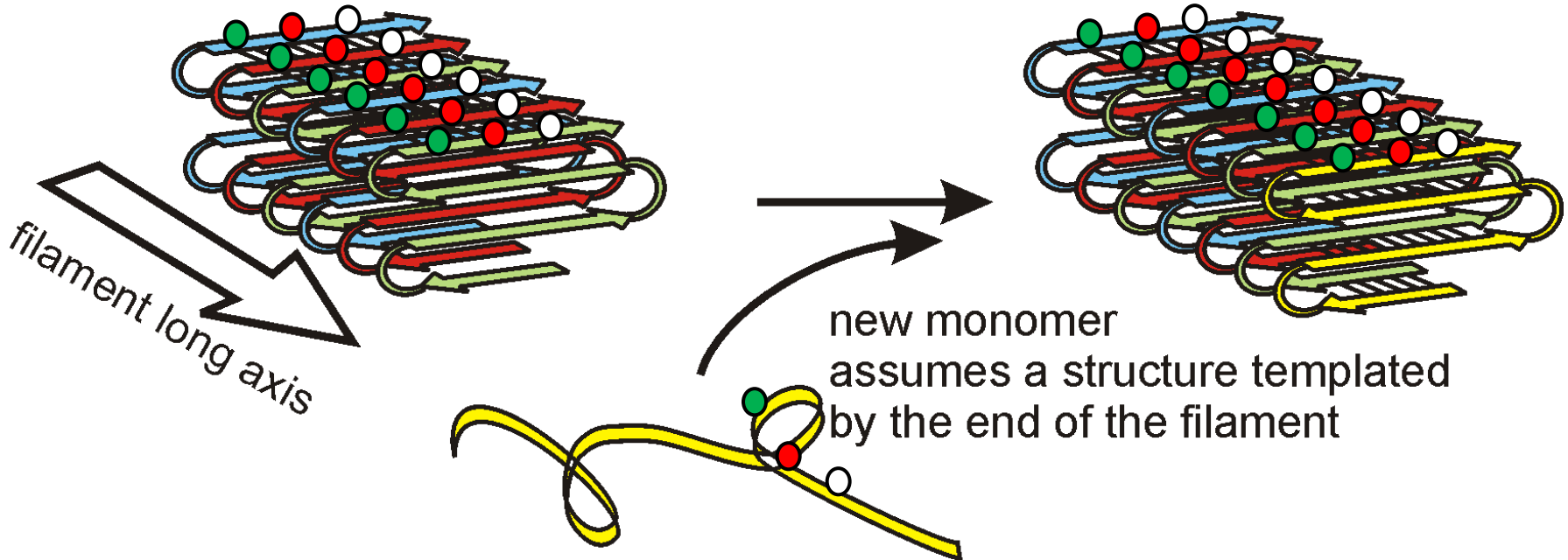
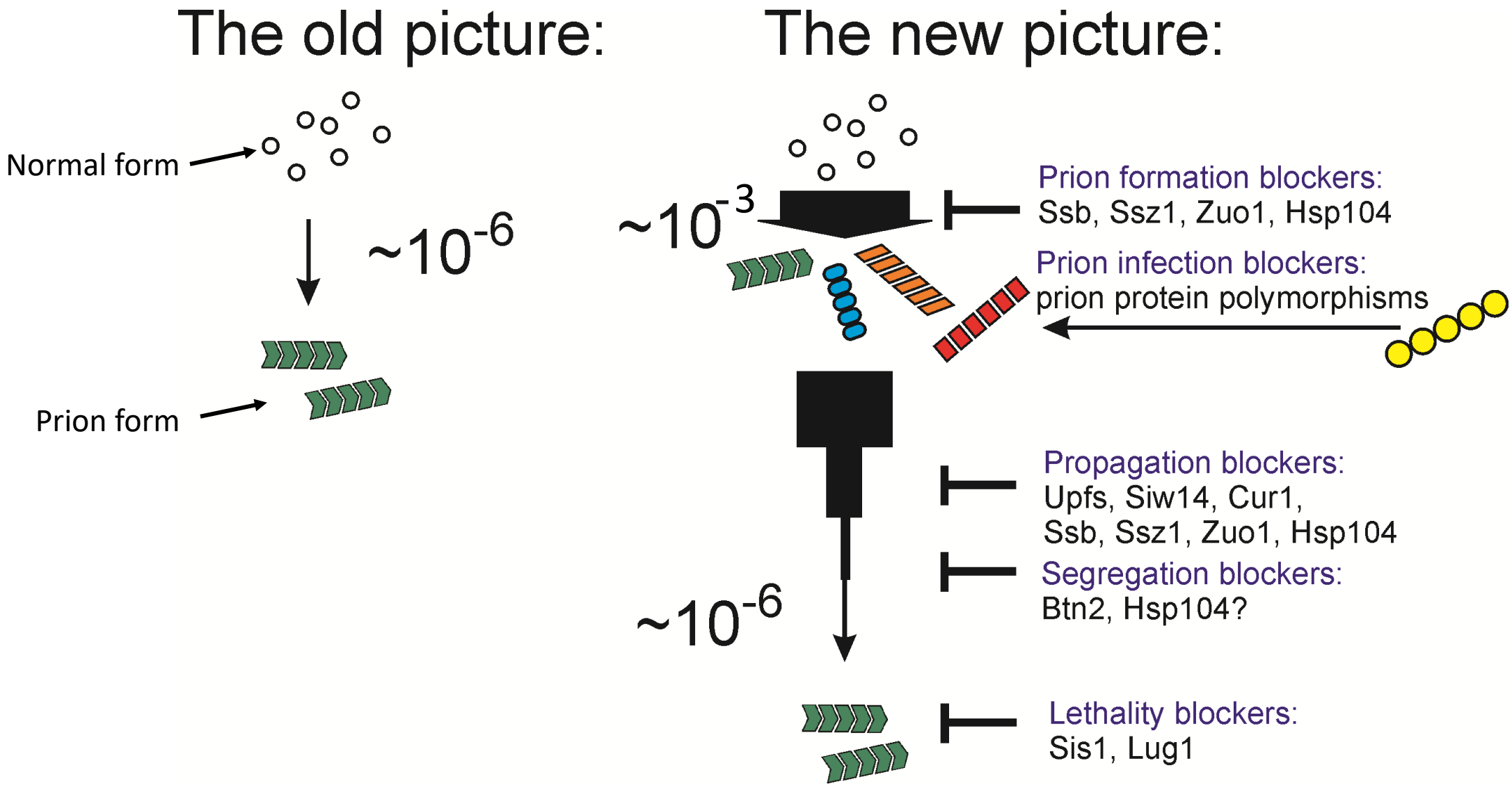
Anti-Prion Systems
Turn a Tsunami of Prions into a Slow Drip

Reed Wickner, Moonil Son, Songsong Wu, Yuho Hayashi, & Herman Edskes

Laboratory of Biochemistry & Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, NIH, Bethesda, MD USA





