Merge

Volume 4 Article 1

2020

How Cellular Metabolic State and the Chaperone Protein Hsp104 Interact to Affect the Spontaneous Formation of the Yeast Prion [URE3]

Demyia Graham

How Cellular Metabolic State and the Chaperone Protein Hsp104 Interact to Affect the Spontaneous Formation of the Yeast Prion [URE3]

Demyia Graham

How Cellular Metabolic States Affect the Spontaneous Formation of Yeast Prions

Overall Aim

My overall aim is to determine how cellular metabolic state can influence spontaneous prion formation rates in baker's yeast (*Saccharomyces cerevisiae*) with or without the Hsp104 chaperone protein present.

Specific Aim 1

We will determine if yeast cells in different phases, i.e. in their growth cycle, such as the exponential phase, the stationary phase, and the declining phase, show spontaneous prion formation rates that are significantly different from one another.

Specific Aim 2

We will determine if spontaneous prion formation rates are different in the KB104 yeast strain lacking the Hsp104 chaperone protein and the PC301u yeast strain containing the Hsp104 chaperone protein, which is known to play a role in facilitating prion propagation [9]. We will compare rates between straits across different phases of the yeast growth cycle.

Background Information

Prions, in general, are transmissible misfolded, pathogenic proteins that can induce abnormal folding of properly folded versions of themselves [2]. Specifically, prions are misfolded infectious self-aggregating proteins in mammals that are associated with neurodegenerative diseases which result in transmissible spongiform encephalopathy such as Creutzfeld-Jacob disease [4,15]. Creutzfeld-Jacob Disease is a fatal neurodegenerative human disease that progresses due to the abnormal formation of a prion protein aggregate [4,15]. Several non-human diseases such as Mad Cow Disease, Chronic Wasting Disease and Scrapie result from misfolded proteins as well. Many farmers' livestock have been affected by

pathogenic prions resulting in livestock being culled [3]. Any knowledge gained in understanding pathogenic prions function will be a significant step in understanding how they evolved into degenerative neural diseases and could lead to treatments that lessen the effects pathogenic prions have on human and animal lives [14].

PRNP is the mammalian gene that encodes the prion protein (PrP) which is found on the surface of numerous cell types [2]. PrP was the first protein discovered that misfolds and aggregates. Later, it was discovered that other proteins do this, so now the term prion is associated with any protein that misfolds into an infectious and self-aggregating form [14]. In mammals, proteins that can misfold into prions are found primarily in the brain and can cause neuronal death when converted to their pathogenic prion form [7]. These misfolded proteins can lead to brain damage when they induce normal cellular versions of themselves to alter their conformation [6]. Prions are a physiological issue due to their ability to induce chain reactions that resulted in the accumulation of large numbers of aberrant structures when they interact with properly folded prion proteins) [8, 10]. Yet, the most dangerous feature of pathogenic prions is that they are extremely difficulty to eradicate. To eradicate them, their protein structure must be denatured to make them nonfunctional. Some cellular process appears to eradicate misfolded prions, and most of the cellular processes capable of this have been found in yeast models that involve chaperone proteins [7].

We will use baker's yeast, *Saccharomyces cerevisiae*, as our model organism. Baker's yeast is also easy to acquire, grow in a laboratory setting. It can produce many colonies in a short time, and there is extensive knowledge available on baker's yeast (*S. cerevisiae* possesses) proteins that can spontaneously misfold and act as pathogenic prions [5]. These prions act on a molecular level exactly like mammalian prions and misfold and self-aggregate into amyloid

