Charles University

Faculty of Science

Study programme: Biology
Branch of study: Biology

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Systém protiváh: antagonistická regulácia rastu a proliferácie u *Schizosaccharomyces pombe* za priaznivých a stresových podmienok

Counterbalances: antagonistic regulation of fission yeast growth and proliferation under favourable conditions and stress

Bachelor's thesis

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Prague, 2019

Aknowledgement

I would like to express my gratitude to my supervisor RNDr. Martin Převorovský Ph.D. for his professional guidance, help and willingness during the preparation of the bachelor's thesis. I would also like to thank the research group from the GenoMik laboratory and namely Mgr. Jarmila Princová for her patience and help with understanding the topic. And last but not least, I would like to thank my friends, who helped me with the language corrections and supported me during the writing process.

Declaration

I declare that I carried out this bachelor thesis independently, and only with the cited sources, literature and other professional sources. I declare that this thesis has not been used to gain any other academic title.

In Prague, 10. 05. 2019

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Abstract

Microorganisms come across dramatically changing conditions in the environment. It is important for them to be agile for a quick and effective response. Signal transduction pathways are essential for this ability. They can sense a broad spectrum of extracellular and intracellular stimuli and regulate a great number of processes in the cell. For unicellular microorganisms, the most essential ability is to sense environmental conditions for proliferation or abnormal stress conditions. One of the most popular model microorganisms, the fission yeast *Schizosaccharomyces pombe*, is used for the signal transduction pathways research. Findings obtained by research on the fission yeast are applicable to other eukaryotic organisms, thanks to the high conservation of the signal transduction pathways between the fission yeast and other eukaryotic organisms. Proliferation-promoting signal transduction pathways promote cell proliferation, growth and mitotic cell cycle in fission yeast. The stress-response signal transduction pathways play an opposite role. They promote cellular defence against stress stimuli and promote the sexual differentiation process alongside meiotic cell cycle. At first sight, the whole machinery may look like a switch mechanism. There is, however, a more complex crosstalk mechanism involved in the regulation machinery between these two types of signal transduction pathways, which will be described in this bachelor´s thesis.

Key words: *Schizosaccharomyces pombe*, proliferation, stress, signalisation, kinase, antagonism, target of rapamycin, protein kinase A

Abstrakt

Mikroorganizmy sa stretávajú s dramatickými zmenami podmienok prostredia. Je pre nich preto dôležité svižne a efektívne na tieto zmeny reagovať. Nevyhnutnými pre túto schopnosť sú signalizačné dráhy. Tie dokážu vnímať široké spektrum vonkajších i vnútorných stimulov a regulovať veľké množstvo bunkových procesov. Pre jednobunkové organizmy je jednou z najdôležitejších schopností vnímanie podmienok prostredia vhodných pre rast, alebo neobvyklých stresových podmienok. Jeden z obľúbených modelových mikroorganizmov používaných na výskum signalizačných dráh je kvasinka *Schizosaccharomyces pombe*. Výsledky získané výskumom na tomto modelovom organizme sú aplikovateľné aj na ďalšie eukaryotické organizmy, vďaka faktu, že signalizačné dráhy sú medzi kvasinkami a ostatnými eukaryotickými organizmami vysoko konzervované. Signalizačné dráhy podporujúce proliferáciu podporujú v kvasinke bunkovú proliferáciu, rast a mitotický bunkový cyklus. Stresové signalizačné dráhy majú opačnú úlohu. Podporujú bunkovú obranu proti stresovým stimulom. Taktiež podporujú proces sexuálnej diferenciácie a meitocký bunkový cyklus. Tento princíp môže na prvý pohľad vyzerať ako mechanizmus prepínača. Ukazuje sa, že v regulácii týchto dvoch signalizačných dráh je prítomný zložitý mechanizmus interakcií, ktoré a vzájomného ovplyvňovania. Tento mechanizmus bude popísaný v tejto bakalárskej práci.

Kľúčové slová: *Schizosaccharomyces pombe*, proliferácia, stres, signalizácia, kináza, antagonizmus, target of rapamycin, proteín kináza A

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List of abbreviations

GPCR	G-protein-coupled receptor
HSE	heat-shock promoter element
HSF	heat-shock factor
HSP	heat-shock protein
MAPK	mitogen activated protein kinase
MAPKK	mitogen activated protein kinase kinase
MAPKKK	mitogen activated protein kinase kinase kinase
PKA	protein kinase A
ROS	reactive oxygen species
SAPK	stress-activated protein kinase
TOR	target of rapamycin
TORC1	TOR complex 1
TORC2	TOR complex 2
UAS1	upstream activation site 1
UAS2	upstream activation site 2

1. Introduction

Fission yeast (*Schizosaccharomyces pombe*) is one of the most popular model organisms used in the studies of eukaryotic cellular and molecular biology. It is an ascomycete unicellular yeast used for beer fermentation by the Swahili. Furthermore, it can be also used for wine deacidification (Gallander, 1977). *S. pombe* cells are cylindrical with hemispherical ends. Haploid cells are 8 – 15 μm long. Diploid cells can be up to 24 μm in length. After reaching the maximal length, fission yeast cells undergo a closed mitosis (Hoffman, Wood and Fantes, 2015). The life cycle of *S. pombe* consists of a vegetative and a sexual phase (Forsburg and Nurse, 1991). The cell cycle consists of a short G1 phase which takes place between mitosis and cell separation. Two unreplicated nuclei are present in the cell during the G1 phase until the septum is degraded and two daughter cells are separated. The S phase is coincident with the presence of the septum. The newly created daughter cells are already in the G2 phase of cell cycle, and contain fully replicated chromosomes. The G2 phase is relatively long and lasts until the next mitosis. In nutrient depleted media and the absence of a mating partner, *S. pombe* cells exit the cell cycle and enter the stationary phase or quiescence state. If a mating partner is present, haploid cells shift from vegetative growth to a sexual cycle (Hoffman, Wood and Fantes, 2015).

Cells undergo many changes in order to survive different external conditions. The ability to constantly respond to environmental changes is even more crucial for microorganisms. The modulation of gene expression through signal-transduction pathways plays a major role in these adaptations. In this bachelor's thesis, I focus on the antagonistically regulated pathways which affect transcription and balance growth alongside the stress response. Examples of signalling pathways that cells use to respond to external changes are discussed in this thesis. Overall, the proliferation pathways promote growth and negatively regulate stress-response genes. Conversely, the stress-response pathways promote stress resistance and repress growth-related genes. As an example of such antagonism, the balance between the target of rapamycin (TOR) and stress-activated protein kinase (SAPK) pathways is depicted in Figure 1. The TOR pathway could be dubbed as a "proliferation pathway" and the SAPK pathway as a "stress-response pathway". When dividing these two types of signalling pathways, it must be kept in mind that they play significant roles in both types of the processes mentioned above.

