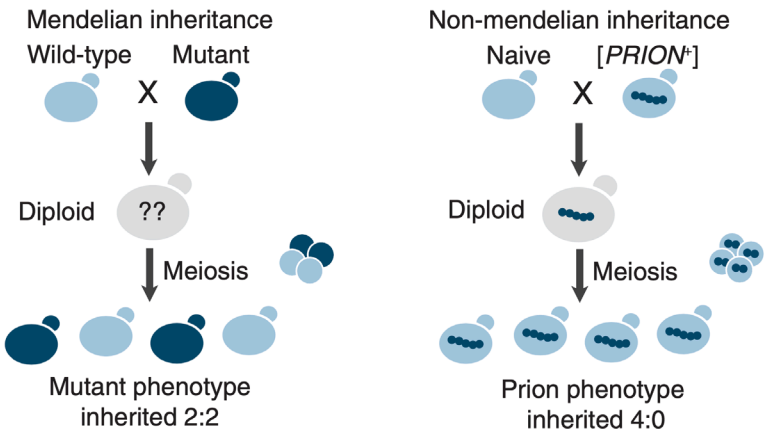

Figure 9. Mendelian vs. non-Mendelian inheritance.

Mutant phenotypes are distributed in a 2:2 ratio when crossed with wild-type and sporulated to produce four haploid offspring, while prion phenotypes, inherited through the cytoplasm, are reflected in all offspring. Adapted from Garcia et al. (2021).

To induce sporulation, diploid yeast was first grown for two days in pre-sporulation (nutrient-rich) liquid media at 25 °C before being transferred to sporulation (nutrient-poor) liquid media. Cells were incubated at 25 °C for six additional days, during which they underwent meiosis to form four haploid spore offspring, encased together by an ascus wall. The ascus was then digested by resuspension of the spores in a chemical solution. The digested and diluted solution was then streaked on a YPD agar plate for dissection under a microscope, where the four spores were separated from one another.

Dissected tetrads were grown on these plates for three days at 30 °C. Tetrads with colonies from all four spores were selected by auxotrophic markers and antibiotic resistance to confirm the presence of the deletion cassette. Gene deletions were confirmed by PCR. Spores containing the deletion cassette were frozen down into 25% glycerol stocks at -80 °C for future experiments.

Luciferase assays

Mutant yeast strains from the sporulation process were transformed with plasmids containing the genes for Firefly and *Renilla* luciferase proteins, which are luminescent reporter genes that emit light quantitatively measurable by a luminometer. Data from this type of assay displays the relative amounts of translation among samples. Both genes are expressed by the same promoter, allowing *Renilla* to be used as an internal control for gene expression levels and plasmid copy numbers. Each strain was transformed with two luciferase plasmids, one containing a Firefly gene composed of standard (common) codons, and one containing a Firefly gene composed of rare codons with fewer available tRNAs with complementary anticodons (Figure 10). These rare codons typically cause a significant reduction in translation due to their difficulty matching with an appropriate tRNA.

Figure 10. Two versions of the Firefly luciferase gene.

The 'normal codon' version of the Firefly gene is composed of codons with abundant complementary tRNAs charged with the appropriate amino acids, while the 'rare codon' version is composed of codons with few complementary tRNAs and are therefore more difficult to match efficiently during translation. Illustration by Eve Weinberg.

Transformed strains were stored in 25% glycerol stocks at -80 °C and streaked onto uracil (URA) dropout agar plates to obtain single, genetically-identical colonies and retain the luciferase plasmids prior to performing the assay. The yeast was grown on plates at 30 °C for two to three days before inoculation of six single colonies per mutation/phenotype combination in 96-well plates containing synthetic complete (SC) -URA liquid media, which has been shown to facilitate even growth in naïve and [BIG^{+}] strains. These cultures were grown at 30 °C for two more days to ensure saturation before dilution of the saturated culture in fresh SC -URA media. OD_{600}, a measure of optical density proportional to culture saturation, was measured until cultures grew to an average OD_{600} of 0.6–0.8 (around 4–5 hours) prior to lysis. Cells were then lysed before addition of Promega Dual-Glo luciferase reagent, which provides a substrate necessary for Firefly luciferase to luminesce. After incubation, the luminescence of

each sample was measured in a luminometer. Promega Stop & Glo reagent was added to quench the Firefly luminescence and to provide a substrate for *Renilla* luciferase, the luminescence of which was also measured in a luminometer following incubation. The outline of this procedure is represented in Figure 11.