fibers, just like prions in mammals [9]. (The prions in baker's yeast are passed on to daughter cells when the yeast cells divide) [13]. These prions cannot be transferred to humans making them completely safe to work with [15]. One of these yeast proteins that misfolds into a prion state is the Ure2 protein (which we have chosen to examine). Therefore, *Saccharomyces cerevisiae* is an appropriate model organism to study spontaneous prion formation rates with. In addition, understanding the formation, propagation, treatment, and eradication of pathogenic prions in yeast will improve our, understanding of how these activities occur in mammals [6]. The URE2 yeast gene encodes the Ure2 protein. The Ure2 is a functional protein that binds the ammonium ion (NH_4^+) in cells and is involved in nitrogen-sensing [7]. The Ure2 protein can misfold into the infectious prion referred to as [URE3]. (In yeast, wildtype genes are usually written in all-caps and proteins are written with only the first letter capitalized, but the misfolded, prion version of the Ure2 protein is written in all-caps and with square brackets. This prion nomenclature is confusing but reflects the fact that the prion state was initially only partially understood as an epigenetic change to the cells [5]. It wasn't until after its nomenclature had been established that it was discovered that [URE3] was the prion form of the Ure2 protein [6].) The [URE3] state of the Ure2 protein is non-functional and no longer able to participate in nitrogen sensing; however, the [URE3] prion will convert all Ure2 proteins it interacts with within the cell into additional infectious [URE3] prions [4]. [URE3] prions will not kill the cell nor dramatically alter its growth cycle. Some research has suggested that the [URE3] prion might be deleterious to yeast cells and might slow down their growth [5]. This is uncertain; while it is certain that, once present, [URE3] will convert all the Ure2 proteins in the cell into [URE3] prions [2, 3, 8].

Chaperone proteins function by assisting other proteins to fold correctly, unfold for degradation, or unfold and refold into their correct conformation [3]. Previous research has established that chaperone proteins can influence how well already-established prions propagate [15]. Chaperone proteins are always present in cells, but their synthesis levels respond to cellular stress [6]. Some conditions that stress the cells, which usually lead to higher instances of protein misfolding, will increase expression of most chaperone proteins [2].

Metabolic states are biochemical processes that occur within living cells that consists of different rates of the catabolism and anabolism of molecules [11]. The exponential phase is a metabolic state when cell division and cell proliferation is occurring rapidly. The static or stationary phase is a metabolic state when there is only slow cell division and the rate of cell division is about equal to the rate of cell death. The declining phase is a metabolic state when there is little or no cell division as a result of cells dying.

Synthetic complete defined medium (SCD) is a minimal medium that contains all the specific nutrients the yeast need to grow, but it contains no extra nutrients. Ureidosuccinic acid (USA) in the medium is required by uracil-deficient cells to synthesize uracil and grow. SCD + NH_4^+ + USA medium does not support the growth of cells defective in uracil synthesis that express the functional Ure2 protein [9]. In the presence of (NH_4^+), functional Ure2 proteins inhibit the expression of the transporter protein required to facilitate the entry of USA into the cells [6]. Without USA, uracil(-) cells cannot synthesize uracil, and as a result of this, they will not grow. That is, prion-free cells defective in uracil biosynthesis with functional Ure2 proteins are unable to make uracil using the USA medium and do not grow. Yeast cells containing the [URE3] prion lack functional Ure2 proteins and are able to grow on SCD + NH_4^+ + USA medium and are able to utilize USA for synthesis of uracil [7]. In summary, in SCD + NH_4^+ +

USA medium, cells defective in uracil biosynthesis without the [URE3] prion present do not grow, but cells defective in uracil biosynthesis with the [URE3] prion present do grow. All the cells we used in these experiments are defective in uracil biosynthesis [6]. Therefore, SCD + NH_4^+ + USA can be used as a selective medium to identify and quantify cells containing the [URE3] prion.

Experimental Approach

Specific Aim 1

Our hypothesis is to determine how cellular metabolic state can influence spontaneous prion formation rates in baker's yeast (*Saccharomyces cerevisiae*). We predict that different metabolic states will influence the rates at which prions spontaneously form. We also predict that yeast cells at different phases in their growth cycle, such as the exponential phase, the stationary phase, and the declining phase, will show spontaneous prion formation rates that vary significantly from one another because of their being in different metabolic states and possessing varied levels of numerous effector proteins. We will start with cultures of prion-free yeast and grow them in the nutrient-rich YPD medium. This medium is a defined medium consisting of yeast extract, peptone, and dextrose (hence YPD) that contains all the nutrients required for growth, reproduction, and survival. A small fraction of cells will spontaneously form prions, but both both prion-free and prion-containing yeast will readily grow in YPD.

We will take samples of the yeast from the YPD medium at the exponential, stationary, and declining phases of growth. These isolates will be plated at a value of one million (10^6) yeast cells on the SCD + NH_4^+ + USA medium and the number of colonies that grow will be counted. Since only prion-containing cells grow on SCD + NH_4^+ + USA, the number of colonies that grow

will indicate how many individual yeast cells out of the original one million spontaneously converted to the prion state by having their Ure2 proteins misfold into [URE3].

