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Syntéza bílkovin za buněčného stresu
Protein synthesis in cellular stress

Bachelor's thesis

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V Praze, 12.8.2015 .....

Martin Cienciala

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Contents

Glossary

Proteins

CAF20	Cap associated factor 20
CBC1	Cap binding complex 1
DOM34	Duplication of multilocus 34
EAP1	eIF4E associated protein 1
EF-2	Elongation factor 2
eIF1	Eukaryotic initiation factor 1
eIF2	Eukaryotic initiation factor 2
eIF2B	Eukaryotic initiation factor 2 B
eIF2α	α subunit of eukaryotic initiation factor 2
eIF4E	Eukaryotic initiation factor 4 E
eIF4E BP	Eukaryotic initiation factor 4 E binding protein
eIF5	Eukaryotic initiation factor 5
ELP	Elongator protein
GCN1	General control nonderepressible 1
GCN2	General control nonderepressible 2
GCN20	General control nonderepressible 20
GCN4	General control nonderepressible 4
GlyRS	Glycyl-tRNA synthetase
HisRS	Histidyl-tRNA synthetase
HOG1	High osmolarity glycerol response 1
HOT1	High-osmolarity-induced transcription 1
HRI	Heme-regulated eIF2α kinase
MetRS	Methionyl-tRNA synthetase
PAB1	Poly(A) binding protein 1
PERK	Protein kinase RNA-like endoplasmic reticulum kinase
PKR	Protein kinase R
PUF3	Pumilio-homology domain family 3 protein
RBP	Ribosome binding protein
RCK2	Radiation sensitivity complementing kinase 2
RNY1	Ribonuclease from yeast 1
RP	Ribosomal protein
RPL22	Ribosomal protein L22
SKO1	Suppressor of kinase overexpression 1

SLF1	Sulfide production 1
SRO9	Suppressor of RHO 3 protein 9
SRX1	Sulfiredoxin 1
TRM	tRNA methyl transferase
URM1	Ubiquitin related modifier 1
XRN1	5’-3’ exoribonuclease 1

Others

$\bullet O_2^-$	Superoxide anion
•OH	Hydroxyl radical
8-oxoG	8-oxo-7,8-dihydroguanine
Cm	2’-O-methylcytidine
GAAC	General amino acid control
GDP	Guanosine-5’-diphosphate
GSH	Glutathione
GTP	Guanosine-5’-triphosphate
H_2O_2	Hydrogen peroxide
$m^2{}_2G$	$N^{2,2}$-dimethylguanosine
m^5C	5-methylcytidine
mcm^5U	5-methoxycarbonylmethyluridine
$NaAsO_2$	Sodium arsenite
NaOCl	Sodium hypochlorite
ncRNA	Non-coding RNA
NGD	No-go decay
NMD	Non-sense mediated decay
ROS	Reactive oxygen species
s^2U	2-thiouridine
TE	Translation efficiency
TOR	Target of rapamycin
uORF	Upstream open reading frame
UTR	Untranslated region

Abstrakt

Mnoho organizmů z různých větví života se často potýká s buněčným stresem zapříčiněným podmíkami vnějšího prostředí. Pro boj s těmito nepříznivými podmínkami se vyvinulo mnoho komplexních mechanismů. Kvasinka *Saccharomyces cerevisiae* je obzvláště dobře vybavená pro eliminaci negativních účinků oxidativního nebo osmotického stresu. Tyto podmínky zpravidla vedou k zastavení produkce proteinů. Kináza GCN2 se považuje za hlavního zprostředkovatele této odpovědi. Zastavení produkce proteinů je také doprovázeno vyšší expresí proteinů, podílejících se na boji se stresem. Tvorbě těchto proteinů předchází sada specializovaných regulačních procesů, fungujících na různých stupních proteinové exprese. Tato práce se pokusí přednést rozmanitost a komplexitu jednotlivých úrovní této regulace.

Klíčová slova: stresová odpověď, inhibice proteinové produkce, Saccharomyces cerevisiae, proteiny stresové odpovědi

Abstract

Environmental stress is a daily bread for organisms across many different branches of life. Very complex response mechanisms have evolved to tackle such insults. Yeast *Saccharomyces cerevisiae* is adapted especially well for counteracting oxidative and osmotic stress. These unfavorable conditions usually lead to inhibition of protein synthesis. The GCN2 kinase is thought to be responsible for this phenomenon. General inhibition of protein synthesis is accompanied by an increase in expression of proteins engaging in stress response. Production of these proteins is often preceded by specialized regulatory processes, that operate on various stages of expression. This thesis will try to present the diversity and complexity of the individual regulatory layers.

Keywords: stress response, inhibition of protein synthesis, Saccharomyces cerevisiae, stress-response proteins

1. Introduction

Yeast *Saccharomyces cerevisiae* is a traditional unicellular eukaryotic model organism, which is used among other things, for studying various signal pathways or conditional responses. This yeast is naturally found on the decomposing fruit, where it usually ferments simple sugars, like glucose or fructose. Such environment is characteristic for changes in osmolarity, because when the fruit dries out, all osmotically active constituents get gradually more concentrated. Evolutionary pressure of the environment thus caused the development of robust mechanisms, which help to fight these changes in osmolarity and allow yeast cells to survive and propagate. Similar mechanisms also evolved as a response to the oxidative nature of our atmosphere. The presence of oxygen, or rather compounds that oxygen can generate, can be very harmful to the cell and, therefore, represents another aspect for a necessary cellular response. Protein synthesis belongs between a broad set of cellular processes, regulated by environmental stress and ultimately defines the regulation outcome.

Protein synthesis is very demanding both in terms of energy consumption and nutrients. It is thus economically advantageous for the cell to regulate translation in specific conditions and only produce proteins, which are currently needed. Mechanisms such as gene expression profiling, modification of transcript stability or translation machinery all conjoin and form the decisive answer of the cell to its environment. In the case of cellular stress, proteins are prone to damage. This fact is one of the reasons why many organisms partially inhibit their protein synthesis and activate an adequate stress response, thus preventing production of defective proteins. By such proposition, translation arrest should forbid all transcripts from being translated. The reality is somewhat different, since transcripts that function in stress induced reactions are translated and, therefore, can consequently enable neutralization of the immediate and persistent effects of unfavorable conditions. Some of the mechanisms participating in the translation inhibition bypass are well described, but the influences of individual elements are still a matter of debate, as are the contributions of different ribosome and protein modifications, which occur as the result of stress induced cell response.

Goal:

1. Compile the newest findings about changes in protein synthesis, in regards to osmotic and oxidative stress response, in the model organism *Saccharomyces cerevisiae*. Give the necessary background information about established theories and known facts.

