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Abstract

Prions are infections proteins that are auto-catalyzing (form by altering a regular protein into the structurally different prion form), and are the cause of many common diseases such as Alzheimer's, Parkinson's, Huntington's, and Bovine Spongiform encephalopathy (or Mad Cow disease). This experiment tested the effect of three different plasmids, pH317, pER62 and pmp46 on prion formation in both wild-type and Urm1 deletion mutants in the yeast *Saccharomyces cerevisiae*. The proposed hypothesis was overexpression of the prion forming protein would increase the frequency of prion formation, as well as yield less sustainable prion amyloids (or prion aggregations) that are easier to cure. Another purpose of this experiment is to investigate how the presence or absence of the URM1 gene, that can form a prion, affects the overall formation of prions. The data showed both that overexpression of proteins was seen to increase prion formation, and that overexpression yielded prions that were less sustainable across generations. Also shown was that deletion mutants yielded higher numbers of prions than their wild-type counterparts. Efforts to cure prions through use of overexpression and guanidine, which inhibits the chaperone proteins associated with dividing prions during cellular division, proved inconclusive because there was no visible difference between any of the three plasmids.

Introduction

Initially, prions were studied because of the threat they posed to food supplies for humans because of the disease they cause in mammals. They are now being studied extensively because of their abilities to mediate non-Mendelian inheritance, produce additional prions, and display

phenotypic variance in yeast (Wickner et al., 2004). They also sparked interest in the scientific community when it was discovered they resisted ultraviolet degradation, which suggests they are not based on nucleotides (Speransky et al., 2001). Yeast prions are generated *de novo* in one out of every 10^6 cells, and there is little chance of isolating a prion from a wild yeast population (Nakayashiki et al., 2005). This incredibly limited distribution is an indication that prions are disadvantageous to their host cells and are selected against (Nakayashiki et al., 2005).

Prions are aggregated amyloid fibrils and can be formed by many different proteins. These are the causative agents for infectious neurodegenerative diseases in humans and non-human animals. There are few non-infectious diseases that result from amyloids such as Alzheimer's disease, Huntington's disease, and Parkinson's disease (Inge-Vechtomov, 2011). One of the defining characteristics of a prion is that the infectious protein creates conformational changes auto-catalytically; in other words, a prion interacts with other soluble proteins to produce more prions. At a sufficiently high protein concentration, they can produce amyloid protein fibrils, which have high resistances to proteases and detergents (Inoue, 2009). These amyloid fibrils are characteristic of mammalian prions (Inoue, 2009). Although the structure of mammalian and yeast prions are similar, there is an extremely low rate of infection when yeast or fungal prions are introduced to mice (Tessier and Lindquist, 2009). This means that any research humans would conduct on non-mammalian prions would be safe for them. The genome of the yeast *Saccharomyces cerevisiae* contains several proteins that can assume a transmissible prion form (Resende et al., 2003). Therefore, it will be the model used to test for prion formation in this experiment.

The URM1 gene encodes the Urm1p protein that functions as an ubiquitin-related modifier and can bind to amyloid-forming prions in *S. cerevisiae*. The protein ubiquitin is used

in cells to regulate proteins by covalently binding to specific sites, tagging them for degradation by other protein complexes called proteasomes (Pedrioli et al., 2008). Ubiquitin-related modifying proteins are part of a family of proteins called ubiquitin-like proteins (UBL's). These proteins have been found to have similar functions as a family of sulfur-carrying proteins in prokaryotes, although these prokaryotic proteins lack the modification functions of prions (Pedrioli et al., 2008). The protein Ure2p and these sulfur-carrying proteins have a similar folding region of β-sheets and covalently modify proteins. Although the Ure2 protein sequence and structure are more similar to the bacterial sulfur carrying proteins than to prion proteins, Ure2p can form prions (Pedrioli et al., 2008). This has led to the suggestion that ancestral members of the UBL family were like the Urm1 protein, which has been called a molecular fossil and indicates it may be conserved among yeasts (Pedrioli et al., 2008). It has been shown in other lab experiments that if the prion-forming protein is over-expressed, the frequency of prion formation increases as well (Wickner et al., 2004). This results from gene over-expression creating an abundance of the precursor proteins for the amyloids, which then become modified into prions (Speransky et al., 2001).