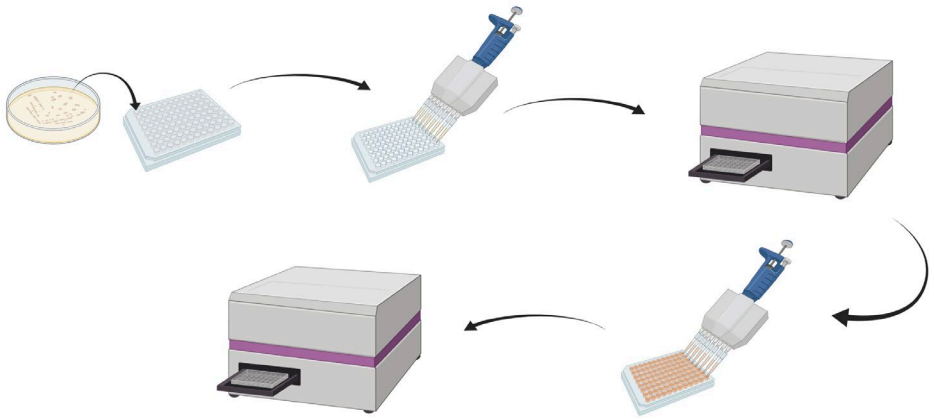

Figure 11. Luciferase assay procedure.

Yeast is selected from single colonies after streaking on an agar plate and inoculated in a 96-well plate with the appropriate liquid media. Cultures in log phase of growth are lysed prior to adding reagents to induce luciferase luminescence, read in a luminometer machine. This figure was created with BioRender.com.

Measurements were normalized in individual wells by dividing Firefly luminescence by that of *Renilla*, then by OD_{600} measurements taken just prior to cell lysis to account for differences in culture density. Averages were then taken across all replicates and [BIG^{+}] averages were divided by the corresponding naïve average to obtain a ratio relative to naïve cell luminescence. Statistical significance was determined between datasets with an unpaired student's t-test.

Results

Previously published data by Garcia et al. (2021) (Figure 12) demonstrates a significant difference in translation between naïve and [*BIG*$^{+}$] states as measured by luciferase assay when using plasmids containing rare ('slow') codons for the Firefly gene. This data reflects a statistically significant increase in translation in [*BIG*$^{+}$] strains when using these rare codons, with a slight but nonsignificant upregulation using normal codons. This finding has characterized the [*BIG*$^{+}$] phenotype as more proficient in translation than the naïve phenotype, especially when utilizing a particularly difficult mRNA transcript to synthesize the protein.

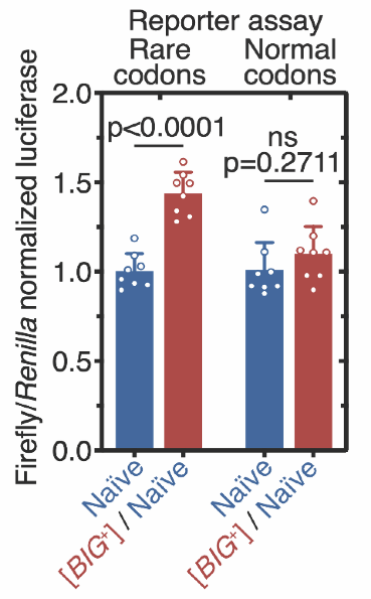

Figure 12. Previously published wild-type naïve and [*BIG*$^{+}$] luciferase assay data. [*BIG*$^{+}$] yeast demonstrates a significant increase in translation as compared to naïve strains when using 'rare' codons to translate Firefly luciferase. Adapted from Garcia et al. (2021).

