Published in final edited form as:

Curr Genet. 2018 June ; 64(3): 571–574. doi:10.1007/s00294-017-0788-2.

Prion propagation and inositol polyphosphates

Reed B. Wickner*, Herman K. Edskes, Evgeny E. Bezsonov, Moonil Son, and Mathieu Ducatez

Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD 20892-0830 USA

Abstract

The [PSI⁺] prion is a folded in-register parallel β -sheet amyloid (filamentous polymer) of Sup35p, a subunit of the translation termination factor. Our searches for anti-prion systems led to our finding that certain soluble inositol polyphosphates (IPs) are important for the propagation of the [PSI⁺] prion. The IPs affect a wide range of processes, including mRNA export, telomere length, phosphate and polyphosphate metabolism, energy regulation, transcription and translation. We found that 5-diphosphoinositol tetra(or penta)kisphosphate or inositol hexakisphosphate could support [PSI⁺] prion propagation, and 1-diphosphoinositol pentakisphosphate appears to inhibit the process.

Keywords

prion; inositol polyphosphate; [PSI⁺]; Sup35; Siw14

Yeast prions were originally viewed as a model for the mammalian transmissible spongiform encephalopathies, particularly the Mad Cow disease epidemic in the UK at that time (Wickner 1994). Now there is increasing evidence that most of the common human amyloidoses have prion-like aspects, and in some cases may be frankly infectious (Jaunmuktane, et al. 2015, Junker and Walker 2011, Kraus, et al. 2013), so the importance of the yeast model is even greater.

While nearly 10 yeast and fungal prions have been described (reviewed in (Wickner, et al. 2015)), the [PSI⁺] and [URE3] prions, self-propagating amyloids of Sup35p and Ure2p (the latter a regulator of nitrogen catabolism) have been most intensively investigated. The folded in-register parallel β -sheet architecture, shown for several, can explain how these amyloids can self-template their structures, and so how they can act as genes composed of protein, with multiple alleles (called prion variants in yeast or prion strains in mammals) (Fig. 1, (Gorkovskiy, et al. 2014, Shewmaker, et al. 2006, Wickner, et al. 2015)).

Overproduction or deficiency of many chaperones and other cell components have been found to result in prion loss (e.g. (Chernoff, et al. 1995, Higurashi, et al. 2008, Kryndushkin, et al. 2008, Sharma and Masison 2008, Troisi, et al. 2015)). Deletion or overexpression of a protein can efficiently eliminate a prion by very indirect means, or, although direct, by

*Corresponding author: wickner@helix.nih.gov, 301-496-3452.

producing a condition that does not occur in the wild. But recently we have proposed a new ‘definition’ of anti-prion system/component, namely a protein that eliminates a prion when expressed at its *normal* level. With this approach several anti-prion systems have been shown, starting with proteins whose curing activity was first shown by overproduction. Hsp104 overproduction cures the [PSI⁺] prion (Chernoff, et al. 1995), and Btn2p or Cur1p overproduction cure the [URE3] prion (Kryndushkin, et al. 2008). But most of the prion variants isolated in strains lacking these curing activities are cured by simply restoring the normal level of the active protein (Gorkovskiy, et al. 2017, Wickner, et al. 2014). In effect, what has been discovered is a large array of [PSI⁺] and [URE3] variants that would normally never appear because the normal levels of the Hsp104 or Btn2p or Cur1p would cure them as they arise.

In a general screen for anti-prion systems using the yeast knockout collection, an *siw14* mutation was found to allow many variants of [PSI⁺] to propagate that could not propagate in *SIW14⁺* cells (Wickner, et al. 2017). However, overexpression of Siw14p did not cure any [PSI⁺] variants isolated in normal cells. Since Siw14p was recently identified as a pyrophosphatase specific for the 5-pyrophosphate-inositol pentakisphosphate (5PP-IP₅, see Fig. 2) (Steidle, et al. 2016), it was suspected that some inositol poly/pyro-phosphates promote prion propagation (Wickner, et al. 2017). Indeed, *arg82* mutants, that lack most of the soluble inositol poly/pyro-phosphate species are unable to propagate the large majority of [PSI⁺] variants. Genetically engineered blockage of various points in the IP pathway led to the conclusion that either IP₆ or 5PP-IP₄ are certainly sufficient for [PSI⁺] propagation, and presumably 5PP-IP₅ can also fulfill this function (Fig. 2). The requirement of [PSI⁺] for the IPs is not eliminated by removing the [PSI⁺]-curing activity of Hsp104, and the [URE3] prion does not have such an IP requirement (Wickner, et al. 2017).