Cytoduction, or cytoplasmic mixing, measures the transmissibility of prions by observing the results of cell mating. Haploid yeast cells exist as one of two mating types or sexes, A and Alpha (Wickner et al., 2004). Haploid cells of opposite mating type can fuse (mate) to become diploid cells. The nuclei of the two cells also fuse which results in a diploid cell (having two sets of chromosomes). Cytoduction is essentially an aborted mating (Wickner et al., 2004). Although karyogamy, or fusion of the two nuclei, does not occur, the two cells fuse and the cytoplasm from both cells is mixed together. If only one of the cells involved in the mating has the gene for the prion, then the recipient cell will be identified as the cell that gains the prion (Wickner et al.,

2004). Although what occurs between the cells during cytoduction is not completely understood, as long as karyogamy is prevented, the cells will appear to join for a certain period of time and will then separate again (Wickner et al., 2004). The two haploid cells will form again, but with the mixed cytoplasmic contents of both cells. This measures transmissibility by visualizing how many of the aggregates can be transferred from one cell to another and from parent to daughter cell. Important in this process, are the chaperone proteins that cleave the prion aggregate so that the daughters of the cell division both receive part of the aggregate. An increased concentration of prion proteins will increase the likelihood of these aggregates forming.

Genetic markers are used to further distinguish between recipient and donor cells and are specific genes that produce recognizable traits that allow the different types of cells to be distinguished by their genotypic strain. For example, the genotype of yeast strains can be written as: MAT A ura2 leu2 (Wickner et al., 2004). This describes a yeast strain that is mating type A, and carries the ura2 and leu2 mutations, which result in a certain nutrient requirement called auxotrophy. The ura2 mutation prevents the cell from synthesizing the base uracil and leu2 prevents the cell from synthesizing the amino acid leucine, which means the MAT A ura2 leu2 strain will grow only in the presence of uracil and leucine (Wickner et al., 2004). Another example strain would be MAT Alpha trp1 his1, which will grow only in the presence of the amino acids tryptophan and histidine (Wickner et al., 2004). This auxotrophic phenotype allows the two strains to be distinguished.

Due to the conservation of URM1 and its relation to ubiquitin and proteasome degradation, the goal of this study is to determine the role of the ubiquitin-related modifier protein URM1 in prion formation in *S. cerevisiae*. My hypothesis is that if the prion exists in cells that have a synthetic abundance of the ubiquitin-related modifier Urm2p, the stability of the

prion will be less than that of a normal prion, thus making it easier to cure, and also that this less stable prion will have a higher frequency of formation. Also, this experiment will investigate the different effects of overexpression in both wild-type and deletion mutants. This research is significant because determining different methods to reduce the stability, thus allowing for easier degradation of the prion and yielding reduced infectivity of prions, which may open doors to treating the diseases associated with them.

Materials and Methods

I. Amplifying DNA and Generating Viable Yeast Colonies

A polymerase-chain reaction (PCR) was performed to amplify the urm1::G418 disruption cassette from a strain in the yeast deletion collection. The purpose is to insert this deletion into the genetic composition of the yeast. This specific cassette contains the G418, or KanMx gene, which is a selected marker that provides resistance to the fungicide Geneticin. The PCR was performed by digesting the yeast cells in 30µL of Zymolymase and then incubating these cells at 37°C for 45 minutes. A solution of cells and PCR primers was created so that each of four tubes contained 50µL of premade Master Mix, 5µL of upstream primer, 5µL of downstream primer, 1µL of cells, and 39µL of water for a total of 100µL. PCR was run on these samples. The products of the PCR were subjected to gel electrophoresis. The first well of the gel was loaded with 6µL of a known DNA ladder and DNA stain, and the next four were loaded with PCR samples. A picture of the gel was taken using an Alpha-Imager system that exposes the gel to ultraviolet light under a special filter.