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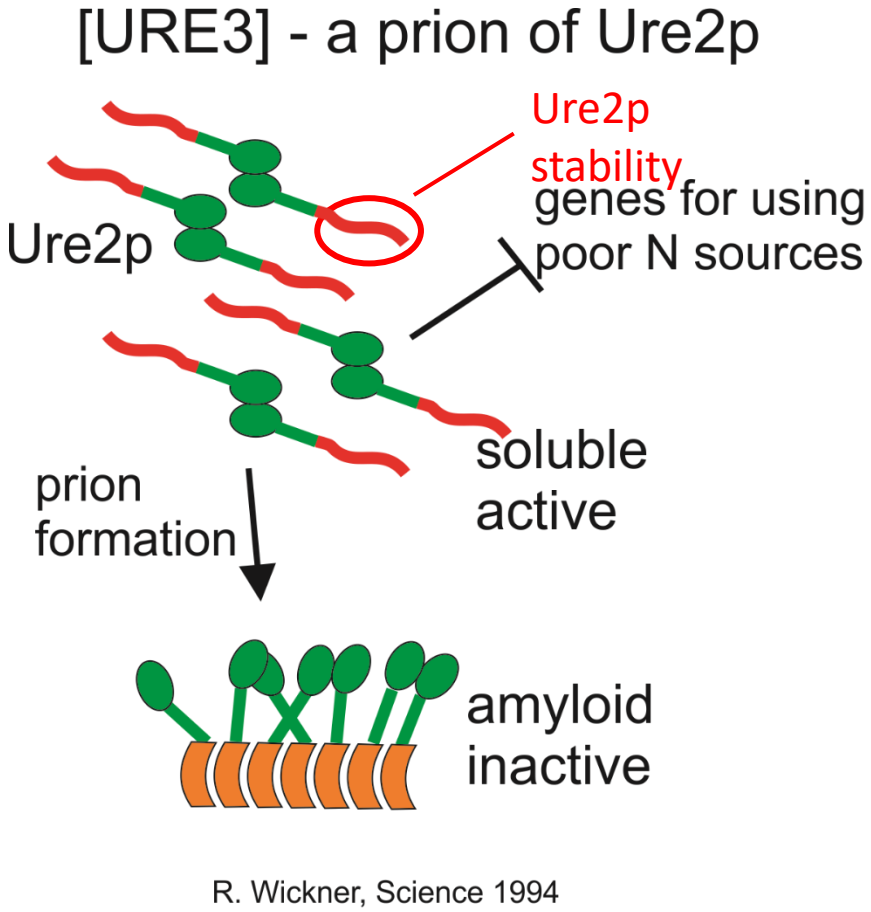
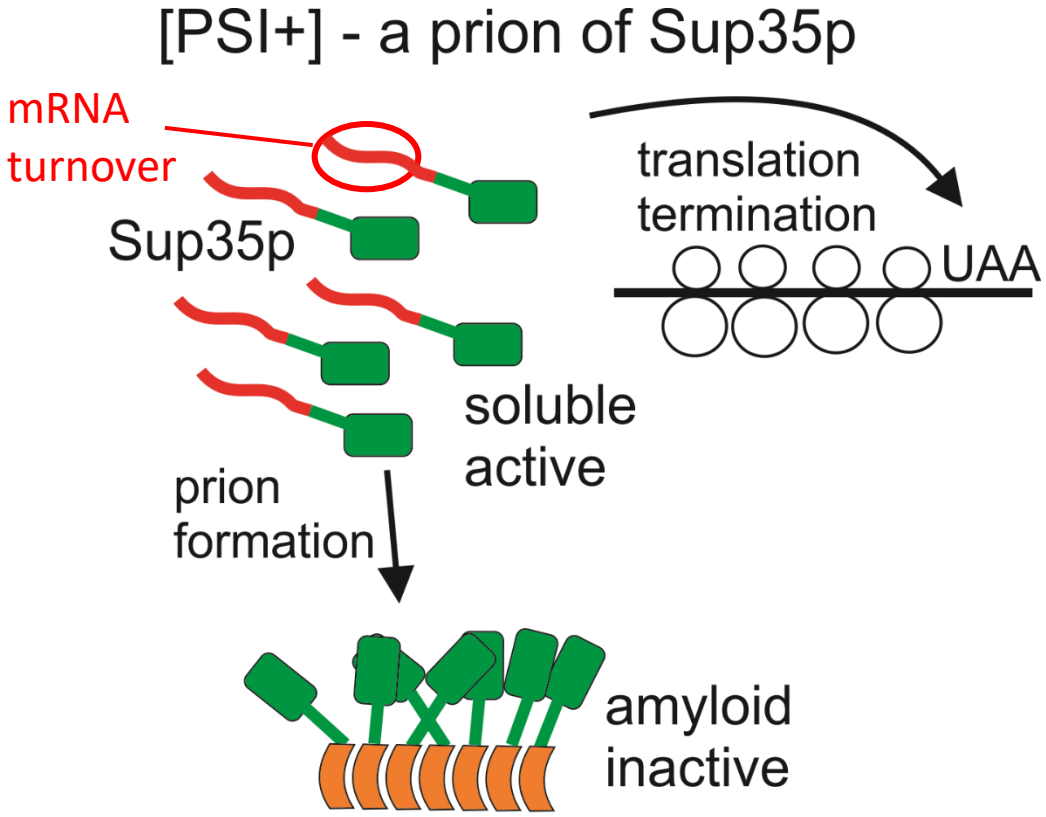
Yeast Prions

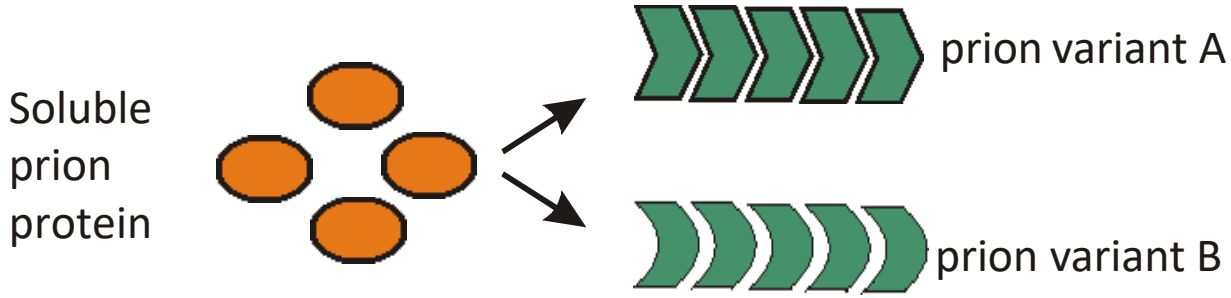


R. Wickner, Science 1994



R. Wickner, Science 1994

Prion variants/strains:

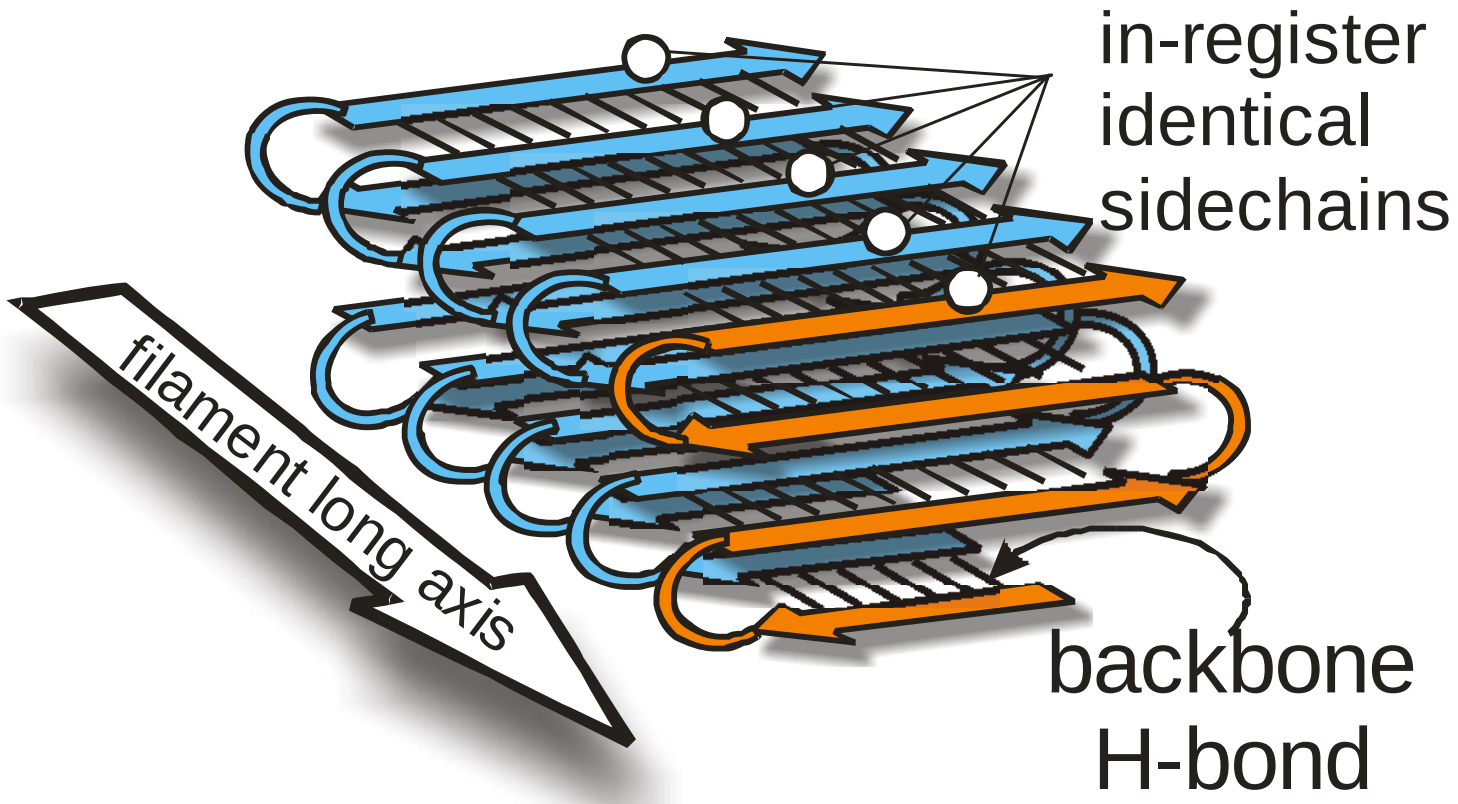


a <u>single</u> protein (amino acid sequence) can form
<u>many</u> different prion variants due to different self-propagating amyloid forms