2. Stress and stress-inducing agents

Oxidative stress can be caused either by extracellular conditions, such as high concentrations of heavy metal salts or can emerge as a byproduct of oxidative phosphorylation, although in minor extent. Yeast *Saccharomyces cerevisiae* is facultative anaerobe and, therefore, has to deal with both situations. Respiration cycle in aerobic organisms offers an opportunity for the emergence of ROS (Reactive Oxygen Species). Molecular oxygen can divert electrons from flavoenzymes that serve as redox mediators, giving rise to the superoxide anion ($\bullet O_2^-$), hydrogen peroxide (H_2O_2) or hydroxyl radical ($\bullet OH$) (Massey et al. 1969). However endogenous levels of ROS are not deleterious for yeast cells, as cellular antioxidant mechanisms readily eliminate them. These consist of enzymes such as superoxide dismutase, catalase or glutathione peroxidase, and small molecules – glutathione (GSH), ascorbate, tocopherols, and carotenoids belong among the most known antioxidants. Antioxidant mechanisms can be overwhelmed in some cases, and inability to cope with such situation can give rise to oxidative stress and subsequent damage. All of the main cellular building blocks suffer from oxidative damage since the cause of reactivity is the unpaired electron of ROS, and the damaging process is as a consequence quite unselective. Oxidative stress and according cellular response can be induced by administration of various organic and inorganic compounds. Application of H_2O_2 or number of other hydroperoxides (cumene hydroperoxide, *tert*-butyl hydroperoxide - individual agents differ in water solubility) belongs among the most frequently used methods. There is also a range of heavy metal salts that can be used. Cadmium, chromium, arsenic, iron and copper salts are typical examples of such reagents and function either by redox cycling reactions or interaction with specific proteins or antioxidants (Wysocki & Tamás 2010). Diamide is a thiol oxidizing agent and causes oxidative stress indirectly, by overloading intracellular GSH pools (Kosower & Kosower 1995). Substances like menadione, paraquat, and antimycin A interact with electron transport chain and cause electron leakage followed by increased level of superoxide generation (Lushchak 2010; Dröse & Brandt 2008).

In the case of osmotic stress, there might be two possible environmental conditions inducing two different responses – hypoosmotic or hyperosmotic, the latter will be the subject of this thesis. Hyperosmotic environment causes water efflux from the yeast cell, which goes hand in hand with a proportional increase of intracellular ions and consequent growth retardation. Salts, such as sodium and potassium chloride are ionic compounds, widely used for stress induction. Since the osmoles to moles ratio is usually high, small quantities of given agent can result in great changes in the osmolarity of the environment. Dissociation of these substances results in a higher concentration of cations that are able to cause cationic stress (Goossens et al. 2001), thereby causing both osmotic and cationic stress response. This situation can be avoided by the usage of glucose, sorbitol or xylitol, which induce an osmotic stress response, but do not cause cationic stress. Sorbitol and xylitol are further advantageous because, unlike glucose, they cannot be metabolized by the cell.

3. Translation inhibition is a general response to stress

3.1 GCN2 pathway activation

Inhibition of general translation and up-regulation of stress-responsive proteins, as a response to unfavorable conditions, is highly conserved throughout evolution. Repression of the first step of translation – initiation, is the most extensively studied regulatory mechanism, where most of the inhibitory effect in eukaryotes, is carried out by General Control Nonderepressible 2 kinase (GCN2), which phosphorylates Eukaryotic Initiation Factor 2 (eIF2) (Hinnebusch 1993). The GCN2 kinase is conserved in eukaryotes, and it is the sole eIF2 kinase in *Saccharomyces cerevisiae*. Mammals have on top of it three more eIF2 kinases: HRI, PKR, and PERK/PEK. GCN2 is a part of well-studied General Amino Acid Control pathway (GAAC), the core regulatory mechanism activated by amino acid unavailability (Mueller & Hinnebusch 1986). Target Of Rapamycin (TOR) is another pathway closely connected to GCN2. It can be inhibited by treatment with rapamycin, which results in translation repression (Cherkasova & Hinnebusch 2003). It had not been elucidated if this effect is a result of GCN2 kinase activation or inhibition of eIF2 dephosphorylation.

As is mentioned above, the GCN2 kinase is activated by amino acid unavailability (Figure 1). Deprivation of amino acids and continuous translation cause depletion of aminoacylated tRNA pools, which is critical for GCN2 activation. Uncharged tRNA can then interact with the Histidyl tRNA Synthetase (HisRS) like regulatory domain of GCN2, which is autoinhibited under normal conditions (Padyana et al. 2005). Binding of the uncharged tRNA induce conformational changes of this enzyme and triggers the protein kinase activity (Dong et al. 2000). Findings of Hinnebusch et al. indicate that there are at least two other proteins, GCN1, and GCN20, that stimulate loading of tRNA onto HisRS region of GCN2 . These two proteins interact with one another in vivo, and their complex adheres on the GCN2 bound to the ribosome (Garcia-Barrio et al. 2000).

Activation of the protein kinase domain leads to phosphorylation of the Eukaryotic Initiation Factor 2 Alpha (eIF2α), on a serine-51 residue (Dever et al. 1992). eIF2α is a regulatory part of eIF2 heterotrimer (α, β and γ subunits). This initiation factor, together with methionyl-tRNA and GTP forms a ternary complex, which has a crucial function in protein synthesis initiation. Ternary complex mediates the interaction of methionyl-tRNA with the small ribosomal subunit and start codon in the associated molecule of mRNA. Methionine is incorporated after successful recognition of the start codon. Incorporation of methionine enables hydrolysis of GTP in eIF2•GTP complex, which is followed by the release of eIF2•GDP.

Guanine nucleotide exchange factor eIF2B can then convert eIF2•GDP back into eIF2•GTP. Activated form of eIF2 can participate in the cycle again (Webb & Proud 1997). Phosphorylated eIF2 is an inhibitor of guanine nucleotide exchange process and thus, an inhibitor of translation initiation. This inhibition works on the basis of competitive interaction between these two substrates, however,

phosphorylated equivalent has a higher affinity for eIF2B factor. Phosphorylation of only a fraction of eIF2 can, therefore, have a strong impact on translation inhibition (Pavitt et al. 1998).