The remaining PCR product was purified using the Promega DNA purification system, and all directions were followed. Wild-type yeast cells were transferred to a single tube of YPAD broth media and allowed to grow overnight. The cells were then centrifuged for five minutes at maximum speed. The media was poured out, leaving only the cells. One milliliter of transformation buffer was then added and the cells were resuspended by mixing with a pipet. This suspension was then transferred to a smaller tube and centrifuged for one minute at maximum speed. The supernatant was removed by pipet, and 200µL of transformation buffer were added. This solution was split into two separate tubes, one for PCR and the other as a negative control. The PCR tube received 100µL of transformation buffer, and all tubes were centrifuged for one minute at maximum speed. The supernatant was removed and all pellets were resuspended with 100µL of transform buffer. Ten microliters of salmon sperm carrier DNA and 500µL of Polyethylene Glycol (PEG) were added to each tube. The cells were mixed by pipetting slowly approximately 10 times. The tubes were incubated at 30°C for 30 minutes, and were then incubated in a 42°C waterbath for 15 minutes. The tubes were centrifuged for one minute at maximum speed. Eight hundred microliters of supernatant were removed, and 300µL of YPAD were pipetted into each tube, mixed with cells, and then transferred to a larger tube. These larger tubes were centrifuged for five minutes at maximum speed. The supernatant was poured off and the cells were mixed with 300µL of water. This suspension was pipetted directly onto a plate and spread using a sterile cell spreader; the plates were incubated at 30°C for three days. The colonies from the PCR plates were re-plated to obtain pure cultures.

While the pure culture plates were incubating, *Eschericia coli* strains, each containing either ph317, pER62, or pmp46 plasmids, were plated on LB + Ampicilin media. These plasmids contain no prion proteins, a mild amount of prion proteins, and an overexpression of prion

proteins, respectively. These plasmids serve to demonstrate how the cells behave without excess prion proteins (the pH317 plasmid), and then at different levels of protein expression (the pER62 and pmp46 plasmids). These plasmids also carry a gene that encode the ability to produce leucine. This is used to select for cells that carry the plasmids. After incubation and growth, these cultures were transferred to broth media. Two hundred and fifty microliters of cell resuspension solution were added to each plasmid tube, and the cells were resuspended by pipetting. The plasmids were then transferred to smaller tubes. Two hundred and fifty microliters of cell lysis solution were added to the tubes, and the solutions were mixed by inverting the tubes several times. Ten microliters of alkaline protease solution were then added to protect the DNA, and the plasmids were mixed by inverting the tubes several times. Three hundred and fifty microliters of neutralization solution were added to the plasmid tubes and the tubes were inverted immediately. A precipitate was observed. The tubes were centrifuged for 10 minutes at top speed. New collection tubes and spin columns were labeled during centrifugation. The liquid was then transferred to the new, corresponding spin columns by pipetting, and the columns were then centrifuged at maximum speed for one minute. The flow-through was discarded and the column was reinserted into the collection tube. Seven hundred and fifty microliters of column wash solution were added to each column, and the columns were then centrifuged for one minute at maximum speed. Then 250μL of column wash solution were added to the columns, and the columns were centrifuged again at maximum speed for two minutes. After the supernatant was poured off, the tubes were centrifuged for one minute at maximum speed to dry. One hundred microliters of nuclease-free water were added to each tube, and the tubes were centrifuged for one minute at top speed. These were stored in the freezer for future use.