Inositol poly/pyro-phosphates are known to have a wide array of functions (Table 1), but none of these functions immediately explain their requirement for [PSI⁺] prion propagation. The need for inositol pyrophosphates for the ‘environmental stress response’, was the most suggestive, but the ability of *kcs1 vip1* mutants (that make no inositol pyrophosphates (Mulugu, et al. 2007)) to support [PSI⁺] indicates that the prion is supported by another IP function (Wickner, et al. 2017). These *kcs1 vip1* mutants do make inositol hexakisphosphate (IP₆), but *kcs1 ipk1* mutants do not make IP₆ and do not support [PSI⁺], implicating IP₆ as another species capable of helping [PSI⁺] propagate (Wickner, et al. 2017). In spite of being able to make IP₆, *kcs1 ddp1* mutants (see Fig. 2) lose [PSI⁺]. These mutants make elevated levels of 1-disphosphoinositol pentakisphosphate (Mulugu, et al. 2007), suggesting that this compound may inhibit [PSI⁺] propagation (Wickner, et al. 2017).

These experiments have revealed an unexpected level of control over prion propagation. However, the mechanism by which these inositol poly/pyro-phosphates exert their positive and negative control is unknown. The inositol pyrophosphates contain high energy pyrophosphate bonds and have been shown able to pyrophosphorylate proteins at certain amino acid residues that are already monophosphorylated (Saiardi, et al. 2004). It is conceivable that this activity is involved in supporting [PSI⁺] by direct modification of Sup35p, but the ability of IP₆, lacking pyrophosphates, to support [PSI⁺] argues against this

idea. IPs are very highly charged molecules and can bind to positively charged regions on certain proteins (e.g., (Macbeth, et al. 2005)), but the prion domain of Sup35p, the part that actually forms the amyloid structure, has very few charged residues, including only two arginines and one lysine in the 123 residues.

It is quite possible that the IPs are acting on some other protein to augment, diminish or qualitatively change its activity in such a way that [PSI⁺] propagation is allowed. Solid resins with IP₆ or 5PP-IP₅ covalently attached were designed and used to purify proteins from *S. cerevisiae* that had specific affinity for one of these molecules (Wu, et al. 2016). Note that these are two of the molecules that we found were each sufficient to support [PSI⁺] propagation. Among the ~40 proteins that bound to both resins were Ssb1 and Ssb2, ribosome – associated Hsp70 chaperones; Hsp26p, a small heat shock protein; and Sse1p, an Hsp70 family member with nucleotide exchange activity. The Ssb proteins (Chernoff and Kiktev 2016, Chernoff, et al. 1999) and Sse1p (Fan, et al. 2007, Kryndushkin and Wickner 2007) are known to affect [PSI⁺] generation and propagation, but preliminary efforts to prove these as targets of the IPs have not yet succeeded. Whatever the mechanism, Siw14p appears to be working by limiting the levels of certain inositol pyrophosphates, preventing the propagation of a group of [PSI⁺] variants that need a high level of these compounds.

This work opens a new avenue for investigation of anti-prion/anti-amyloid modalities. Do human amyloidoses involve IPs in some way? The IP biosynthetic pathways in yeast and humans are very similar, and most pathogenic human amyloids studied have, like yeast prions, the folded in-register parallel β -sheet architecture (reviewed by (Tycko 2014, Tycko and Wickner 2013). Of course, even [URE3] is not affected by IP deficiency, so there need not be direct carry-over of these results to mammals. Nonetheless, these results again highlight the familiar theme that studies of yeast, rapidly and cheaply carried out, can form the basis for understanding of human disease processes.