Because of the increase in translation activity in [*BIG*$^{+}$] cells as compared to naïve cells, specifically when utilizing rare codons and the involvement of RQC in translation

efficiency and progression, it was hypothesized that removal of RQC-related genes would affect the [*BIG*$^+$] translation phenotype. To analyze the difference in translation occurring in [*BIG*$^+$] versus naïve cells, luciferase measurements were normalized to naïve averages for both plasmid versions respectively (Figure 13).

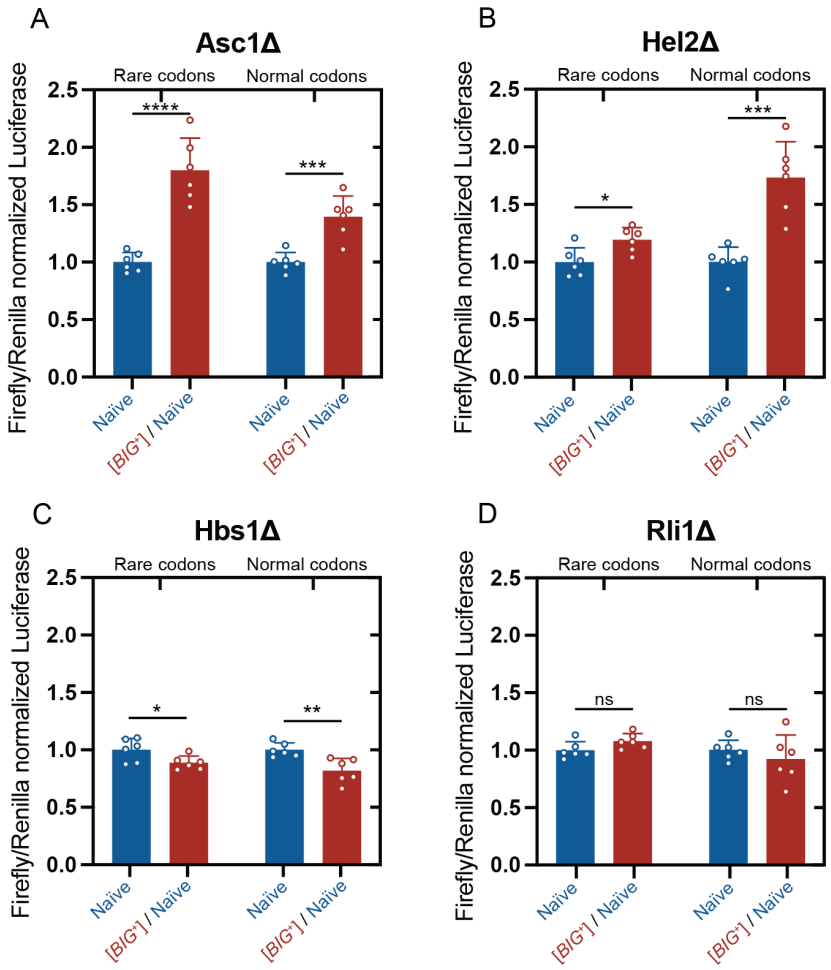

Figure 13. Mutant luciferase assay graphs.

Relative translation levels in [*BIG*$^+$] mutant strains are normalized to mutant naïve translation levels within each codon type.

The most striking deviations from the trend observed in previously published data occurred in both *hbs1*Δ and *rli1*Δ mutants. [*BIG*$^+$] *rli1*Δ mutants were measured to have a nearly equal amount of translation as the corresponding naïve mutants, with no

statistically significant difference between the data sets for naïve and [*BIG*$^{+}$] for either plasmid type (Figure 13D). Assays of *hbs1*Δ mutants showed a marked decrease in translation of [*BIG*$^{+}$] strains as compared to naïve for both plasmids, with statistically significant drops for each (Figure 13C). The nonsignificance between [*BIG*$^{+}$] and naïve measurements in the *rli1*Δ data, in addition to the inverse relationship of [*BIG*$^{+}$] to naïve translation phenotypes in the *hbs1*Δ data, represent significant deviations from the pattern of translation measurements in non-mutants.