Next, the overexpression plasmids were transformed into both wild-type and Urm1Δ mutants. One milliliter of yeast transformation buffer was added to each of eight new tubes (four for Urm1Δ cells and four for wildtype cells). A large colony of cells was selected from each plate and added to a tube. These cells were then washed with the buffer, which was promptly removed by pipetting. The cells were resuspended in 400µL of Tris+EDTA+Lithium Acetate (TE+LiAc). Four new tubes were obtained, one to contain only cells and no transformational DNA, one for the ph317 plasmid, one for the pER62 plasmid, and one for the pmp46 plasmid. First, 10µL of the specific plasmid were added to the appropriate tube, and the tube with only cells received the same volume of TE+LiAc. One hundred microliters of cells and 10µL of carrier DNA were then added to each of these four tubes. Six hundred microliters of PEG were added to each tube and mixed by pipetting. These tubes were incubated at 30° C for 30 minutes, and then incubated in a water-bath of 42° C for 15 minutes. The tubes were centrifuged for one minute at maximum speed and the supernatant was removed. The pellet was resuspended in 300µL of sterile water and the total volume was spread on a plate of SD-Leucine media. Leucine dropout was added to the media to select for those cells that carried that plasmids. These were incubated at 30° C for 3 days.

II. Prion Induction and Stability

Next, the induction of prions within the cells was started by obtaining six large tubes. Three were used for Wild-type colonies and three were used for urm1Δ mutant colonies. Each was filled with five milliliters of liquid YPAD media. A single pure colony from the pER62 plasmid cultures previously plated was added to each tube. The six tubes were incubated in a shaker incubator, at 30°C for three days. The cultures were then diluted by a factor of 20, using 950µL of distilled water and 50µL of cells. The absorbency of optical density of these cultures

was determined by putting them in a spectrophotometer. The values were recorded. The two colonies corresponding to the largest absorbency values that were measured at a wavelength of 550nm, were used to create a serial dilution ranging from 10^7 to 10^2. These values have a scale of 1.0 DO is equivalent to $2.0*10^7$ cells. Each dilution was plated on SD+USA+His+Trp media (containing ureidosuccinate (or USA), histidine, and tryptophan), except the 10^2 cultures that were plated on YPAD. These plates were incubated at 30°C for two days. It is important to not incubate the plates for too long because it has been shown that prolonged incubation can generate a heavier oligomer (Thual et al., 1999). This heavy oligomer refers to the amyloid, and refers to the idea that a heavier oligomer will be more difficult to cure or degrade. Also, proper incubation time is important because it is definitively known that deletion mutants grow more slowly, are sensitive to temperature, and often fail to establish a mitotic spindle, so over incubating the plates may alter the results (Pedrioli et al., 2008). The number of yeast colonies on each USA plate was counted and recorded. This process was repeated for the pmp46 and ph317 plasmids.

These colony counts represent the number of prion positive units present at a specific dilution. In order to determine whether these values were corrected to represent the number of USA^+ cells present per one million cells (10^6). The method to correct the original colony counts depends on the number of colonies counted for the 10^2 dilutions. If the value is less than one hundred, one hundred is divided by that value, and the resulting factor is multiplied to all higher dilution colony counts. If the value is one hundred or greater, that value is then divided by one hundred to receive a decimal value. The higher dilutions are multiplied by a number that will make the dilution become 10^6, and this number is divided by the decimal value.

III. Prion Maintenance and Curing

Next, USA^+ were selected from the prion induction experiment and the stability of the prions was determined. Protein stability was ascertained by growing the prion-containing strains on rich media (with all nutrients) for several days and then transferring several colonies to the SD+USA+His+Trp media. Even with very stable prions, some loss occurs over many cell divisions. Four [URE3] colonies that carried the urm1 mutation and four [URE3] colonies of the URM1 wild type were tested. Each strain was streaked on SD-Leu (Synthetic dextrose with Leucine dropout mix) media in order to isolate single colonies, which were then transferred to the prion testing plates. The characterizing phenotype of the [URE3] prion strain is the ability to grow in the presence of a metabolite called ureidosuccinic acid, or USA, (in place of the nucleic acid uracil; Ross et al., 2005). Ten colonies from each streak were then transferred to other SD-Leu plates and arranged in a grid orientation. The grid was of four rows and five columns. Four of these grid plates were created, at least one for each plasmid tested, and two deletion mutants and two wildtype colonies. These four plates were allowed to incubate at 30°C for three days. These served as the master plates and were replica plated twice. First onto a SD+Ura+His+Trp (or Synthetic dextrose with added uracil, histadine, and tryptophan), and a second time onto SD+USA+His+Trp. The second plate used USA instead of uracil because uracil allowed all cells to grow equally and served as a positive control to demonstrate successful replica plating. The USA is selective for prion growth only. The replica plates were incubated with the master plates at 30°C for three days. The results were then photographed and the growth levels were compared.