Acknowledgments

The authors thank John York (Vanderbilt University) for enlightening discussions. This work was supported by the Intramural Program of the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health.

References

- Bolger TA, Folkmann AW, Tran EJ, Wentz SR. The mRNA export factor Gle1 and inositol hexakisphosphate regulate distinct stages of translation. *Cell*. 2008; 134:624–633. doi: [PubMed: 18724935]
- Chernoff YO, Kiktev DA. Dual role of ribosome-associated chaperones in prion formation and propagation. *Curr Genet*. 2016; doi: 10.1007/s00294-016-0586-2
- Chernoff YO, Lindquist SL, Ono B-I, Inge-Vechtomov SG, Liebman SW. Role of the chaperone protein Hsp104 in propagation of the yeast prion-like factor [psi⁺]. *Science*. 1995; 268:880–884. doi: [PubMed: 7754373]
- Chernoff YO, Newnam GP, Kumar J, Allen K, Zink AD. Evidence for a protein mutator in yeast: role of the Hsp70-related chaperone Ssb in formation, stability and toxicity of the [PSI⁺] prion. *Mol Cell Biol*. 1999; 19:8103–8112. doi: [PubMed: 10567536]
- Dubois E, Scherens B, Vierendeels F, Ho MMW, Messenguy F, Shears SB. In *Saccharomyces cerevisiae*, the inositol polyphosphate kinase activity of Kcs1p is required for resistance to salt

