Innovation for Treatments on Creutzfeldt-Jakob Disease - CSFY project

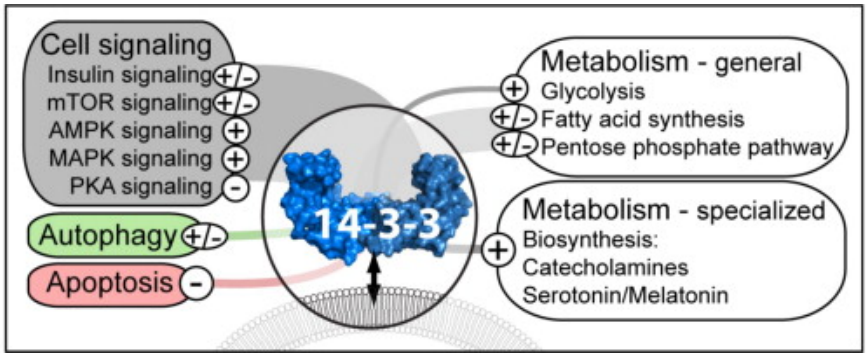
SCIENCE FAIR LOGBOOK- Part Two 2025-2026

January 18th, 2026

Goal of Project

- Innovating ideas on how to treat the prion disease, Creutzfeldt-Jakob disease, before conditions in a patient become critical.
- *Creutzfeldt-Jakob disease is the most common prion disease found in humans, being fatal yet under-researched.* Not only is it unable to be recognized by the immune system as a threat, but no existing treatments have been found to silence the disease completely. **The purpose of this investigation is to innovate solutions on how to treat Creutzfeldt-Jakob disease before a patient's condition becomes critical.**
- How are we to accomplish this feat? As a matter of fact, many existing solutions do in fact exist regarding the treatment of CJD… (as per part one of this logbook)

Summary of Research



<u>14-3-3 PROTEINS -</u>
An Overview

- 14-3-3 proteins are molecules which have remained unchanged throughout evolution, (Conserved regulatory molecules) showing that they are extremely important for biological processes (such as gene expression, signal transduction, and cellular metabolism. They live within within eukaryotic cells (cells with a nucleus)
- They bind with SIGNALING PROTEINS such as kinases, phosphatases, and transmembrane receptors
- Signaling proteins are important because they act as messengers to coordinate activities like growth and immunity. They trigger cellular responses from signals

received by hormones and neurotransmitters to transfer information within cells and between cells.
- Both kinases and phosphates communicate and control a cell's behavior, hence what they are signaling proteins.
- This function can allow 14-3-3 proteins to play a role in a wide range of vital regulatory processes (Important functions; allow cells to stay healthy (regulate activities like growth, metabolism, and cell survival.)

In summary…**The protein 14-3-3 can bind those specific signaling proteins together, which can assist in processes which keep organisms healthy and alive.**

What is it doing for CJD? - An Overview
(check part one of logbook for in depth study)

- 14-3-3 proteins are found in abundance within the neuron. When proteins mutate, and prions form, creating spongiform degeneration will those proteins be released
- An antibody of a 14-3-3 protein reacted with cerebrospinal fluid (which is a clear fluid around the brain and spinal cord which serves protection)
- Patients with CJD had this 14-3-3 protein.
- Meanwhile healthy individuals or individuals with other diseases such as Alzheimer's did not have this protein.
- This helps us differentiate between these two diseases (CJD and Alzheimer's) which have very similar symptoms. Therefore, allowing proper treatment as needed in order to maximize a patient's health before symptoms arise.
- This was done through an immunoassay of 14-3-3 (a procedure for detecting or measuring specific proteins or other substances through their properties as antigens or antibodies, defined by google dictionary)

In summary…it is agreed that 14-3-3 proteins should be done if CJD is suspected in a patient. However, there are some limitations.

<u>Pentosan polysulfate (PPS) - what it is and what its doing for CJD</u>
An Overview

- Pentosan polysulfate (PPS) is a commonly used medication to treat bladder pain, relieving the symptoms of pain and discomfort that come with it.
- However, Pentosan polysulfate (PPS) has shown some results in mice, prolonging the incubation periods those infected with prion diseases

- PPS seemingly prolongs the lives of patients who take it, however does not halt the neurodegeneration done by the prions
- While usually taken in pill form for bladder pain, it in injected directly into the brain of patients with CJD

There are apparently four possible situations

- The estimate for the expected survival of a patient is wrong, as previous patients may have been diagnosed late in the course of their disease.
- These patients survived longer due to aggressive treatments for other conditions (like pneumonia) (however data suggests this isn't a likely case)
- It is a simple chance finding due to the very small sample
- PPS actually works in treating CJD!

Ian Bone says the following about the study conducted

"There are some things the families can take away from this. There will now be more research, particularly animal research, because we need to measure the drug's penetration into the brain. Also, the concerns over possible dangerous side effects seem to be groundless. And there is limited evidence of prolongation of life."

In summary…it is a promising experiment, but not yet a definitive treatment

January 26th, 2025

Emerging roles of 14-3-3γ in the brain disorder - PMC.

- 14-3-3 proteins are found in the brain, and are associated with the brain's function as well as disorders
- It is expressed in the neurons of cells, and are produced during the brain's development (this implies it has significance in the brain's development)
- The expression of this protein as per various brain disorders (such as CJD) also shows an impact on the brains plasticity (that being the brain's ability to form new connections in its structure as per external responses such as learning and injury)

What does this review cover?