Differences in the results of *asc1*Δ mutants included a significant increase in translation using both codon versions, as opposed to solely the rare codons as seen in non-mutant data (Figure 13A). In *hel2*Δ mutants, both sets of codons again produced a significant increase in [*BIG*$^{+}$] translation; however, the more significant increase in translation was seen using normal codons (Figure 13B). These results demonstrate less deviation from non-mutant data than *hbs1*Δ and *rli1*Δ mutants, while retaining the overall pattern of an increase in [*BIG*$^{+}$] translation phenotype over naïve.

Discussion

The luciferase assay data revealed a striking change in the translation phenotype of [*BIG*$^{+}$] *hbs1*Δ and *rli1*Δ mutants when compared to corresponding naïve mutants. The *hbs1*Δ mutants revealed no significant difference between [*BIG*$^{+}$] and naïve translation phenotypes, and *rli1*Δ mutants demonstrated a significant decrease in [*BIG*$^{+}$] translation phenotype, both in the normal and rare codon reporters. The fact that the removal of these proteins disrupted the translation phenotype of non-mutant [*BIG*$^{+}$] yeast to such an extent suggests that both proteins likely play some important role in the normally enhanced translation of [*BIG*$^{+}$] cells. The loss of the translation increase could mean that these proteins play a central role in the translation regulation or efficiency that results in the characterized translation phenotype in [*BIG*$^{+}$] yeast.

Given that *asc1*Δ and *hel2*Δ mutants did not demonstrate a significant change in translation, it is less clear whether they are involved in the translation phenotype of [*BIG*$^{+}$] yeast. Both sets of mutants revealed an increase in [*BIG*$^{+}$] translation as compared to naïve yeast, similar to the phenotype observed in published wild-type (WT) data. The *asc1*Δ mutants followed this pattern closely, with a more significant difference when using rare codons. However, the increase in [*BIG*$^{+}$] translation was overall more significant using both plasmids, which could suggest an overall enhancement of [*BIG*$^{+}$] translation upon loss of this protein. The *hel2*Δ mutants inverted the WT pattern, with a more significant increase in translation using ‘normal’ codons. This result could also indicate some change to the translation mechanism in [*BIG*$^{+}$] but is difficult to interpret.

Given the roles of each of these proteins in the RQC pathway, some preliminary ideas about their implication in the [*BIG*$^{+}$] phenotype can be formed. Because Asc1 and Hel2 are involved in upstream steps in the pathway, their deletion could be causing an increase in translational readthrough despite any ribosome stalling that may occur, resulting in the observed increase in translation of luciferase transcripts. This also suggests that the increased translation phenotype in nonmutant [*BIG*$^{+}$] yeast relies on faithful translation, as translational readthrough is likely to include translation errors and if increased readthrough was part of the [*BIG*$^{+}$] mechanism, nonmutant data would more closely resemble that of these mutants. In addition to this, because the primary understanding of Asc1 and Hel2 protein function is that they assist in the targeting of stalled ribosomes, the single deletions investigated in this thesis could be compensated for by the redundant function of the other protein; therefore, a less significant impact on translation is observed in single mutants but could be observed more strongly in a double deletion mutant.

Given that Dom34, Rli1, and Hbs1 are implicated in a complex involved in ribosomal subunit dissociation, deletion of these proteins is likely to interfere more with the progression of translation and therefore decrease successful protein synthesis if a ribosome has stalled but is unable to be removed from the transcript, reducing translation efficiency. This is consistent with the observed decrease in translation of luciferase transcripts in these [*BIG*$^{+}$] mutants. This also supports the theory that RQC activity in [*BIG*$^{+}$] yeast may be altered in a way that contributes to enhanced efficiency in protein synthesis as well as production of accurate protein products, and that these are both important for the observed [*BIG*$^{+}$] translation phenotype. Because all three

proteins work together to perform ribosomal subunit dissociation, double deletions could also affect this process more profoundly and result in more striking impacts on translation phenotype once a more significant portion of the complex has been removed. Additional investigation would be required to ascertain whether the observed effects are due to a lack of a role for a given protein in the [*BIG*$^{+}$] phenotype, or instead due to the context of the protein's function and deletion.

