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Research paper

Clearance of yeast prions by misfolded multi-transmembrane proteins

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ABSTRACT

Accumulation of misfolded proteins in the endoplasmic reticulum (ER) induces the stress response to protect cells against toxicity by the unfolded protein response (UPR), heat shock response (HSR), and ER-associated degradation pathways. Here, we found that over-production of C-terminally truncated multi-transmembrane (MTM) mutant proteins triggers HSR, but not UPR, and clearance of yeast prions [*PSI*$^+$] and [*URE3*]. One of the mutant MTM proteins, Dip5ΔC-v82, produces a disabled amino-acid permease. Fluorescence microscopy analysis revealed abnormal accumulation of Dip5ΔC-v82 in the ER. Importantly, the mutant defective in the GET pathway, which functions for ER membrane insertion of tail-anchored proteins, failed to translocate Dip5ΔC-v82 to the ER and disabled Dip5ΔC-v82-mediated prion clearance. These findings suggest that the GET pathway plays a pivotal role in quality assurance of MTM proteins, and entraps misfolded MTM proteins into ER compartments, leading to loss-of-prion through a yet undefined mechanism.

1. Introduction

Prions are transmissible agents caused by the self-propagating conformational change of proteins [1]. According to the "protein only" hypothesis [1], the prion protein (PrP) is the sole agent responsible for causing numerous infectious diseases including scrapie (sheep), bovine spongiform encephalopathy (BSE, cow), chronic wasting disease (deer, elk) as well as kuru and Creutzfeld-Jacob disease (humans). In *Saccharomyces cerevisiae*, prions have also been characterized as non-Mendelian inheritable elements, notably [*PSI*$^+$], [*URE3*] and [*PIN*$^+$] [2–4]. Molecular and genetic studies of these yeast prions have greatly facilitated the elucidation of the molecular basis for prion conversion and propagation.

The yeast prion [*PSI*$^+$] [2] is the amyloid-like structure of the eRF3 polypeptide release factor, Sup35, which is essential for terminating protein synthesis at stop codons (reviewed by Ref. [5]). [*PSI*$^+$] cells are marked by an altered catalytic protein conformation of Sup35 whereby the Sup35 protein is converted from a soluble, active state to an aggregated, inactive state. When Sup35 is in the [*PSI*$^+$] state, ribosomes exhibit an increased rate of stop codon readthrough, causing a non-Mendelian trait easily detected by nonsense suppression [6]. The biological (or functional) significance of [*PSI*$^+$] and other yeast prions is not well understood. It is speculated that heritable prion-encoded information has been harnessed during evolution to confer selective advantages [7].

Several host factors are known to be involved in prion maintenance and propagation. Of these, the best characterized is the heat shock protein Hsp104, which facilitates the propagation of yeast prions by breaking apart amyloid filaments to generate prion seeds, which are transmissible to daughter cells [8,9]. It is also known that Hsp104 overproduction causes loss of [*PSI*$^+$] prion phenotypes in yeast [8]. Moreover, Hsp70/Ssa1 and Hsp40/Sis1 are also known to affect yeast prion propagation and clearance [10,11].

We have conducted genome-wide screens for prion-eliminating factors or mutants using a multi-copy expression system in yeast [12,13]. These screens revealed known factors, such as Hsp104, Hsp70, and Hsp40, their mutant forms, and novel host factors [12,13]. Some factors are glutamine/asparagine rich proteins of known and unknown prions [12,14], while other factors are

Abbreviations: ER, endoplasmic reticulum; MTM, multi-transmembrane; UPR, unfolded protein response; ERAD, ER-associated degradation; TMD, transmembrane domain; GET, guided entry of tail-anchored protein; TA, tail-anchored; HSR, heat shock response; HSE, heat shock element; UPRE, UPR element; ERAC, ER-associated compartment.

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non-prion proteins [13]. One of these, Gpg1, is a Gγ subunit mimic implicated in G-protein glucose signaling, and cures yeast prions when overproduced [13] by an unknown mechanism. These findings suggest that yeast prion proteins might undergo reversible phenotypic changes under certain conditions.