- *Introduced the various brain disorders reported to be involved with 14-3-3γ*
- *Summarize the changes of 14-3-3γ expression in each brain disease.*
- *Discuss the potential of 14-3-3γ for treatment*
- *Importance of research on specific 14-3-3 isotypes for an effective therapeutic approach.*

More Information of 14-3-3 protein

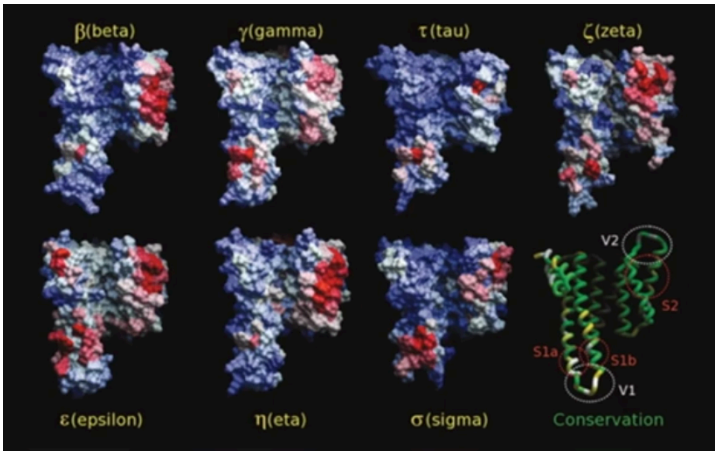
- Extracted from bovine (cow) brain in 1960
- Involved in the making of neurotransmitters (as they activate tyrosine and tryptophan hydroxylases, which make said neurotransmitters)
- They are involved in many cell functions! (including including cell survival, growth, differentiation, migra-tion, and signaling)
- They are able to bind (connect) with some intracellular (inside the cell) proteins such as transmembrane receptors, cytoskeletal proteins, and signal-transducing proteins, such as kinases and phosphatases
- This function allows for the following to happen; the regulation of transcription, cell-cycle control, protein trafficking, metabolism, signal transduction, stress response, and apoptosis

So these proteins are very very important! (wow)

MORE INFORMATION

14-3-3 Protein (2017)

- As we know, proteins play a big role in all of our bodily and cell functions from structure, development, survival, growth, and death
- The family of 14-3-3 proteins are highly conservative. This means that members of said family are very similar in their structure.
- They are found in all eukaryotic cells
- They serve as a ADAPTER CHAPERONE MOLECULE, (a type of accessory protein that prevents misfolding whilst also moving) and help in cellular functions by moving from the cytoplasm to the nucleus of a cell (and vice versa)
- Humans have seven different versions or ISOFORMS of the 14-3-3 protein



But how do they relate to neurological disorders?

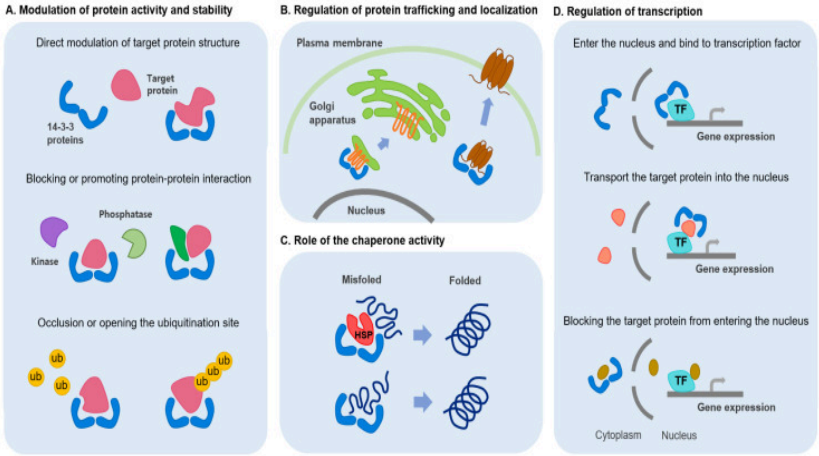
- Under normal conditions, a 14-3-3- protein (like 14-3-3 sigma) performs its usual function (in this case, serving as a blockage for any tumors)
- However, if there is a problem, that protein becomes harmful (say for example the protein 14-3-3 gamma, acting as an oncogene and is able to create cancer, rather than suppress it)
- When the gene is normal, its normal function takes place. When there is a mutation, an oncogene is activated, increases that person's risk to disease such as cancer
- The more mutations (and losses) lead to a higher chance of that patient obtaining that disease (such as cancer)
- Many factors can affect why a gene mutation includes external factors, age, and genetics.

Example - Cancer

- The 14-3-3 sigma protein has a teammate known as P53, and also suppresses tumors.
- They are activated when a cell's DNA is damaged. P53 will express a large amount of 14-3-3 sigma, leading to the cells death (apoptosis) in order to prevent that broken DNA from transmitting to new cells (daughter cells)
- However, when there is a mutation, the P53 is also mutated and decreases 14-3-3 production. Apoptosis therefore does not happen, and the cell transfers that broken DNA, continuing to mutate leading to tumors and potentially cancer.

MORE FUNCTIONS OF 14-3-3 PROTEINS

- A) The proteins can be structurally altered by interacting with a 14-3-3 protein, either by blocking or promoting interactions with other proteins.
- B) regulates trafficking/localize of binding proteins
- - C) interact with chaperone proteins (assist other proteins in proper folding, assembly,



and transport within cells, while preventing harmful aggregation, such as HSP) or can act like chaperone proteins themselves

- D) 14-3-3 proteins regulate gene expression by transporting the target protein to the nucleus, or by blocking the target protein from entering the nucleus. Section D shows the NLS (nuclear localization sequence) of the 14-3-3 protein, which does just that

HOW DO THEY RELATE TO CJD?

Creutzfeldt-Jakob Disease (CJD) is a fatal degenerative brain disorder caused by a misfolded protein, PrPSc prion (scrapie isoform of the prion protein), with the symptoms of confusion, depression, abnormal body sensations, autonomic nervous sys-tem disorders, and dementia (directly from website)

- 14-3-3 proteins have been thought to serve as a biomarker for the diagnosis of CJD
- Finding 14-3-3 proteins in cerebrospinal fluid was often seen as an indication of CJD, concluding that its presence serves as a useful diagnostic tool for the disease
- However, as always, things change…
- In other neurological diseases, (Rasmussen's encephalitis, Schilder's disease, or diffuse large B-cell lymphoma), the 14-3-3 protein was not found
- This may then mean that the presence of 14-3-3 proteins ISN'T because of cell death causing it to leak everywhere, but rather the way in which the proteins actually fold within the brain
- While all isomers of 14-3-3 were found, two were found in specific; 14-3-3 beta and gamma, more specifically now 14-3-3 gamma, be used as a DETECTION for CJD.