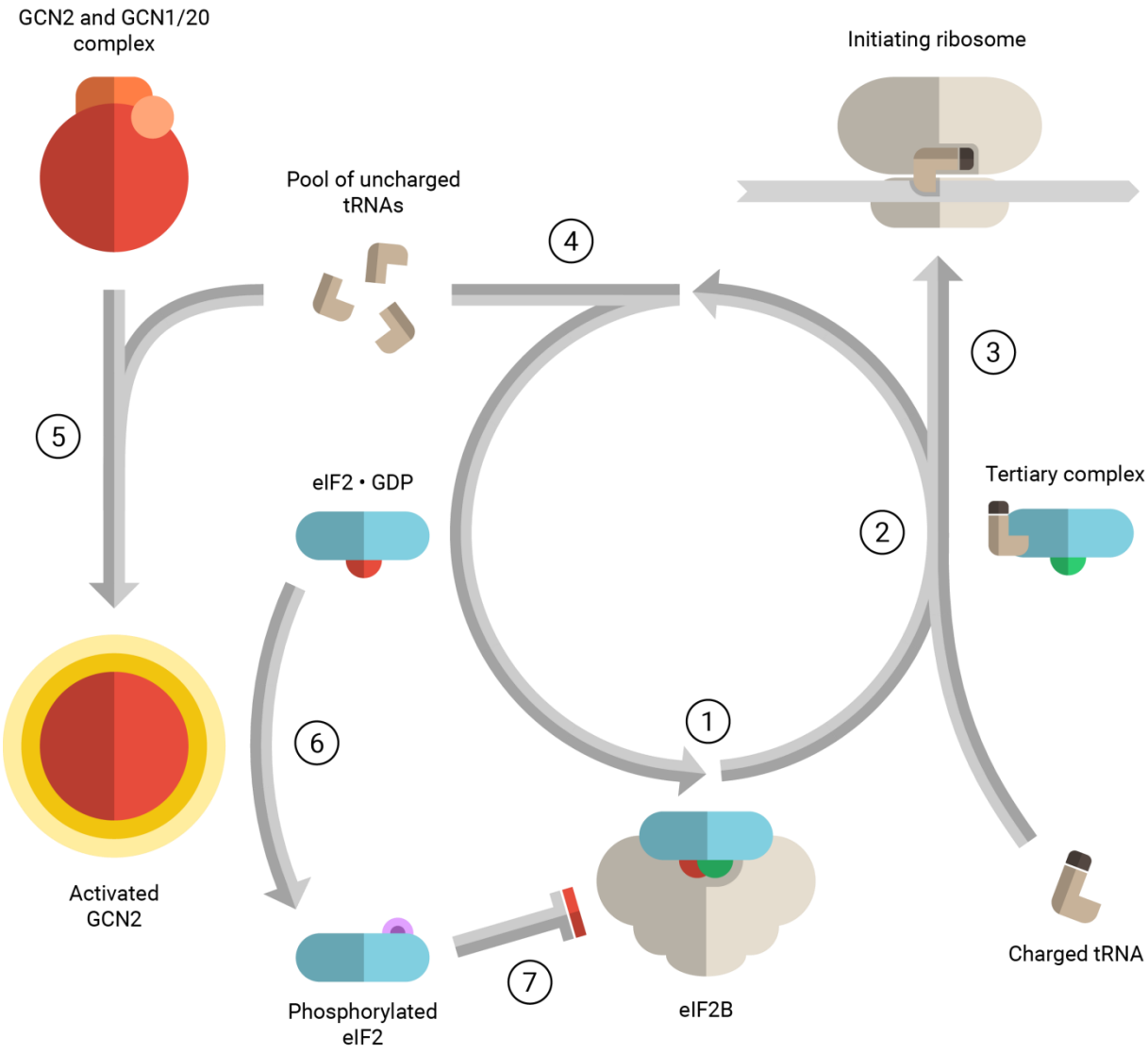

Fig. 1. Schematics of GCN2 pathway activation.

1. Under normal circumstances, eIF2B is able to exchange GDP for GTP and activate eIF2. **2.** Activated eIF2 can create a ternary complex with charged tRNA. **3.** The ternary complex can participate in translation initiation and mRNA scanning. Successful recognition of start codon leads to hydrolysis of GTP and uncoupling of eIF2•GDP complex. **4.** Stress can increase the pool of uncharged tRNAs. **5.** High levels of uncharged tRNAs can activate GCN2. The complex of GCN1/20 proteins helps with loading of uncharged tRNAs to GCN2. **6.** Activated GCN2 can phosphorylate eIF2. **7.** Phosphorylated eIF2 function as a negative regulator of eIF2B and thus indirectly inhibits translation initiation.

An important part of the GAAC pathway is effector gene called *GCN4*. It encodes a protein, which has many roles in stress responses but is acknowledged mainly for its capability to work as a transcription factor (Natarajan et al. 2001). Translation of the GCN4 transcript is regulated by four Upstream Open Reading Frames (uORFs), which keep GCN4 transcript from being translated under normal conditions. From four uORFs present on the GCN4 mRNA, only the first has a positive regulatory function, therefore if amino acids are abundant, ribosomes dissociate from the transcript after reinitiating on one of the three remaining uORFs. In the end, GCN4 transcript is rarely translated. The situation changes under high concentration of uncharged tRNAs. Decrease in the number of ternary complexes leads to limited rate of translation initiation; consequently, ribosomes can scan through three repressor uORFs and reinitiate translation at the following *GCN4* start codon with much higher probability (Mueller & Hinnebusch 1986). Many of the genes targeted by GCN4 are associated either with the biosynthesis of amino acids, transmembrane amino acid transport or autophagy proteins. This connection affirms the irreplaceable role of GCN4 in starvation response. GCN4 is also a vital component in response to oxidative and osmotic stress (Mascarenhas et al. 2008; Pascual-Ahuir et al. 2001).

3.2 How different stresses activate GCN2 pathway/inhibit translation

3.2.1 Oxidative stress

Treatment with hydrogen peroxide inhibits protein synthesis in a dose-dependent manner(Shenton et al. 2006), but the exact mechanism of activation is not known. However, there are some proposed ways, which could explain yeast stress response to some degree. When analyzing stress responses, we must bear in mind, that there is no universal oxidative stress agent and although the general response to oxidative stress will be similar, it will differ slightly in details for each oxidative agent(Temple et al. 2005). This is clearly visible in the example of two eIF4E Binding Proteins (eIF4E BP), Cap Associated Factor 20 (CAF20) and eIF4E Associated Protein 1 (EAP1). Eukaryotic initiation factor 4E (eIF4E) is an important component of cap-dependent translation initiation and its blockage can result in protein synthesis inhibition, these proteins, therefore, act as important regulatory factors (Mascarenhas et al. 2008). Cells treated with diamide or cadmium needed EAP1, but not CAF20, for an appropriate answer to oxidative stress. Conversely, EAP1 was not needed in the response to H_2O_2 treatment (Mascarenhas et al. 2008).

Experiments done by Mascarenhas et al. demonstrated, that exposure of prototrophic yeast cells to hydrogen peroxide induces GCN2 pathway, hence ruling out the possibility of hydrogen peroxide negative effect on amino acid uptake (Mascarenhas et al. 2008). Inhibition of translation, therefore, does not stem from impaired amino acid transport but is probably a manifestation of a natural cellular response to oxidative stress. The translation inhibition could be, to some extent, a consequence of damaged translation machinery and other vital components connected to it. Free amino acids are needed for the synthesis of novel proteins, and their deterioration could lead to an increased concentration of uncharged tRNAs (Grant 2011). Stadtman & Levine described the sensitivity of amino acids to oxidative stress (Stadtman & Levine 2003). Treatment of yeast cells with a mild concentration of hydrogen peroxide resulted in increased levels of corrupted amino acids. Although the frequency of affected phenylalanine residues was around one in a thousand, the cumulative effect of damage to all amino acids could potentially activate the GCN2 pathway and cause translation halt (Stadtman & Levine 2003). High susceptibility to oxidative damage was shown even in the instance of tRNA synthetases and tRNAs, which are essential for proper protein synthesis as well (Takahashi & Goto 1990; Thompson et al. 2008).