<u>Animals:</u>
Incubation period
Brain regions affected
Ease of crossing species barrier

<u>Yeast:</u>
Intensity of phenotype
Stability
Sensitivity to chaperones (↑ or ↓)
Lethal, toxic, mild
Ease of crossing intra or inter species barrier

Infectious amyloid of Ure2, Sup35 and Rnq1 prion domains all have folded, parallel in-register, β-sheet architecture



Shewmaker, F., R. B. Wickner and R. Tycko, 2006 PNAS **103:** 19754 - 19759.
Baxa, U., R. B. Wickner, A. C. Steven, D. Anderson, L. Marenkov, M. Yao & R. Tycko, 2007 Biochemistry **46:** 13149 - 13162.
Wickner, R. B., F. Dyda & R. Tycko, 2008 PNAS **105:** 2403 - 2408.
Shewmaker, F., D. Kryndushkin, B. Chen, R. Tycko & R. B. Wickner, 2009 Biochemistry **48:** 5074-5082.
Shewmaker, F., E. D. Ross, R. Tycko & R. B. Wickner, 2008 Biochemistry **47:** 4000-4007.
Kryndushkin, D., R. B. Wickner & R. Tycko 2011 J. Mol. Biol. 409: 263 - 277.
Gorkovskiy, A., K. Thurber, R. Tycko & R. B. Wickner 2014 PNAS 111:E4615-22.

Yeast prions [URE3] and [PSI+] are pathogens:

- Claims of marginal growth advantage of prion - containing strains have not been reproducible. The question cannot be answered this way:

  A. Only mild variants are examined

  B. Impossible to judge representation of various conditions in the yeast niche

- Even the <u>mildest</u> [URE3] and [PSI^{+}] are rare in wild strains indicating they are harmful.
- Prion-forming ability is not conserved, even within *Saccharomyces:*

  >Ure2p of *S. castellii, Candida glabrata, Kluyveromyces lactis,* cannot form [URE3]

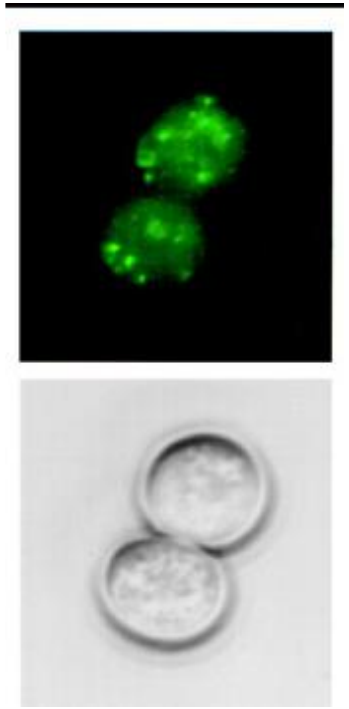
  >Sup35p of *S. pombe, Ashbya gossypii, Aspergillus nidulans, Cryptococcus neoformans*…… cannot form [PSI+] Edskes et al. *Genetics* **198:** 605 – 616 (2014).

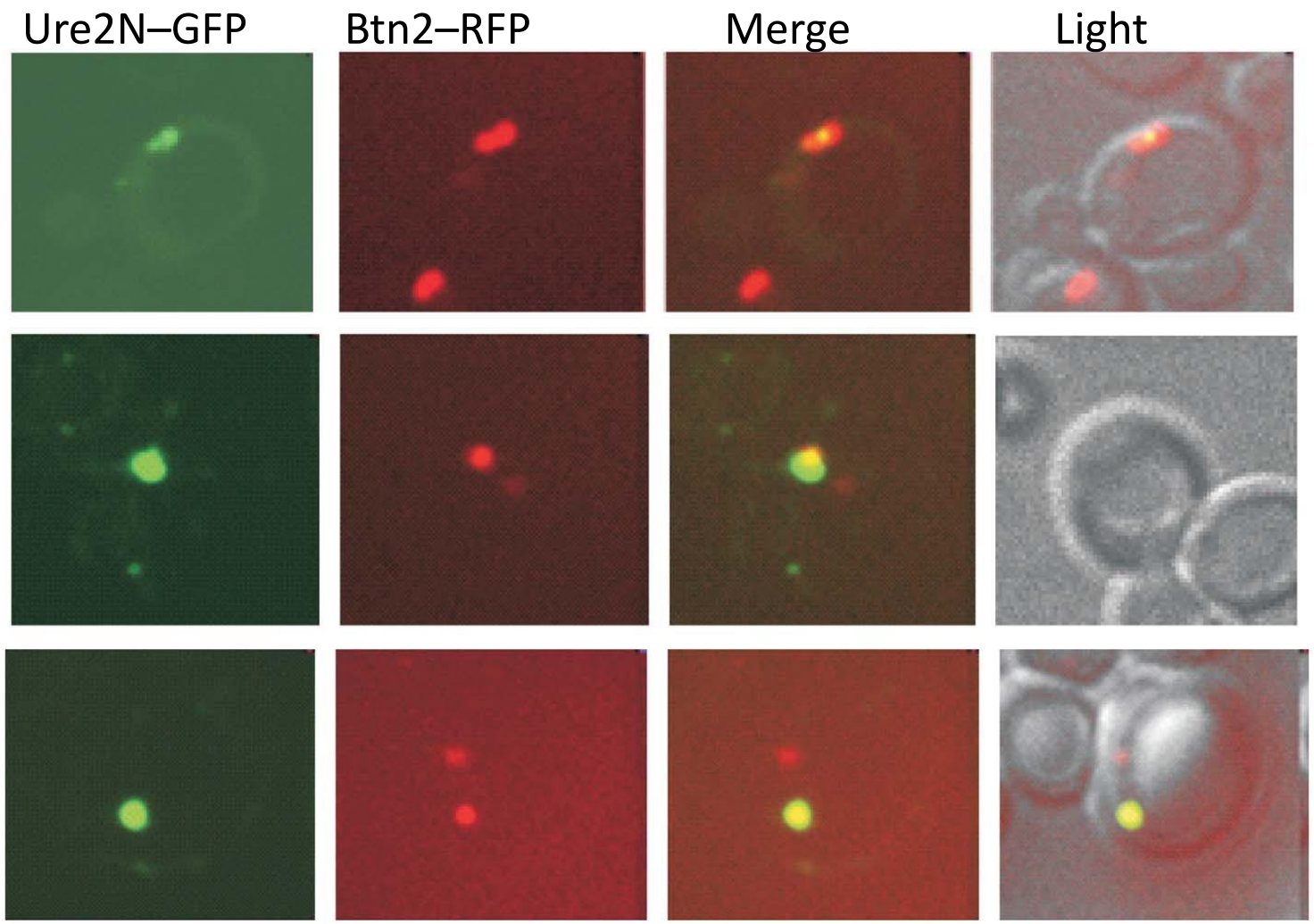
- "Prion domains" have non-prion functions, explaining their preservation.
- Rapid evolution of prion domains has produced species barriers, apparently to protect against infection: Wild *S. cerevisiae* have intra-species barriers to [PSI+] transmission.
- Most isolates of [PSI+] and [URE3] are lethal or highly toxic.
- Beneficial prion [Het-s] has only one variant, pathological prions ([PSI+] and [URE3]) have many.
- Reported stress – induction of [PSI+] has not been reproducible. Westergaard & True Mol. Microb. **92:**698 - 715
- Cells sense infection with [URE3] or [PSI+] as a stress. Jung & Masison, 2000; Schwimmer & Masison, 2002

Anti-prion systems: curing most prions in normal cells.