RT-QuIC: a new test for sporadic CJD | Practical Neurology

SUMMARY OF CJD (AS PER THE ARTICLE)

These diseases are associated with the creation of misfolded proteins (PrPSc) from cellular protein known as prion protein (PrPC). PrPSc is able to bind to PrPC to induce misfolding and the creation of additional PrPSc, which can then induce further PrPC misfolding and subsequent aggregation into fibrils and plaques. Thus small amounts of PrPSc are able to propagate throughout the central nervous system.

- *85% are sporadic, the majority of the remaining cases being genetics (a small amount of iatrogenic)*

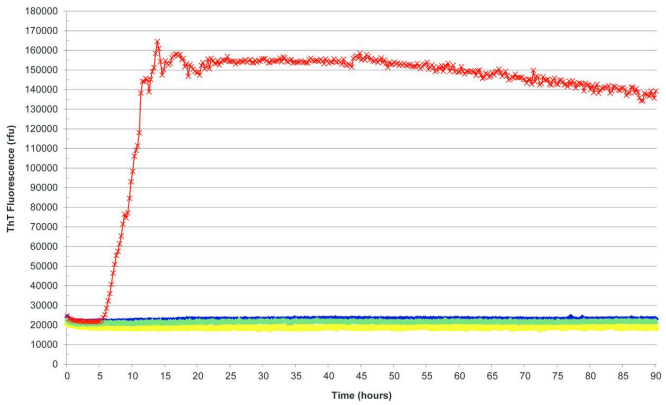
- *Sporadic CJD shows signs of rapidly developing dementia, and death occurs in 6 months*

The diagnosis can be difficult in the early stages and is made on the presence of a series of clinical features and distinctive abnormalities in supportive investigations, such as triphasic waves on electroencephalogram, high signal in the basal ganglia on MR scan of the brain, and the detection of cerebrospinal fluid (CSF) ***14-3-3*** *and other markers of acute neuronal damage such as tau protein in CSF*

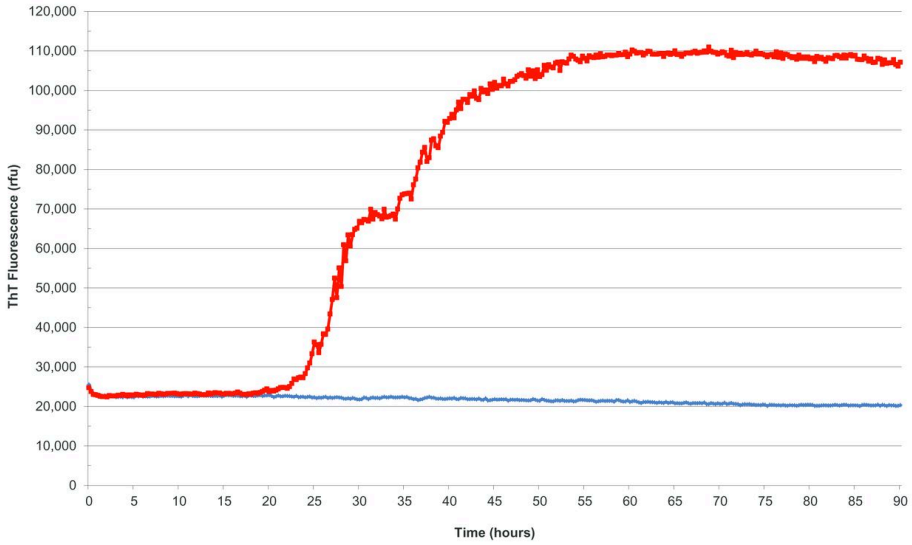
- ***However, they are faulty as they are not specific to CJD // they can be used as a sign for any relating diseases and conditions***

What is RT-QuIC?

- Real time quaking induced conversion or RT-QuIC, has been a way for clinical diagnoses of CJD
- transmissible spongiform encephalopathies are often a sure fire sign or PrPsC, **so detecting it is often the first logical step**
- This test does just that! By taking a fluorescent die called thioflavin T, is able to bind to aggregates proteins
- **CSF (cerebrospinal fluid) is extracted, and mixed with rPrP (Recombinant Prion Protein, which is used for research)**
- **If misfolded prions are detected they bind to the rPrP, causing the formation of long fibrils, indicating the presence of disease**
- The time between the creation of the fibrils is called the lag phase (it can last up to 30 hours!)
- Once they do this, they bind to the dye, which lights up under certain lighting
- This allows for real time tracking of the diseases progression
- The rate of which the aggregation occurs follows a sigmoidal curve,
- This curve indicates that initially, there's little to no aggregation, then it accelerates, and finally, it levels out as the system reaches a maximum point of aggregation (clumping)
- Sensitivities (test those with the disease) 69%-89%
- Specificity (test those without the disease) 99%-100%



The figure shows the RT-QuIC reactions // ThT, thioflavin T. detected as per sporadic Creutzfeldt-Jakob disease brain homogenate as positive control (red), Alzheimer's disease brain homogenate (yellow). This shows CJD detects high prion aggregation



This one shows the "lag phase" of prion aggregation as per sporadic CJD (red) and another unrelated prion disorder (blue)

Despite high performance and accuracy, the process is very long (taking up to 90 hours, so about 3.75 days). An improved version of RT-QuIC called RT-QuIC (IQ) uses hamster rPrP as a substrate, providing similar results but much less observational time (30 hours)

January 28th, 2026

Summary of Background Research

EXISTING TREATMENTS - (previous document)

- Gene regulation/crispr **(pg 16 - 23)**
- Environmental factors **(pg 23 - 24)**
- Unfolding prion proteins from PrPSC to PrPC by the N-Terminus **(pg 24-25)**
- Protein 14-3-3 **(this document)**
- intraventricular pentosan polysulfate **(pg. 67-68)**
- RT-QuIC **(this document)**

SUMMARY OF THOUGHTS

Humans don't actually need the prion protein for survival. Which is why most studies explore ways to silence the prion protein gene or the prion protein to prevent the possibility of a misfolding. Hence the ideas of genetic expression, regulations, and the efforts to see how prions affect the brain and the cells.