Pre-initiation and initiation of translation are not the only stages affected by oxidative stress. Experiments with Δ*GCN2* yeast strains implicate that translation elongation could be just another process sensitive to the presence of oxidative stress. Shenton et al. showed, that translation rate in Δ*GCN2* strains stays unaffected under amino acid starvation but is inhibited by oxidative stress (Shenton et al. 2006). Slower rates of protein synthesis could be either consequence of global oxidative damage or fairly selective impairment of elongation step. Ribosome transit time increased by 50% in response to stress caused by hydrogen peroxide, supporting the latter claim(Shenton et al.

2006). Some of the elongation factors are among targets of S-thiolation, which protects proteins from oxidation. This association could imply an innate sensitivity of these factors to oxidative stress and another way of stress response regulation(Shenton & Grant 2003).

3.2.2 Osmotic stress

Osmotic stress, just like oxidative stress, causes a rapid drop in global protein synthesis(Uesono & Toh-e 2002). Unfortunately, as in the case of oxidative stress, exact mechanisms behind translation inhibition induced by hyperosmotic environment are not known.

Uesono et al. investigated the extent, to which osmotic stress affects two most probable control stages in protein synthesis, initiation, and elongation. They showed that changes in translation rates can be explained mostly by impairment of translation initiation (Uesono & Toh-e 2002). Inhibition of initiation was common response both for sorbitol and salt treatment, demonstrating that initiation is not impaired due to sodium toxicity, but instead due to high osmolarity. Effects on elongation were observed too. Elongation Factor 2 (EF-2) is an important part of translation machinery that mediates the GTP-dependent ribosome translocation during elongation stage of the protein synthesis. Phosphorylation of EF-2 leads to inhibition of the protein synthesis(Ryazanov et al. 1988). Phosphorylation of the EF-2 in yeast is carried out by RCK2(Teige et al. 2001). For activation of the kinase activity, RCK2 must be phosphorylated by High Osmolarity Glycerol response 1 (HOG1), this happens shortly after an increase in osmolarity of the environment. HOG1 is analogous to GCN4 because of its capability to activate a range of stress response components, but it does so in the instance of osmotic stress.

Unlike oxidative stress, osmotic stress inhibits amino acid uptake. Inhibition of amino acid uptake could give rise to decreased levels of amino acids and, therefore, could activate the amino acid starvation response (Norbeck & Blomberg 1998). However, experiments done on yeast strain with mutated serine-51 residue in eIF2α showed, that inhibition of translation initiation in response to osmotic stress was equivalent in both mutant and wild-type strains, indicating minor role of the GCN2 pathway in osmotic dependent translation inhibition. Nonetheless, comparison of prototrophic and auxotrophic strains showed a slight difference in translation inhibition in the hyperosmotic environment, indicating the minor influence of amino acid depletion (caused by the repression of amino acid uptake) on translation initiation. This behavior could be also explained by an impaired uptake of uracil and glucose, which was also proven to inhibit protein synthesis and is present in hyperosmotic conditions (Uesono & Toh-e 2002).

4. mRNA and its dynamics

4.1 Stress-induced decay/stability enhancement of mRNA

The half-life of mRNA also denoted as mRNA stability, is one of many attributes describing dynamics of mRNA behavior in the cell. This attribute represents a time, in which half of the total pool of specified mRNA in a cell gets degraded. The increase of mRNA stability usually results in a higher chance of mRNA translation and vice versa. Alteration of mRNA stability was described as a part of an answer to osmotic and oxidative stress (Romero-santacreu et al. 2009; Molina-Navarro et al. 2008).

The half-life of mRNA molecule is usually associated with the length of its poly(A) tail. Poly(A) tail is a structure on the 3‘ end that protects mRNA against degradation. It is therefore, in this sense, functionally related to 7-methylguanosine cap at the 5‘ end of the mRNA. Poly(A) tail and 7-methylguanosine cap are also crucial for efficient initiation of protein synthesis. Poly(A) tail is a substrate for various exonuclease complexes in the cytosol, and its removal is usually necessary if mRNA is to be degraded. Subsequent mRNA decay can be carried out either in 5‘ → 3’ or 3‘ → 5‘ direction, where the former is the most abundant. Degradation in 5‘ → 3‘ direction requires decapping of the mRNA on the 5‘ end of the molecule, which enables mounting of the 5’-3’ Exoribonuclease 1 (XRN1) exonuclease that cleaves up the mRNA molecule completely. Alternative 3‘ → 5‘ degradation can be executed by exosome complex. But not all mRNA decay is dependent on deadenylation of the mRNA molecule. An example of additional mechanisms affecting a range of mRNAs is Non-Sense Mediated Decay (NMD). NMD can prevent translation of unspliced pre-mRNAs. With the help of RNA surveillance proteins UPF1, UPF2, and UPF3, NMD can recognize Premature Terminating Codon (PTC) in mRNA sequence. NMD then directs the Upf-bound mRNA to already mentioned XRN1 dependent degradation pathway (Baker & Parker 2004).

Newly described co-translational degradation seems to be more frequent than previously thought. It is dependent on XRN1 exonuclease, which follows the last active ribosome and gradually degrades the transcript. It was shown that co-translational degradation could have implications in stress-responsive alteration of mRNA stability (Pelechano et al. 2015). Pelechano et al. investigated the phenomenon in yeast treated with low levels of H_2O_2 and found out, that there indeed exists a difference in susceptibility to this kind of decay. For example genes connected with ribosomal biogenesis and translation increased their ribosome protection, whereas genes important for budding and cell cycle showed the opposite. However, authors emphasize that increased ribosome protection does not directly imply higher protein production.

There is a competition between cap binding proteins and decapping complex. The decapping complex is able to proceed only if the 5‘ mRNA cap is free of any interfering proteins. Stability of mRNA is therefore negatively altered if translation initiation alone is inhibited (Schwartz & Parker 1999). This is because unbound mRNAs are more susceptible to degradation. However, if the yeast

cell is subjected to stress, the result is quite the opposite. Stress inhibits both translation and deadenylation and accordingly increases the stability of most mRNAs. Deadenylation is the first, and rate-limiting step in mRNA degradation and majority of consequent mRNA decay pathways depends on it (Decker & Parker 1993). Stress-response transcripts are among stabilized mRNA as well (Romero-santacreu et al. 2009; Molina-Navarro et al. 2008).However, stabilization of stress-response transcripts is not mediated through stress-response pathways, such as HOG1 pathway in the instance of hyperosmotic stress but is a result of the already mentioned global increase in mRNA half-life. Stabilization of mRNA during stress is similar to translation stalling under the same conditions, as both of these safe-switches give cell time to determine whether it should change gene expression to overcome the stress or wait, in the instance of transient stress situations.