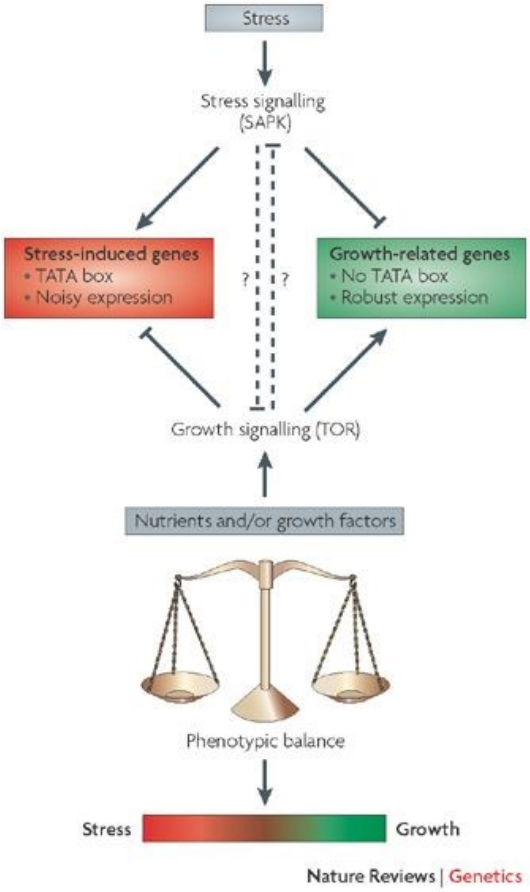

Figure 1 - Balancing the expression of growth- and stress-related genes. Adapted from (López-Maury, Marguerat and Bähler, 2008).

2. Stress conditions and responses to them by *Schizosaccharomyces pombe*

As mentioned above, cells must respond to different types of stress conditions during their life cycle. Below, I show some of the major stress conditions a single cell organism, such as *S. pombe*, can come across.

2.1. Heat stress

S. pombe, as many other organisms, respond to high temperatures by the induction of the synthesis of a set of proteins called heat-shock proteins (HSPs) (Lindquist, 1986). In non-stressed cells HSPs function as molecular chaperons. For example, they help maintain newly synthesized proteins in an unfolded state or they assist in the folding of unfolded proteins. The function of HSPs is to limit heat stress-induced damage by isolating partially heat-damaged proteins, preventing protein aggregation and helping either the reactivation or degradation of unfolded proteins. HSPs are highly conserved and can be grouped into six major families: Hsp100, Hsp90, Hsp70, Hsp60, Hsp40 and small HSPs (Toone and Jones, 2004). Hsp90 has been shown to positively regulate the activity of the cyclin dependent kinase Cdc2 controlling Wee1 and Mik1 kinases (Muñoz and Jimenez, 1999). These findings suggest that Hsp90 has a role in controlling the cell cycle.

In *S. pombe* stress induced transcription of heat-shock genes requires the activation of the heat-shock factor (HSF). In non-stressed cells HSFs are maintained as monomers in the cytoplasm and nucleus by the chaperons Hsp70, Hsp90 and Hdj1. In response to an increased level of unfolded proteins, HSFs are relieved from chaperone control allowing their trimerization and relocalisation to the nucleus. In the nucleus HSFs bind to the HSE heat-shock promoter elements and activate the transcription of genes related to heat shock (Toone and Jones, 2004).

2.2. Oxidative stress

Reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-) or hydroxyl radicals (-OH) are generated by multiple processes in the eukaryotic cell (for example by the incomplete reduction of oxygen to water in the mitochondrial respiratory chain or by exposure to UV). To deal with this constant low-level generation of ROS, a set of antioxidants and enzymes are produced to keep the cell in a state of redox balance. Oxidative stress occurs when this balance is disrupted by increases in ROS that are caused, for example, by exposure

to redox-active chemicals (e.g. H_2O_2) or to UV light. Increased ROS levels can induce DNA modification and strand breaks (Toone and Jones, 2004). They can also damage proteins and lipids (Sies, 1986).

In *S. pombe* the transcriptional response to oxidative stress is mediated by at least two transcription factors, called Pap1 and Atf1. While Pap1 is important for the response to low levels of H_2O_2, Atf1 is more important for the response to high levels of H_2O_2 (Quinn *et al.*, 2002). In response to low levels of H_2O_2, Pap1 quickly accumulates to nucleus. As the concentration of H_2O_2 in the cell rises, Pap1 is actively inhibited from entering the nucleus and the transcription factor Atf1 begins to play a more significant role. As H_2O_2 levels rise, Atf1 is increasingly phosphorylated by the Sty1 stress-response kinase as H_2O_2 levels rise (Quinn *et al.*, 2002).

2.3. Osmotic and salt stress

Exposure of cells to high osmolarity leads to the collapse of ion gradients over the plasma membrane and dehydration, resulting in decreased viability. Exposure to high concentrations of various salts leads to salt stress induced by the toxic effects of high ion concentrations.

In *S. pombe* the primary response to osmotic stress is the accumulation of the major compatible osmolyte – glycerol. Glycerol increases the internal osmolarity and allows the uptake of H_2O from the environment (Toone and Jones, 2004). The reduction of dihydroxy-acetonephosphate is required for glycerol production. In eukaryotic cells, this is performed by glycerol-3-phosphate dehydrogenase. In *S. pombe* genes *gpd1* and *gpd2* encode glycerol-3-phosphate dehydrogenase. The expression of *gpd1* is induced by osmotic stress and is important for osmoregulation. The expression of *gpd2* is constitutive (Ohmiya *et al.*, 1995). The transcriptional response to osmotic stress in *S. pombe* is regulated by the major stress-activated protein kinase Sty1 (Chen *et al.*, 2002).

The reduction of toxic ion levels, or salt stress is controlled by specific membrane and vacuolar ion pumps alongside regulators of the plasma membrane potential (Toone and Jones, 2004).

2.4. Endoplasmic reticulum stress

The endoplasmic reticulum provides an oxidizing environment for the folding of proteins. It is the site of lipid and sterol biosynthesis and it acts as a storage for intracellular calcium. Endoplasmic reticulum stress, or the so-called unfolded-protein response, can be generated by calcium depletion, inhibition of N-linked glycosylation or reduction of disulphide bonds. In *S. pombe*, unfolded-protein response controls factors affecting lipid metabolism, protein folding, vesicle trafficking, vacuolar protein sorting, glycosylation, cell wall biogenesis and protein degradation (Toone and Jones, 2004).

This type of stress is not the main focus of my thesis, so I will not elaborate upon it.

2.5. Nutritional stress

In this review I will also include condition when there are not enough nutrients present in the medium as a stress condition. In this case, wild type cells begin their sexual differentiation process and create dormant spores. *S. pombe* cells form *h*+ and *h*- mating types. The genome of the *h*+ mating type cells contains a P sequence, so they produce P-factor pheromones. On the contrary, the genome of *h*- mating type cells contains an M sequence for the production of M-factor pheromones. It is also known that cells of each mating type have receptors specific to the opposite mating type pheromones on their surface. Opposite mating type cells sense each other via the mating type pheromone pathway. After successful paring they conjugate and form a zygote. The zygote develops into an ascus that contains four dormant haploid ascospores (Toone and Jones, 2004).

3. Pathways involved in stress response

For this thesis I chose three major proliferation and stress response pathways. Their brief characteristics can be found below.

