Projektexamenskod: BBTX11-23-01

Avdelningen för Systembiologi
Institutionen för biologi och bioteknik, Chalmers tekniska högskola

Projektförslag för kandidatarbete inom inst. Kemi och kemiteknik och Biologi och bioteknik

Titel: Cellular mechanisms of protection against protein misfolding in neurodegenerative disease. Role of redox regulation and calcium signaling.

Bakgrund

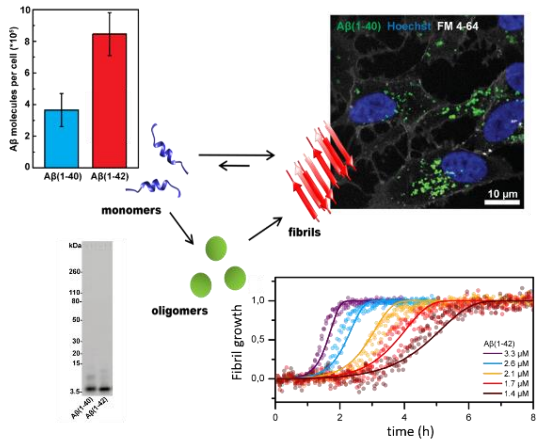
Protein biosynthesis is an energetically demanding process and it is regulated in response to environment (nutrient availability, stress …). Proteins must be folded in order to perform their functions. To adapt to this, cells have exquisite molecular machineries, for example molecular chaperones that aid in protein folding and in maintaining proteins in a folded state.

Under stress conditions, proteins may unfold or fail to fold correctly. This misfolding of proteins can cause protein aggregate formation which is problematic in the brain, being the underlying cause of several severe and incurable age-related disorders including Alzheimer's and Parkinson's diseases as the motor neuron disorder Amyotrophic Lateral Sclerosis (ALS). The pathophysiology of ALS is also characterized by increased oxidative stress. Different types of protein aggregates occur in most neurodegenerative disease including ALS.

Interestingly, we found that a particular type of anti-oxidant protein, peroxiredoxin, was absolutely required for molecular chaperones to engage age-related aggregates. Peroxiredoxins function as H_2O_2-controlled chaperones that slow down aging and decrease the incidence of age-related disease in organisms ranging from yeast to mice.

TDP-43 is an RNA binding protein that has been identified as a major component of the protein aggregates characterizing ALS. By expressing TDP-43 in baker's yeast, several novel regulators of ALS have been identified in genome-wide screens, notably genes encoding ribosomal proteins and regulators of so-called stress granules. These are a type of beneficial inclusions where mRNA and translational components are stored transiently under unfavorable conditions until translation can resume.

The exact relationship between beneficial aggregates and pathophysiological TDP-43 aggregates is still mostly unclear. Our previous research in yeast show that the absence of the peroxiredoxin Tsa1 results in increased TDP-43 toxicity and aggregation.



Our ongoing studies suggest firstly that Tsa1 may be involved in transfer RNA (tRNA) modification. This since Tsa1 shares negative genetic interactions with several tRNA modification enzymes, suggesting a functional interaction. In fact, cells lacking one of these enzymes display a strong growth defect upon H_2O_2 which cannot be overcome by overexpression of the *TSA1* gene, suggesting that Tsa1 may elicit H_2O_2 resistance through modifying tRNAs. Interestingly, tRNA modification enzymes have also been linked to ALS pathology.

Problembeskrivning

With a global increase in life expectancy, the

prevalence of neurodegenerative disease, for instance ALS, is expected to increase. These diseases are characterized by, among other things, progressive loss of neurons. As of today, these diseases cannot be cured. Neurodegenerative diseases can be linked both oxidative stress and accumulation of toxic proteins aggregates in neurons. In ALS, these aggregates consist mainly of the protein TDP-43. Previous studies performed in yeast suggest that the absence of the peroxiredoxin Tsa1 result in increased TDP-43 toxicity.

We will study:

1. **Role of tRNA modification enzymes in TDP-43 aggregation**: Using TDP-43 plasmids and fluorescence microscopy we will study if Deg1 and other tRNA modification enzymes have a role in TDP-43 aggregation.
2. **Role of calcium signaling in TDP-43 aggregation:** Calcium signaling has turned out to be a crucial modifier of neurodegeneration as well as the toxicity of humanized yeast expressing neurodegenerative disease-related proteins. A key component in this signaling cascade, that governs calcium transport inside cells and many other cellular processes, is the protein phosphatase calcineurin that dephosphorylates a number of target proteins. Calcium signaling is closely coordinated with e.g. nutrient signaling cascades such as protein kinase A (PKA), that in yeast regulates e.g. growth and stress-resistance as a function of nutrient levels. We have recently shown that Tsa1 redox signaling targets PKA via a novel mechanism. PKA- and calcium-dependent signaling are closely coordinated in mammalian cells but whether calcium and peroxiredoxin signaling intersect is not yet known. We will study the role of calcineurin in TDP-43 toxicity and its connection with the peroxiredoxin Tsa1.

Genomförande /Viktiga moment/teknikinnehåll

1. **PCR amplification** of both plasmids. **DNA electrophoresis** to check if the amplify is well done.
2. **Plasmid extraction** from bacteria through growing bacteria in LB medium + ampicillin and extraction using a midiprep kit.
3. **Yeast transformation** with both plasmids and growth in selective medium.
4. **Growth curves** and characterization of TDP-43 expressing humanized yeast using **spot tests** with **estradiol** (to induce TDP-43 expression) and different stress conditions.
5. Protein analysis including **Western Blot** and biochemical techniques to extract insoluble protein aggregates.
6. **Fluorescence microscopy** and live cell confocal microscopy imaging. Image analysis including cell tracking.

Speciella förkunskapskrav: …

Basic microbiological techniques and image analysis including cell tracking is meritorious

Möjlig målgrupp: Bt

Gruppstorlek: 4-6 studenter

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