Certain mRNAs are not stabilized under stress. Quite conversely, stress causes various changes in their behavior that lead to lowered translation rate of these mRNAs. Ribosomal Proteins (RP) transcripts are often mentioned, with relation to mRNA destabilization and stress response. There was a reported decrease in RP pre-mRNAs in hyperosmotic conditions, interpreted as a result of lowered transcription rate of RP genes (Bergkessel et al. 2011). However Garre et al. showed that it is not the lowered transcription rate that is responsible, but high levels of RP pre-mRNA degradation (Garre et al. 2013). They subjected Δ*UPF* and Δ*XRN1 S. cerevisiae* strains to hyperosmotic stress and observed no decrease in RP pre-mRNA levels, indicating a possible role of pre-mRNA degradation in the response to hyperosmotic conditions. Their results also suggest that primarily NMD is accountable for the change in pre-mRNA levels. This could be due to reduced activity of nuclear exosome or spliceosome complexes under osmotic stress. Both of these possibilities result in higher level of unspliced or otherwise impaired pre-mRNA. Reduced stability was also observed in the instance of mature RP mRNA, but the effect of unstable RP pre-mRNA greatly surpasses one, that is caused by the mature RP mRNA destabilization. Low RP pre-mRNA levels would be beneficial in stress conditions because RP mRNA accounts for the majority of translated mRNAs. Free ribosomes could be utilized in the translation of crucial stress response mRNAs.

4.2 Influence of UTRs on mRNA translation

It has been established that levels of given mRNA in the cell do not have to correlate with a production rate of protein they are encoding. As a result, some mRNAs can be translated at very low levels, and some need significantly higher levels just for detectable protein synthesis. We can describe this mRNA property with Translation Efficiency (TE) index, TE value is condition dependent and can, therefore, differ substantially. For example SRX1, which encodes sulfiredoxin, the very important protein in cellular response to oxidative stress is seldom translated under normal circumstances. TE of SRX1 transcript goes up in the orders of magnitude, immediately after treatment with peroxide, despite the mRNA levels being the same as in untreated cells. We can see the opposite tendency in the

instance of Poly(A) Binding protein 1 (PAB1). TE of PAB1 mRNA rapidly decreases after exposure to peroxide and consequently, production of PAB1 protein decreases as well(Gerashchenko et al. 2012).

It is not fully understood, what influences TE of individual transcripts. However, we can reason, that transcripts regulated on the translational level must have a distinct sequence in it. This theoretical sequence could have various properties by itself or could attract proteins that might further influence dynamics of associated mRNA molecule. Predominantly studied segments of mRNA, regarding translation regulation, are 5‘ Untranslated Region (UTR) and 3’ UTR.

A typical example of uORF controlled stress inducible transcript is GCN4, which was already described in section 3.1 GCN2 pathway activation. Lawless et al. showed, that uORFs are statistically under-represented in the sequences of 5‘ UTR and proposed, that tolerated uORFs should thus have some specific function in regulation. One of the suggested uORF function that could play a role in stress response is temporal stalling of ribosomes on the transcript. uORFs would, therefore, serve as stress inducible “waiting rooms” for ribosomes. Furthermore, uORFs have weak stop codons, and they do not completely impair the translation of subsequent ORF. There is also an interesting connection between relative up-regulation of translation and length of 5‘ UTR in mRNA sequences. Experiments show that mRNAs that are up-regulated under stress conditions have significantly longer 5‘ UTR sequences (Lawless et al. 2009). Gerashchenko et al. observed a dramatic increase in the frequency of ribosomes bound to uORFs under oxidative stress conditions. They explain this phenomenon by an increase in elongation time, which was observed in yeast after peroxide treatment (Shenton et al. 2006). The same behavior could also be due to more frequent translation initiation at non-AUG codons. Translation initiation factors eIF1 and eIF5 mediate the recognition of start codon and are proven to be sensitive to oxidative stress (Gerashchenko et al. 2012).

Another part of mRNA molecule with possible regulatory function is 3‘ UTR. Shalgi et al. analyzed different 3’ UTR oligo-sequences. Results of this study indicate, that presence of specific sequences in 3‘ UTR of the transcript and their position, may affect its stability and TE. Although results of the study are rather abstract, they could be easily stretched to encompass the possibility of selective stress response 3’ UTRs dependent regulation (Shalgi et al. 2005). Shalem et al. analyzed the influence of 3’ UTR sequences on the stability of the harboring transcript. They analyzed a synthetic library of mRNAs, varying only in their 3‘ UTR sequences (Shalem et al. 2013). Stability of individual transcripts differed in the order of magnitude. The absence of different promoters or ORF sequences in examined transcripts eliminated their possible influence on examined dynamics. Most important feature of 3‘ UTRs determined by their sequence, is, however, their ability to function as a binding site for various Ribosome Binding Proteins (RBPs).

4.3 mRNA binding proteins

Response to hyperosmotic stress involves translation of osmo-mRNAs during global translation halt. New evidence indicates that Cap Binding Complex 1 (CBC1) is involved in the process (Garre et al. 2012). CBC1 is an RNA-binding protein that is able to interact with 7-methylguanosine cap structure of newly transcribed mRNA and is therefore related to eIF4E to some extent. Translation initiation is generally eIF4E dependent under normal conditions, and CBC1 is consequently rarely used. Garre et al. proposed that eIF4E dependent translation initiation is nearly shut under hyperosmotic conditions and translation is initiated in a stress-resistant CBC1 dependent manner (Garre et al. 2012). Their claim is supported by many experiments, which, for example, show the low viability of Δ*CBC1* strain in hyperosmotic medium and also significantly delayed association of osmo-mRNAs with polysomes. The role of CBC1 dependent initiation of translation was further affirmed by cultivation of temperature sensitive *S. cerevisiae* strain $CDC33^{ts}$ in the hyperosmotic medium at 37 °C. CBC1 dependent initiation of translation was activated and overcame the effect of temperature inhibited growth of $CDC33^{ts}$ mutant. Other stress conditions might also require the presence of CBC1, but no examinations were done so far.

SLF1 is another RBP, which was proved important for response to oxidative, but not osmotic stress (Kershaw et al. 2015). Experiments show a significant overlap between SLF1 mRNA targets and mRNAs that are being readily translated in stressed cells - f.e. superoxide dismutase, thioredoxins, and glutaredoxins mRNAs, suggesting a possible role of SLF1 in stress induced selective mRNA translation. In accordance with this proposition, Δ*SLF1* strain was hypersensitive to peroxide treatment. A noticeable AU repeat tendency in 3‘ UTRs of SLF1 target mRNAs, highlights the complexity of stress induced translation control, considering these repeats tend to destabilize mRNA (Shalgi et al. 2005). Ability to bind both 40S ribosomes and specific mRNAs, indicate a potential role of SLF1 as an adaptor protein. Kershaw et al. also investigated SRO9 protein, which showed reduced capability of altering levels of its target mRNAs when compared to SLF1. The presence of PUF3 target sequence in SLF1, but not in SRO9 is rather interesting too.