3.1. The protein kinase A signalling pathway

The protein kinase A (PKA) pathway is the major proliferation-promoting glucose-sensing pathway in the fission yeast. It is a G-protein mediated signalling pathway. The heterotrimeric G-proteins are composed of G_α, G_β and G_γ subunits that are bound to seven transmembrane helix receptors (G-protein-coupled receptors – GPCRs) in the plasma membranes of eukaryotic cells. Here they transduce extracellular signals into intracellular response (Gilman, 1987). The binding of a ligand to the receptor facilitates the change of GDP for GTP on the G_α subunit leading to dissociation of G_α from the G_β-G_γ dimer. The G_α subunit is then able to regulate downstream effectors, including adenylate cyclase, ion channels, mitogen-activated protein kinases and phospholipase C (Neves, 2002).

In fission yeast, the seven-transmembrane Git3 protein occupies the role of the glucose-sensing putative GPCR receptor (Welton and Hoffman, 2000). It directly activates the G_α subunit of the G-protein - the Gpa2 protein. After its activation, Gpa2 binds to the N-terminus of the adenylate cyclase (Cyr 1) (Welton and Hoffman, 2000). The binding of Gpa2 activates the adenylate cyclase in order to generate cAMP from ATP (Kawamukai *et al.*, 1991; Welton and Hoffman, 2000). The intracellular presence of the cAMP is required for the activation of PKA (DeVoti *et al.*, 1991). The PKA heterotetramer consists of two regulatory subunits (Cgs1) and two catalytic subunits (Pka1) (Yu, Li and Young, 1994). While PKA is inactive, the regulatory subunits remain bound to the catalytic subunits. The cAMP binding to the regulatory subunits promotes the dissociation of the catalytic subunits from the regulatory subunits and activates the protein kinase A (Maeda *et al.*, 1994). The catalytic subunits then accumulate in the nucleus. There, they affect numerous transcription factors responsible for different cellular processes (Matsuo, McInnis and Marcus, 2008). The PKA signalling pathway can be seen in Figure 2.

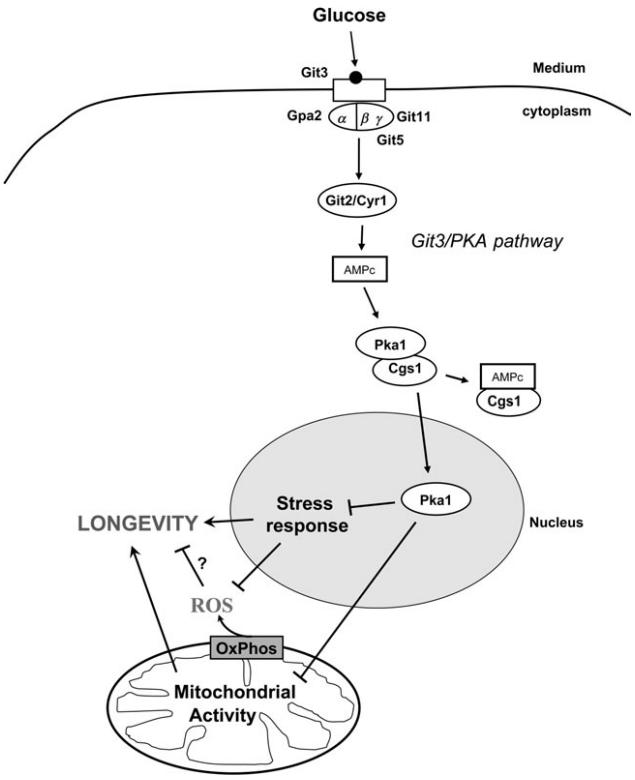

Figure 2 - The PKA signalling pathway model. Adapted from (Roux *et al.*, 2009)

3.2. The TOR signalling pathway

TOR (target of rapamycin) is a highly conserved serine/threonine kinase, which regulates cell growth and metabolism in response to environmental changes. Two TOR homologs were identified in *Saccharomyes cerevisiae* by studying a potential anticancer drug in mutants resistant to rapamycin (Heitman, Movva and Hall, 1991). Rapamycin is an antibiotic that inhibits growth of mammalian and budding yeast cells. However, in fission yeast, it does not inhibit vegetative growth (Weisman, Choder and Koltin, 1997).

In *S. pombe*, rapamycin inhibits sexual development under starvation conditions by impairing mating function (Weisman, Choder and Koltin, 1997). The TOR signalisation consists of two distinct complexes - TOR complex 1 (TORC1) and TOR complex 2 (TORC2). The protein kinase Tor1 binds to the Rictor ortholog component Ste20 of TORC2. The protein kinase Tor2 binds to the Raptor ortholog component Mip1 of TORC1 (Wullschleger, Loewith and Hall, 2006; Matsuo *et al.*, 2007). The simplified TOR signalisation model alongside its impact to the cell, which is discussed in later sections of this chapter, can be seen in Figure 3.

3.2.1. The Tor1 (TORC2) pathway

The fission yeast serine/threonine kinase Tor1 is not essential for cell viability. However, it affects the G1 arrest, fertility, the morphology of the cell and cell stress response. Therefore, *tor1*-deletion mutants are sterile and cannot properly arrest in the G1 phase under nutrient limiting conditions (Matsuo *et al.*, 2003). It has been shown that TORC2 also promotes transition from G2 to M phase in the life cycle of *S. pombe* (Ikeda *et al.*, 2008). The deletion of *tor1* results in elongated morphology and sensitivity to various types of stressors (Kawai *et al.*, 2001; Weisman and Choder, 2001).

TORC2 phosphorylates the Gad8 kinase via Tor1 and both Tor1 and Gad8 kinases are required for survival under stress conditions (Matsuo *et al.*, 2003; Hartmuth and Petersen, 2009). After its activation, the Gad8 kinase phosphorylates various proteins. Examples of downstream phosphorylation targets of Gad8 kinase are, the ribosomal protein S6 kinase Psk1 (Du *et al.*, 2012; Nakashima *et al.*, 2012) or the Taf12 subunit of SAGA complex co-activator (Laboucarié *et al.*, 2017). Taf12 is phosphorylated early upon nutrient defficiency. In its phosphorylated state, it modulates the sexual differentiation process (Laboucarié *et al.*, 2017). The TORC2-Gad8 pathway is also involved in the silencing of some specific chromosomal regions (Cohen *et al.*, 2018) and other processes that not described in this thesis.

3.2.2. The Tor2 (TORC1) pathway

In contrast to the Tor1 kinase, the fission yeast Tor2 kinase is essential for vegetative growth and represses sexual differentiation under nutrient favourable conditions (Alvarez and Moreno, 2006; Matsuo *et al.*, 2007). Cells defective in Tor2 kinase, arrest in G1 phase even in nutrient-rich medium and initiate sexual development. It has also been shown, that the expression of nitrogen starvation-responsive genes *mei2* and *ste11* that are important for sexual development is repressed by Tor2 function (Matsuo *et al.*, 2007). The Tor2 kinase physically interacts with the Rheb GTPase Rhb1 which is responsible for the Tor2 kinase activation (Urano *et al.*, 2005; Uritani *et al.*, 2006; Nakashima, Sato and Tamanoi, 2010).

The examples of Tor2 phosphorylation targets in *S. pombe* are the RNA polymerase III transcription repressor Maf1 (Michels, 2011), autophagy regulator Atg13 (Kohda *et al.*, 2007) and the two AGC family kinases Sck1 and Sck2 (Nakashima *et al.*, 2012). Both Sck1 and Sck2 kinases have an overlapping function with the PKA catalytic subunit and are therefore involved in the cAMP pathway regulation (Jin *et al.*, 1995; Fujita and Yamamoto, 1998). One of the

common targets of both TOR kinases is the ribosomal S6 protein kinase Psk1 (Nakashima *et al.*, 2012).