We will then calculate the spontaneous [URE3] prion formation rate in these cells by dividing the number of CFUs on the SCD + NH_4^++ USA plates by the number of cells originally plated. That is, spontaneous [URE3] formation rate is defined as the fraction of cells that have their Ure2 proteins spontaneously misfold into the [URE3] prion state without having an outside [URE3] prion introduced to them.

We will obtain and compare spontaneous [URE3] prion formation rates for cells in three different metabolic states – exponential phase, stationary phase, and declining phase of cell growth.

We predict that spontaneous prion formation rates will be highest in the declining phase of yeast growth as a result of the cultures being deficient in nutrients. In this stressful environment, the PC301u cells will produce more chaperone proteins and other proteins involved in protein folding and misfolding and will be more likely to generate prions.

Specific Aim 2

We-will use two yeast strains, PC301u and KB104 that are both prion-free. Both strains are deficient in uracil synthesis, so that growth on SCD + NH_4^+ + USA plates can be used as a marker for the presence of the [URE3] prion. The two strains are genetically identical except that KB104 has had its HSP104 gene deleted and PC301u retains that HSP104 gene. [5].
Previous research has established that the Hsp104 protein aids [URE3] prion propagation in cells that already contains prions, which is why we chose to focus on the contribution of this specific chaperone protein [6]. If chaperone protein levels are affecting the rates of spontaneous prion formation, we hypothesize that Hsp104 will be one of the chaperones playing a role. In cells

lacking the Hsp104 protein, we hypothesize that we will see altered rates of spontaneous formation at each phase of cell growth compared to cells with functional Hsp104. This will also provide insight on how Hsp104 proteins and metabolic states interact to influence the rates of spontaneous prion formation in *S. cervesaie* cells.

Both strains (PC301u expressing Hsp104 chaperone protein and KB104 unable to express Hsp104) will be initially free of any prions. Both will grow readily in YPD medium, which supports growth of both prion-free cells and cells that spontaneously form the [URE3] prion. During incubation, we expect that about one in every 100,000-yeast cell should spontaneously convert Ure2 protein to [URE3] prion [6]. To identify and quantify the few rare cells that contain the [URE3] prion, one million cells will be isolated from the culture at the 4, 8, 24, 72, and 100 hours and plated on SCD+ NH_4^++ USA medium [8]. Yeast colonies that grow on the SCD+ NH_4^++ USA medium will then be counted. The rate of spontaneous formation at each growth phase assayed can be calculated as the number of colonies divided by the total number of cells that were originally plated. This experiment will be repeated a total of four times and the average taken for the spontaneous prion formation rates during the three different metabolic states. Hsp104 does not cause prions but in its presence prion proteins are more likely to occur. As a result, we expect the spontaneous prion formation rate to be lower during the exponential growth phase in the KB104 yeast strain (which lacks the Hsp104 protein) than in the PC301u strain that contains Hsp104. In this environment, the KB104 cells should be less likely to convert their proteins into prions because the cells have ample nutrients in a non-stressful environment and the Hsp104 chaperone.

Methodology

YPD plates of KB104 and PC301u cultures were taken out of the cold room. One colony of each was placed in a test tube containing 5mL of YPD liquid medium and placed in the incubator overnight. After overnight growth, cell concentrations were calculated using the TC10 cell counter from BioRad. KB104 and PC301u were each diluted to a concentration of 5×10^5 c/mL in 5 mL of YPD and then placed in a New Brunkswick Innova shaking incubator at 30 C. Cell counts were performed on a BioRad TC10 Counter as follows. Ten microliters of diluted cells were pipetted onto the TC10 slide and the number of cells were counted by the machine which reported the count in cells/mL. Original cell concentration was calculated as TC10 count * dilution factor.

Cells were collected from the YPD medium at the exponential, stationary, and declining phases of growth at 4, 8, 20, and 100 hours post inoculation to determine spontaneous [URE3] prion formation rates. Cell concentration at each time point was determined using the TC10 counter.