Inhibition of mitochondrial protein production is another part of selective translation in oxidative stress. As mitochondria are the main source of intrinsic ROS in the cell, restricted production of new mitochondrial proteins should prevent further damage to the cell. These transcripts often have PUF sites in their 3‘ UTR, recognizable by PUF family RBPs. All PUF protein family members are known to destabilize or repress translation of target mRNAs. They are therefore the main candidates for mediation of the negative feedback. New data show that PUF3 protein negatively impacts the greatest number of examined mRNAs (Rowe et al. 2014). Observation of reduced down-shift of mRNAs from polysomal to monosomal fraction, after exposure of Δ*PUF3* strain to oxidative stress, supports the PUF3 position as a translation repressor. Quite surprising is the occurrence of increased robustness of Δ*PUF3* strain to peroxide, which authors explain as a consequence of

increased translation of various ROS detoxifying enzymes, bound by PUF3 under normal circumstances.

4.4 Non-coding mRNA modulate ribosomal functioning

More and more recent studies point out the significance of small non-coding RNA (ncRNA) molecules in translation modification. ncRNAs are exceptional regulators, as they function in the form of RNA and does not have to be translated to regulate cellular processes. Yeast, however, does not possess required machinery to utilize miRNA and siRNA and must manage without this type of translation regulation (Houseley & Tollervey 2008).

It was therefore surprising when Pircher et al. discovered 18-nucleotide long small ncRNA, which influences ribosomes directly and is crucial for osmotic response in *S. cerevisiae* (Pircher et al. 2014). This short ncRNA is derived from *TRM10* locus, which encodes tRNA methyltransferase and probably emerge as a consequence of TRM10 mRNA cleavage. Previous experiments with Δ*TRM10* strain showed deficient growth in hyperosmotic conditions that could be rescued by introducing plasmid containing *TRM10* locus. To elucidate if it was because of the renewed production of tRNA methyltransferase or reintroduction of the 18-mer ncRNA, synonymous mutations were introduced to the sequence of ncRNA. The newly created plasmid was not able to rescue the deficient growth, although active tRNA methyltransferase was present. Such evidence supports the role of *TRM10* derived 18-mer in osmotic stress translation regulation.

Most of the ncRNAs were associated with ribosomes in vivo and were present in both stressed and unstressed cells. The main difference was in the ribosome fraction localization. In stressed cells, ncRNA was bound to polysomes, whereas, in unstressed cells, the majority was bound to untranslating monosomes. This ncRNA is thought to support the osmotic stress response through inhibition of translation initiation. Accordingly, Δ*TRM10* strain has 2.1-fold higher polysome:monosome ratio and an increased level of protein synthesis in hyperosmotic conditions.

5. tRNA

5.1 Stress-induced cleavage and up/down-regulation of tRNA synthesis

The genome of S. cerevisiae encodes 295 different tRNAs that can be used differentially in accordance with the environment or general status of the cell. Various tRNA differ in specific ways, but the most evident distinction is their amino acid and codon specificity. Anticodon loop determines both the amino acid and codon specificity and also, is the area mostly connected with tRNA degradation. Anticodon loop is probably the target of endonucleases because it is the most exposed part of the tRNA structure. Stress-induced degradation of tRNA in the instance of oxidative stress was described by Thompson et al. (Thompson et al. 2008). Unfortunately, there were no observations made associating tRNA degradation and osmotic stress yet.

Nuclease that is responsible for the tRNA cleavage in anticodon loop was termed Ribonuclease from Yeast 1 (RNY1) and is localized mainly in vacuoles, under normal conditions (Thompson & Parker 2009b). Subjection of *S. cerevisiae* cells to oxidative agents cause the release of RNY1 into the cytosol. Considering the observed degradation of rRNA in the same conditions, it is possible that RNY1 negatively influences protein synthesis in general. The author further demonstrated that mRNA is also vulnerable to RNY1 activity. If RNY1 indeed affects mRNA, it could offer cell just another level of mRNA specific stress induced translation regulation, as some sequences would be innately more susceptible. Strain with a deletion in the gene for RNY1 ribonuclease was vulnerable to peroxide treatment, this implicates a possible role of RNY1 in oxidative stress translation regulation (Thompson et al. 2008).

Studies on mammals show that stress-induced angiogenin dependent tRNA degradation is capable of global translation inhibition (Czech et al. 2013). The situation in yeast is different because it was proved that stress-induced degradation of tRNA is not high enough, to influence the rate of protein synthesis by tRNA depletion (Thompson & Parker 2009a). However, experiments done by Zhang et al. show, that tRNA cleaved by RNase A can inhibit translation in vitro (Zhang et al. 2009). In order to work, cleaved tRNA has to keep its tertiary structure, as cleaved tRNA that was denatured consequently, lost its ability to inhibit translation. This effect was to be expected since correctly folded tRNAs have a naturally higher affinity for translation machinery and tRNA binding proteins. They have therefore higher chance to stall the steps of protein synthesis and potentially induce GCN2 pathway.

Study engaging the topic of tRNA regulation describes the stress-specific control of tRNA production (Pang et al. 2014). Increased levels of specific tRNAs, combined with matching modification, f.e. methylation, could be used for preferential synthesis of stress response proteins, which often need unusual tRNAs (Chan et al. 2010). Oxidation and alkylation showed similar up- and

down-regulation of 58 tRNAs and opposing in 18 tRNAs. This result could imply a general stress-induced regulation of tRNA production, with minor differences between individual stresses.

Novel 5' sequencing method enabled Pelechano and co-workers to characterize codon and terminator specific ribosomal stalling sites (Pelechano et al. 2015). Decreased rate of ribosomal run-off and inhibition of both translation elongation and termination was already described as a part of the response to peroxide but was not explained (Shenton et al. 2006). Since the ribosomal pausing showed variability between alternative codons for the same amino acids, authors propose that the regulation is probably performed on the basis of charged tRNA availability. Strain Δ*RNY1* was subsequently treated with H_2O_2, which led to no detectable stalling at sites identified earlier. The presence of RNY1 ribonuclease is, therefore, essential for this newly characterized ribosomal pausing.

5.2 Stress-induced tRNA modification and codon biased translation

Ribonucleotides in mRNA, rRNA and especially tRNA belong among the most frequently modified molecules in the cell. Yeast *Saccharomyces cerevisiae* also has really substantive arsenal of modifications, where nucleotides in tRNA alone can be modified on 36 positions in 25 different ways. Many of these modifications are highly conserved across species. Surprisingly, they seem to be non-essential, since the loss of genes encoding enzymes responsible for them usually does not lead to severe phenotypes. Mechanisms in which tRNA modifications affect translation are still not understood to a great extent, however increasing numbers of studies suggest a possible role of some modifications in proteome profile alterations. Modifications of tRNA ribonucleotides were also shown to influence ribosome binding affinity, frame-shifting and misreading, therefore influencing speed and fidelity of the translation both in stress and normal conditions (Motorin & Helm 2010).

Secondary structure of the tRNA resembles a cloverleaf, with three main loops: D-loop, anticodon loop, and TΨC loop. The anticodon loop and 3' terminal CCA sequence, which conjugates with specific amino acid, are essential for managing incorporation of correct amino acid into the newly synthesized protein. From all tRNA motifs, anticodon loop seems to be the one with the highest modification diversity. Anticodon segment spans from 34th to the 36th nucleotide of the tRNA sequence. Position number 34 is called wobble position and is known for frequent modifications, that promote or restrict wobbling (Phizicky & Hopper 2010).