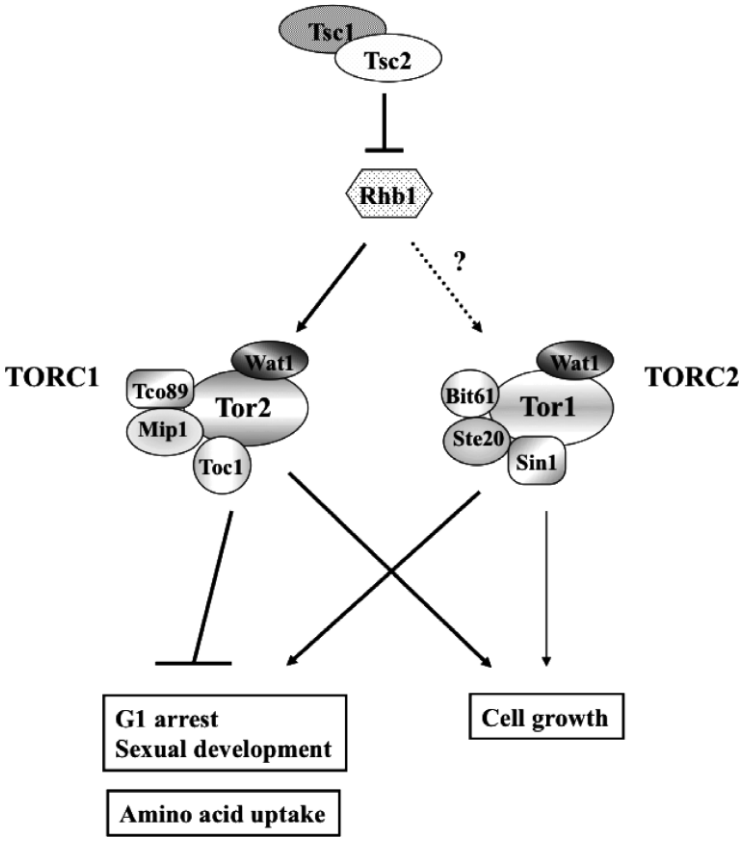

Figure 3 - The simplified TOR signalisation model. Adapted from (Otsubo and Yamamato, 2008).

3.3. Mitogen activated protein kinase cascades

The mitogen activated protein kinase cascades are conserved in all eukaryotic organisms. They affect many intracellular processes such as gene expression regulation or other nuclear or non-nuclear cytoplasmic activities (Schaeffer and Weber, 1999). Typically, mitogen activated protein kinase pathways are constructed of three kinase components: the mitogen activated protein kinase itself (MAPK), it's activator MAPK kinase (MAPKK), and the MAPKK activator MAPK kinase kinase (MAPKKK). However, every MAPK module can signal independently (Schaeffer and Weber, 1999).

3.3.1. The Sty1 stress activated protein kinase pathway

The stress activated protein kinase pathway (SAPK) represents the MAPK transduction pathway subfamily important for response to different types of stress stimuli, e.g. heat stress or oxidative stress. Therefore, it is not triggered by the presence of a mitogen, but by the presence of a stressor. However, the field of cellular sensors responsible for the detection of most stressors is not well explored (Pérez *et al.*, 2019). The detection and response to oxidative stress induced by hydrogen peroxide is an exception to this. In this case, a specific multistep phosphorelay module composed by two sensor kinases Mak2 and Mak3, the phosphotransfer protein Mpr1, and the response regulator Mcs4 react to the stressor upstream of the SAPK pathway itself (Nguyen *et al.*, 2000; Buck *et al.*, 2001; Quinn *et al.*, 2002). The activation of the SAPK pathway occurs via binding of the phosphorelay system protein Mcs4 to the Wis4 and Win1 MAPKKKs (Shieh *et al.*, 1997). These two kinases then redundantly physically interact and phosphorylate with the Wis1 MAPKK (Degols & Russell, 1997).

The N-terminal domain of the Wis1 MAPKK contains a nuclear export signal (NES) and a MAPK-docking site. It is actively exported from the nucleus to recruit the Sty1 MAPK and "wait" for phosphorylation. Activated Wis1 phosphorylates the MAPK Sty1 and also promotes its dissociation (Nguyen et al., 2002). The activated Sty1 translocates into the nucleus (Gaits *et al.*, 1998), where it phosphorylates the Atf1 transcription factor. The activation of the Atf1 transcription factor also leads to the increase of its transcript and protein levels (Lawrence *et al.*, 2007). Increased Atf1 levels lead to increased transcription of Atf1 target genes and in oxidative stress resistance. In the nucleus Atf1 can form a homodimer or a heterodimer with the transcription factor Pcr1 (Watanabe and Masayuki Yamamoto, 1996). This dimer is primarily involved in the induction of Sty1-dependent genes (Eshaghi *et al.*, 2010) and has

higher binding affinity than either homodimer complexes (Kon *et al.*, 1997). Although, there are genes solely dependent on Atf1 binding (Eshaghi *et al.*, 2010).

The Sty1 SAPK has also a notable role in the post-transcriptional steps of gene expression. It significantly stabilises the *atf1* mRNA transcript (Rodríguez-Gabriel *et al.*, 2003). It also phosphorylates the RNA-binding protein Csx1 that plays a major role in post-transcriptional stabilisation of *atf1* and other transcripts (Rodríguez-Gabriel *et al.*, 2003). The overview of the Sty1 SAPK pathway signalisation is visualised in Figure 4.

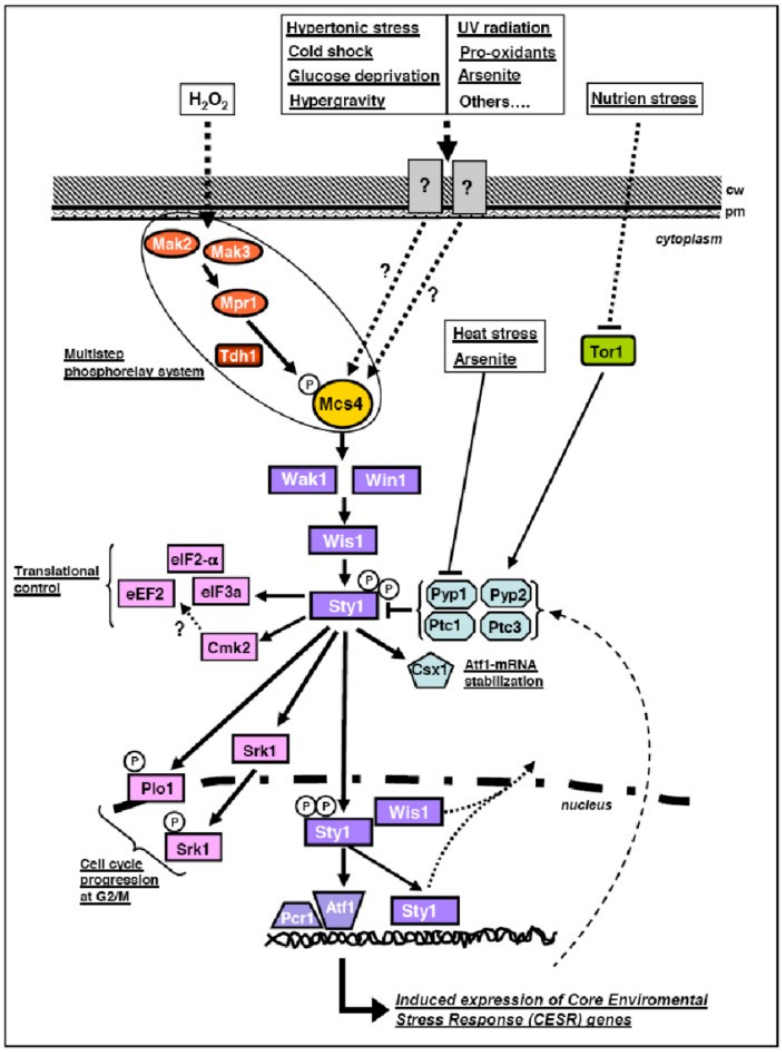

Figure 4 - The stress response SAPK Sty1 pathway signaling model. Adapted from (Perez & Cansado, 2010).