Recent findings suggest a functional interplay between the stress response and prion formation [15]. In yeast, Tsa1/Tsa2 Peroxiredoxins are ubiquitous antioxidants, protect cells against oxidative stress, and prevent the de novo appearance of [*PSI*$^+$] by protecting the ribosomal machinery against oxidative damage [15]. Under overwhelming oxidative stress, [*PSI*$^+$] prion formation is induced to provide a mechanism that promotes genetic variation and phenotypic diversity through stop codon readthrough, uncovering genetic traits that aid survival. This attractive idea suggests that cells are equipped with a system to prevent prion formation in several detrimental environments.

Cells maintain multiple mechanisms to prevent the accumulation of misfolded proteins in the endoplasmic reticulum (ER), which is known as ER stress and induces a stress response. The most well characterized response is a transcriptional pathway known as the unfolded protein response (UPR), which functions to upregulate ER chaperones [16]. When protein folding still fails, despite the activity of UPR, cells rapidly eliminate abnormally folded proteins by the ER-associated degradation (ERAD) machinery [17]. Heat shock response (HSR) is also induced by the accumulation of abnormal proteins, resulting in production of various chaperone proteins and ubiquitin-proteasome factors via the transcriptional activation of the heat shock element (HSE) by the transcription factor Hsf1 [18]. HSR can relieve ER stress independently of UPR, however, the mechanism of HSR activation by abnormal proteins is unknown [19].

Here, we find that over-production of multi-transmembrane (MTM) mutant proteins triggers clearance of yeast prions [*PSI*$^+$] and [*URE3*]. These proteins are truncated at transmembrane domains (TMDs) and are fused to a vector-coded peptide. Analysis by genetic mutation revealed that overexpression of Hsp104 is not primarily involved in this prion-clearance phenomenon. Instead, the abnormal accumulation of misfolded MTM mutant proteins in the ER triggered prion clearance, independent of UPR and HSR, probably through the GET (Guided Entry of Tail-anchored protein) pathway, which functions to integrate tail-anchored (TA) proteins in the ER [20]. The surveillance and clearance mechanisms for misfolded MTM proteins are unknown. We report a crucial role for the GET pathway for the quality assurance of MTM proteins and a physiological link between this surveillance system and a prion clearance pathway in yeast.

2. Materials and methods

2.1. Strains

S. cerevisiae strains used in this study are: NPK294 ([*PSI*$^+$] [*PIN*$^+$] isogenic with NPK50 [12]), NPK265 ([*PSI*$^+$] [*PIN*$^+$] *MATa ade1-14 leu2Δ0 ura3-197 his3Δ200 trp1-289*), NPK302 ([*URE3*] [*pin*$^-$] *MATa PD-ADE2 his3 leu2 trp1 kar1 PD-CAN1*), NA99 (*MATa his3Δ200 ura3Δ0 gap1*::*URA3*) (Regenberg et al., 1998 [21]), NA100 (*dip5*::*KanMX* derivative of NA99) [21], NA103 ([*PSI*$^+$] [*PIN*$^+$] *get3*::*kanMX* isogenic with NPK265).

2.2. Plasmids

pMCZ-Y is a *URA3*-marked 2-μ plasmid bearing the *KAR2 UPRE* fused to *lacZ*, kindly provided by Dr. K. Mori (Kyoto University) [22]. KAR2HSE-CYC1-lacZ is a *URA3*-marked 2-μ plasmid kindly provided by Dr. N. Kohno (Nara Institute of Science and Technology) [23]. Other expression plasmids used in this study were constructed from pRS400 series vectors (Stratagene; see Ref. [24]) carrying the *GAL1* and *GPD* promoters at the *Sac*I-*Bam*HI site and the *CYC* terminator at the *Xho*I-*Kpn*I site. The *DIP5* and *GET3* sequences were amplified by PCR using the following primers and cloned into the *Bam*HI-*Xho*I site: P1 (TTTGGATCCATGAAGATGCCTCTAAAG, all the sequence shown here are from 5′ to 3′) and P2 (TTTCTCGAGCTAGAAGATATTACCC) for wild-type *DIP5*; P1 and P3 (TTTCTCGAGTTAGATCTTTGGCGCTTTACC) for *Dip5ΔC*; P1 and P4 (CGCCTCGAGTCAGGCTGCGCAACTGTTG) for *Dip5ΔC-v82*; P5 (CGCCCCGGGATGGATTTAACCGTGGAAC) and P6 (CGCCTCGAGCTATTCCTTATCTTCTAAC) for *GET3*. The *Dip5-v82*, *TM7-v82* and *TM9-v82* fragments were amplified by PCR using primers: P1 and P7 (GATCCCGGGGGAAGATATTACCCAAAA) for *Dip5-v82*; P1 and P8 (GATCCCGGGGGCAACCACGAATGGAG) for *TM7-v82*; and P1 and P9 (GATCCCGGGGTGATCCTGAAGACACATTCA) for *TM9-v82*. These fragments were then cloned into the *Bam*HI/*Sma*I site of pRS413GP Dp or pRSGAL1p-Dip5ΔC-v82. To construct the Dip5(HA) variant, a *Bgl*II site was created between TMDs 1 and 2 of Dip5 using the mutagenic primer P10 (CAACCGGACCAGATCTACCCGTCAATAAG), which did not alter the amino acid sequence, and the *Bam*HI-digested segment of the HA tag was inserted into the *Bgl*II site.