The data collected from rare codon luciferase reporters may also provide an additional indication of how RQC activity is implicated in the [*BIG*$^{+}$] phenotype, as rare codons are one of the known triggers for inducing ribosome stalling and therefore RQC activity. In the context of non-mutant strains, the more significant increase in luciferase reporter translation when utilizing rare codons could point to involvement of RQC activity in producing this particular phenotype of the prion. The impact on translation of this rare codon reporter as compared to that of the normal codon in mutant reporter assays may then demonstrate how RQC pathway disruption affects the [*BIG*$^{+}$] phenotype in mutants.

The lack of significant impact on the rare codon translation phenotype in *asc1*Δ mutants may suggest a relatively minor influence of Asc1 on RQC activity contributing to the [*BIG*$^{+}$] phenotype. In contrast, there is a less significant increase of rare codon reporter translation in *hel2*Δ mutants when compared to non-mutant data, which could indicate that Hel2 specifically is important for RQC pathway initiation in [*BIG*$^{+}$] yeast. Support for this idea also comes from findings demonstrating preferential interaction of Hel2 with collided stalled ribosomes as opposed to stalled monosomes (Ikeuchi et al.,

2019), which connects to the previously discussed higher frequency of polysomes and therefore increased capacity for ribosome collisions in [*BIG*$^+$] yeast.

A significant impact on rare codon translation was also observed in *hbs1*Δ and *rli1*Δ mutants, though the impact on reporter translation was similar between rare and normal codon reporters. This could mean that while both proteins appear to be crucial for the overall translation phenotype observed in [*BIG*$^+$] yeast, they are less specifically involved in RQC pathway initiation, which is consistent with their known roles in the pathway overall.

While the data strongly suggest some involvement of RQC proteins in the [*BIG*$^+$] translation phenotype, it will be necessary to repeat these experiments. The data shown here are compared to previously published WT data by the Garcia lab, while ideally, they would be presented alongside a WT control measured in the same assay. This proved difficult in the experimental time frame due to issues using previously frozen glycerol stocks of WT strains. We are currently troubleshooting this problem, and success will provide more credibility to collected data and open more opportunities for data analysis.

For all mutants, additional experiments (such as those discussed below) would be required to draw any specific conclusions about their involvement in the translation phenotype of [*BIG*$^+$] cells. However, the results of this assay are encouraging for further investigations into these specific proteins and the RQC pathway overall.

Future directions

A necessary future direction for this investigation, as mentioned, is the creation of reliable WT control strains in both naïve and [*BIG*$^+$] phenotypes with which to

perform luciferase assays. The current data is encouraging for future investigations of RQC proteins, but not definitive about their effects on the [*BIG*$^{+}$] translation phenotype. Reliable WT controls would also introduce the option to analyze the assay data in other normalization schemes that could be informative, such as normalizing all mutant data (naïve and [*BIG*$^{+}$]) to a WT naïve control to consider changes in translation relative to the non-mutant phenotype. However, WT controls measured in the same assay as the mutants are necessary to accomplish this and therefore remain a priority.

Growth assays evaluating the difference in growth rate between naïve and [*BIG*$^{+}$] mutants are planned for this project and will provide another informative set of data. These assays measure the optical density of yeast over time as they grow, in order to assess differences in growth rates. Given that proliferation rate is another phenotype altered in [*BIG*$^{+}$], and that translation is also closely tied to cellular growth, this information would further inform our conclusions about RQC involvement in [*BIG*$^{+}$] phenotypes. These assays would also incorporate stress conditions such as the inclusion of cycloheximide (a translation elongation inhibitor) in the media, allowing further manipulation of the cell's ability to synthesize proteins.

A direction of this investigation that has proved difficult to pursue thus far is collecting data using *dom34*Δ mutants. In naïve yeast, these mutants are not possible to create using the yeast mating and diploid sporulation technique used for the other four sets of mutants due to the inability of homozygous *dom34*Δ mutants to undergo meiosis, which is the process by which yeast sporulates (Davis & Engebrecht, 1998). However, attempts to produce these mutants in both naïve and [*BIG*$^{+}$] crosses resulted in successful sporulation of [*BIG*$^{+}$] diploids only, indicating that the prion phenotype may

somehow suppress this meiotic defect in *dom34*Δ mutants and providing further evidence of interaction between the [*BIG*$^{+}$] phenotype and this RQC protein.