3.3.2. The Pmk1 mitogen activated protein kinase pathway

The protein kinase Pmk1 represents another member of the MAPK family. It is an extracellular signal regulated MAPK that affects cell morphology and cell integrity (Toda *et al.*, 1996). The Pmk1 pathway responds to different types of stresses, e.g. oxidative stress, osmotic stress,

nutritional stress or cell wall damage (Madrid *et al.*, 2006; Takada *et al.*, 2007). The Pmk1 protein kinase upstream MAPK components are the Pek1 MAPKK and the Mkh1 MAPKKK (Sengar *et al.*, 1997; Sugiura *et al.*, 1999). The Rho2 GTPase and the Pck2 protein kinase C ortholog are the main regulators of the Pmk1 pathway in *S. pombe*. Other GTPases, such as Rho1, Rho5 and Cdc24 GTPases are also involved. (Toda, Shimanuki and Yanagida, 1993). However, it can be branched with other elements acting upstream (Barba *et al.*, 2008). It was also shown, that some specific types of stress conditions (e.g. nutritional stress) require functional proteosynthesis for the proper activation of the Pmk1 MAPK pathway (Madrid *et al.*, 2013). However, the exact proteins that require *de novo* synthesis remain unknown. The visualised overview of the Pmk1 MAPK pathway signalisation can be found in *Figure 5 - The cell integrity MAPK Pmk1 pathway signaling model. Adapted from (Perez and Cansado, 2010).*Figure 5.

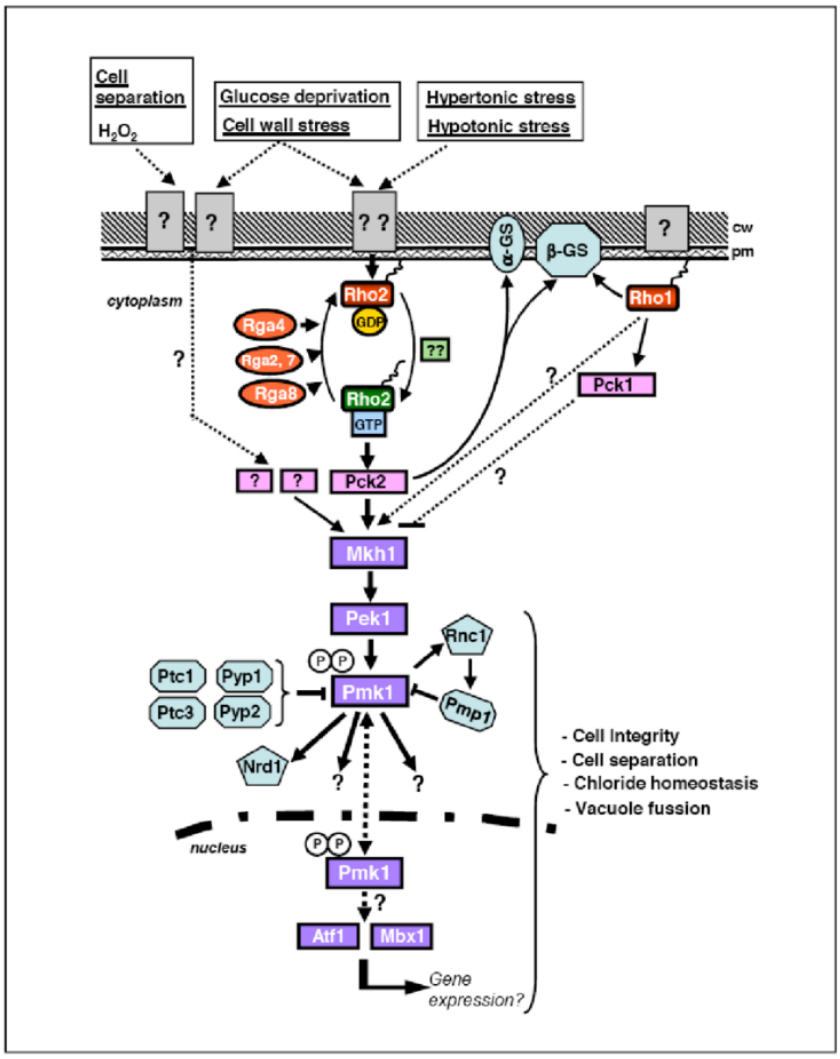

Figure 5 - The cell integrity MAPK Pmk1 pathway signaling model. Adapted from (Perez and Cansado, 2010).

4. Crosstalk and antagonism in the regulation of gene expression

Signal transduction pathways that have a crosstalk interaction relationship regulate a common target in a similar or opposite manner.

An antagonistic interaction means a situation in which two or more signal transduction pathways regulate a certain gene in the opposite manner. Basically, there are two types of interactions:

1. The activation of one pathway and the deactivation of another leads to change of gene expression of a certain gene. This type of interaction happens at the promoter region of the gene, where the transcription factors are regulated by different signal transduction pathways.
2. The activation of one pathway leads to the attenuation of another, resulting in a change of gene expression of a certain gene.

4.1. The PKA pathway and the SAPK pathway antagonistically regulate the *fbp1* gene expression

In the majority of eukaryotic organisms, the lack of glucose sources triggers a biochemical process called gluconeogenesis. The fission yeast is not an exception. Using this process, cells can create glucose from some other stored biomolecules such as glycerol or amino acids. The essential enzyme that catalyses this process is the fructose-1, 6- bisphosphatase.

In *S. pombe*, the fructose-1,6-bisphosphatase is encoded by the *fbp1* gene (Navas and Gancedo, 1996). The gene has two upstream activation sites. The upstream activation site 1 (UAS1), located approximately 900 bp from the transcriptional start site, contains a cAMP response element (CRE) and is the binding site for the Atf1-Pcr1 heterodimeric activator (Neely and Hoffman, 2000). The upstream activation site 2 (UAS2), located approximately 250bp from the transcriptional start site, contains a stress response element (STRE) and recruits the Rst2 transcription activator (Hirota, Hoffman and Ohta, 2006). Both of these regions are regulated by both PKA and stress-response MAPK pathways (Neely and Hoffman, 2000). Overall, the *fbp1* gene expression is attenuated by these pathways under physiological conditions and activated under different types of stress conditions.