Protein	Prion affected	Generation Frequency in mutant	Mechanism	Reference
Btn2	[URE3]	5x ↑	curing prion by sequestration of amyloid filaments	(Kryndushkin, et al. 2008, Wickner, et al. 2014)
Cur1	[URE3]	5x ↑	curing by unknown mechanism ???	(Kryndushkin, et al. 2008, Wickner, et al. 2014)
Hsp104	[PSI+] (& [URE3])	13x ↑	blocking generation and curing; mechanism controversial	(Chernoff, et al. 1995, Hung & Masison, 2006, Gorkovskiy, et al. 2017)
Siw14	[PSI+]	2x ↑	Inositol pyrophosphatase lowers 5PP-IPs that some variants need	(Wickner, et al. 2017)
Upf1,2,3	[PSI+]	10-15x ↑	curing by complex formation with Sup35p	(Son and Wickner 2018)
Ssb, Ssz1, Zuo1	[PSI+] & [URE3]	10-15x ↑	Ribosome-associated chaperones block generation and cure by facilitating folding of nascent proteins	(Chernoff, et al. 1999, Kiktev, et al. 2015, Son and Wickner 2020)

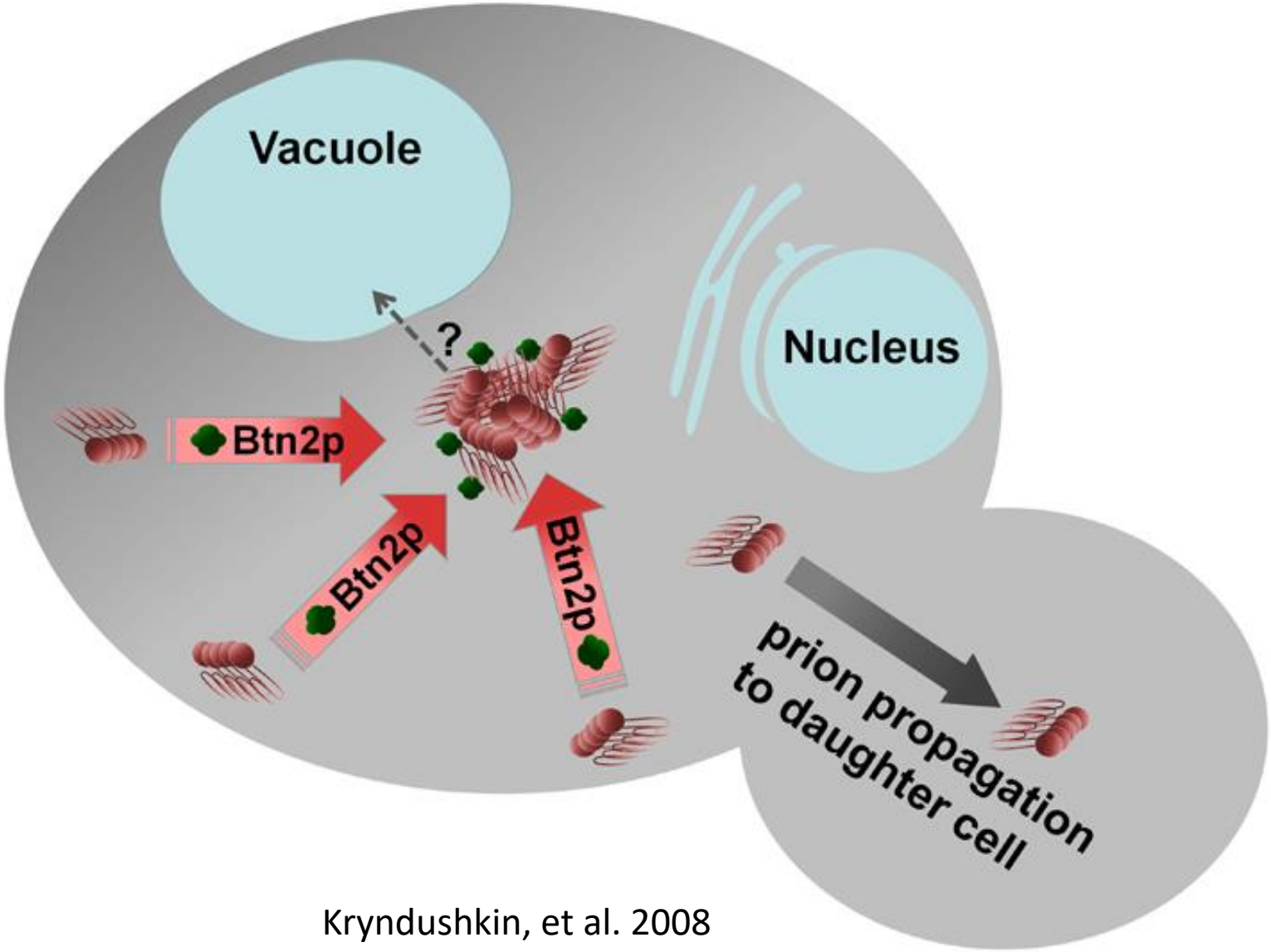
During curing of [URE3] by Btn2p overproduction,
Ure2N-GFP and Btn2-RFP co-localize:





Kryndushkin, D., Shewmaker, F., and Wickner, R. B. (2008) Curing of the [URE3] prion by Btn2p, a Batten disease-related protein, *EMBO J. 27, 2725 - 2735.*

Btn2p cures [URE3] prion by sequestering amyloid filaments



Kryndushkin, et al. 2008

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Ssb, Ssz1, Zuo1	[PSI+], [URE3]	10-15x ↑	Ribosome-associated chaperones block generation and cure by facilitating folding of native proteins	(Chernoff, et al. 1999, Kiktev, et al. 2015, Son and Wickner 2020; Jay-Garcia et al. 2023)

De novo generation of [PSI+] in strains lacking multiple anti-prion components: 1000- to 5000-fold increase in prions arising in ssz1Δ upf1Δ hsp104T106M mutant

Host	Spontaneous [PSI+] / 10^6 cells	Induced [PSI+] / 10^6 cells
WT	0.3	11
hsp104^{T106M}	3	570
ssz1Δ	13	1,600
upf1Δ	13	1,800
ssz1Δ hsp104^{T106M}	490	44,000
upf1Δ hsp104^{T106M}	470	60,000
upf1Δ ssz1Δ	480	46,000
ssz1Δ upf1Δ hsp104^{T106M}	520	49,000

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Most prions arising in *ssz1*Δ *upf1*Δ *hsp104*^{T160M} are cured by restoring any ONE gene

New variant type: 
cured by
any <u>one</u> of the 3 systems.

Anti-prion systems work <u>independently</u>.

Strain	[PSI+] / Total transformants			
	Vector	p*SSZ1*	p*UPF1*	p*HSP104*
suh [*PSI*+]1	64/65	30/92****	20/35**	33/52**
2	82/85	34/80****	53/86**	43/55*
3	51/55	15/31*	19/33*	10/15
4	84/90	54/100**	C	46/59
5	50/52	25/51**	39/84**	68/76
6	74/75	56/102***	24/59****	65/84*
7	78/80	C	8/34****	C
8	41/45	45/65	57/97*	53/58
9	37/38	19/33*	10/15	29/42*
10	52/54	14/33**	3/6	13/15