- stress, cell wall integrity, and vacuolar morphogenesis. *J Biol Chem.* 2002; 277:23755–23763. doi: [PubMed: 11956213]
- Fan Q, Park K-W, Du Z, Morano KA, Li L. The role of Sse1 in the de novo formation and variant determination of the [PSI⁺] prion. *Genetics.* 2007; 177:1583–1593. doi:
- Gorkovskiy A, Reidy M, Masison DC, Wickner RB. Hsp104 at normal levels cures many [PSI⁺] variants in a process promoted by Sti1p, Hsp90 and Sis1p. *Proc Natl Acad Sci USA.* 2017; 114:E4193–E4202. doi: [PubMed: 28484020]
- Gorkovskiy A, Thurber KR, Tycko R, Wickner RB. Locating folds of the in-register parallel -sheet of the Sup35p prion domain infectious amyloid. *Proc Natl Acad Sci USA.* 2014; 111:E4615–E4622. doi: [PubMed: 25313080]
- Higurashi T, Hines JK, Sahi C, Aron R, Craig EA. Specificity of the J-protein Sis1 in the propagation of 3 yeast prions. *Proc Natl Acad Sci USA.* 2008; 105:16596–16601. doi: [PubMed: 18955697]
- Jaunmuktane Z, Mead S, Ellis M, Wadsworth JD, Nicoll AJ, Kenny J, Launchbury F, Linehan J, Richard-Loendt A, Walker AS, Rudge P, Collinge J, Brandner S. Evidence for human transmission of amyloid-pathology and cerebral amyloid angiopathy. *Nature.* 2015; 525:247–250. doi: [PubMed: 26354483]
- Junker M, Walker LC. Pathogenic protein seeding in Alzheimer's disease and other neurodegenerative disorders. *Ann Neurol.* 2011; 70:532–540. doi: [PubMed: 22028219]
- Kraus A, Groveman BR, Caughey B. Prions and the potential transmissibility of protein misfolding diseases. *Ann Rev Microbiol.* 2013; 67:543–564. doi: [PubMed: 23808331]
- Kryndushkin D, Shewmaker F, Wickner RB. Curing of the [URE3] prion by Btn2p, a Batten disease-related protein. *EMBO J.* 2008; 27:2725–2735. doi: [PubMed: 18833194]
- Kryndushkin D, Wickner RB. Nucleotide exchange factors for Hsp70s are required for [URE3] prion propagation in *Saccharomyces cerevisiae*. *Mol Biol Cell.* 2007; 18:2149–2154. doi: [PubMed: 17392510]
- Lee YS, Mulugu S, York JD, O'Shea EK. Regulation of a cyclin-CDK-CDK inhibitor complex by inositol pyrophosphates. *Science.* 2007; 316:109–112. doi: [PubMed: 17412959]
- Luo HR, Huang YE, Chen JC, Saiardi A, Iijima M, Ye K, Huang Y, Nagata E, Devreotes P, Snyder SH. Inositol pyrophosphates mediate chemotaxis in *Dictyostelium* via pleckstrin homology domain=PtdIns(3,4,5)P₃ interactions. *Cell.* 2003; 114:559–572. doi: [PubMed: 13678580]
- Macbeth MR, Schubert HL, Vandemark AP, Lingam AT, Hill CP, Bass BL. Inositol hexakisphosphate is bound in the ADAR2 core and required for RNA editing. *Science.* 2005; 309:1534–1539. doi: [PubMed: 16141067]
- Mulugu S, Bai W, Fridy PC, Bastidas RJ, Otto JC, Dollins DE, Haystead TA, Ribeiro AA, York JD. A conserved family of enzymes that phosphorylate inositol hexakisphosphate. *Science.* 2007; 316:106–109. doi: [PubMed: 17412958]
- Odom AR, Stahlberg A, Wenthe SR, York JD. A role for nuclear inositol 1,4,5 trisphosphate kinase in transcriptional control. *Science.* 2000; 287:2026–2029. doi: [PubMed: 10720331]
- Saiardi A, Azevedo C, Desfougères Y, Portela-Torres P, Wilson MSC. Microbial inositol polyphosphate metabolic pathway as a drug development target. *Adv Biol Regul.* 2017; doi: 10.1016/j.jbior.2017.09.007
- Saiardi A, Bhandari R, Resnick AC, Snowman AM, Snyder SH. Phosphorylation of proteins by inositol pyrophosphates. *Science.* 2004; 306:2101–2105. doi: [PubMed: 15604408]
- Saiardi A, Sciambi C, McMaffery JM, Wendland B, Snyder SH. Inositol polyphosphates regulate endocytic trafficking. *Proc Natl Acad Sci USA.* 2002; 99:14206–14211. doi: [PubMed: 12391334]
- Sharma D, Masison DC. Functionally redundant isoforms of a yeast Hsp70 chaperone subfamily have different antiprion effects. *Genetics.* 2008; 179:1301–1311. doi: [PubMed: 18562668]
- Shen X, Xiao H, Ranallo R, Wu WH, Wu C. Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science.* 2003; 299:112–114. doi: [PubMed: 12434013]
- Shewmaker F, Wickner RB, Tycko R. Amyloid of the prion domain of Sup35p has an in-register parallel -sheet structure. *Proc Natl Acad Sci USA.* 2006; 103:19754–19759. doi: [PubMed: 17170131]
- Steger DJ, Haswell ES, Miller AL, Wenthe SR, O'Shea EK. Regulation of chromatin remodeling by inositol polyphosphates. *Science.* 2003; 299:114–116. doi: [PubMed: 12434012]