To test these prion colonies for curing abilities, the USA plates were examined. The four best growing colonies were determined and marked. The colonies on the master plates that

corresponded to the marked USA colonies were determined, and used to streak on plates that were YPAD with added guanidine. These were streaked two per plate, for a total of eight plates. These plates were allowed to grow at 30°C for one day. Following incubation, these plates were replica plated back onto SD+USA+His+Trp plates, and were incubated at 30°C for three days. The overall growth of these replica plates was then analyzed.

Results

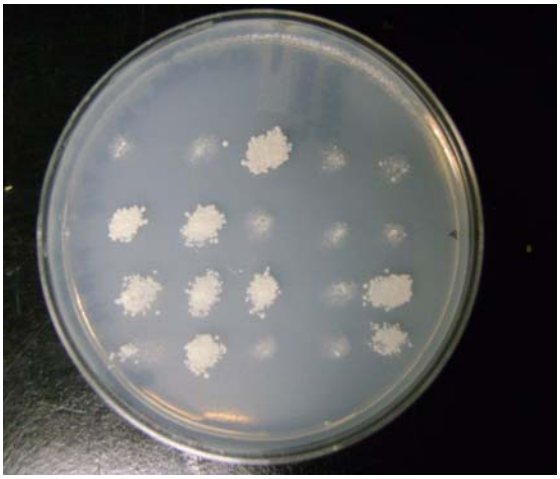
The frequency of prion formation was recorded for each of the three plasmids, for both the wild-type and Urm1Δ cultures. The number of counted colonies was then corrected to represent the number of prion positive cells per million cells. Table 1 shows the corrected values, the plasmids, colonies, and dilutions they correspond to. Table 1 shows the hyperinducing plasmid, pmp46, consistently has values that are higher than the other two plasmids. In regards to the pER62 plasmid, Table 1 shows the wildtype colonies have values similar to, or less than the Urm1Δ cultures. The pmp46 plasmid shows Wt, 2 plates had upwards of 70,000 USA^+ units per million cells, and that while the Urm1Δ colonies has lower average USA^+ units, the Urm1Δ colonies had a higher maximum value of 97452 USA^+ units per million cells, (Table 1). The pH317 plasmid shows the lowest values of all three plasmids, with a maximum of 281 USA^+ units per million cells (Table 1). Table 1 also shows the Urm1Δ, 1 the lowest values of the four groups, and that the other three groups have similar values.

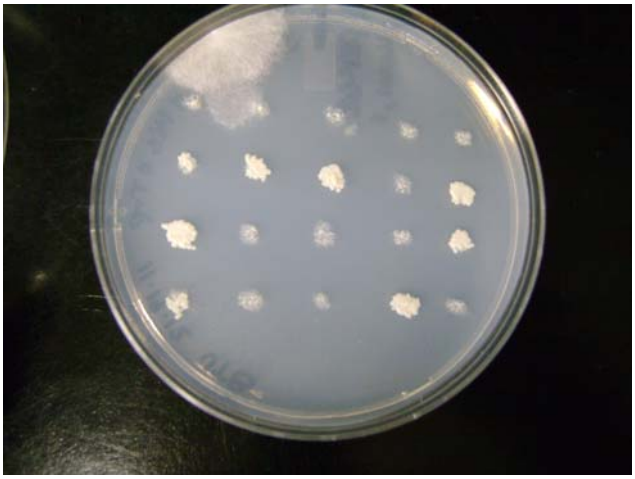
The replica plating technique was used to test the prion colonies obtained in the first part of the experiment by creating duplicate colonies and determining the intensity of growth by visual qualifications. The scale of growth intensity ranged from (+++), which represented very intense growth comparable to the positive control plate, down to (+), which represented very