To construct fluorescent-tag fusions to Dip5 derivatives, EGFP sequence was first amplified from plasmid pKT128 [25] by PCR using primers P11 (CGCCTCGAGGAAATTCGCTTATTTAG) and P12 (CGGGTC GACGGTGACGGTGCTGGTTTAATTAAC), and digested with *Sal*I and *Xho*I, followed by cloning into the *Sal*I-*Xho*I site of pRS413GPDp (ARS/CEN, *HIS3*). Then, the Dip5-v82 and Dip5ΔC-v82 sequences were cloned into the *Bam*HI-*Sal*I site using *Bam*HI-*Sal*I digests of PCR fragments amplified with primers P1 and P13 (GCCGTCGACGAA-GATATTACCCAAAA) for Dip5-v82, and P1 and P14 (GCCGTCGACG GCTGCGCAACTGTTGGG) for Dip5ΔC-v82, giving rise to pRS413 GPDp-Dip5-v82-EGFP and pRS413GPDp-Dip5ΔC-v82-EGFP. To construct the pKT-DsRed plasmid, the *DsRed* sequence was amplified by PCR from plasmid pDsRed-Monomer-N1 (Clontech) using primers P15 (CGCTTAATTAACATGGACAACACCGAGGAC) and P16 (CACTCCGG CTCCCAGTAGGGCGCGCCGCG), and their *Sal*I-*Xho*I digests were cloned into the *Sal*I/*Xho*I site of pKT128. To construct the pRS416 Sec63-DsRed plasmid, the *SEC63* sequence was first amplifying by PCR from genome DNA using P17 (CGCGGATCCGGAAACCTTGCAAT-CAG) and P18 (CGCGTCGACTTCTGGTGATTCATCATC), and its *Bam*HI-*Sal*I digests were cloned into the *Bam*HI-*Sal*I site of pRS416. Next, the *DsRed* fragment amplified by PCR using P15 and P16 was cloned into *Sal*I-*Xho*I site of pRS416Sec63.

Plasmid pRS413GPDp-Hsp104 was constructed as follows: the *Bam*HI-*Xho*I fragment encoding *HSP104* was isolated from p*HSP104* [13], and cloned into pRS413GPDp.

2.3. Culture manipulations

The yeast media used were YPD (1% yeast extract, 2% polypeptone, 2% dextrose) and synthetic complete medium containing glucose (SC) (0.67% yeast nitrogen base without amino acids [DIFCO] and 2% dextrose), sucrose (SSuc) (0.67% yeast nitrogen base without amino acids [DIFCO] and 2% sucrose) or galactose (SGal) (0.67% yeast nitrogen base without amino acids and 4% galactose), supplemented with adenine, leucine, uracil, histidine, tryptophan, or 5-FOA when required. Yeast cells were grown at 30 °C in YPD, SC or SGal media with the appropriate supplements. The *dip5* and *get3* null strains were constructed by transformation with PCR products of the *dip5*::*KanMX* and *get3*::*KanMX* sequences (American Type Culture Collection catalog no. 4013435; see Ref. [26]). The *gap1* null strain was constructed by transformation with PCR products of the *gap1*::*URA3* sequence, which was made by substituting *URA3* for *KanMX* in the *gap1*::*KanMX* strain.