Because of this, another approach such as CRISPR-mediated gene replacement is required to create these mutants, which was attempted but not completed during the span of this thesis due to experimental challenges. As discussed, this protein is involved in the heart of the RQC pathway along with several other proteins in ribosomal subunit dissociation. The ability to consider the data from a *dom34*Δ mutant alongside mutants of the other related proteins will strengthen the conclusions drawn and consideration of the functional context of these proteins.

Because of the demonstrated differences in translation phenotype of the deletion mutants, another informative future direction for this project is the creation and testing of multi-deletion mutants. This would include creating mutant strains with combinations of deletions, such as *asc1*Δ*hel2*Δ or *dom34*Δ*hbs1*Δ*rli1*Δ mutants. These groupings would delete multiple proteins associated with similar functions or with one another in the pathway, examining the possibility that a single deletion is compensated for by another protein with a redundant function. Deleting a group of associated proteins would give more compelling data to determine whether it is the complexes involved in the RQC pathway or individual proteins associated with RQC that are involved in the [*BIG*$^{+}$] mechanism.

Another direction that would specifically test the involvement of RQC would utilize DNA constructs with a sequence designed to induce ribosome stalling between two reporter genes, as illustrated in Figure 14. These constructs have been used by Brandman et al. (2012) in similar reporter assays to study RQC activity. The relative

amounts of products from the translation of each reporter gene would be analyzed by flow cytometry, yielding data similar to that of the luciferase assays. Though the rare codon reporter in luciferase assays may act as an RQC trigger and provide similar data to these constructs, these stalling sequences specifically designed to trigger for RQC activity provide an additional way to investigate the role of the overall pathway in the [*BIG*$^{+}$] phenotype directly, as opposed to the roles of individual proteins within the pathway.

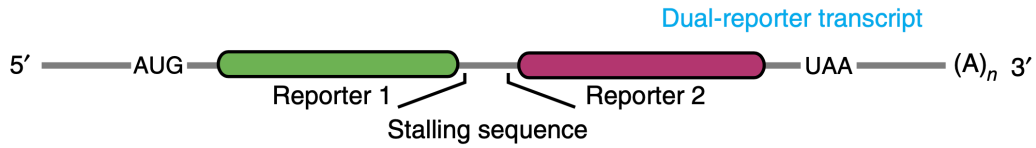

Figure 14. Dual-reporter transcript with an RQC-inducing stalling sequence.

The amounts of each reporter gene measured in an assay will give a relative measurement of translation of each gene. Adapted from Brandman & Hegde (2016).

Conclusions

The data collected using deletion mutants in [*BIG*$^{+}$] and naïve yeast confirms the hypothesis that protein quality control is involved in the mechanism of this prion. Though the assays performed with these mutants do not reveal the exact nature of the mechanism, they display a clear perturbation of the established translation phenotype upon removal of RQC proteins. This provides a strong basis for additional investigation into this pathway and the details of how it may interact with the [*BIG*$^{+}$] prion. Future experiments aimed at uncovering the molecular interactions will be among the first to contribute to characterization of the prion's mechanism, furthering not only the understanding of how [*BIG*$^{+}$] exerts its influence but the knowledge on prions as epigenetic mechanisms and how protein quality control may be impacted by them.

Glossary

[*BIG*$^{+}$]: A prion protein found in yeast, the prion form of the enzyme Pus4; acts as an epigenetic mechanism to produce a phenotype with increased growth rate and cell size

DNA: Deoxyribonucleic acid; the double-stranded molecules that store genetic information in the nucleus (in eukaryotic cells)

Codon: A set of three bases in DNA or RNA that correspond to the matching 'anticodon' of a tRNA carrying a specific amino acid

Culture: Yeast grown in liquid media

Epigenetics: The study of processes that alter gene expression without changes to DNA sequence; includes heritable, non-genetically-based phenotypes

Eukaryote: An organism of the biological domain *Eukaryota*, including both yeast and mammals