The volume of each culture needed to give 10^6 cells was calculated from the cell count determined above. The appropriate volume of cells was placed in an eppendorf micro-centrifuge tube and spun for 1 minute in a Fisher AccuSpin micro-centrifuge. The supernatant was aspirated off, 100µL of deionized water was added to the micro-centrifuge tube, and the pellet was re-suspended gently with pipetting. The solution was spread on SCD + NH_4^+ + USA agar plates with a glass hockey stick. Each plate was labeled with the strain, incubation time, and the number of cells plated. The SCD + NH_4^++ USA agar plates were then placed in a Fisher incubator for seven days at 30°C. To quantitate spontaneous prion formation rates, the number of

colonies that grew on SCD+ NH_4^++ USA agar plates were divided by the total number of cells plated.

Results

Prion protein concentration is a key factor for prion propagation of yeast prions and is dependent on the expression levels of a number of molecular chaperones [9]. Hsp104 has been shown to play a significant role in prion propagation in yeast [4]. Hsp104 has a bystander effect assisting prion propagation when they are present, while also seeming to reduce the rate at which the first prion proteins spontaneously mis-fold [4,13]. As shown in figure 1, when cells lack the Hsp104 chaperone protein, their rate of spontaneous prion formation after 20 hours of growth is significantly higher than when Hsp104 is present. Preliminary results at other time points suggest that in the absence of Hsp104, the spontaneous generation rates of prions are more stable at time points prior to 20 hours. Due to bacterial contamination of the PC301u strain during our experiments, a repeat of the PC301u experiments needs to be conducted to determine if spontaneous prion formation rates are consistently higher in the KB104 yeast containing strains.

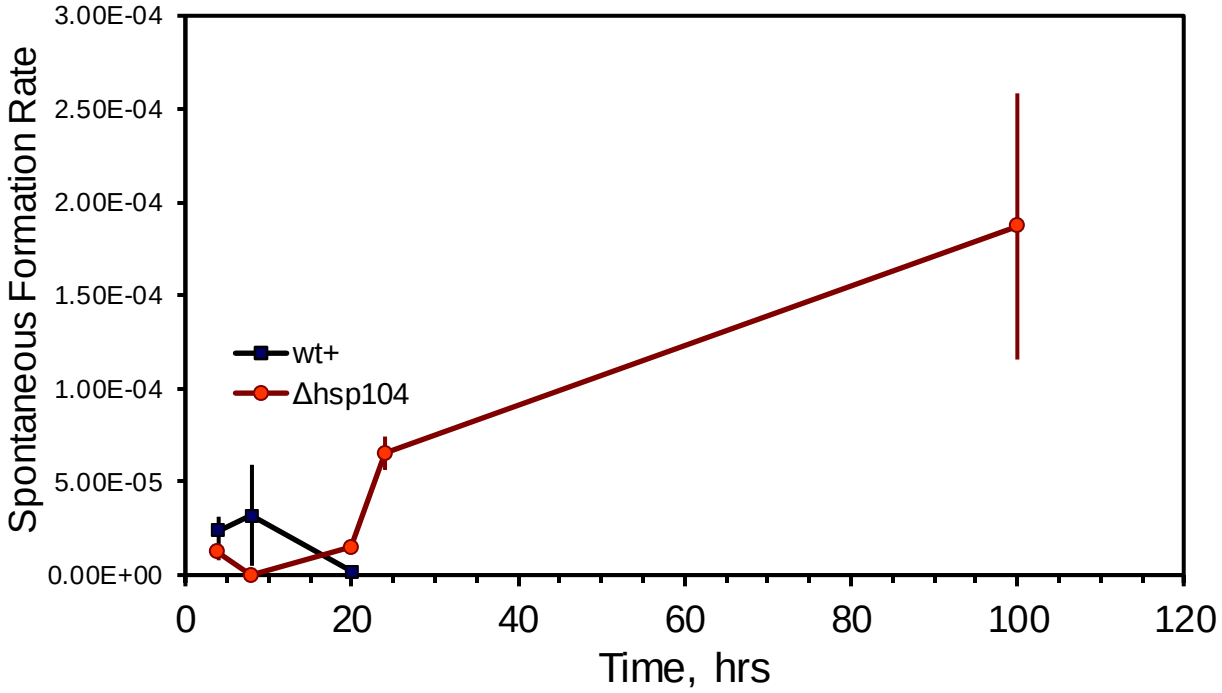

Figure 1: Spontaneous prion formation rates are the lowest in the first 20 hours and the highest after 20 hours in the KB104 strain lacking the Hsp104 gene (labeled Δhsp104 on the graph.) The two strains used were PC301u, labeled wt+ on the graph as it is wildtype, and KB104, labeled Δhsp104 on the graph as it has had the HSP104 chaperone protein gene deleted.