The best-known tRNA modification connected to stress response is methylation. Pang et al. described occurrence of various modifications after exposure to different stresses, including oxidative stress (Pang et al. 2014). This study used hydrogen peroxide, sodium arsenite ($NaAsO_2$) and sodium hypochlorite (NaOCl) as oxidative agents. Induced profiles of tRNA modifications differed for each agent used. Treatment with H_2O_2 increased the frequency of modifications m^5C, Cm, and $m^2{}_2G$, whereas levels of these modifications decreased or were unaffected by treatment with other agents. Methylation of tRNA is catalyzed by Transfer RNA Methyltransferase (TRM) protein family. TRM1,

TRM4, and TRM7 account for $m^2{}_2G$, m^5C and Cm modifications respectively. Increased sensitivity to peroxide was observed in Δ*TRM4* and Δ*TRM7* strains. Strain ΔTRM1 did not show any signs of sensitivity, despite the elevation of $m^2{}_2G$ modification after treatment, indicating that $m^2{}_2G$ modification is not essential for peroxide stress response. Authors explain that increased levels of modified tRNA might be needed for effective response to oxidative environment, because a higher concentration of modified tRNA would be beneficial for TE of stress-response mRNAs, which often harbor rare codons. Differential response to various oxidative agents is clearly visible, as only TRM4 deletion strain for NaOCl and TRM1, TRM4, TRM7 and TRM9 deletion strains for $NaAsO_2$, showed sensitivity. Nucleotides at positions 34 and 37 are the most frequently modified and have the greatest influence on translational efficiency in stress conditions, given their location near anticodon segment. However, modifications on the body of tRNA also proved to be important, since examined Δ*TRM44* strain was sensitive to NaAsO2. TRM44 is thought to catalyze only the Um modification at position 44 of the yeast tRNA (Kotelawala et al. 2008).

Preferential translation of stress-response mRNAs with specific codons was finally demonstrated three years after the broad description of modifications, again by Chan et al. (Chan et al. 2013). m^5C modification was found in 34 species of tRNA so far. However, only one type of tRNA, $tRNA^{Leu(CAA)}$, has m^5C modification at position 34, directly in the anticodon segment (Dunin-Horkawicz et al. 2006). Exposure of *S. Cerevisiae* cell to H_2O_2 results in 70% increase in m^5C modified tRNA. The presence of m^5C modification on wobble location was shown to enhance translation of UUG enriched mRNAs by the introduction of 4xTTG linker upstream of Firefly luciferase construct. Therefore, expression of this luciferase was dependent on translation efficiency of the UUG sector. An experiment comparing Δ*TRM4* mutant and wild-type cells showed a 10-fold and 24-fold reduction of reporter signal under normal conditions and after treatment with H_2O_2 respectively. Control Δ*TRM4* strain with a construct, where the TTG linker region was exchanged for four random codons, did not show any of these effects. Lack of these effects suggests that stress responsive TRM4 dependent increase of m^5C in $tRNA^{Leu(CAA)}$ notably helps in the translation of transcripts encoded by TTG rich genes. This raised a question if there are oxidative stress response genes enriched with TTG codon. Authors found a pair of ribosomal protein homologs RPL22A and RPL22B, where 100% of leucine is encoded by TTG codon in RPL22A whereas in the instance of RPL22B, it is only 34%. Treatment with hydrogen peroxide increased production of RPL22A, despite levels of both transcripts remained the same. This behavior could be explained as a consequence of combining preferential translation of UUG enriched transcripts in an oxidative environment with increased levels of methylated $tRNA^{Leu(CAA)}$. Increased levels of methylated $tRNA^{Leu(CAA)}$ could be caused either by elevated activity of TRM4 or selective degradation of the unmethylated counterpart. Moreover, peroxide sensitivity arises if RPL22A is lost, indicating its crucial role in stress response and also highlighting the importance of dynamic ribosome composition changes, in response to different conditions.

Another important modification up-regulated in response to oxidative and osmotic stress is the addition of 5-methoxycarbonylmethyl (mcm^5) and 2-thio (s^2) groups to uridine at position 34 of yeast tRNAs, mainly $tRNA^{Gln(UUG)}$, $tRNA^{Glu(UUC)}$ and $tRNA^{Lys(UUU)}$. This modification is catalyzed by Ubiquitin Related Modifier 1 (URM1) and Elongator Protein (ELP) pathways and stabilizes binding of modified tRNA in position A inside a ribosome, consequently enhancing efficiency and fidelity of protein synthesis. It is also vital for expression of genes rich in CAA, GAA and AAA codons. Strains lacking necessary machinery for mcm^5 and s^2 modifications, show severely impaired phenotypes and double mutants are not viable (Björk et al. 2007). Mechanism of the interplay between these deletions and emergence of phenotypes was not elucidated yet. Recent findings show that mcm^5s^2 modification could play a significant role in stress signaling since treatment with various chemical agents, including H_2O_2 and $NaAsO_2$, decreased the levels of mcm^5s^2 modified tRNA. Hypomodified tRNAs are aminoacylated inefficiently and thus, can result in induction of GCN2, general inhibition of protein synthesis and subsequent GCN4 activation, which leads to transcription up-regulation of stress-responsive genes (Zinshteyn & Gilbert 2013).

Gingold et al. analyzed the mRNA expression profiles from *S. cerevisiae* (Gingold et al. 2012). They calculated codon usage in transcripts from yeast exposed to osmotic, oxidative and alkylating agents, and confirmed, that usage of rare codons increases in individual stress conditions and continuously changes during the stress recovery phase (Gingold et al. 2012). This trend was observed in every investigated organism, implying it might be a conserved response. Codons with the highest rise in representation increased its abundance up to 140% when compared with the reference condition. Usage of individual amino acids in protein synthesis remained roughly the same, and so did the frequency of used nucleotides. These notable changes, therefore, cannot be explained by neither the need to change the composition of proteome nor changes in nucleotide availability. Surprising was the similarity in codon usage when comparing different stresses. Authors explained that it could be simply caused by expression of general response proteins, which are similarly expressed in many stress conditions. To test the hypothesis, they picked stress specific response genes and compared them. Remarkably, even in this analysis, codon usage was very similar. The only apparent difference was the negative correlation between frequency of used codons and availability of their cognate tRNAs. Genes encoding general stress response usually used regular codons.

6. Ribosomal adaptation to stress and stress-inducible proteomes

6.1 Newly discovered ribosomal functions

Ribosomes are the final executors of protein synthesis and are therefore obvious targets of translational regulation with regards to stress. The conception of the ribosome as a passive translator of genetic information is getting more and more obsolete, as new lines of evidence emphasize the dynamic nature of this cellular component.