Most cases of CJD (85%) are also sporadic (spontaneous); there is no way to know for sure when it will occur. Therefore, these methods of gene regulation/expression allow opportunities for a very small percentage of patients with CJD.

QUESTION

How can we tackle prion disease once it has been diagnosed within a patient?

- 14-3-3 proteins
- intraventricular pentosan polysulfate.
- RT-QuIC

These are all methods which help in the detection/diagnosis of CJD. However, what are we able to do after?

METHODS

1) Unfolding the prions.
- if the problem is being created from the misfolding of prions, would unfolding them back into their regular shape solve the issue?
- This would be done by disrupting or breaking the bond of the fold
2) Changing the prions through their environment
- Environmental factors

FINAL THOUGHTS

1. They discovered that small environmental changes may lead to their unfolding since tertiary and solvation forces are weak.
2. They discover that when a prion protein is in a metastable state, it may be more prone to misfolding.
3. They may create non-native bonds, which furthers their misfolding.
4. PrP^Sc is still very compact, despite folding (suggesting structure from said non-native bonds)
5. Temperature can affect the stability of prion protein (such as thermal deregulation) however the entire structure does not fall apart completely (due to the contacts between non-native bonds)
6. They restated their belief that the C-terminus of a protein is crucial for its stability. So much so, they call it “the achilles heel of prion stability.

Another thing I noticed is that the structure of the PrP^C and the PrP^Sc is also very crucial in understanding the processes and mechanisms of prion disease. However, these mechanisms and structures are still unknown when it comes to this disease. I think it is important to dive into these structures. The role they play, how they interact, how they differ, etc. That will help us understand what to tackle when it comes to our experiment.
But what crucial information have I learned do I think I could use?

1. The fact that tertiary contacts and solvation forces are weak, so small environmental changes could affect them. (such as temperature)
2. Yet due to non-native contacts and unforeseen stable structure, it may not unfold completely.
3. The metastable state is when it seems best to act on it.
4. The C-terminus is a place to target, being the 'achilles heel'

My ideas that I mentioned above earlier regarding environmental factors and somehow allowing PrP^c to help mend PrP^Sc is something I'm looking into.
Prions are able to react, adapt, and change over time based on their environment and other factors. They also touched on prion strains, and mentioned that when said prion strain was moved, it was able to do just that in order to survive in its new host. These are factors which help a prion strain thrive. Becoming immune to certain vaccines and drugs, and adapting when it is moved to a new host, are all evolutionary benefits a prion strain has so it will survive.
We see how in the study above, certain environmental and host factors also affect prion strains and their survivability. It seems all these factors play in the prions favour. **That is important to remember.**

These changes prions are seen to make all come down to its need to survive and thrive. The way it adapts to different environments by changing its shape is so the prion proteins are able to survive, like any other organism would.

FINAL REALIZATION
So we see that prion disease is of course very complex and difficult to treat. Due to the way prions adapt to new environments, the way they are structured, are immune to protease, have no DNA, and the way the disease is contracted spontaneously, all contribute to the difficulty surrounding these types of disease. And while this disease is very rare, that contributes to a lack of research which in turn contributes to a lack of knowledge and understanding.

Most studies focus on handling the prion protein or PrP^c before it misfolds into the actual PrP^Sc form. This is done through things like gene regulation/expression, epigenetics, genetic editing (CHARM, CRISPR, etc) This is a plausible and understandable solution, **as when the prion protein actually misfolds, there isn't a lot you can necessarily do to treat the disease since unfolding it within the brain is risky and difficult.** However, since CJD (the most common prion disease in humans and the one I am tackling) most often is sporadic (80%), meaning it is contracted spontaneously. **So there often is no way to know if a person will contract CJD; how will you then treat it early on?**

IDEAS

1. Dwell on the structure; study, analyze, and see if any part of it can be manipulated to break it down (such as the C-terminus, or the achilles heel of prion structure)
2. Slightly more complicated idea…taking a unfolded/healthy prion protein, and use its structure to somehow help a misfolded protein

YEAST AND HUMAN PRIONS

(pg. 39-40)

- Prions are not only found in animals, where they can cause extremely harmful neurodegenerative diseases; they can also be found in fungi such as yeast (Saccharomyces cerevisiae)
- Like human prions, these fungal prions are also able to change their conformation on their own terms. Adopting multiple shapes/conformations, and causing other proteins to do the same. (they are able to exist in one or more self-propagating alternative conformations)

(pg. 42-43)

- Animals such as humans do not have this certain chaperone, but we do have others such as Hsp 40, 70, and 110 which all help with the disaggregation of prions by stopping them from misfolding in the first place
- If Hsp104 is introduced (exogenous Hsp104) from another source, it may be able to team up with these chaperones and promote disaggregation in humans and therefore help with treating diseases associated with amyloids
- But there is still doubt that this would work at all, as scientists worry that Hsp104 alone may not be enough. These limitations may mean that other treatments will have to be used alongside this introduction of a fungal chaperone.
- The study was to show and explain findings on how certain chaperones in fungi and humans can work together, and then perhaps lead to certain therapeutic treatments for certain diseases associated with them (amyloid diseases, such as CJD)

Hsp104 is a chaperone (which is defined as a protein which helps the other proteins) specifically to fungi such as yeast. This chaperone is interesting, because rather than preventing the misfolding of proteins like chaperones Hsp40, 70, and 110, by working

together, sharing information, etc, it takes the already aggregated proteins due to stress such as heat, toxins, etc, and helps it to unfold and keep the fungi safe.