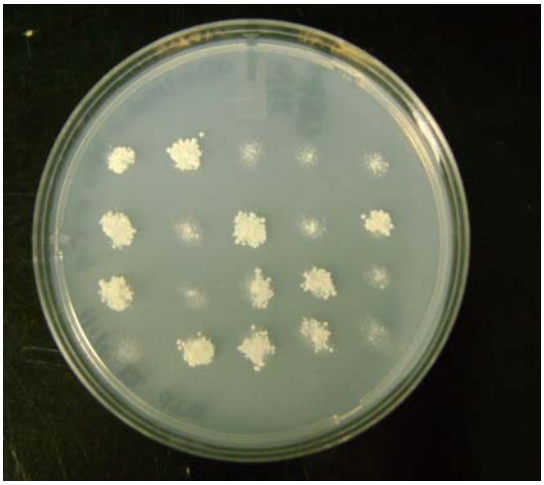
little growth, as well as (-) which indicates that minimal to no growth was observed. Although there may appear to have been growth, these may have just been dead cells that were localized from the replica plating process. Fig. 1A, Fig. 1B, Fig. 1C, and Fig. 1D are all photographs of the replica plates that were quantified by visual assessments on the (+++) to (-) scale. This scale can best be seen in Fig. 1B, where there are differences in the size of the colonies, where the colonies that grew that are distinct in their size ranges. Fig. 1A and Fig. 1B show 8 (+++) prion positive colonies were maintained through replica plating (Table 2). This is the highest number of conserved prion positive colonies among the three plasmids (Table 2). In regards to the ph317 plasmid, the Urm1 mutant colonies show very intense grow and very large clusters, whereas the wild-type colonies show various sizes and intensities (Fig. 1A and Fig. 1B). The pER62 and pmp46 plasmids show to have higher (-) colony growth than the pH317 plasmid (Table 2). The colonies that grew on the pER62 plate have only one (+++) of intense growth, and much more mild growth ((++) and (+)) (Fig. 1C and Table 2). The replica plates that were done on YPAD and guanidine media all yielded no visible cell growth.

Plasmid	Colony	Dilution	Number of Colonies	Multiply by	Divide by	Corrected Value of USA+ colonies per million cells
pH317	Wt 2	10^6	296	1	1.32	224
		10^5	31	10	1.32	235
		10 ^2	132	becomes 1.32		
	Wt 3	10^6	420	1	1.51	278
		10^5	28	10	1.51	185
		10^4	3	100	1.51	199
		10 ^2	151	becomes 1.51		
	Urm 1, 1	10^6	280	1	1.53	183
		10^4	3	100	1.53	196
		10 ^2	153	becomes 1.53		
	Urm 1, 2	10^6	388	1	1.38	281
		10^5	31	10	1.38	225
		10 ^2	138	becomes 1.38		
pER62	Wt 2	10^4	104	4	1	416
		10 ^2	25	4	1	100
	Wt3	10^4	103	4.3	1	442.9
		10^3	10	4.3	1	43
		10 ^2	23	4.3	1	98.9
	Urm 1, 1	10^4	129	100	3.15	4095
		10^3	1	1000	3.15	317
		10 ^2	315	becomes 3.15		
	Urm 1, 3	10^4	79	6.666666667	1	527
		10^3	19	6.666666667	1	127
		10 ^2	15	6.666666667	1	100
pmp46	Wt 2	10^4	956	100	1.24	77097
		10^3	99	1000	1.24	79839
		10 ^2	124	becomes 1.24		
	Wt 3	10^4	424	100	3.35	12657
		10^3	192	1000	3.35	57313
		10 ^2	335	becomes 3.35		
	Urm 1, 1	10^4	808	100	1.64	49268
		10 ^2	164	becomes 1.64		
	Urm 1, 2	10^4	778	100	1.57	49554
		10^3	153	1000	1.57	97452
		10 ^2	157	becomes 1.57		

Table 1. Data shows the number of colonies counted for each plate and the corrected number of USA^+ colonies per million cells.

A. 

C. 

B. 