There are many morphological formations present in ribosome structure. One of them is the ribosomal stalk. This complex is made up of three phosphoproteins; P0 is the scaffold protein and binds two heterodimers P1/P2. The stalk is not essential for the ribosomal function under normal conditions. Instead, it mainly regulates the activity of the ribosome (Jiménez-Díaz et al. 2013). Eukaryotes generally have a big pool of unbound P1 and P2 monomers in the cytosol, which are thought to be periodically switched for those, bound to the ribosome. The connection between P1/P2 proteins and GCN2 kinase, with regards to eIF2 phosphorylation, was examined (Jiménez-Díaz et al. 2013). In their experiment, Jiménez-Díaz and co-workers examined three strains, two of them were lacking individual genes for P1 (P67) and P2 (P45) phosphoproteins and the third strain was double deletant (P4567). Cultivation of these strains in the hyperosmotic environment should cause activation of the GCN2 kinase, eIF2 phosphorylation, and subsequent translation inhibition. Interestingly, this was the case only for one of the mutant strains – P67. There is a difference between P1 and P2 proteins, P1 protein gets quickly degraded in the absence of its moiety. Therefore only one strain, P67, could have a substantial cytoplasmic pool of free phosphoproteins. Expression of P2 protein in the two remaining strains (P45 and P4567) was enough to raise the basal phosphorylation of eIF2 to levels present in the wild type strain. In vitro experiments show, that P1 and P2 phosphoproteins are able to activate GCN2 kinase even more efficiently than uncharged tRNA – the natural activator of the GCN2 pathway. P1 and P2 phosphoproteins activate the GCN2 kinase probably in the same way as the uncharged tRNA does since strains with a mutation in HisRS region did not respond to any of these activators.

As was mentioned in the section 2. Stress and stress-inducing agents, ROS cause unspecific damage to all structures in the cell, including mRNA. Damaged mRNA lacks the ability to produce a functional protein. This can be a big handicap as cell has only a finite number of active ribosomes,. Influence of oxidized mRNA on translation and its elimination by active ribosomal response was described (Simms et al. 2014). Oxidized ribonucleotide 8-oxo-7,8-dihydroguanine (8-oxoG) was shown to stall translation in eukaryotic extracts. Peptide bond formation was measured to be more than three orders of magnitude slower than with undamaged transcripts. Almost no effect on the accuracy of anticodon incorporation was observed. Authors presume that translation inhibiting effects of oxidized mRNAs could be a result of faulty anticodon to codon interaction because the recognition of

stop codons by protein factors was only slightly affected. Oxidized ribonucleotide probably affects the decoding center of the ribosome, since treatment of yeast cells with paromomycin restores the ribosomal capability to elongate. Paromomycin binds the decoding center of the ribosome and induces a conformational change similar to one that is present when cognate tRNA is bound. Oxidized mRNAs are thought to be the target of No Go Decay (NGD). NGD is highly dependent on Duplication Of Multilocus protein (DOM34). Deletion in gene encoding DOM34 led to inoperative NGD pathway and accumulation of oxidized mRNA.

6.2 Stress inducible proteins and protein buffers

There are many characterized, and incomparably more uncharacterized stress response proteins encoded in the genetic information of yeast *Saccharomyces cerevisiae*. Proteins are the main instruments directly affecting cellular activities and, therefore, influence the future course of cellular development. Two proteins connected to osmotic and oxidative stress response have been investigated recently.

Oxidative stress was shown to induce glycyl-tRNA synthetase (GlyRS). It serves as a mediator for coupling of glycine with appropriate tRNA, making it essential for protein synthesis. There are two genes encoding GlyRS in *Saccharomyces cerevisiae* – *GRS1* and *GRS2*. Only one of them, *GRS1*, encodes GlyRS that is active under normal conditions, conversely, GlyRS encoded by *GRS2* seems to be silent. Oxidative stress, caused by H_2O_2 treatment, seems to induce expression of *GRS2*, whereas transcription of *GRS1* is slightly inhibited (Chen et al. 2012). Results suggest that GRS2 could function as a helper GlyRS in the instance of GRS1 incapacitation.

Osmotic stress induces expression of protein connected to protein synthesis (Gomar-Alba et al. 2012). Response to osmotic stress is mainly under the control of the HOG1 pathway, which has many downstream effectors. An example of such effectors could be High-Osmolarity-induced Transcription 1 (HOT1) and Suppressor of Kinase Overexpression 1 (SKO1) transcription factors, which also regulate expression of the YHR087W protein. They do so under the condition of osmotic stress. This study induced osmotic stress by addition of glucose to the growing medium to final concentration of 20%. Protein YHR087W is distributed throughout the whole cell and is at least partially present in ribosomal fractions. Analysis of the protein sequence indicated a possible role of YHR087W protein in transcription, but later experiments disproved this claim. In silico analysis showed that proteins fulfilling the conditions to bind with YHR087W protein are mainly connected either stress response or translation. Deletion of *YHR087W* gene results in lower growth rate under osmotic conditions and accordingly, increased levels of gene dosage or YHR087W transcript result in better osmotic stress resistance (Jiménez-Martí et al. 2011). Connection with translation machinery is apparent as the TAP analysis showed the interaction of YHR087W with several translation-associated proteins. It was also shown that Δ*YHR087W* strain is more sensitive to translation inbitors. The

absence of YHR087W protein results in decreased translation rate of certain osmo-responsive proteins, making YHR087W a candidate for selective translation regulator.

Proteins can be protected from oxidative damage by incorrect incorporation of methionine residue (Netzer et al. 2009). Misacylation of tRNA with methionine in *Saccharomyces cerevisiae* was described (Wiltrout et al. 2012). Misacylation of tRNA is not very common because most of the tRNA synthetases are very accurate. Methionyl tRNA synthetase (MetRS) is the only exception, with misacylation rate of about 1% in mammals and 0.5% in *Saccharomyces cerevisiae*. The error rate can increase up to 10% under innate immune activation or chemically triggered oxidative stress (Netzer et al. 2009). Methionine residues are usually positioned strategically, near the active sites of the protein. This position enables them to neutralize ROS before potential impairment of the protein function. Misacylation occurs mainly on the tRNAs mediating incorporation of polar or charged amino acids, which makes sense, as substitution of hydrophobic amino acid for methionine could result in misfolded protein. Polar or charged amino acids are usually found on the surface, or active sites of proteins and substitution for methionine is therefore less problematic.

7. Conclusion

The complicated nature of interactions between individual regulatory components of stress-response protein production is the main problem that needs to be resolved. Stability of the transcripts encoding stress-response proteins and their active degradation or promotion for translation seemingly have a big role in influencing the final rate of protein production. The rate of protein production can also be affected by the preferential translation of certain transcripts using codon biased translation, as in the example of RPL22A protein. The combination of codon biased translation with stress-inducible changes in tRNA production and utilization give the issue just another level of complexity. All of these changes can be stress-specific and can function on a level of one distinct protein. This degree of control enables the cell to benefit from an array of stress specific protein profiles that can bypass the barrier of translation inhibition. These proteins can be additionally modified to increase their resistance against stressful conditions. Systems with this level of complexity can be comprehended only by bottom-up strategy. Luckily there is a discipline employing this approach – synthetic biology.

8. References

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