The role of accelerated protein turnover in adaptation to mistranslation

Ph.D. thesis summary

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Szeged

2017

Introduction

Protein synthesis is one of the major cellular processes. As proteins are essential building blocks of the cells, the fidelity of translation has a substantial impact on cell survival. Errors in protein synthesis can reduce the organisms' viability and they serve as an onset of genetic diseases.

Errors can occur during the whole process of protein synthesis, from the incorporation of amino acids to protein folding, even during posttranslational modification. Recent studies have indicated, that error rates during protein production are three to five orders of magnitude higher than during DNA-replication. We asked if protein production is such error prone, how can organisms still maintain the homeostasis of cellular processes?

Two type of quality-control mechanisms exist based on the direction of processes: one that reduce the rate at which errors occur (error prevention) and one that limits the harmful effects if an error has already been made (error mitigation). Although the major types of

quality control processes have been identified, the relative contribution of these pathways to safeguarding the integrity of biological information is remained largely unknown.

Our aim was to investigate this problem systematically by taking advantage of a previously engineered bakers' yeast strain, on which we analyzed the effects of ambiguous amino acid decoding, as a specific type of mistranslation.

Aims

We integrated evolutionary biology, biochemical and functional genomics tools to answer the following questions:

1. Can fitness loss due to mistranslation be compensated in *Saccharomyces cerevisiae* cells during a laboratory evolutionary experiment?
2. What are the underlying mechanisms of fitness loss caused by mistranslation?
3. What type of genetic- and physiological changes are involved in the adaptation to mistranslation?

4. What are the evolutionary side-effects of adaptation to mistranslation?

Methods

- Transforming the *Candida albicans* $tRNA_{CAG}^{serine}$ gene on a single copy vector into *Saccharomyces cerevisiae* in order to generate a strain with high mistranslation rate
- Measuring mistranslation rate with β-galactosidase assay
- Laboratory evolution in liquid culture involving strains with low and high mistranslation rate
- Assaying fitness of yeast strains based on growth rate measuments
- Transforming yeast cells in order to switch from high to low mistranlation rate
- Measuring the rate of protein aggregation using a fluorescent mCherry tagged human protein (von Hippel–Lindau protein, VHL)
- Quantification of β-galactosidase protein level using Western blot

- Quantification of $tRNA_{CAG}^{serine}$ expression by Northern Blot
- Quantification of protein synthesis rate using Pulse-labeling method
- Quantification of proteasome chymotrypsin-like activity using a fluorogen peptide substrate (s-LLVY-MCA)
- Assaying glucose uptake rate by measuring glucose content of the culturing media
- Measuring chronological lifespan by a series of agar plate based viability assays
- Inferring the extent of ribophagy by scoring the location of a GFP tagged ribosomal protein (Rpl25p) in the citoplasm and in the vacuole
- Microarray based gene expression profiling
- Whole genome sequencing using Illumina platform

Results

1. The fitness defect due to mistranslation can rapidly be mitigated during a relatively short period of laboratory evolution

We used a previously engineered *Saccharomyces cerevisiae* strain as a model to investigate the effects of mistranslation on cellular fitness. We expressed the *Candida albicans* $tRNA_{CAG}^{Ser}$ gene from a single copy vector in diploid bakers' yeast cells in order to increase the mistranslation rate experimentally. The engineered construct misincorporates serine at leucine sites encoded by the CUG codon. Using β-galactosidase assay, we confirmed, that expression of $tRNA_{CAG}^{Ser}$ increase the rate of mistranslation in bakers' yeast cells. The expression of the mutant tRNA caused a 62% reduction in β-galactosidase activity. Accordingly this resulted in a significant increase in mistranslation rate.

Measuring the growth rate of yeast populations expressing the $tRNA_{CAG}^{Ser}$ construct indicated that elevated mistranslation rate reduced the fitness of yeast populations by 40% compared to wild type populations

with low mistranslation rate. Next we initiated a laboratory evolutionary experiment with strains having low and high mistranslation rate, 11-11 parallel replicates each, for approximately 250 generations. After the evolution, significant fitness improvement (13.3%-51.7%) was detected in all the parallel evolved lines with high mistranslation rate compared to the ancestral state. These results indicate that fitness defect due to mistranslation can rapidly be mitigated during a relatively short period of laboratory evolution.

2. Reducing the rate of protein aggregation was one of the main targets for natural selection during laboratory evolution

Henceforth, we focused on the underlying mechanisms of fitness loss caused by mistranslation. It is known, that fitness cost of mistranslation could partly be due to protein misfolding, protein aggregation, and consequent induction of cellular toxicity. To shed light on the possible link between mistranslation and protein aggregation, we used a fluorescent riporter system. We measured the distribution of a fluorescently tagged

aggregation prone human protein (VHL, von Hippel–Lindau) to infer the changes in protein aggregation propensity during the course of laboratory evolution. We demonstrated, that elevated mistranslation rate increased the rate of protein aggregation in the ancestral state. In contrast, all the 11 parallel evolved lines showed significantly reduced protein aggregation propensity. These results indicate that reducing the rate of protein aggregation was one of the main targets for natural selection during laboratory evolution.