* $p<0.05$, ** $p<10^{-2}$, *** $p<10^{-3}$, **** $p<10^{-4}$

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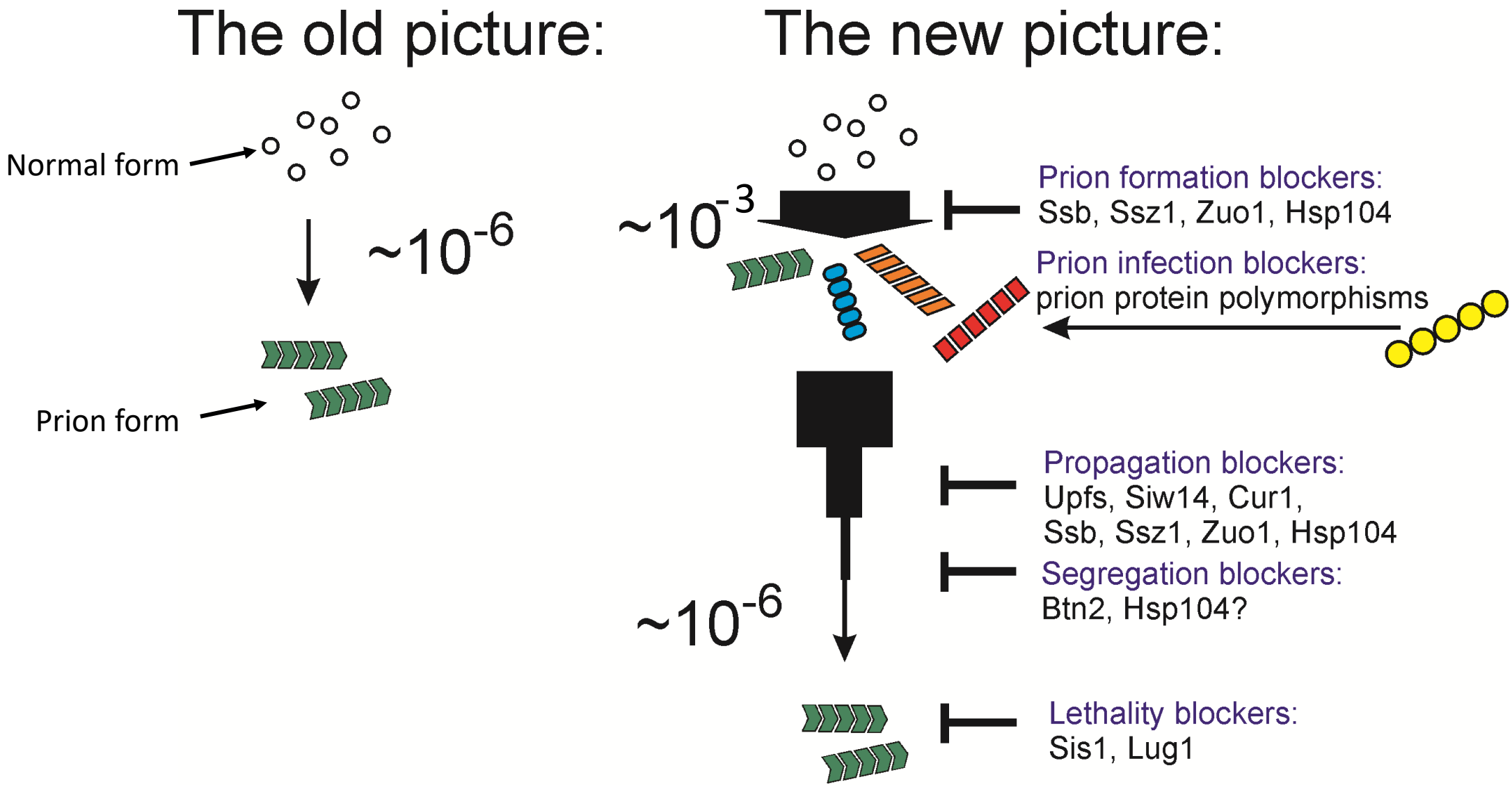
Three reasons for very frequent [PSI+] appearance in multiple mutants:

Increased generation of prion variants stable in w.t. strains

Failure to cure variants sensitive to **only one** system

Failure to cure variants curable by **any one** of these systems

NEW



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[Like DNA repair]

Hunt for human anti-prion systems:

pGAL-[human ORF] bank of 14,913 human gene ORFs

Test for their curing yeast prions [PSI+] or [URE3].

19 human proteins cure yeast prions.

Focus on 6 human curing proteins that have yeast homologs.

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Curing of [PSI+] and [URE3] by human proteins and their yeast homologs

Human Gene	Human Protein Curing		Yeast Gene	Overexpression curing		Deletion curing	
	[PSI+]	[URE3]		[PSI+]	[URE3]	[PSI+]	[URE3]
BAG5	+	+	*SNL1*	–	+ (BD)*	–	–
BAG4	–	+					
PATL1	+	+	*PAT1*	–	–	inviable	
DNAJA1	+	–	*YDJ1*	enhanced	+**	–	–
PRPF19	–	+	*PRP19*	–	–	inviable	
KRI1	–	+	*KRI1*	–	–	inviable	

+ = cures
– = no curing

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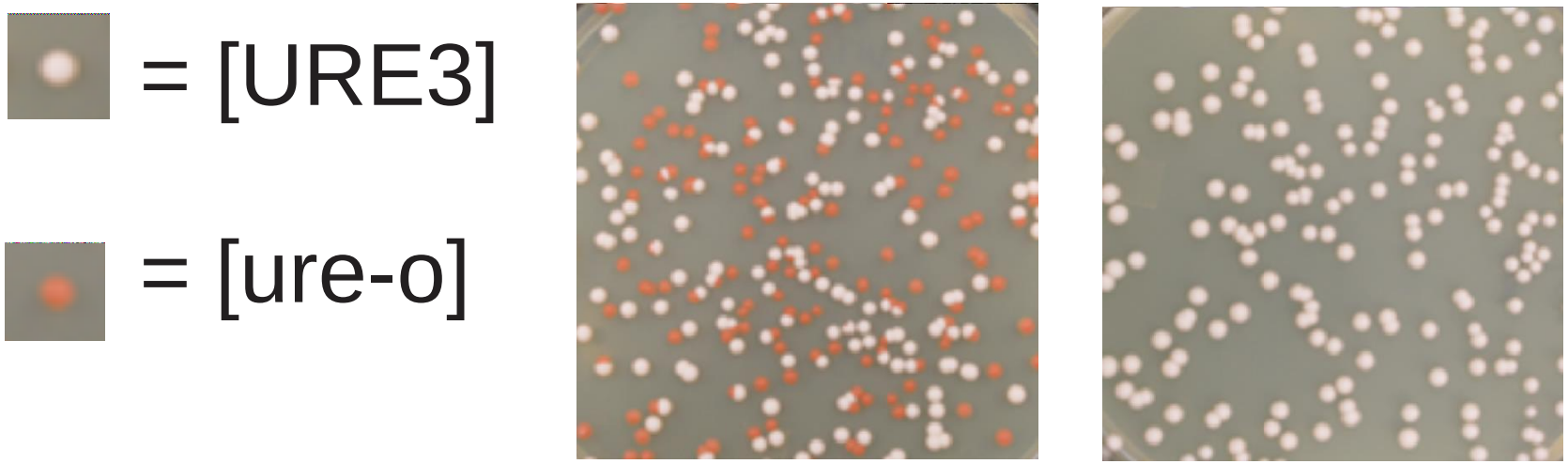
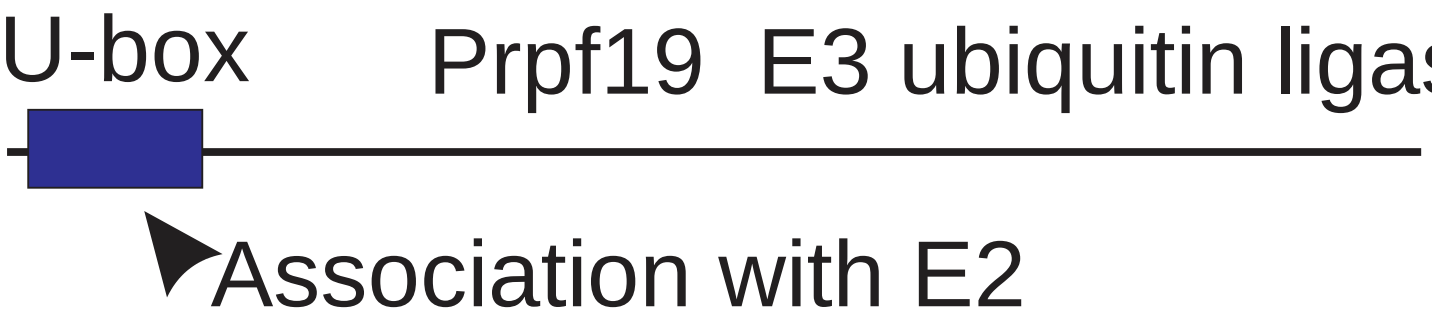
*D. Masison group

**Moriyama & Wickner, 2001

Human E3 ubiquitin ligase cures [URE3] :





