The Mechanism of Large-Scale CAG Repeat Expansion

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Abstract

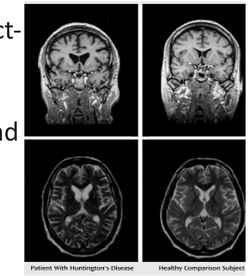
Nearly 30 genetic diseases are caused by the repetition of a short sequence of 2-9 base pairs, known as microsatellites. These microsatellite repeats occur in humans normally; however disease symptoms become apparent once a certain repeat threshold is reached. Usually, these diseases follow a phenomenon known as genetic anticipation, in which each subsequent generation experiences increased disease severity and earlier onset, which was found to correlate with microsatellite repeat length. To study these expansions, budding yeast (*S. cerevisiae*) strains were used in genetic experiments to determine a protein's effect on CAG repeat expansion. Preliminary data suggest that Rmi1 is required for expansions to occur, Los1 protects against expansions, and Gpb1 does not have a role in expansion.

Background

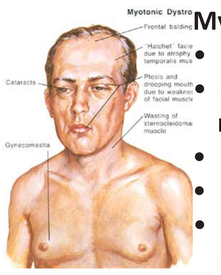
Huntington's Disease, Myotonic Dystrophy, and 7 other genetic diseases are caused by an expanded CAG repeat. Though CAG repeats occur normally, it is only once a certain threshold is reached that disease symptoms become apparent. Disease severity is also correlated with increasing repeat length.

Huntington's Disease

- 5-7 people per 100,000 affected
- neurodegenerative disorder, causing physical, cognitive, and psychological problems
- normal: 16-20 repeats
- premutation: 27-35 repeats
- disease: 36 or more repeats



Brain scan of an individual with Huntington's Disease (left) and a control (right).



Myotonic Dystrophy

- 1 in 8,000 people affected
- characterized by progressive muscle wasting and weakness
- normal: 5-34 repeats
- premutation: 35-49 repeats
- disease: 50-2000 or more repeats

Despite this knowledge, the mechanism of CAG repeat expansion is still unclear. By establishing the role of specific proteins, we can determine which cellular processes may be involved in causing or preventing expansions.

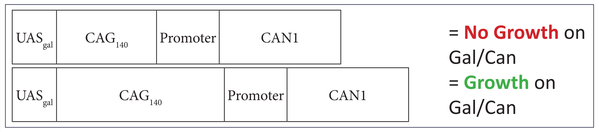
Research Question

Which proteins are required for and which proteins protect against large-scale CAG repeat expansion?

Materials and Methods

Yeast Experimental System

The *CAN1* cassette is an experimental yeast system that selects for cells that have experienced large-scale CAG repeat expansion. Cassette-containing cells will not grow on canavanine and galactose containing media, unless an expansion occurs.

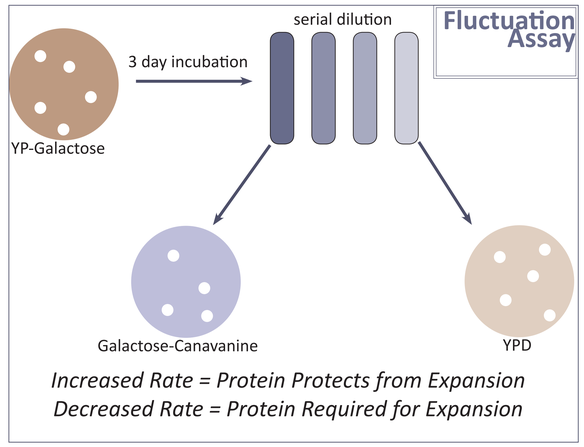


Fluctuation Assay

A fluctuation assay was performed to calculate the expansion rate. After growth on YP-Galactose, dilution, and plating onto selective and non-selective media, the number of colonies was counted on day 3, day 4, and day 5 for YPD, gc60, and gc200, respectively. Expansions were confirmed by PCR. Canavanine resistance rate and expansion rate were calculated using Drake's method.