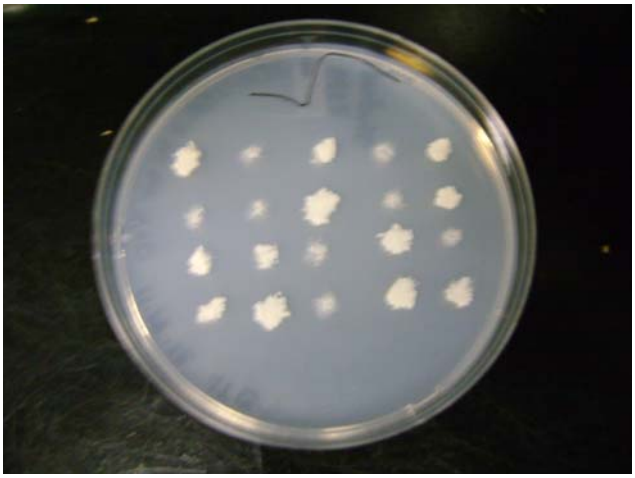
D. 

Fig. 1. Photographs of the replica plating. A and B represent the pH317 plasmid, with A showing Urm1 mutant colonies, and B showing wild-type colonies. C shows growth for the pER62 plasmid. D shows the growth for the pmp46 plasmid.

Plasmid	Type of Colony	(+++)	(++)	(+)	(-)
pH317	Urm 1	8	1	7	4
	Wt	5	5	6	4
pER62	Urm 1	1	6	3	10
pmp46	Wt	5	4	3	8

Table 2. The number of colonies representing various growth intensities. Each value shown is based out of 20. .

Discussion

The pH317 plasmid was the known empty plasmid and served as the negative control. When looking at the results seen in Table 1, the values for all four colony groups are relatively consistent with each other. These values tend to be atypical of what is expected from the negative control and may have resulted from spontaneous induction. These greater than anticipated values may have resulted from successful incubation of partially formed prions, which would still form fully developed amyloids because the entire protein is not required in prion formation (Ross et al., 2004). Also, these may have resulted from spontaneous formation because it has been shown the [URE3] prion can be produced *de novo* in a strain that it was previously absent from. (Masison et al., 1997).

The pER62 was the test plasmid in this experiment. Table 1 shows the two colony groups that best demonstrate the results are Wt3 and Urm1, 1. These two groups best demonstrate the expected results because the lower dilution cell counts were roughly ten-fold less than those of the higher dilution. As seen in Table 1, The Wt3 group had approximately 43 USA^+ colonies per million cells for the 10^3 dilution, and had 448 USA^+ colonies per million cells for the 10^4. This shows the ten-fold increase between dilution factors, as expected. In relation to the Urm1,1 group however, these values are ten-fold less, meaning the highest cell count was 4095 USA^+ per million cells. This shows the deletion mutants yielded more prion formation than the wild-type colonies did.

The pmp46 plasmid served as the hyper-inducer, which was used because it is known that overexpression of the gene tends to increase the frequency of prion formation (Ross et al., 2005). The pmp46 plasmid groups all yielded the highest observed values of USA^+ colonies per million cells. This is consistent with other research that shows the [URE3] has an increased frequency of

the prion when the gene coding the prion protein is over-expressed. (Masison et al., 1997). Table 1 shows the Urm1, 2 mutants had the highest corrected value overall, while the Wt3 group had the lowest count observed for this plasmid. The results of these three plasmids, showed that as the number of available prion forming genes increases, the number of prions increased as well, supports the hypothesis that increased expression increases formation. Multiple experiments have also been done that show this to be true, that over-expressing the Ure2p protein domain resulted in an increase in prion formation (Ross et al., 2005).