(pg. 41-42)

- Prions which are found in fungus behave like cytoplasmic non-Mendelian genetic elements
- This means that the genetic material is found in the cytoplasm of a cell, rather than the nucleus. This does not follow the typical medallion genetic pattern
- But prions do not have genetic material; the article is referring to the fact that these fungal prions can act like a heritable trait, by passing down their traits to induce misfolding.
- It ensues a chain sequence of misfolded prion proteins. This is considered cytoplasmic non-Mendelian genetics BECAUSE that trait is being passed down without any transfer of genetics.

- They helped discover how prions have no DNA or RNA but misfold all on their own (protein-only nature), the basics of protein strains, and the role that chaperones have in the propagation of prions.
- And yet they do have their differences, like how the fungal prion misfoldings help the organism rather than harm it.

(pg. 43-44)

- We see that in fungal prions, including prion strains such as [PSI+], [URE3] and [PIN+] have a specific structure of beta sheets
- Discussing specifically the structure, the B-sheets in amyloids seems to be the reason as to why a prion can be self templating (why the shape of the prion can be transferred to another prion, creating a chain reaction of misfolds)
- Hence, explaining how these prions act as genes in yeast.
- But how is the idea of prion strains and the non-mendelian behaviour of fungal prions connected? Well, as we know these different strains have different characteristics. These differences impact the way it transmits and spreads itself, and therefore impact certain trains of other proteins. This is all done through the beta-sheet structure (which ensured stability and also effective self-templating)
- And this ability to self-template (the ability of different prions to pass along their different traits to other proteins) reinforces the idea of these prions acting as genes.
-

More on prion strains in bakers yeast (Saccharomyces cerevisiae)

- [PSI+]- related to a protein called SUP35. This protein plays a role in translation termination. PSI leads to something called nonsense suppression- which leads to incomplete proteins
- [URE3]- associated with a protein called Ure2, which regulates nitrogen utilization. When this prion is present, it disrupts this utilization, which means that different nitrogen sources must be used.
- [PIN+]- links to a protein of the name Rnq1. this prion enhances the reproduction of other prions (the propagation)

They all follow non-mendelian behaviours, making them interesting to a fungi protein structure.

Btn2/Cur1 can help heal the prion strain URE3. These two proteins recognise misfolded prions, and target them to prevent their accumulation, and therefore their spread. They do this through **DEGRADING the protein, or INHIBITING THE PROPAGATION.**

- PSI+ is a variant which affects the translation of proteins, which can lead to non-functional proteins
- While it can be lethal if in large amounts, however it is often mild and can allow the yeast to handle stressful conditions.
- The other strain URE3 is related to nitrogen metabolism, and like PSI+, depending on the quantity it can either be lethal of helpful
- The study suggests that because these variants are rare, they could have severe detriments (such as toxicity or decrease in reproduction) to yeast despite their beneficiary properties.
- However, in a surprising twist, the protein variant [Het-s] is beneficial to the host.
- It participates in something called heterokaryon incompatibility which can help an organism distinguish its cells from different ones with a different genetic background, preventing the spread of harmful genetic elements in that organism
- This difference highlights the complexity of the fungal protein structure.

FINAL THOUGHTS

What I am trying to accomplish with my experiment is to deal with PrPSC once a patient has already contracted it. The protein is already misfolded, and the patient has been diagnosed. **Is there a way to unfold this prion> To stop it from**

spreading/propagating? What can be done? I summarized everything in my notebook, and here are some key points I found interesting.

KEY POINTS

- They can be beneficial towards certain stressors yeast can face (such as temperature) which can trigger misfolding-while that is detrimental that folding can actually be a way for the yeast to survive.
- Similar to amyloid structures, which have helped in developments for human amyloid disease (such as CJD)
- They can also also have variants and chaperones

- These prions self template and self propagate (non-mendelian)
- This self templating is done through the B-Sheet structure of said prion.
- They have certain prion strains which do damage, but also systems which fight against that (Btn2/Cur1 can help heal the prion strain URE3)
- HET-S acts on heterokaryon incompatibility
- Certain stressors lead to its misfolding

Things like Btn2/Cur1 which work towards fighting against the URE3 protein strain. They recognize the misfolded protein, target them, and work to either DEGRADE or INHIBIT THE PROPAGATION of said prion.

<u>MY FIRST IDEA (pg. 47-49)</u>

If we take these proteins, and insert them exogenously within a system of yeast that has been ridden with misfolded proteins, would the introduction of these proteins fight against that propagation and degrade the protein, therefore ceasing its reproduction? If the prions stop reproducing, then they are unable to form protein aggregates, quelling the disease. (I may have to somehow remove the Btn2/Cur1 from the test on yeast, to see if it will work. Perhaps removing that protein will be a more honest replica of human prion protein systems, leading to a better connection)

The concept of introducing something from outside its system has been thought of with the chaperone HSP104, in order to have mammalian chaperones work together and perhaps fight the prion misfolding. Could it not be done with other structural elements of the fungal system? And if this method of exogenously inserting these proteins works, that same logic could be applied to the human brain. Backing off again the idea with the HSP104 chaperone, it was thought to insert that within a mammal in order for it to work

with their mammalian chaperones. **Could the protein Btn2/Cur1 be used the same way?**

QUESTION- Can the protein Btn2 and Cur1 be used exogenously like the chaperone protein Hsp140 to cease the aggregation of the prion protein, therefore stopping or slowing neurodegeneration?