Spot Assay

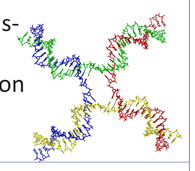
A spot assay was done by creating 1:5 serial dilutions and plating onto YPD plates, galactose-canavanine 60µg/mL (gc60) plates, and gc200 plates.

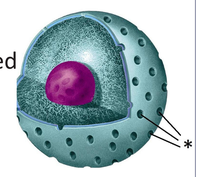


Increased Rate = Protein Protects from Expansion
Decreased Rate = Protein Required for Expansion

Proteins of Interest

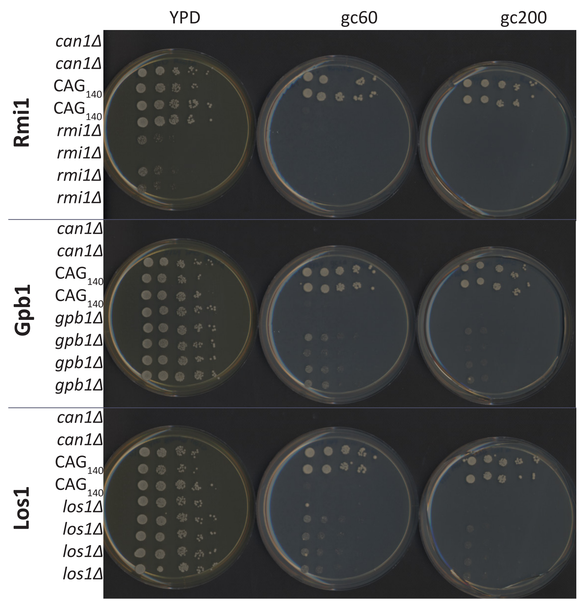
Rmi1	•Implicated by previous work with homologous recombination •Part of STR complex which dissolves Holliday junctions •Expect a decrease in expansion rate
Los1	•Implicated through an unbiased genetic screen •Nuclear pore protein involved in the transport of tRNAs
Gpb1	•Implicated through an unbiased genetic screen •Multistep regulator of CAMP-PKA signaling



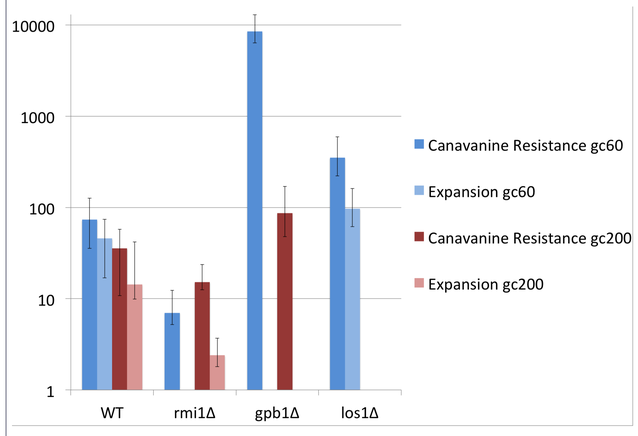


Results

Spot Assay Results



Fluctuation Assay Results



- Increased canavanine resistance rate for *los1Δ* corresponded with increased expansion rate.
- *gpb1Δ* canavanine resistance was only largely affected on low canavanine concentration. Expansion rates were not calculated, but very few expansions were seen during PCR confirmation.
- Canavanine resistance was decreased for *rmi1Δ*. Expansion rate is assumed to be $<10^{-7}$ for gc60 because no expansions were visualized during PCR confimation.

Next Steps

- Confirm increase in expansion rate for *los1Δ*
- Determine whether Los1 affects small-scale repeat expansion
- Determine whether Los1 affects expansion rate in non-hairpin forming repeats, such as GAA

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