All of the replica plates that were done on media containing uracil were not pictured in Fig. 1. This was because, since uracil was on the media, all cells could grow, not just those containing prions. The media containing uracil was used as a positive control to show the replica plating was successful. USA is ureidosuccinate, and is an important factor in identifying prions. The normal function of the Ure2 protein is involved in the repression of nitrogen catabolites (Ross et al., 2005). This nitrogen repression mechanism allows cells to take in poor nitrogen sources when otherwise deficient (Fernandez-Bellot et al., 2000). This means that the presence of [URE3] leads to cells gaining the ability to intake USA (Edskes et al., 1999). Overall, this nitrogen catabolite pathway is significant to this experiment because it allows all cells containing a prion to have the ability to absorb USA from the medium and convert it to the nucleotide uracil. (Nakayashiki et al., 2005). Table 2 shows the pH317 plasmid had the most sustained growth, especially in the Urm1 mutant colonies (Fig. 1A and Fig. 1B). The Urm1 mutant showed more colonies of sustained growth than the wild-type colonies. The pER62 and pmp46 plasmids had higher negative (-) values than either pH317 plasmid colony (Fig. 1C, Fig. 1B, and Table 2). This supports the hypothesis that the more genes expressing the prion protein there are available, the less stable the prions may be, thus meaning less sustainability over generations. Table 2

shows the pER62 plasmid to have more negative colonies than the pmp46 plasmid. This does not support the hypothesis because the plasmid with less prion protein gene had less sustainable prions. In part, this may be due to the pmp46 plasmid expressing so much prion protein, the amyloid may have simply been larger than those in pER62, and hence may have been harder to degrade.

The curing experiment that was done on YPAD and guanidine media showed no visible cellular growth, suggesting that all prion-containing cells were cured of their prions, and thus could not grow. Guanidine is a chemical that interferes with the natural chaperone proteins of the Ure2 prion proteins. One such chaperone protein Hsp104, functions by partitioning the prions in the dividing cells by severing the prion fibers and allowing equitable division for each daughter cell. (Halfmann et al., 2012). This means to effectively cure the cells with prions, there must be enough of the chaperon proteins to inhibit the cleavage of prion aggregates, and thus overexpressing the prion proteins the chaperones assist will increase the amount of chaperone proteins. This is known as prion curing by means of overexpression. It poses a plausible method for curing because more chaperone proteins would yield larger amyloids, and the larger aggregate would only be passed onto one of the daughter cells after division, and the other cell would be amyloid free (Speransky et al., 2001). This would not just benefit the pER62 and pmp46 plasmids however, because many smaller amyloids can be seen to eventually coalesce into a much larger aggregate naturally (Speransky et al., 2001). Hence the pH317 was also seen to be cured because its smaller amyloids naturally created a larger one that was prevented from cleavage during cellular division, and results in prion free cells. It is important to not however that other experiments have shown even though these cells may have been cured of the prions, it

has been shown that the [URE3] amyloid does has reversible curability, but these cured strains may form the amyloid again (Masison et al., 1997).

Overall, the results support the idea that overexpression of the protein increases frequency of formation, (Fig. 1), as well as the idea that an excess of prion protein will yield less sustainable prions. The curing aspect of the hypothesis is not strongly supported because there was not a discernable difference between the plasmids in regards to curing effects. These effects may be further fine tuned by adjusting the levels of guanidine in the media. Human error is always an issue in experiments, and should be accounted for justly. It is known for certain that prion proteins are found in mammals and yeast, can transmit diseases, and code for heritable traits. (Namy et al., 2008). It is known that a regular cellular conformation that can become the non-functional and thus cause ailments and malfunctions in host cells (Namy et al., 2008). This makes it important research to continue in regards to human medicine because it has been shown that the presence of prions creates several phenotypes, and affect the hosts' ability to survive (Namy et al., 2008). It is reasonable to suggest that if the normal function of cellular prion proteins is for neuroprotection, then loss of this function by prion conversion might contribute to the prion-induced neurodegeneration (Harris and True, 2006). As it currently stands, a diagnosis of an amyloidosis is equivalent to a death sentence, because the human diseases that involve prions tend to be fatal (Inge-Vechtomov, 2011). Thus it this and further research are significant, not only to better aid in understanding the interactions between aggregates and inheritance-chaperone proteins, but also the possibility of modeling the mammalian diseases in yeasts may provide new insight into the molecular mechanism of their pathogenesis and the development of treatment approaches (Oshervich et al., 2004; Inge-Vechtomov, 2011).

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