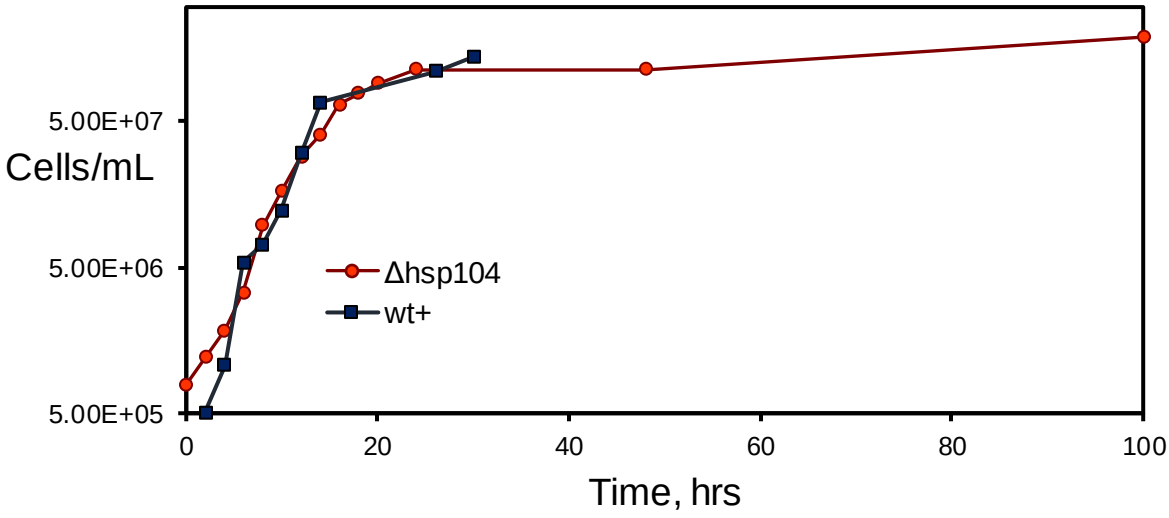

Figure 2: There is no significant difference in the growth rates of the KB104 (labeled Δhsp104) and PC301u (labeled wt+) yeast strains. This suggest that the Hsp104 chaperone protein does not affect the yeast strains growth rates.

Discussion

In both yeast strains, the exponential growth phase ended post 21 hours and the stationary phase lasted for approximately 48 hours. The cultures reached their maximum growth rate at approximately 70 hours. At this time point, the yeast cells entered their declining phase. In both strains, the declining phase was not documented because of time restraints and some bacterial contamination problems. I was unable to collect all the data I originally intended to collect. Additionally, we believed that the TC10 Counter was counting yeast cells present whether dead or alive, which made the data difficult to interpret. To address this problem, we started mixing the dilution factor with trypan blue, which stains dead cells blue thus allowing us to get an accurate count of the cells that are dead versus those still alive. However, the TC10 Counter reported that only nine to eleven percent of the cells are viable or alive. This needs to be investigated further. Counts can be done manually with a hemocytometer, using cells in the presence and absence of trypan blue. We can do a visual count of live versus dead. We might also use a spectrophotometer to get a total cell count. We will then compare living and dead cell proportions using these various methods.

The preliminary results in Figure 1 show that metabolic states do appear to affect rates of spontaneous prion formation. Samples taken during the first 20 hours were in the exponential phase of growth. These cells had a lower rate of spontaneous prion formation than in stationary and lagging phases of growth (beyond the 20-hour mark). We only had data for this comparison in the cells lacking the Hsp104 protein, but if cells lacking the Hsp104 protein had different rates of spontaneous [URE3] prion formation in different metabolic states, we expect to see the same pattern in cells which express the Hsp104 protein, although this will need to be confirmed. Certainly Figure 2 shows that the lack of the Hsp104 protein appears to have no effect on growth

rates of yeast when compared to an identical strain expressing Hsp104. That is, the absence of Hsp104 does not affect the metabolic states of the yeast so dramatically as to alter its pattern of growth rates.

