Prions

Other molecules besides organelle DNA are inherited in non-Mendelian patterns. Examples of non-Mendelian patterns of inheritance extend beyond the inheritance of organelle DNA. Certain DNA and RNA plasmids, for example, are inherited in non-Mendelian patterns, but the strangest cases of non-Mendelian inheritance is the inheritance of endogenous proteins with altered structures; these proteins are known as prions. Prions can be inherited in yeast and the filamentous fungus *Podospera anserina*, and most of our discussion will focus on two elements in yeast known as [URE3] and [PSI+].

Prions were first proposed to cause spongiform encephalopathies in mammals
Prions were first discovered as the etiological agents in transmissible spongiform encephalopathies such as scrapie in sheep and goats, bovine spongiform encephalopathy (BSE or mad cow disease) in cattle, and Creutzfeldt-Jacob disease (CJD), Gerstmann-Straussler-Schneinker syndrome (GSS), fatal familial insomnia and kuru in humans. Collectively, these diseases have been explained by the prion hypothesis. This hypothesis posits that the infectious agent is an abnormal form of a cellular protein known as PrP. During the course of the prion disease, a protease resistant, aggregated form of PrP, designated PrPSc,
accumulates in the brain. The model proposes that the presence of PrPSc, which has the same sequence as PrP but a different structure, will recruit normal PrP into the abnormal structure to spread the disease. The disease is thought to spread when animals are fed food containing brain material. Alternatively, with rare genetic forms of the disease, individuals inherit a mutant form of PrP that has a higher probability of spontaneously folding into a protease resistant prion form. In 1997, Stanley Prusiner, a neurologist at UCSF, won the Nobel Prize in Physiology and Medicine for his work leading to the prion hypothesis. Below are several results for this class of diseases that are consistent with the prion hypothesis. (1) No nucleic acid has ever been shown to be associated with the purified Scrapie infectious agent. Purified PrPSc can cause Scrapie in many animals including mice. (2) Familial spongiform encephalopathies in humans are associated with mutations in the PrP gene. About 10% of human prion diseases are hereditary, and these are associated with mutations in the PrP gene. Individuals affected by GSS, for example, contain a Pro to Leu mutation in their PrP gene. Transgenic mice that carry a mouse PrP gene with the GSS lesion will spontaneously develop spongiform disease. Normal mice carrying the wild-type PrP gene do not. (3) Mice lacking the PrP gene are normal, but are resistant to Scrapie. All of these results are consistent with the prion hypothesis.
Further evidence for the prion hypothesis
Evidence of a different type supporting the prion hypothesis has come from experiments in fungi. The yeast element \([URE3]\), for example, can now be explained by the prion hypothesis. In yeast, mutations in the nuclear gene for aspartate transcarbamylase, an enzyme in the pyrimidine biosynthetic pathway that produces ureidosuccinate from carbamyl phosphate and aspartate, cause an auxotrophic phenotype that can be rescued by growing on exogenous ureidosuccinate in low nitrogen.

Low Nitrogen

\[
\begin{align*}
\text{Ure} 2p & \quad \text{Chn} 3p \\
& \quad \text{(Transcriptional Regulator)} \\
& \quad \text{Dal5p} \\
& \quad \text{(ureidosuccinate transporter)} \\
& \quad \text{ureidosuccinate} \quad \text{ureidosuccinate} \\
\text{in} & \quad \text{out}
\end{align*}
\]

High Nitrogen

\[
\begin{align*}
\text{NH}_3 & \quad \text{Ure} 2p \\
& \quad \text{Chn} 3p \\
& \quad \text{(Transcriptional Regulator)} \\
& \quad \text{Dal5} \\
& \quad \text{ureidosuccinate} \\
\text{out}
\end{align*}
\]
Yeast growing on a rich nitrogen source, such as ammonia, repress transcription of enzymes and transporters needed for the utilization of poor nitrogen sources. The Ure2 protein senses the presence of a rich nitrogen source and blocks the action of the Gln3 protein, a positive transcriptional regulator of many genes whose products facilitate the utilization of poor nitrogen sources. One of the genes that Gln3p regulates is \textit{DAL5}, which encodes a protein that can import ureidosuccinate into the cell. Thus, cells mutant for aspartate transcarbamylase will not grow on exogenous ureidosuccinate in the presence of ammonia because Gln3 is inactivated by Ure2 protein. In the absence of active Gln3, the Dal5 transporter is not produced.

Aspartate transcarbamylase mutant grown on exogenous ureidosuccinate

<table>
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<tr>
<th>URE2 genotype</th>
<th>nitrogen</th>
<th>growth</th>
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<tbody>
<tr>
<td>+</td>
<td>-NH3</td>
<td>+</td>
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<tr>
<td>+</td>
<td>+ NH3</td>
<td>-</td>
</tr>
<tr>
<td>ure2-</td>
<td>+ NH3</td>
<td>+</td>
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<tr>
<td>[URE3]</td>
<td>+ NH3</td>
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In the 1970s, Lacroute identified mutations in the gene $URE2$ on the basis of this phenotype: mutants that would now grow in the absence of the aspartate transcarbamylase gene and the presence of ammonia and ureidosuccinate. $ure2$ mutations segregate 2:2 as a Mendelian trait. Other "mutants" with the same phenotype called $[URE3]$ were also isolated, but these elements are not inherited in a Mendelian pattern. For example, crosses between wild type and $[URE3]$ mutants generated diploids that were $[URE3]$. Hence, the mutant appeared dominant. Sporulation of the $[URE3]$ diploids, however, usually generated 4 $[URE3]$; 0 wild-type spores, although other inheritance patterns were also seen. This inheritance pattern of $[URA3]$ is clearly non-Mendelian. These experiments suggested that $[URE3]$ was mitochondrial, but when mitochondrial DNA was lost in these strains, $[URE3]$ was still present. Lacroute carried out these experiments in the early 1970s and didn’t have a good explanation for $[URE3]$.

In the 1990s, Reed Wickner tested the idea that $[URE3]$ was a prion form of the Ure2 protein. Several experiments supported the prion hypothesis. First, increasing the amount of Ure2 protein by 10 fold increases the frequency of spontaneous $[URE3]$ mutants generated by 100 fold. Second, yeast cells containing $[URE3]$ can be
cured of this element by growth in low concentrations of guanidine, a protein denaturant. Third, the normal Ure2 protein is susceptible to protease, but resistant in a [URE3] background. Fourth, the URE2 gene, and thus the Ure2 protein, was also shown to be required for the activity of [URE3]. [URE3] is lost when placed in a ure2 mutant background.

Additional evidence for the prion hypothesis comes from [PSI], which is likely to be a prion form of the Sup35 protein, a translational terminator. Like [URE3], [PSI] is a non-Mendelian element that requires the presence of the SUP35 gene for its effects. The [PSI] phenotype occurs at a much higher frequency in yeast that overexpress Sup35 protein, similar to the relationship between [URE3] and the Ure2 protein. Removal of the gene encoding the chaperone protein Hsp104 cures yeast of [PSI]. The involvement of a protein-folding chaperone in the maintenance of [PSI] argues strongly that [PSI] is a protein structure. These genetic and biochemical results are all consistent with [URE3] and [PSI] being prion forms of the Ure2 and Sup35 proteins. It is interesting that the portions of the Ure2 and Sup35 proteins involved in prion formation are distinct from the regions involved in their normal cellular functions and are not required for their normal function. Moreover, the prion-forming regions are conserved
in organisms other than *S. cerevisiae*, suggesting that this region has some important function. Current models propose that the epigenetic control of functional protein levels though prion formation can have a selective advantage.