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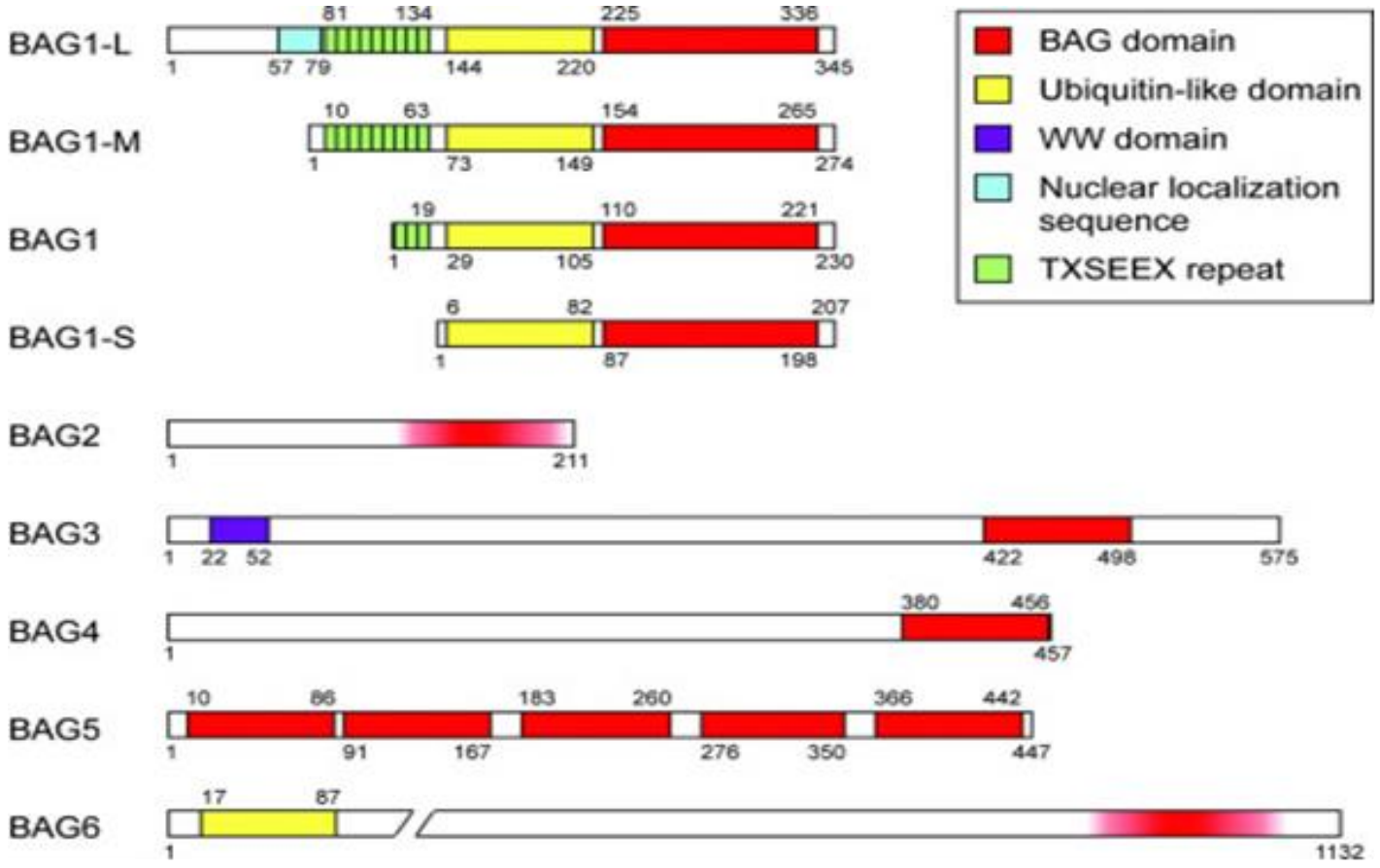
Bag5 expressed in yeast cures [URE3] and [PSI+]

Cures [URE3]	Cures [PSI+]
+	–
+	–
+	–
+	–
+	–
+	–
+	+
–	–



Briknarova´ et al., 2002

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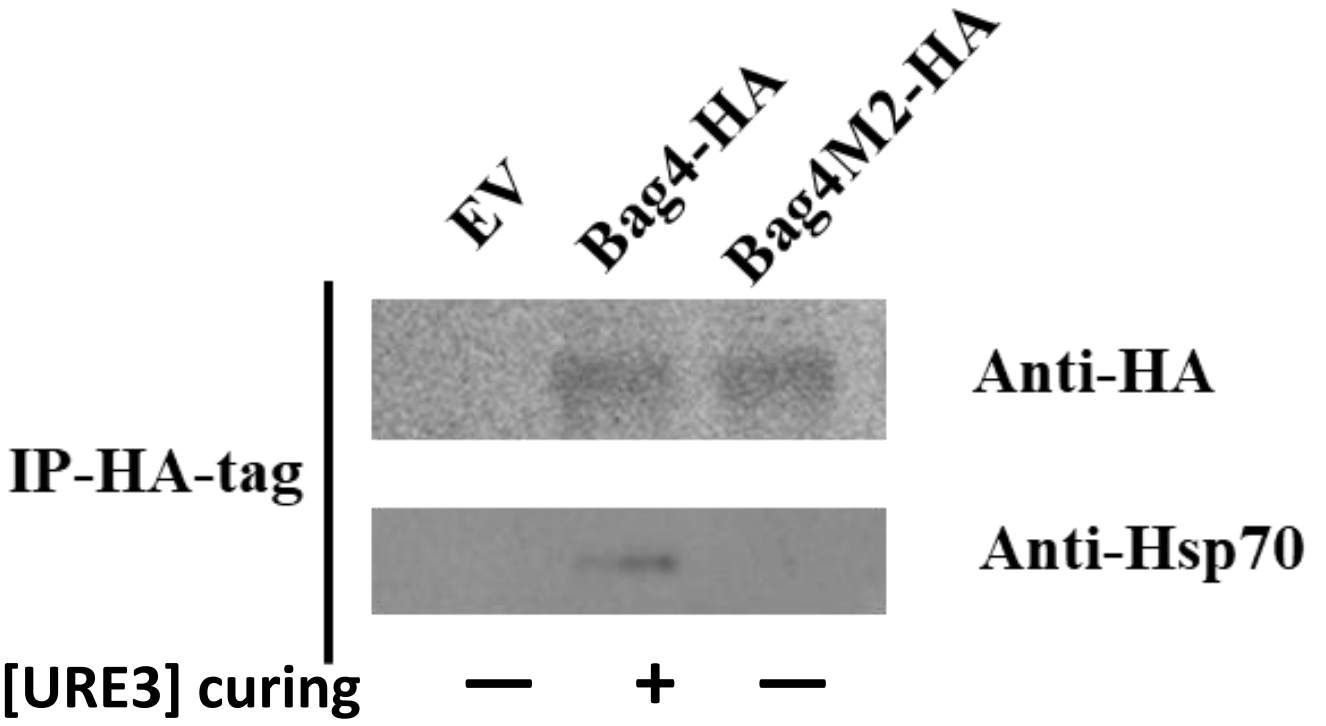
Bag4 interacts with yeast Hsp70s to cure the [URE3] prion



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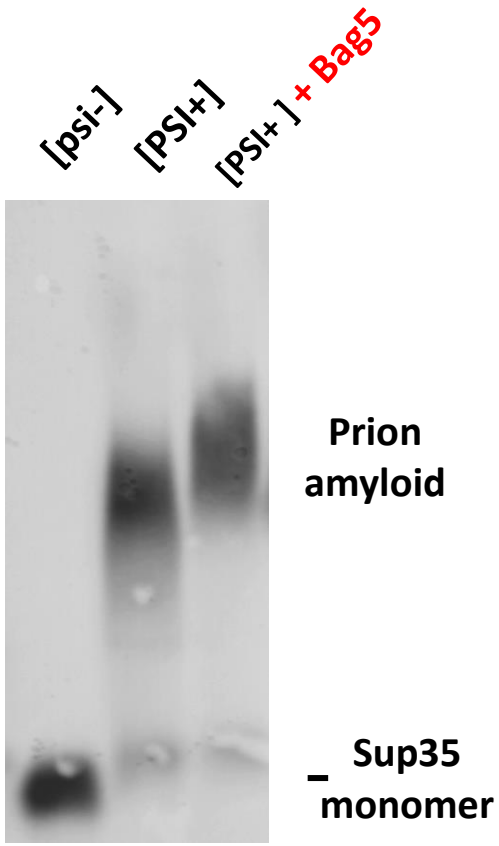
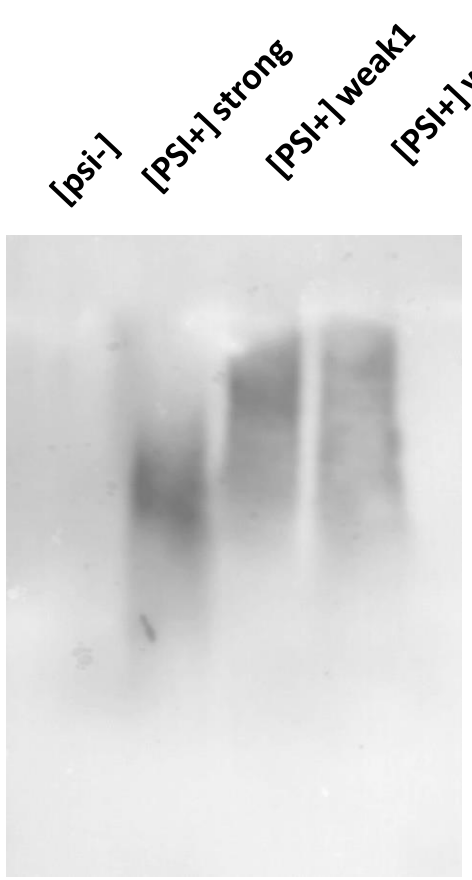
Expression Bag5 increase the polymers size of [PSI+]