MY SECOND IDEA

Using hydrolysis (or catabolism), we can take a polymer (such as a protein) and break the bond into its monomer units (amino acids). Prions have the same amino acid structures as their healthy protein counterparts, it is simply the shape which causes them to become infectious. If we have a prion and perform hydrolysis on it to break the bond, it would be assumed that only the prion would be destroyed. **Since these prions cause other healthy proteins to misfold, causing a large sum of brain cells to die, breaking down those prions early in the process may stop the disease from spreading.**

I am rethinking the idea regarding the protein hydrolysis, as if we were to apply that to the actual brain **a) those proteins would be extremely difficult to distinguish between and b) actually performing hydrolysis on them would be seemingly impossible because of it.** However, my original question still stands on how we can deal with this disease once it has contracted. Due to the fact that CJD is spontaneous, there is no way to detect or predict when an individual may contract it.

MY THIRD IDEA

So, is it possible there is a way to detect and destroy the spore of this disease, or any sort of beginning infection before the proteins continue to misfold? And overall, the next steps of this project involve taking my soon to be completed research and narrowing it down into something more specific. Additional experiments and future studies can be something else I look into, as well as connecting the project to the way it serves, protects, and helps people.

QUESTION: Is there an inbetween stage between the contraction of CJD and the misfolding of proteins in the brain? Are there ways we can kill or weaken its effect before it infects the proteins of the brain, preventing neurodegeneration?

- In sporadic CJD (85% of cases), symptoms include things such as imbalance/incoordination, memory loss, and impaired thinking

- There are also psychiatric symptoms such as depression or anxiety.
- However, once symptoms do appear, CJD will progress very quickly and within a few months it will be considered fatal.

- The reason for this is because the incubation period of CJD is extremely long and can take decades for symptoms to appear.
- Key hallmarks of CJD include rapid progression of dimension/involuntary muscle movement. These symptoms will worsen over time, and vision as well as the ability to move and speak is lost. They may also enter a coma.
- It resembles Alzheimer's, but the difference is that symptoms take weeks not years to form

The incubation period for CJD is actually extremely long and not able to be known since no symptoms are present. And once they do, it is already too late for the patient. There are ways to check for CJD and various unspecific symptoms which will be present, but again once symptoms appear the patient has nearly six months to live.

This may mean that seniors (who are most susceptible to CJD) should go under the multiple tests that doctors can provide such as urine samples, blood samples, MRI's, EEGS, etc, so that if that misfolded protein is present the family and the doctors know, and we can deal with the disease before symptoms even show. In addition to this, it could lead to other diagnoses such as RPD, as well as other neurological complications. However this involves spreading awareness on CJD, and making it known to the public so they can protect themselves and their family members.

I think I'm going with my first idea.

QUESTION

Can the protein Btn2 and Cur1 be used exogenously like the chaperone protein Hsp104 to cease the aggregation of the prion protein, therefore stopping or slowing neurodegeneration?

HYPOTHESIS

If Btn2 and Cur1 proteins are introduced exogenously into the human brain from saccharomyces cerevisiae then will the aggregation of mammalian prions in the human

brain slow because Btn2 and Cur1 helps degrade and inhibit the propagation of the prion strain URE3 found in bakers yeast.

February 12th, 2026

HSP104 | SGD

- A high shock protein (meaning they react to external factors/stressors such as heat, ethanol, and sodium arsenite)
- Described as a disaggregase (a molecular chaperone behaving like an enzyme to break down/reverse the aggregation of proteins, making them functional)
- It works with/ cooperates with Ydj1p (Hsp40) and Ssa1p (Hsp70) to refold/reacitivate protein aggregates
- The stressors of heat, ethanol, and sodium arsenite (and of course others) make it so proteins become tangled. Hsp 104 helps to detangle them, turning them into a liquid form so they can disperse and function properly

- Hsp 104 has been studied in yeast models for human diseases, such as Creutzfeldt-Jakob disease (and Huntingson disease)
- In YEAST there are three types of proteins strains, and three related proteins

[PSI+] : Related to the protein Sup35p (impacts how genetic information is expressed)
[PIN+]: Related to the protein Rnq1p (similar to PSI)
[URE3] : Related to the protein Ure2 (regulates nitrogen metabolism)

- If HSP104 is deleted, these prion strains are not able to be multiplied (propagated) .
- In order for that prion to be inherited by a daughter cell, it must be broken down. Without that fragmentation, the aggregate becomes large and is not able to be passed to the daughter cell effectively
- With Hsp40 and Hsp70 does Hsp104 break down these strains into smaller fragments. Those smaller fragments go on to convert other proteins into prions
- If HSP is underexpressed/deleted, that break down can't happen, preventing the transfer of those prions (however, its a double edged sword, because a large toxic aggregate can still have negative impacts on those yeast cells)
- If HSP104 is overexpressed, it can cure the yeast cells of the [PSI+] prion strain (reduces clumping/protein aggregation because it just breaks it down so well)
- In mice that were genetically modified to give Huntington's disease, extra HSP104 let them live longer. (what could that mean for humans?)

In YEAST, you want a balance of Hsp104, as you don't want the prion strain to go extinct in the yeast cell because while they are harmful, they help yeast cells adapt and thrive in very harsh environmental stress. So a "goldilocks" amount of HSP104 ensures it stays regulated, but not extinct.

February 13th, 2026

What did I do and learn today? Well, I fixed my method and problem on the science fair to-do list, so it is exactly what I want it to be.
(here's a snippet)

Final Method

If we take these Btn2/Cur1 proteins, and insert them exogenously with a system that has been ridden with misfolded proteins, would the introduction of these proteins fight against that propagation and degrade the protein, therefore ceasing the aggregation? And if those proteins stop forming aggregates, would the disease be silenced?

The concept of introducing something from outside its system has been thought of with the chaperone HSP104, by having mammalian chaperones work together and fight prion misfolding. Could it not be done with other structural elements of the fungi? Using what we know about the HSP104 chaperone, could the protein Btn2/Cur1 be used the same way?

QUESTION- Can the protein Btn2 and Cur1 be used exogenously like the chaperone protein Hsp104 to cease the aggregation of the prion protein, therefore stopping or slowing neurodegeneration?

Btn2 and Cur1 are proteins that would be needed to be delivered to mammalian cells. This differs from Hsp104 because that is a molecular chaperone - not a protein.

Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants | PNAS

- URE3 prion is the mutated form of Ure2 (performs nitrogen catabolism) in yeast
- Btn2 and Cur1p can cure this prion when overexpressed
- ***Cur1 is a paralog or Btn2, meaning its from the same species from the same ancestor and formed from gene duplication***
- Btn2 **colocalizes** (is present at the same time as) Ure2 aggregates
- URE3 prions will get larger in size if Btn2 and Cur1 aren't present - it can be cured when the two proteins are back to at least a normal amount

- Btn2 and Cur1 are members of the *hook protein family*
- They are **metazoans** (members of kingdom animalia as they are multicellular heterotrophic eukaryotes) which use **microtubules** (small hollow protein tubes in a cell) to move things like organelles or even aggregates in them
- Those organelles and aggregates are the cargo which are moved by the motor (motor proteins) kinesins and dyneins with ATP. The microtubules are the tracks
- The hook proteins Btn2 and Cur1 HOOK the motors and cargo together

Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants - PMC

How do Btn2 and Cur1 work?

- Sequestration is how Btn2 and Cur1 work
- They gather the prion aggregates in a single location, prevent their division to daughter cells, and thus there is no more prion aggregation because it is no longer being passed down!
- This is suggested/what is assumed to be happening as Btn2 is colocalized (is present at the same time as) Ure2 aggregates
- Both proteins are needed for efficient curing

Inside Job: Methods for Delivering Proteins to the Interior of Mammalian Cells - ScienceDirect

- 7/10 of the top selling drugs are made of proteins (biologicals)
- Their large size, structural complexity, and molecular diversity often results in them being able to recognize receptors that challenge and evade (basically harm) smaller molecules
- However! There is an issue. Most proteins are not actually able to get through the phospholipid bilayers of these mammalian cells!
- The limitation has prevented disease causing and relating receptors that proteins would have been able to deal with
- Because of this, there is a lot of work being done to get over this hurdle.
- **Often through the exogenous proteins to be delivered to cells (intracellular delivery) in mammalian (human) cells**
- These can be natural or engineered

- There are methods which transport nucleic acids (DNA or RNA, which are able to make/encode proteins) into cells
- CATIONIC LIPIDS which are positively charged molecules that make liposomes to carry said nucleic acids
- VIRUSES as they can infiltrate cell walls naturally (probably wouldn't want to use that…)
- Breaking open the cell membrane to get what's needed inside the cell
- **CELL-PENETRATING PEPTIDES (CPPS) are short peptide chains that help proteins enter cells (sometimes to specific cells)**
- PROTEIN RESURFACING changes the surface of the proteins used to allow them to fit in better
- Using proteins from TOXINS as they can naturally penetrate the cell barriers
- CHEMICALLY MODIFIED PROTEINS which change the shape with chemicals to allow them to better fit

- NANOPARTICLES which surround and capture proteins within them like a capsule to help transport them better

It is also possible to carry nucleic acids and proteins at the same time which can be important for things like CRISPR where the Cas9 enzyme and RNA must be delivered at the same time

February 17th, 2026

The question remains…would it be better to exogenously introduce proteins into the brain through nucleic acids that encode for proteins or directly inserting the protein itself? **Well, as we know, the brain is a very delicate organ. If we inserted new DNA to the brain, how would that itself impact the brain?** Would it cause more complications than simply inserting the protein as is? I think inserting the protein by itself contains less risk with the same effect. Of the many ways the article lists on how to insert proteins, CPPS seem the least invasive or risky. I think I will pursue my final stretch of research on that.

February 20th, 2026

- Directly inserting the proteins is a far simpler way to introduce exogenous proteins to mammalian cells
- But with the hydrophilic surface of the cells, protein transfers are limited
- **BIOLOGICS is when medicines are derived from living organisms such as plants, animals, cells, etc than making them artificially with chemicals**
- With the rise of biologics in the pharmaceutical industry, many methods have been uncovered in order to insert exogenous proteins into mammalian cells

Cell-Penetrating Peptides (CPP's)

- Relatively short peptides (less than 20) that can attach to a protein and help it get over the phospholipid bilayer of a cell.
- Very straightforward for direct delivery of the protein to the cell.
- **But between common examples of cpp's, shows a common trend of high cationic residues (positively charged amino acids) in particular lysine and arginine, two types of amino acids.** (this includes Tat peptide from the trans-activating protein Tat of HIV-1, and the third helix of the homeodomain of antennapedia called penetratin)

- Cationic residues react in nice ways when met with the phospholipid bilayer of a mammalian cell by **engaging with negatively charged glycosaminoglycans (GAGs)such as heparin sulfate and chondroitin sulfate on the surface of cells.** Specialized glycoproteins (a class of proteins where carbohydrates groups are attached to the peptide chain) called proteoglycans, have a core protein which is attached to the GAGs.

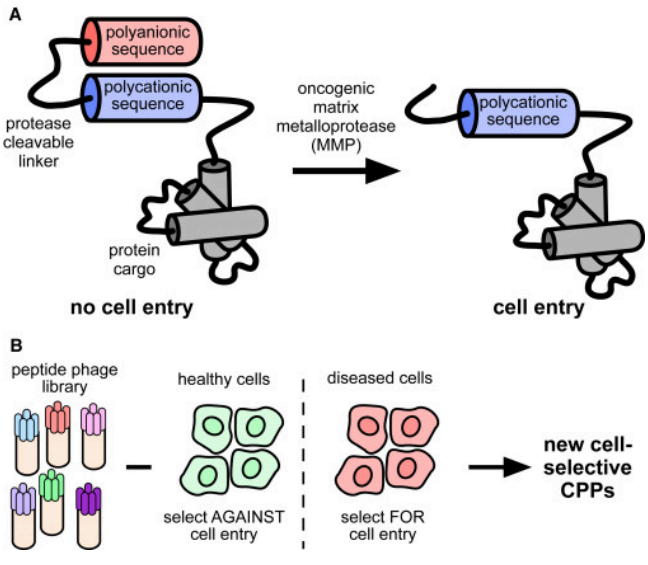
- ***glycosaminoglycans*** *are long, negatively charged polysaccharide found on a cell's surface.*
- ***chondroitin sulfate and heparin sulfate*** *is a sulfated GAG.*
- *They have functions in regulating cell growth, proliferation (rapidly protruding parts of a cell), promotes cell adhesion, anticoagulation, and wound repair*
- Polypeptides with more arginine are better at transportation than lysine
- They react with the proteoglycans through **bidentate interactions.** This means the bond happens in two places, making it stronger (and the transportation better)
- The cell will then engulf and internalize those cells (endocytosis)