3. Reduction of mistranslation is not exclusively responsible for adaptation

With the help of a biochemical assay, we detected a small, but significant decrease in the misreading activity of the $tRNA_{CAG}^{Ser}$. We suspect that the reduction in $tRNA_{CAG}^{Ser}$ expression might be responsible for this pattern. However we hypothesize that neither the reduction of mistranslation rate nor the reduction of $tRNA_{CAG}^{Ser}$ are exclusively responsible for adaptation.

4. The evolutionary adjustment of proteome homeostasis to mistranslation was achieved through acceleration of protein turnover

Applying biochemical tests, we demonstrated that both the translation- and proteasomal degradation rate was significantly increased in the evolved lines with high mistranslation rate compared to the ancestor. We have quantified the rate of protein synthesis by measuring the incorporation of radioactively labelled amino acids. To quantify the chymotrypsin-like proteasomal activity, we have used s-LLVY-MCA, a fluorogenic peptide substrate based assay. We could conclude that the evolutionary adjustment of proteome homeostasis to mistranslation was achieved through acceleration of protein turnover, a process determined by the combined rates of protein synthesis and ubiquitin-proteasome system mediated degradation.

5. Large-scale chromosomal rearrangements are genetic basis of adaptation

We also performed whole genome sequencing on the 11 parallel evolved lines in order to reveal the

genetic basis of adaptation to mistranslation. The genomic analysis demonstrated that adaptation to mistranslation induced large-scale chromosomal duplication and deletion events in the evolved lines. These large-scale genomic changes simultaneously altered the dosage of genes involved in ribosomal biogenesis, ribosome assembly, and rRNA processing and could function as a genetic basis for accelerated protein synthesis. In more than 90 % of the evolved lines we also have identified copy number loss in two genes encoding deubiquitinating enzymes (UBP3, UBP5). These genes are involved in the deubiquitilation of the proteins diverting them away from the proteasome system. Accordingly, reduced dosage of these genes can increases the fraction of proteins destined for destruction, resulting an increased protein degradation rate.

We also investigated the effect of genomic mutations on gene expression levels. We focused on two evolved lines, both of which carried typical large-scale chromosomal rearrangements. The analysis included only those genomic segments which were unaffected by

copy number changes during the course of laboratory evolution. We observed increased expression of genes involved in ribosomal RNA processing, ribosome biogenesis and amino acid biosynthesis. These results also support the role of accelerated protein synthesis in the adaptation to mistranslation.

6. Increased energetic demand is an evolutionary side-effect of adaptation to mistranslation

As both translation and proteasomal degradation are highly energy consuming processes, accelerated protein turnover must imply higher energetic cost to the cells. Measuring the glucose uptake of the ancestor and the evolved lines we demonstrated that, evolved cells generally internalized more glucose molecules than the ancestor cells in order to fulfil the energetic demand of accelerated protein turnover. We also demonstrated that - due to the increased energetic demand - evolved lines generally showed decreased fitness and reduced chronological lifespan upon nutrient limitation. As an evolutionary trade-off, adaptation to mistranslation reduced the capacity of evolved lines to degrade mature

ribosomes. This might led to the loss of viability and faster extinction rate of the evolved populations under starvation.

Summary

This work provides several lines of evidence that accelerated protein turnover is a fast and effective method for adaptation to mistranslation. However, due to it's high energetic demand, this strategy might be only feasible in nutrient-rich environments, such as our experimental settings. Therefore it is, this work does not states that accelerated protein turnover is the only safeguarding mechanism against the detrimental effects of mistranslation. Translation fidelity may vary across environments and organisms might have evolved different defence mechanisms against protein synthesis errors.

List of publications

MTMT number: 10046224

1.1. Publication related to the Ph D. thesis

Kalapis D, Bezerra A R, Farkas Z, Horvath P, Bódi Z, Daraba A, Szamecz B, Gut I, Bayes M, Santos MA, Pál Cs. Evolution of Robustness to Protein Mistranslation by Accelerated Protein Turnover PLOS BIOLOGY Nov 6;13(11):e1002291 (2015)
IF: 8.66

1.2. Other publication

Szamecz B, Boross G, Kalapis D, Kovács K, Fekete G, Farkas Z, Lázár V, Hrtyan M, Kemmeren P, Groot Koerkamp M J, Rutkai E, Holstege F C, Papp B & Pál Cs. The genomic landscape of compensatory evolution PLOS BIOLOGY Aug 26; 12(8):e1001935 (2014)
IF: 9.34