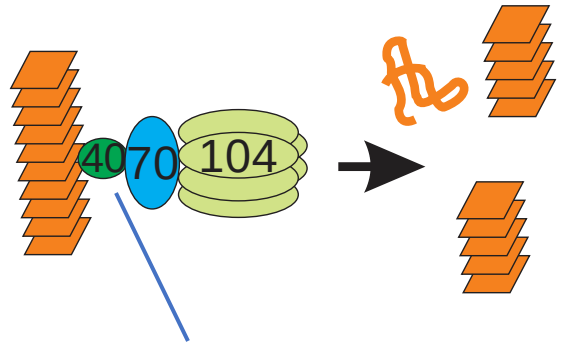


Overproduction of Bag5 blocks prion polymer cleavage



Weak [PSI+]s amyloids are harder to cleave, easier for Bag5 to cure than strong [PSI+]

Hsp104-70-40 amyloid cleavage machine



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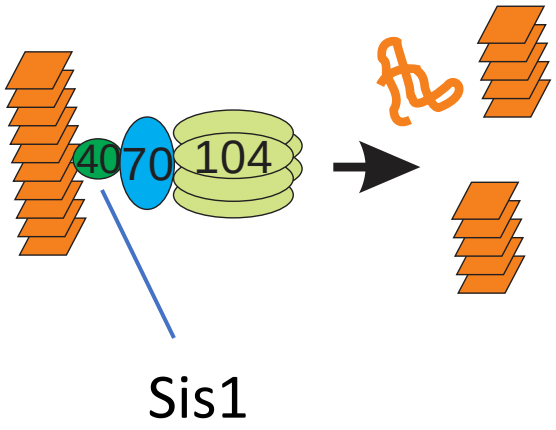
Both Sis1 and Bag bind to Hsp70's nucleotide-binding domain.

We find: Overproduction of Sis1 blocks prion curing by Bag proteins.

<u>Hypothesis:</u> **Bag proteins block Hsp104-70-40 filament cleavage by blocking Sis1 association with Hsp70s**.



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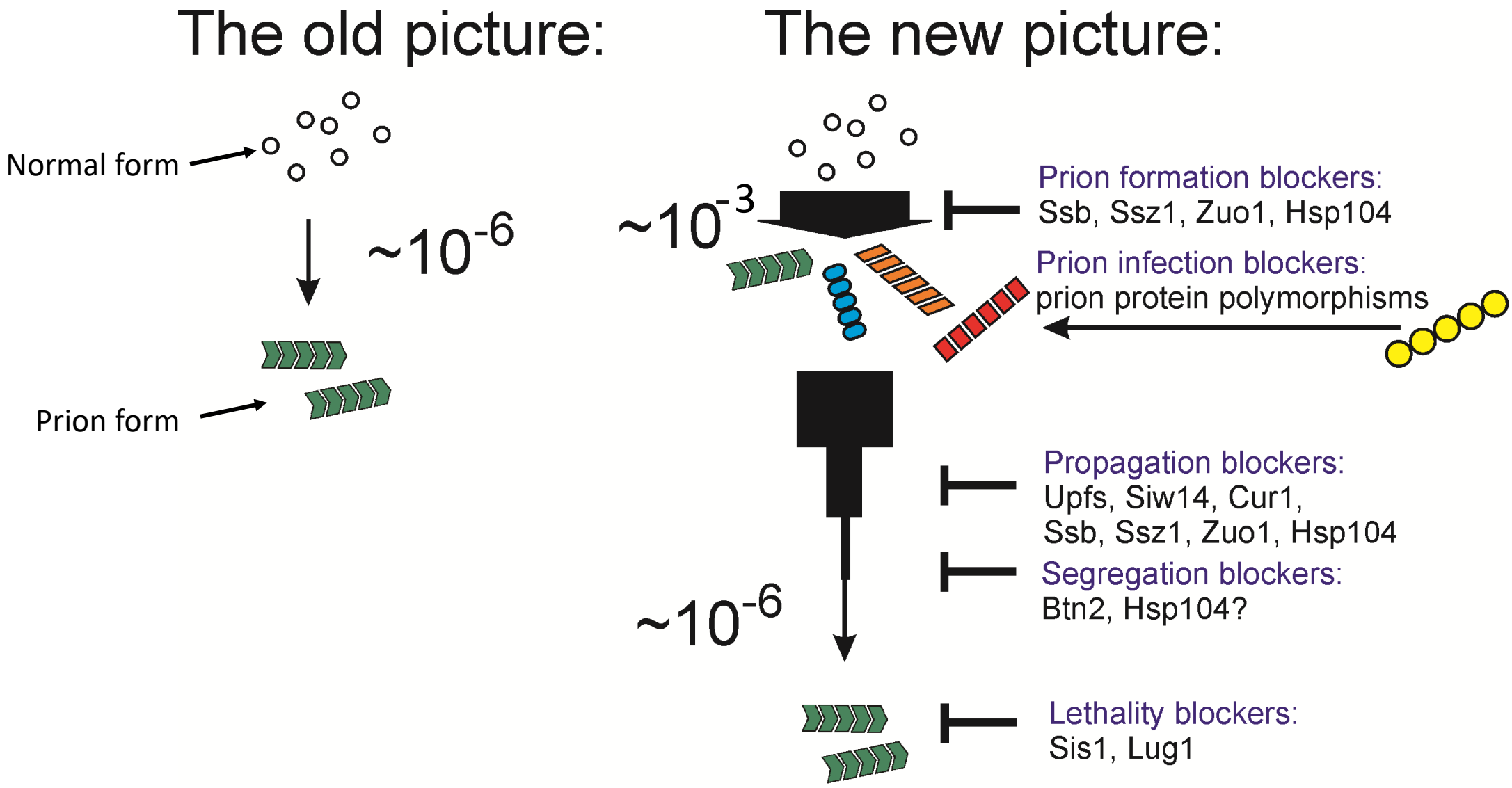
Human proteins can cure yeast prions and this may be useful in finding human anti-prion proteins useful in dealing with amyloid diseases, much like we manipulate the cellular, humoral and innate immune systems to deal with viral, bacterial and parasite infections.



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[Like DNA repair]

THANKS:

Present Lab Members:
Herman Edskes
Yuho Hayashi

Collaborators:
Rob Tycko, Kent Thurber, NIH
Dan Masison, Mike Reidy, NIH

Past Postocs:
Moonil Son [Pusan Natl. Univ.]
Songsong Wu [Southwest Univ. Chongqing, PRC]
Dmitry Kryndushkin
Eric Ross [Colorado State]
Anton Gorkovskiy
Amy Kelly [USDA]
David Bateman [FDA]
Frank Shewmaker [NIH]

Conclusions:

1) Multiple anti-prion systems work on each prion (like DNA repair systems).

2) Defects in a single anti-prion system => 2x to 15x higher prion appearance frequency.

3) Multiple defects in anti-prion systems show up to 5000x higher prion appearance.

4) Normal cells are constantly fighting to prevent prions arising or propagating, or infecting from another cell or from being toxic.

5) Just as we use the various immune systems against bacteria, viruses and parasites, we hope to use anti-prion/anti-amyloid systems to fight prion/amyloid diseases.