- A limitation that exists however, is that these polycationic CPPs like to bind and enter really any mammalian cells. This means that the cells (in our case, the protein cells in the brain) may not get targeted like we want
- Because of this, the cargo they carry can go to undesirable cells which could lead to unwanted side affects

- An example of how CPPs has been made more specific includes cancer-cell activated CPPs
- These cells have three components;

Polycationic CPP to penetrate the cell membrane
Polyanionic biopolymer- binds to the polycationic CPP to stop it at a certain point from entering healthy cells

- The polyanionic biopolymer binds to the cationic CPP and stays linked when no cancer associated proteases are present. This keeps the cpps from penetrating any cells including healthy ones
- But when cancer is detected, it releases a protease, which disconnects the link between the cationic and anionic parts of the cpps. And they separate, the cationic cells able to penetrate cell membranes
- This means the cationic cells are able to be targeted specifically by cancel cells and act upon them only



The protein BTN2 has cationic parts to it, but is not necessarily considered a high cationic protein.

- However, the thought process and research continues. It has been thought possible to mutate some proteins to add or replace amino acids with cationic residues to increase their positive charge, which increases the ability to bond with negatively charged aspects of the cell membrane, making the bond stronger.
- When arginine is used as a replacement for amino acids, it is called arginine grafting.
- Take for example RNase, an enzyme which can destroy RNA found within cells. When it is modified with the cation arginine, they can enter the cells and can cause the death of said cell.
- Something to keep in mind however, is the fact that the proteins may get trapped in small compartments within the cells called endosomes. This could prevent the movement of the proteins, meaning they don't act to their full capacity
- Scientists are still trying to navigate around this obstacle to more desirable areas of a cell such as the nucleus.

Supercharged proteins

- These are proteins modified to have a lot of cations
- **The main benefit they have is that they not only can penetrate mammalian cells, but are stable and therefore won't aggregate or fold**

- Most proteins actually aren't able to go through this type of genetic mutation
- So instead, scientists have been looking into using NANOBODIES
- Nanobodies are proteins derived from camels and llamas that have structures that allow them to bond to other molecules due to their unique beta-sheet structure and CDRs (complementary determining regions)
- Cationic resurfacing can be done to these nanobodies, and can effectively enter the mammalian cells

CONCLUSION

Btn2 and Cur1 proteins are able to silence misfolded proteins that can form within saccharomyces cerevisiae by gathering the prion aggregates in a single location. Btn2 and Cur1 prevent aggregated proteins from dividing into daughter cells, which stops their traits from being passed down, ceasing aggregation within the yeast. This sequestering done by the proteins can help mammalian cells fight off prion diseases as well, however, Btn2 and Cur1 proteins are not present within them. But using the practices of biologics, it is possible to extract Btn2 and Cur1 proteins from saccharomyces cerevisiae, and insert them into mammalian cells within the human brain exogenously. This would sequester those proteins the same way it does in the fungi.

Cell penetrating peptides are one of the simplest ways to introduce exogenous proteins such as Btn2 and Cur1 into mammalian cells. These small peptides attach to the desired protein, assisting in its transportation. The cationic residues on CPP's (such as arginine and lysine) bind to the phospholipid bilayer of a mammalian cell by engaging with negatively charged glycosaminoglycans (GAGs) such as heparin sulfate and chondroitin sulfate on the surface of cells. Through this bond, the protein which is attached gets engulfed by the cell through endocytosis. From this point, the Btn2 and Cur1 proteins are expected to act as they usually would within yeast cells, and sequester the protein aggregates.

LIMITATIONS

A limitation that exists however, is that these polycationic CPPs like to bind and enter most mammalian cells. This means that the cells we want the protein to bind to, may not get targeted like we want because they are so general. Because of this, the cargo they carry can go to undesirable cells, which could lead to unwanted side effects.

Efforts have been made to make CPPs more specific. An example of this includes cancer-cell activated CPPs. These cells have a few components; Polycationic CPP to penetrate the cell membrane, and polyanionic biopolymer, which binds to the polycationic CPP to stop it from entering healthy cells. The polyanionic biopolymer binds to the cationic CPP and stays linked when no cancer associated proteases are present. This keeps the CPPs from penetrating any cells including healthy ones. But when cancer is detected, it releases a protease, which disconnects the link between the cationic and anionic parts of the CPPs. And they separate, the cationic cells able to penetrate cell membranes. Meaning the cationic cells are able to be targeted specifically by cancer cells and act upon them only.

FUTURE STUDY

Applying what we know about cancer-activates CPPS, it may be possible to apply that same knowledge to prion diseases. When misfolding is occurring within the brain, then will the Btn2 and Cur1 proteins be released in order to sequester them. That way, healthy proteins are not being targeted. Diagnosing CJD within patients also becomes less strenuous because if the Btn2 and Cur1 proteins are inserted and work like cancer activates CPPs, they will handle them accordingly rather than festering into a larger issue.

However the doubt remains on how the brain will react to foreign entities inside of it, such as these Btn2/Cur1 proteins, as well as potentially nanobodies. Will the brain/immune system attack these foreign entities? How are we supposed to infer the potential risks of biologics, especially to something as delicate as the human brain? These questions raise the possibilities for future studies and works to be conducted upon prion disease and exogenous proteins.