4.1.1. Atf1

As mentioned above, the transcription factor Atf1 is one of the major Sty1 SAPK phosphorylation targets. In case of the *fbp1* gene expression regulation, it is the transcription activator binding to UAS1 as a phosphorylated dimer with the Pcr1 transcription factor. It is regulated by both PKA and Sty1 SAPK. The PKA catalytic subunit Pka1 inhibits the dimer binding to the UAS1, while the SAPK activity promotes its binding. Therefore, Atf1/Pcr1 transcription activator binding is present in glucose defficient conditions and the presence of glucose prevents the formation of this bond (Neely and Hoffman, 2000). The same authors showed that the PKA activity cannot overcome the stress-induced Sty1 effect.

4.1.2. Rst2

Rst2 is the product of the *rst2* gene which encodes a zinc finger DNA binding transcription factor. It acts as a transcriptional activator that binds to the STRE upstream elements. Typical examples of this activity are the *ste11* and *fbp1* gene expression control mechanisms. In case of *fbp1* gene expression regulation, Rst2 binds to the UAS2 upstream regulator element. The activity of the transcription factor is not regulated at the transcription level, but by its phosphorylation via the PKA catalytic subunit Pka1. In its phosphorylated form, Rst2 undergoes nucleocytoplasmic shuttling. Therefore, it is no more able to affect gene expression regulation on the transcriptional level in the nucleus. Only unphosphorylated Rst2 can regulate the *fbp1* gene expression on the transcriptional level under nutrient-starved conditions (Higuchi, Watanabe and Yamamoto, 2002). The PKA-Rst2 regulatory network is shown in Figure 6.

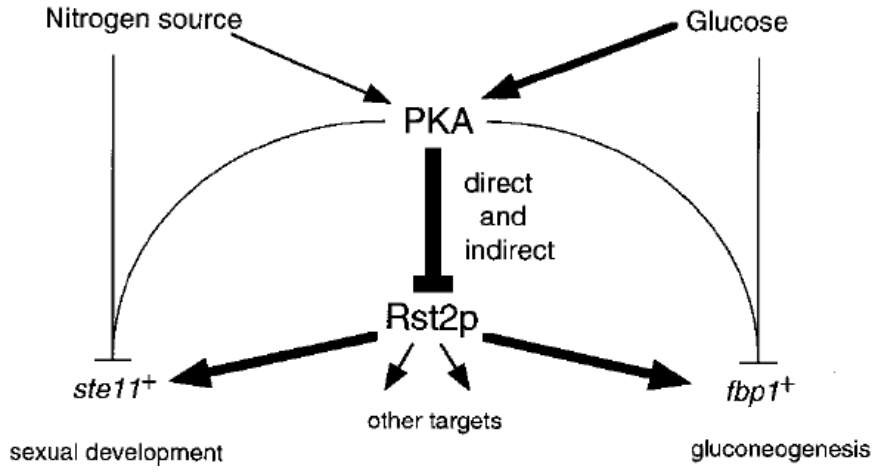

Figure 6 - Rst2 transcription factor regulation and targets. Adapted from (Higuchi, Watanabe and Yamamoto, 2002).

4.2. Pmk1 crosstalk in *Schizosaccharomyces pombe*

Apart from the role of the Pmk1 protein kinase in cell integrity, it also works as one of the cellular switches between fermentative and respiratory metabolism. Therefore, it was shown to interact with other major signal transduction pathways in *S. pombe*.

4.2.1. The crosstalk between the Pmk1 and the PKA pathways in response to glucose availability in the environment

The other glucose-responding pathway in *S. pombe* is the cell integrity MAPK Pmk1 pathway. However, it's response is strictly dependent on functional PKA pathway upstream regulation components (Madrid *et al.*, 2013). Furthermore, the activation is independent from upstream regulating GTPases mentioned in chapter 3.3.2 in response to glucose limitation (Madrid *et al.*, 2013). The same authors suggest the existence of unknown additional upstream elements that activate the cell integrity MAPK cascade via Pck2 under glucose limiting conditions. They also claim that the activation of the cascade is dependent on functional proteosynthesis, as described in chapter 3.3.2. Different carbon sources were tested by the same authors to see whether the activation is carbon source specific. They show that the activation of the Pmk1 MAPK in the absence of glucose appears to be due to the lack of this particular carbon source.

4.2.2. The crosstalk between the Pmk1 and the Sty1 MAPK pathways

The activation of a MAPK pathway usually leads to an increase in the transcript level of the protein phosphatases that inactivate the MAPK pathway. It was shown that activated Pmk1 and Sty1 protein kinases both trigger an increase in the transcript level of the Pyp1, Pyp2 and Ptc1 protein phosphatases. Both protein kinases are therefore considered to regulate themselves and each other (Takada *et al.*, 2007).

The other crosstalk between these two pathways in *S pombe* happens in the regulation of the transcript levels of transcription factor Atf1 (Takada *et al.*, 2007) . The same author showed a notable role of the Pmk1 MAPK in the Atf1 phosphorylation during the cell wall stress.

One of the differences between these two MAPKs is in their ability to change their localisation in the activated state. Whereas Sty1 accumulates in the nucleus after its activatory phosphorylation (Gaits and Russell, 1999), the Pmk1 kinase normally does not change its localisation when activated under stressed conditions (Madrid *et al.*, 2006).

4.3. The TOR signalling crosstalk

As described above, the two fission yeast complexes responsiible for maintaining the TOR signalisation have a broad spectrum of effects. In this sub-chapter I elaborate on some of the examples of interactions between TOR complexes and other major signal transduction pathways in fission yeast. I will also discuss how the two complexes interact with each other.

4.3.1. Crosstalk between two TOR complexes

Even though the TOR complexes are studied by many research groups, not much is known about the crosstalk interactions between the TORC1 and TORC2 signalisation complexes. The crosstalk between the two TOR complexes is maintained via the PP2A protein phosphatase. It was so far described by Portantier *et al.* (2017). The PP2A protein phosphatase functions downstream of TORC1, which means that its activation is TORC1 dependent. In its active state, PP2A negatively regulates the TORC2 pathway through direct dephosphorylation of protein kinase Gad8. Because the Gad8 kinase is the major regulator of the transcription of sexual differentiation genes, the PP2A protein phosphatase prevents the sexual differentiation process (Portantier *et al.*, 2017).

Another type of crosstalk between the two TOR complexes was suggested by research on mammalian cells via the ribosomal protein S6 kinase S6K1 (Xie and Proud, 2013).

4.3.2. The crosstalk between TOR complexes and the Pmk1 MAPK cell integrity pathway

Firstly, the interaction between TORC2 and the cell integrity pathway was shown. One of the main TORC2 targets, the protein kinase Gad8, regulates the levels of the Pck2 protein kinase C ortholog in *S. pombe* during the cell wall stress and glucose deprivation. Gad8 contributes to *de novo* Pck2 synthesis essential for the Pmk1 activation in some specific conditions (Madrid *et al.*, 2013, 2016).

Furthermore, the TORC1 was also shown to affect the *de novo* Pck2 synthesis in response to specific types of stress. A TORC1 target, the ribosomal protein S6 kinase Psk1 seems to be another crucial activator of the synthesis. However, the effect of Psk1 depends on its interaction with the TORC2 components (Madrid *et al.*, 2016).

