Original research / Orijinal araştırma

Yeast prions

Maya prionları

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Abstract

Prions are responsible for neurodegenerative diseases. Altered protein conformation induces normally folded proteins to adopt infective prion form. The prion protein appears to be responsible for different diseases with different pathologies. Propagating prions are viable in *S. cerevisiae* cells; therefore, these eukaryotic cells provide a unique system to study prion propagation and protein disaggregation to investigate prion phenomena. Several research groups work on prions and related diseases and *S. cerevisiae* is a tractable model. This review focuses on [PSI$^+$], [URE3] and [RNQ$^+$] prions.

Keywords: Prion, PSI, URE3, PIN, Sup35, Ure2, Rqn1

Özet

Prionlar nörodejeneretif hastalıklardan sorumludurlar. Değişmiş protein konformasyonu normal proteinlerin enfektif prion haline geçmesini indükler. Prion protein farklı patolojideki hastalıkların oluşumundan sorumludur. Biriken prionlar *S. cerevisiae* hücrelerinde görselleştirilebilirler, bunun için bu ökaryotik hücreler prion konusunu araştırmada prion birikimi ve protein ayrışması için önemli bir sistem oluşturur. Birçok araştırma grubu prion ve ilgili hastalıkları çalışmaktadırlar ve *S. cerevisiae* uygun bir modeldir. Bu derleme [PSI$^+$], [URE3] ve [RNQ$^+$] prionları üzerine yoğunlaşmıştır.

Anahtar sözcükler: Prion, PSI, URE3, PIN, Sup35, Ure2, Rqn1

Protein Folding

Molecular chaperones recognize and selectively bind to non-native protein structures. Then molecular chaperones guide the substrate protein to reach its functional correctly folded form. Energy requirement for binding and folding process is compensated by ATP

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hydrolysis energy. Molecular chaperones consist of several highly conserved families of unrelated proteins. Many chaperones are also Hsps. Major Hsp families of *S. cerevisiae* cytosol are Hsp70 (Ssa1-4, Ssb1-2, Ssz1), Hsp40 (Sis1, Ydj1), Hsp90 (Hsc82, Hsp82), Hsp100 (Hsp104) and co-chaperones (Sti1, Cpr6, Cpr7, Cns1) (1-5).

Hsp70 has unique structure to fold non-native protein substrates. N-terminal has a cavity to hydrolyze ATP and then the domain transmits the energy to C-terminal domain where hydrophobic forces help the substrate to fold correctly. *S. cerevisiae* cytosol has at least nine Hsp70s. There are several redundant forms of molecular chaperones exist even in the same compartment of a cell. Possible explanation to this question is different protein-protein interaction of these molecules provide distinct function to the cell (1-5).

Hsp40 facilitates substrate protein folding by submitting the substrate to Hsp70 substrate binding domain. Hsp40 interacts with Hsp70 through its J domain. Hsp40 interaction increases Hsp70 ATP hydrolysis rate.

Hsp100 coordinates with Hsp70-Hsp40 complex for dissolving the aggregates. Hsp100 pull down a peptide from aggregate and pass the substrate from its central hole. At the end of the hole Hsp70-Hsp40 complex acts on the substrate to bring it to its native state. Hsp90 displays similar Hsp70 activities but unlike Hsp70 Hsp90 does not act on new released peptides from ribosomes. Hsp90 coordinates with Hsp70 and with co-chaperones (1, 3). Details of structure and function of Hsps can be found in a recent comprehensive review (1).