During the experiments, some of the PC301u cultures got contaminated with bacteria. We could not collect spontaneous prion formation data from those cultures, and time ran out before we could re-do those experiments. The lack of data due to these contamination problems meant could not do a proper comparison of spontaneous [URE3] prion formation rates in cells expressing the Hsp104 protein to those lacking the Hsp104 protein. Figure 1 shows we only have spontaneous [URE3] prion formation rate data for the PC301u cells with Hsp104 protein during exponential phases of growth. As a result, we cannot yet conclude if presence of Hsp104 contributes to or hinders spontaneous [URE3] prion formation rates in the stationary or declining phases of growth.

Future Directions

The PC301u yeast strain in some of our experiments was contaminated by bacteria. This contamination is the reason why there are no data points available for PC301u past the 20-hour mark. Antibiotics are not traditionally used in yeast cultures, but if bacterial contamination persists, a commonly used antibiotic like ampicillin may be added in future experiments. Because of time restrictions, we were not able to evaluate if the low levels of our yeast cells' viability has anything to do with the rate of spontaneous prion formation. Furthermore, more research can be done on documenting what happens to prion formation rates in yeast cells when they are put into a stressful environment such as lack of nutrients during exponential phases of growth. Future studies can measure Hsp104 levels at different time during growth to see if there is any correlation between Hsp104 levels and spontaneous prion formation rates. Future studies

can also investigate spontaneous prion formation levels in strains with different chaperone proteins knocked out.

References

1. Bagyinszky E, Giau VV, Youn YC, An SSA, Kim S. Characterization of mutations in PRNP (prion) gene and their possible roles in neurodegenerative diseases. Neuropsychiatr Dis Treat. 2018;14(14):2067–2085. pmid:30147320

2. Sakudo A, Onodera T. Prion protein (PrP) gene-knockout cell lines: Insight into functions of PrP. Front Cell Dev. Biol. 2015;2(75):1-18. pmid:25642423

3. Atkinson CJ, Zhang K, Munn AL, Wiegmans A. Prion protein scrapie and the normal cellular prion protein. Prion. 2016;10(1):63-82. pmid:26645475

4. Sanchez-Juan P, Bishop MT, Croes EA, Knight RS, van Duijn CM et al. A polymorphism in the regulatory region of PRNP is associated with increased risk of sporadic Creutzfeldt-Jakob disease. BMC Med Genet. 2011;22(73):1-6. pmid:21600043

5. Wickner RB. Yeast and fungal prions. Cold Spring Harb Perspect Biol. 2016;8(9):e023531. pmid:27481532

6. Wickner RB, Edskes HK, Bateman D, Kelly AC, Gorkovskiy A. The yeast prions [PSI+] and [URE3] are molecular degenerative diseases. Prion. 2011;5(4):258-262. pmid:22052353

7. Chen LJ, Sawyer EB, Perrett S. The yeast prion protein Ure2: Insights into the mechanism of amyloid formation. Biochem Soc Trans. 2011;39(5):1359-1364. pmid:21936815

8. Crapeau M, Marchal C, Cullin C, Maillet L. The cellular concentration of the yeast Ure2p prion protein affects its propagation as a prion. Mol Biol Cell. 2009;20(8):2286-2296. pmid:19225154

9. Ngo S, Chiang V, Ho E, Le L, Guo Z. Prion domain of yeast Ure2 protein adopts a completely disordered structure: a solid-support EPR study. PLoS One. 2012;7(10):e47248. pmid:23077577

10. Toombs JA, McCarty BR, Ross ED. Compositional determinants of prion formation in yeast. Mol Cell Biol. 2010;30(1):319-332. pmid:19884345

11. Wickner RB, Edskes HK, Kryndushkin, D, McGlinchey, R, Bateman D et al. Prion Diseases of Yeast: Amyloid Structure and Biology. Semin Cell Dev Biol. 2011;22(5):469-475. pmid: 21345375

12. Kaplan S. How a history of eating human brains protected this tribe from brain disease? The Washington Post. 2015.

13. Mabbott NA. How do PrP^{Sc} Prions Spread between Host Species, and within Hosts? Pathogens. 2017;6(4):60. pmid:29186791

14. Asante EA, Smidak M, Grimshaw A, Houghton R, Tomlinson A, Jeelani A, et al. A naturally occurring variant of the human prion protein completely prevents prion disease. Nature. 2015;522(7557):478-481. pmid:26061765

15. Sikorska B, Liberski PP. Human prion diseases: from Kuru to variant Creutzfeldt-Jakob disease. Subcell Biochem. 2012;65:457-96. pmid:23225013