The cell integrity pathway regulates the TORC2-Gad8 signalling on two levels. The Pmk1 upstream regulator Pck2 phosphorylates the Gad8 protein kinase to negatively regulate the physical interaction with TORC2 (Du *et al.*, 2016). Therefore, TORC2 is unable to control

the Gad8 activation. The Pmk1 itself also acts upstream of the TORC2 pathway. It negatively regulates the major TORC2 activator, the Ryh1 GTPase (Tatebe, Morigasaki and Shiozaki, 2010), and therefore negatively regulates TORC2 signalling. Ryh1 GTPase, however, also regulates the cell integrity pathway through a TORC2-dependent mechanism (Madrid *et al.*, 2016).

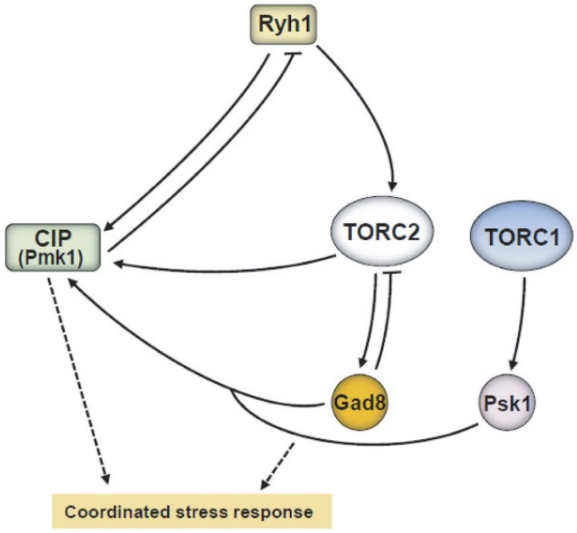

Figure 7 - Crosstalk interactions between TOR and Pmk1 signalling in fission yeast. Adapted from (Madrid *et al.*, 2016).

4.3.3. The crosstalk between the TORC2 and PKA pathways in response to glucose availability in the environment

The TORC2 pathway, as one of the main stress response pathways, also responds to nutrient stress. Cohen, Kupiec and Weisman (2014) observed a decrease in the TORC2-dependent Gad8 phosphorylation and Gad8 activity in glucose limiting conditions. They suggest that glucose is required, as it is the most effective carbon source for the activation of the TORC2-Gad8 cascade. The previously described fact of functional PKA pathway requirement for the Pmk1 MAPK pathway response to glucose availability in the environment also applies to the TORC2-Gad8 cascade. While the presence of glucose is sensed by the GPCRs, glucose absence is sensed by the AMP dependent kinase Ssp2 (Matsuzawa *et al.*, 2012). This PKA pathway module was shown to positively regulate and genetically interact with the Gad8 kinase and therefore it activates the TORC2-Gad8 cascade (Cohen, Kupiec and Weisman, 2014).

4.3.4. SAGA complex co-activator regulation

Co-activators are proteins or multi-subunit protein complexes that participate in various actions, such as nucleosome remodelling, histone modification and recruitment of general transcription

factors (Koutelou, Hirsch and Dent, 2010). In *S. pombe,* the SAGA co-activator complex consists of 19 subunits, from which at least two, Gcn5 and Taf12, are involved in the switch from proliferation to sexual differentiation regulation via TOR signalisation (Laboucarié *et al.*, 2017).

In non-stressing conditions, Gcn5 directly represses the expression of sexual differentiation transcription factors (Helmlinger *et al.*, 2008). As TORC1 is the pathway to prevent the sexual differentiation process in its activated state, it was shown that the process is also dependent on the Gcn5 SAGA complex subunit which seems to act downstream of the TORC1 pathway (Laboucarié *et al.*, 2017). The same authors prove that the Gcn5 subunit acts also downstream of TORC2, but it requires additional effectors to induce sexual differentiation.

The previously outlined Taf12 SAGA complex subunit activation has also an impact on the sexual differentiation regulation. It acts downstream of the TORC2-Gad8 pathway and is activated via phosphorylation upon nutrient starvation. In its activated state, it modulates the sexual differentiation process in nutrient-depleted conditions. The Taf12 subunit is also a target of the PP2A protein phosphatase activity (Laboucarié *et al.*, 2017). As the PP2A function is TORC1 dependent, the Taf12 regulation seems to be a crosstalk target of both TOR pathways that regulate the sexual differentiation process.

4.4. The crosstalk on the regulation of the Ste11 transcription factor level

In a nutrient-starved state, haploid *S. pombe* cells begin the sexual differentiation process. They arrest in the G1 phase, conjugate and form diploid zygotes. The zygotes undergo meiosis and form asci containing four haploid spores. This process includes many gene products in the fission yeast cell, and therefore must by highly regulated.

The Ste11 transcription factor plays the role of a major sexual differentiation regulator on the transcriptional level. The Ste11 target genes contain a common regulation TR-box motif in their 5'-upstream regions (Sugimoto *et al.*, 1991). The 5'-upstream region of the *ste11* gene also contains the TR box motif. According to this fact, Ste11 appears to regulate its own transcription through this TR box (Kunitomo *et al.*, 2000).

The *ste11* transcription is repressed under nutrient-rich conditions (Sugimoto *et al.*, 1991). Also, under nutrient-rich conditions, the Pat1 kinase phosphorylates Ste11 (Li and McLeod, 1996). The phosphorylated form of Ste11 recruits the Rad24 protein, which inhibits the Ste11 transcription factor accumulation in the nucleus (Kitamura *et al.*, 2001). As St11

stimulates the expression of itself, this nuclear exclusion also reduces the level of *ste11* expression (Qin *et al.*, 2003). The phosphorylation of Ste11 also prevents it from binding to DNA (Kjærulff *et al.*, 2007). The same author also showed the Ste11 phosphorylation by the cyclin dependent kinase. This phosphorylation also ensures that Ste11 is active only in the G1 phase of the cell cycle.

The Ste11 level increases upon nitrogen starvation (Sugimoto *et al.*, 1991). This increase in the *ste11* expression level is dependent on the major signal transduction pathways mentioned in this thesis. The overview of the Ste11 transcription factor regulation by major signal transduction pathways in fission yeast is depicted in Figure 8.