Mechanism: The process by which a biological event occurs; often includes a series of interactions between molecules and results in a specific effect

Mutant: A strain of yeast in which the genotype has been manipulated and deviates from wild-type

Phenotype: Physically or externally observed traits

Plasmid: A circular piece of DNA that is easily inserted into cells, facilitates expression of a desired gene in cells

Polysome: A string of ribosomes simultaneously translating one mRNA transcript

Prion: A protein with a structure different from that of another with the same amino acid sequence; often referenced in comparison to a 'native' protein conformation

Ribosome: Protein complexes that synthesize proteins by linking amino acids together according to RNA sequence

RNA: Ribonucleic acid; the single-stranded 'messenger' molecule transcribed from DNA

RQC: Ribosome-associated protein quality control pathway; a cellular response to ribosome stalling that allows the cell to recycle translation components

Strain: A genetic line of yeast

Translation: The process by which ribosomes link amino acids together according to RNA sequence to form protein products

tRNA: A type of RNA with tertiary structure, functions to escort amino acids to ribosomes during translation

Wild-type/WT: The standard geno- and phenotype found in a given species in nature (used specifically in reference to genotypes in the context of this investigation)

YPD: Yeast extract peptone dextrose; a nutrient-rich, standard media for yeast growth

Bibliography

Arnez, J. G., & Steitz, T. A. (1994). Crystal structure of unmodified tRNA(Gln) complexed with glutaminyl-tRNA synthetase and ATP suggests a possible role for pseudo-uridines in stabilization of RNA structure. *Biochemistry*, *33*(24), 7560–7567. https://doi.org/10.1021/bi00190a008

Brandman, O., & Hegde, R. S. (2016). Ribosome-associated protein quality control. *Nature Structural & Molecular Biology*, *23*(1), 7–15. https://doi.org/10.1038/nsmb.3147

Brandman, O., Stewart-Ornstein, J., Wong, D., Larson, A., Williams, C. C., Li, G.-W., Zhou, S., King, D., Shen, P. S., Weibezahn, J., Dunn, J. G., Rouskin, S., Inada, T., Frost, A., & Weissman, J. S. (2012). A ribosome-bound quality control complex triggers degradation of nascent peptides and signals translation stress. *Cell*, *151*(5), 1042–1054. https://doi.org/10.1016/j.cell.2012.10.044

Byers, J. S., & Jarosz, D. F. (2014). Pernicious Pathogens or Expedient Elements of Inheritance: The Significance of Yeast Prions. *PLOS Pathogens*, *10*(4), e1003992. https://doi.org/10.1371/journal.ppat.1003992

Chakrabortee, S., Byers, J. S., Jones, S., Garcia, D., Bhullar, B., Chang, A., She, R., Lee, L., Fremin, B., Lindquist, S., & Jarosz, D. F. (2016). Intrinsically Disordered Proteins Drive Emergence and Inheritance of Biological Traits. *Cell*, *167*(2), 369-381.e12. https://doi.org/10.1016/j.cell.2016.09.017

Davis, L., & Engebrecht, J. (1998). Yeast dom34 mutants are defective in multiple developmental pathways and exhibit decreased levels of polyribosomes. *Genetics*, *149*(1), 45–56. https://doi.org/10.1093/genetics/149.1.45

Doma, M. K., & Parker, R. (2006). Endonucleolytic cleavage of eukaryotic mRNAs with stalls in translation elongation. *Nature*, *440*(7083), 561–564. https://doi.org/10.1038/nature04530

Franco, M. K., & Koutmou, K. S. (2022). Chemical modifications to mRNA nucleobases impact translation elongation and termination. *Biophysical Chemistry*, *285*, 106780. https://doi.org/10.1016/j.bpc.2022.106780

Garcia, D. M., Campbell, E. A., Jakobson, C. M., Tsuchiya, M., Shaw, E. A., DiNardo, A. L., Kaeberlein, M., & Jarosz, D. F. (2021). A prion accelerates proliferation at the expense of lifespan. *ELife*, *10*, e60917. https://doi.org/10.7554/eLife.60917