Yeast Prions

Damaged, mutant, and misfolded proteins tend to aggregate. Some protein aggregates infect normal proteins, propagates in cell and divide in daughter cells. These infectious proteins adopt distinct functional and conformational states of a cellular protein. The infectious proteins convert the normal form of the protein into abnormal isoform. These altered proteins form aggregates. The self-propagating infectious aggregates are named prion. Reed Wickner described two prions ([PSI^{+}], [URE3]) in *S. cerevisiae* as non-Mendelian elements in 1994 since they do not follow typical transmission mechanism (6). *In vitro* experiments from purified protein proved "protein only" hypothesis for prion formation (7-9). Other proteins form amyloids but only prion proteins have the ability to infect and the nature of self-propagation still remains elusive. In this hypothesis yeast prions are denoted within brackets by capital letters. This demonstration means that the prion is transmitted to daughter cells inheritably (10).

The protein aggregation causes several diseases, namely Alzheimer's disease, Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, bovine spongiform encephalopathy, scrapie, cystic fibrosis and Gaucher's disease. These diseases and subsequent appearance of new variant diseases have highlighted the importance of research in this area (1). Further, propagation of yeast prions is dependent on the levels

of molecular chaperones (11). Ssa1/Ssa2 chaperones interact with [PSI$^+$] and Hsp104 chaperone plays essential role in prion propagation and in prion maintenance (12-14).

The three extensively studied prions in the yeast *S. cerevisiae* are [PSI$^+$], [URE3] and [RNQ$^+$]. These self-replicating prions are misfolded forms of the Sup35, Ure2, and Rnq1 proteins, respectively. In addition to these prions, there are also potential prion forms of yeast, i.e. NEW1, MCA1, SWI1 identified by biochemical and genetic techniques (15, 16). Another known yeast prion is Podospora anserina prion [Het-s]. The prion negatively regulates heterokaryon formation between incompatible strains (5,17,18). This review is focuses on *S. cerevisiae* prions [PSI$^+$], [Ure3] and [RNQ$^+$] prions.

Yeast prion [PSI$^+$]

[PSI$^+$] is an aggregated form of Sup35 ([psi−]). The protein consists of 685 amino acids. Sup35 identifies stop codons and terminate translation along with Sup45. Sup35 is an essential protein and cells lacking SUP35 gene is not viable. A reporter assay system for [PSI$^+$] relies on its function of translation termination. Since prion form of Sup35, [PSI$^+$], causes read-through of stop codons, a yeast auxotroph for adenine is used to monitor presence of [PSI$^+$]. The yeast phenotype has a premature stop codon that interrupts the ADE1 gene (19). Yeast [psi−] cells terminate translation at the premature ston codon and therefore, cannot produce much adenine. They can only grow on adenine rich medium and accumulation of an intermediate at adenine biosynthesis pathway gives cells a red color (20). Yeast [PSI$^+$] cells occasionally read through the premature stop codon in ADE1, this enables them to grow in the absence of adenine. In contrast to [psi−] cells, the [PSI$^+$] cells appear white or pink in color in the presence of rich medium depending on the nonsense suppression strength of a particular strain (19).

Sup35 protein non-essential N-terminal domain, so called prion domain, is responsible for [PSI$^+$] appearance and maintenance. C-terminal domain itself performs Sup35 function and terminates translation (21-24). The N-terminal is rich in glutamines (Q) and asparagines (N) and implicated both in prion conversion (25, 26). A comparison of amino acid residues of Sup35, Ure2 and Rnq1 is shown in Figure 1. The sequences are rich in Q and N residues.

Yeast prion [*URE3*]

S. cerevisiae can utilize alternative nitrogen sources in the absence of preferred nitrogen sources such as glutamine, asparagine, or ammonium. GLN3 protein induces transcription of specific enzymes when preferred nitrogen sources are unavailable and URE2 protein repress the transcription of the enzymes when preferred nitrogen sources abundant in the environment (27). Ure2 has similar N-terminal amino acid composition as Sup35. Ure2 represses some enzymes in nitrogen metabolism and functions as peroxidase. Ure2 prion form, [URE3], maintains its enzymatic activity but loses its regulatory function in nitrogen metabolism (6, 28).