- Steidle EA, Chong LS, Wu M, Crooke E, Fiedler D, Resnick AC, Rolfes RJ. A novel inositol pyrophosphate phosphatase in *Saccharomyces cerevisiae*: Siw14 protein selectively cleaves the -phosphate from 5-diphosphoinositol pentakisphosphate (5PP-IP5). *J Biol Chem*. 2016; 291:6772–6783. doi: [PubMed: 26828065]
- Troisi EM, Rockman ME, Nguyen PP, Oliver EE, Hines JK. Swa2, the yeast homolog of mammalian auxilin, is specifically required for the propagation of the prion variant [URE3-1]. *Mol Microbiol*. 2015; 97:926–941. doi: [PubMed: 26031938]
- Tsui MM, York JD. Roles of inositol phosphates and inositol pyrophosphates in development, cell signaling and nuclear processes. *Adv Biol Regul*. 2010; 50:324–337. doi:
- Tycko R. Physical and structural basis for polymorphism in amyloid fibrils. *Protein Sci*. 2014; 23:1528–1539. doi: [PubMed: 25179159]
- Tycko R, Wickner RB. Molecular structures of amyloid and prion fibrils: consensus vs controversy. *Accounts of Chem Res* in press. 2013
- Wickner RB. [URE3] as an altered *URE2* protein: evidence for a prion analog in *S. cerevisiae*. *Science*. 1994; 264:566–569. doi: [PubMed: 7909170]
- Wickner RB, Beszonov E, Bateman DA. Normal levels of the antiprion proteins Btn2 and Cur1 cure most newly formed [URE3] prion variants. *Proc Natl Acad Sci USA*. 2014; 111:E2711–E2720. doi: [PubMed: 24938787]
- Wickner RB, Kelly AC, Bezsonov EE, Edskes HE. Prion propagation is controlled by inositol polyphosphates. *Proc Natl Acad Sci USA*. 2017; 114:E8402–E8410. doi: [PubMed: 28923943]
- Wickner RB, Shewmaker F, Bateman DA, Edskes HE, Gorkovskiy A, Dayani Y, Bezsonov EE. Yeast prions: structure, biology and prion-handling systems. *Microbiol Mol Biol Rev*. 2015; 79:1–17. doi: [PubMed: 25631286]
- Worley J, Luo X, Capaldi AP. Inositol pyrophosphates regulate cell growth and the environmental stress response by activating the HDAC Rpd3L. *Cell Reports*. 2013; 3:1476–1482. doi: [PubMed: 23643537]
- Wu M, Chong LS, Perlman DH, Resnick AC, Fiedler D. Inositol polyphosphates intersect with signaling and metabolic networks via two distinct mechanisms. *Proc Natl Acad Sci USA*. 2016; 113:E6757–E6765. doi: [PubMed: 27791083]
- York JD, Odom AR, Murphy R, Ives EB, Wente SR. A phospholipase C-dependent inositol polyphosphate kinase pathway required for efficient messenger RNA export. *Science*. 1999; 285:96–100. doi: [PubMed: 10390371]
- York SJ, Armbruster BN, Greenwell P, Petes TD, York JD. Inositol diphosphate signaling regulates telomere length. *J Biol Chem*. 2005; 280:4264–4269. doi: [PubMed: 15561716]

Fig. 1.

Conformational templating mechanism based on the folded in-register parallel β -sheet architecture of yeast prion amyloids (Wickner, et al. 2015). Favorable interactions between identical amino acid side chains (hydrogen bonds or hydrophobic interactions) maintain the in-register architecture, and drive a monomer joining the end of an amyloid filament to acquire turns/folds at the same locations as those of molecules already in the filament. This conformational templating, analogous to DNA's sequence templating, allows these proteins to be genes (Wickner, et al. 2015). Different prion variants (with the same protein sequence) are hypothesized to have the folds/turns at different locations.

Fig. 2.

Biosynthetic pathways of the soluble inositol poly/pyro-phosphates (IPs) (reviewed in (Saiardi, et al. 2017, Tsui and York 2010). IP₆ and 5PP-IP₄, and probably 5PP-IP₅, are capable of helping [PSI+] propagate (green arrows), and 1PP-IP₅ may inhibit [PSI+] propagation (red blockage symbol) (Wickner, et al. 2017).

Table 1

Some of the Functions of inositol poly/pyro-phosphates.

Function	Reference
Endocytic trafficking	(Saiardi, et al. 2002)
Environmental stress response, histone deacetylation	(Dubois, et al. 2002, Worley, et al. 2013)
RNA editing	(Macbeth, et al. 2005)
Translation termination	(Bolger, et al. 2008)
Phosphate assimilation	(Lee, et al. 2007)
mRNA export	(York, et al. 1999)
Chemotaxis	(Luo, et al. 2003)
Telomere length	(York, et al. 2005)
Transcription	(Odom, et al. 2000)
Chromatin remodeling	(Shen, et al. 2003, Steger, et al. 2003)