Garcia, D. M., & Jarosz, D. F. (2014). Rebels with a cause: Molecular features and physiological consequences of yeast prions. *FEMS Yeast Research*, *14*(1), 136–147. https://doi.org/10.1111/1567-1364.12116

Ikeuchi, K., Tesina, P., Matsuo, Y., Sugiyama, T., Cheng, J., Saeki, Y., Tanaka, K., Becker, T., Beckmann, R., & Inada, T. (2019). Collided ribosomes form a unique structural interface to induce Hel2-driven quality control pathways. *The EMBO Journal*, *38*(5), e100276. https://doi.org/10.15252/embj.2018100276

Jarosz, D. F., Brown, J. C. S., Walker, G. A., Datta, M. S., Ung, W. L., Lancaster, A. K., Rotem, A., Chang, A., Newby, G. A., Weitz, D. A., Bisson, L. F., & Lindquist, S. (2014). Cross-kingdom chemical communication drives a heritable, mutually beneficial prion-based transformation of metabolism. *Cell*, *158*(5), 1083–1093. https://doi.org/10.1016/j.cell.2014.07.025

Juszkiewicz, S., Speldewinde, S. H., Wan, L., Svejstrup, J. Q., & Hegde, R. S. (2020). The ASC-1 Complex Disassembles Collided Ribosomes. *Molecular Cell*, *79*(4), 603-614.e8. https://doi.org/10.1016/j.molcel.2020.06.006

Karikó, K., Muramatsu, H., Welsh, F. A., Ludwig, J., Kato, H., Akira, S., & Weissman, D. (2008). Incorporation of Pseudouridine Into mRNA Yields Superior Nonimmunogenic Vector With Increased Translational Capacity and Biological Stability. *Molecular Therapy : The Journal of the American Society of Gene Therapy*, *16*(11), 1833–1840. https://doi.org/10.1038/mt.2008.200

Prusiner, S. B. (1982). Novel proteinaceous infectious particles cause scrapie. *Science (New York, N.Y.)*, *216*(4542), 136–144. https://doi.org/10.1126/science.6801762

Shao, S., Brown, A., Santhanam, B., & Hegde, R. S. (2015). Structure and assembly pathway of the ribosome quality control complex. *Molecular Cell*, *57*(3), 433–444. https://doi.org/10.1016/j.molcel.2014.12.015

Shoemaker, C. J., Eyler, D. E., & Green, R. (2010). Dom34:Hbs1 promotes subunit dissociation and peptidyl-tRNA drop-off to initiate no-go decay. *Science (New York, N.Y.)*, *330*(6002), 369–372. https://doi.org/10.1126/science.1192430

Shoemaker, C. J., & Green, R. (2012). Translation drives mRNA quality control. *Nature Structural & Molecular Biology*, *19*(6), 594–601. https://doi.org/10.1038/nsmb.2301

Shorter, J., & Lindquist, S. (2005). Prions as adaptive conduits of memory and inheritance. *Nature Reviews Genetics*, *6*(6), 435–450. https://doi.org/10.1038/nrg1616

Sitron, C. S., Park, J. H., & Brandman, O. (2017). Asc1, Hel2, and Slh1 couple translation arrest to nascent chain degradation. *RNA (New York, N.Y.)*, *23*(5), 798–810. https://doi.org/10.1261/rna.060897.117

Tyedmers, J., Madariaga, M. L., & Lindquist, S. (2008). Prion Switching in Response to Environmental Stress. *PLOS Biology*, *6*(11), e294. https://doi.org/10.1371/journal.pbio.0060294

Van den Elzen, A. M. G., Schuller, A., Green, R., & Séraphin, B. (2014). Dom34-Hbs1 mediated dissociation of inactive 80S ribosomes promotes restart of translation after stress. *The EMBO Journal*, *33*(3), 265–276. https://doi.org/10.1002/embj.201386123

Winz, M.-L., Peil, L., Turowski, T. W., Rappsilber, J., & Tollervey, D. (2019). Molecular interactions between Hel2 and RNA supporting ribosome-associated quality control. *Nature Communications*, *10*(1), 563. https://doi.org/10.1038/s41467-019-08382-z