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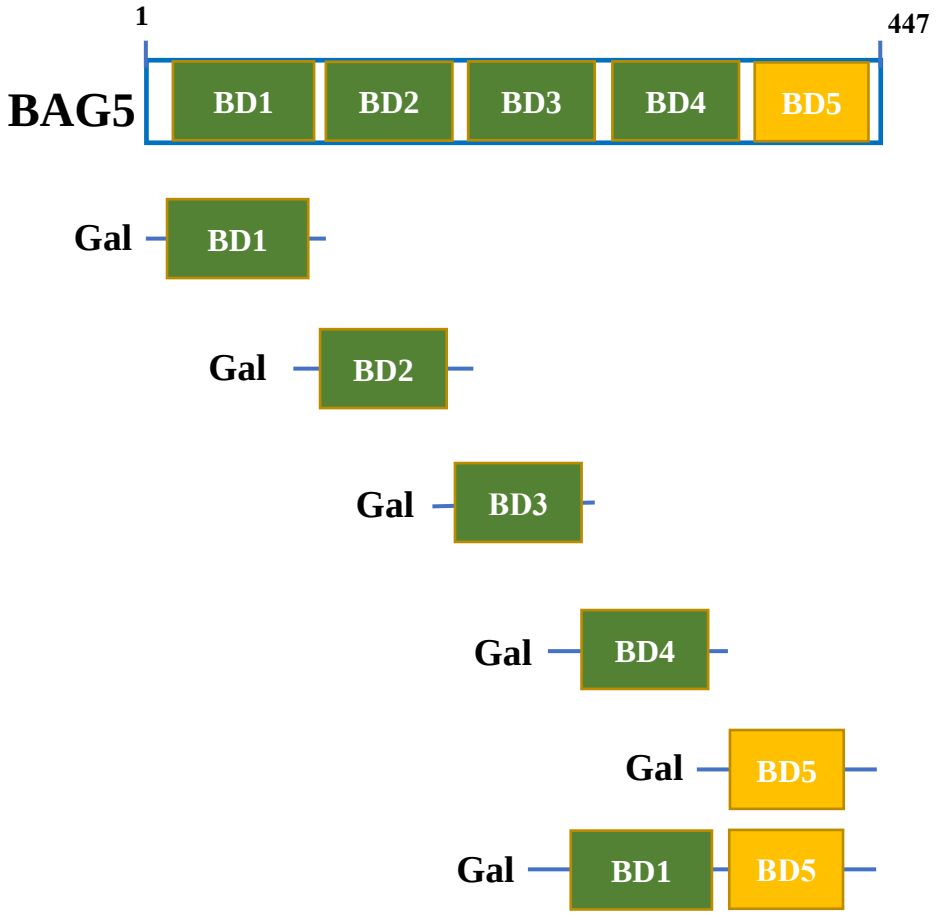
6) Ssz1, Zuo1, Ssb1/2 (ribosome-associated chaperones)
Upf1,2,3 (nonsense-mediated decay proteins)
Btn2, Cur1 (HOOK family proteins)
Siw14 (inositol pyro-,poly-phosphate pyrophosphatase)

Human homologs known

No one Bag domain of Bag5 cures [PSI+]

No one Bag domain is necessary

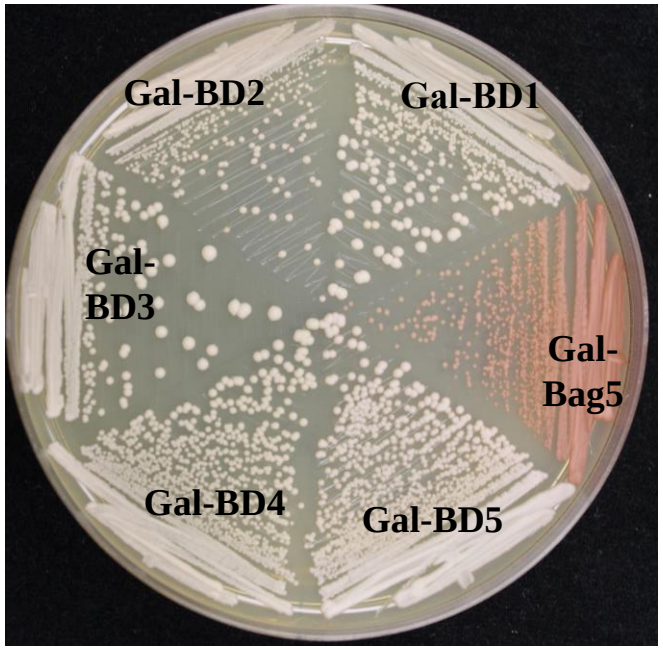
Two Bag domains can suffice to cure [PSI+]



Curing [PSI+]

+
–
–
–
–
–
+

779-6A [PSI+]



Gal medium

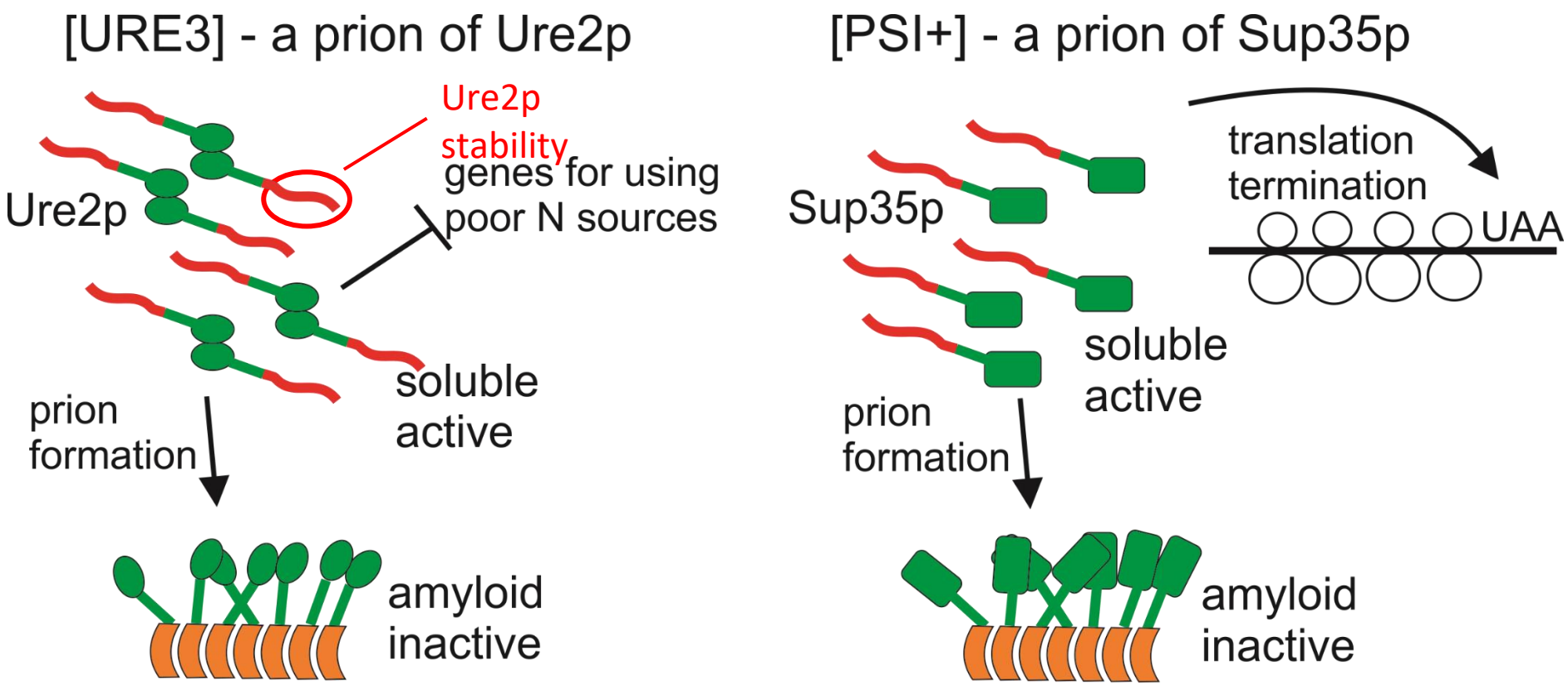
Songsong Wu, Herman Edskes, RBW, *PNAS, in press*

Prpf19 promotes ubiquitination and degradation by the proteasome of polyQ
S. Chen, X. Huang, K. Talbot & H.Y.E. Chan, Cell Death Dis. 2021 Feb; **12**(2): 136

Bag5 inhibits parkin and enhances dopaminergic neuron degeneration
Kalia, S. K., Lee, S., Smith, P. D., Liu, L., Crocker, S. J., Thorrinsdottir, T. E., Glover, J. R., Fon, E. A., 513 Park, D. S., & Lorzano, A. M. (2004) *Neuron* **44,** 931 - 945. 514

Bag5 binds to and inhibits *parkin,* and can promote aggregation of synuclein in neuron-derived tissue culture cells
De Snoo, M. L., Friesen, E. L., Zhang, Y. T., Earnshaw, R., Dorval, G., Kapadia, M., O'Hara, D. M., 515 Agapova, V., Chau, H., Pellerito, O.*, et al.* (2019) *Cell Death Dis.* **10,** 907. 516

Bag2, Bag3 and Bag5 each also interact with LRRK2 (leucine-rich repeat kinase 2), whose mutation can cause Parkinson's disease.
Beilina, A., Rudenko, I. N., Kaganovich, A., Civiero, L., Chau, H., Kalia, S. K., Kalia, L. V., 517 Lobbestael, E., Chia, R., Ndukwe, K.*, et al.* (2013) *Proc Natl Acad Sci U S A* **111,** 2626 - 2631.


[URE3] - a prion of Ure2p
Ure2p
Ure2p stability
genes for using poor N sources
soluble active
prion formation
amyloid inactive
[PSI+] - a prion of Sup35p
Sup35p
translation termination
UAA
soluble active
prion formation
amyloid inactive