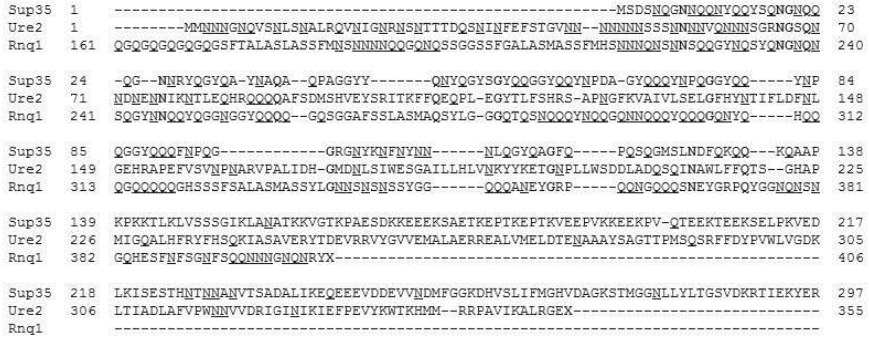

Figure 1. Comparison of Q/N-rich regions of Sup35, Ure2 and Rnq1 primary structures. N and Q residues dominate which are required for prion propogation and these residues are underlined. Conserved residues in all three proteins are shown in bold. Blast, NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) was used for comparison.

Yeast prion [*PIN*$^+$]

Database research for other possible prion forms elucidated several proteins. Rnq1 has similar prion-type sequence composition like other two proteins. The function of Rnq1 is elusive but display prion characteristics therefore, named "rich in aspargine (N) and glutamine (Q)". [PIN$^+$] is misfolded form of Rnq1. The [PIN$^+$] abbreviation corresponds to the initials of [PSI$^+$] inducibility [18]. RNQ1 aggregates enhance [PSI$^+$] propagation. This supports the postulated cross-seeding mechanism (9, 18).

Prevention of Aggregation and Prions

There is growing number of patents and literature on diagnostic and therapeutic aspects of prion diseases. There are several patents for the treatment of prion related disease by chemical agents. Yeast is a good model to monitor the progress of prion propagation and drug target for the treatment of prion diseases.

A labeling method for protein aggregation was developed and tested for Sup35, β-amyloid protein, huntingtin, α-synuclein, Bovine spongiform encephalopathy. The method recognizes the mentioned aggregates (29). Determination of prion was employed by another group (30).

Distinguishing normal and prion proteins is also an important diagnostic tool. This is achieved for PrP by employing molecular chaperone Hsp60 in *S. cerevisiae*. PrP is a pathogenic agent causing a series of neurodegenerative diseases. Labeled Hsp60 binds to normal for of PrPc and this helps distinguishing normal protein from prion form (PrPSc) (31). Another high throughput testing of bovine spongiform encephalopathy, Creutzfeldt-Jakob disease, scrapie was patented. The method based on serial cyclic amplification of prion (32).

Several molecular chaperones alone or with coordinating chaperones employed to prevent protein aggregation by different research groups (33). Protein aggregation is mainly due to hydrophobic protein-protein association. Compounds patented to prevent this undesired interaction (34). An application was patented that screen chemical agents with anti-prion activity. The screening employs [PSI^{+}] detecting method by Ade1-14 allele of the ADE gene (35).

Current and Future Development

There is a great need for assays to detect protein aggregates. Protein aggregation in solution or in different tissues may not always visual. New biochemical tests are required to detect infected animals rapidly. Several cases require rapid detection of prion seeds i.e. in mad cow diseases, in neurodegenerative diseases. Unfortunately the results can be observed after couple decades. There is no general method to test all different prion proteins and not all prion types are identified at early stage yet. Understanding a common prion propagation mechanism and assay development for this mechanism is essential to prevent aggregation based diseases.

Acknowledgements

This work was funded through a seed grant from the Turkish National Academy of Sciences (TUBA-GEBIP, TUTAR 2008-29).

Conflict of Interest

The author declare no conflict of interest.

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