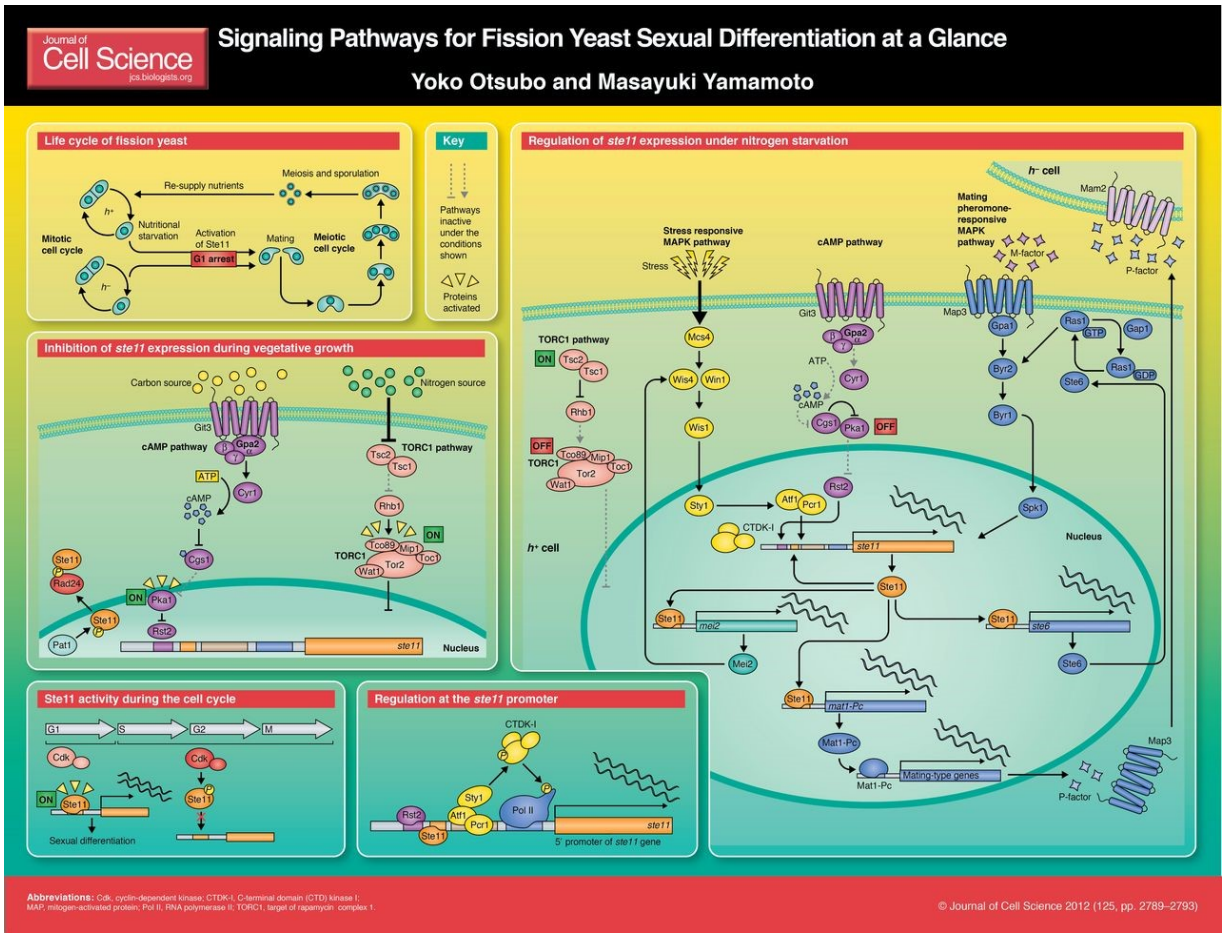

Figure 8 - The Ste11 transcription factor regulation overview. Adapted from (Otsubo and Yamamoto, 2012).

4.4.1. PKA and TORC1 crosstalk on the *ste11* transcription regulation

As mentioned in chapter 4.1.2, Rst2 is the major activator of the *fbp1* transcription. It is also known as the major activator of *ste11* transcription (Higuchi, Watanabe and Yamamoto, 2002). In case of *ste11* transcription regulation, the mechanism of Rst2 regulation by the PKA pathway is similar to that of the *fbp1* regulation. The activated PKA catalytic subunit, Pka1,

phosphorylates the transcription factor Rst2. The phosphorylated form of Rst2 is excluded from the nucleus and can no longer activate *ste11* transcription (Higuchi, Watanabe and Yamamoto, 2002). In addition, the PKA pathway was also shown to inhibit the nuclear accumulation of the Ste11 transcription factor (Valbuena and Moreno, 2010).

The TORC1 kinase, Tor2, regulates the *ste11* transcription in a similar manner. The Ste11 transcription factor physically interacts with active Tor2 (Alvarez and Moreno, 2006) and Ste11 is excluded from the nucleus by this interaction (Valbuena and Moreno, 2010). The same authors also showed the analysis of temperature-sensitive *tor2* mutants. According to their results, *tor2* temperature sensitive mutants arrest in the G1 phase and initiate the sexual differentiation process even in nutrient-rich medium in the restrictive temperatures. They also showed increased *ste11* expression in these conditions.

Valbuena & Moreno described the synergy between the PKA and TORC1 pathways in the *ste11* gene expression regulation. They also showed that the PKA pathway has a bigger impact than the TORC1 pathway on the *ste11* gene expression regulation (Valbuena and Moreno, 2010).

4.4.2. Role of the Sty1 SAPK pathway in the *ste11* transcription regulation

The stress-response Sty1 MAPK also plays a significant role in the *ste11* transcription regulation. A number of different SAPK pathway components are essential for the G1 arrest and the initiation of sexual differentiation under nitrogen starvation. The essential components are Wis1 MAPKK, Sty1 MAPK, and also the major target of the SAPK pathway, the Atf1 transcription factor (Shiozaki and Russell, 1996). The Atf1 transcription factor is also involved in direct regulation of the 5′-regulation site of the *ste11* gene (Watanabe and M Yamamoto, 1996). The activated Atf1 forms a heterodimer with the Pcr1 transcription factor, as described above. The heterodimer binds to the 5′-regulation site of the *ste11* gene and acts as a transcription activator (Watanabe and M Yamamoto, 1996). Sty1 also phosphorylates the cyclin dependent kinase-like kinase under nitrogen starvation, which subsequently phosphorylates the C-terminal domain of the RNA polymerase II as shown in Figure 8 (Phatnani and Greenleaf, 2006).

5. Discussion and conclusion

In this thesis, I have reviewed the main proliferation-promoting and stress-response pathways in fission yeast. However, we cannot properly divide them to these two groups. Some of the pathways (in this review the PKA and the TORC1 pathways) truly promote proliferation more than the other ones, but they also have a stress response activity, at least by sensing nutrient depletion. Also, stress-response classified pathways are at least indirectly responsible for proliferation promotion.

Overall, PKA and TORC1 (proliferation-promoting pathways in this thesis) promote cell proliferation in nutrient favourable conditions by activating the proliferation factors on various levels. Their targets also actively repress the sexual differentiation process by regulation of the sexual differentiation-promoting factors (described in chapter 4.4.1). Components of different proliferation-promoting pathways are also able to repress or attenuate the activity of the stress-response pathways.

On the other hand, both activating and repressing functions of proliferation-promoting pathways are reduced in nutrient limiting conditions. Stress-response pathways are getting less repressed and more active by sensing stress conditions. They activate the stress-response and sexual differentiation factors (described in chapters 3.3 and 4.4.2). This activation occurs directly or indirectly by repressing the activity of the proliferation-promoting pathways' elements (described in the chapter 4.3.2).

Apparently, the crosstalk between these two types of signal transduction pathways in *S. pombe* cells is present in order to preserve the balance between proliferation and stress-response. By default, cells proliferate until the level of nutrients hits a critical minimum concentration, or until the presence of a stressor. We can therefore say, that the activity of proliferation-promoting pathways is superior to the activity of stress-response pathways in physiological conditions (hypothesis of the author). However, it is essential for cells to keep the stress-response pathways in an attenuated state and not to abolish the transcription of their elements completely, so that they keep themselves agile responsive to changes in the environment.

The antagonistic interaction system that was described in this thesis has many benefits for the cells. For example, it can prevent genome enlargements and accumulation of unnecessary transcription factors (hypothesis of the author). Therefore, it speeds up the cells' response to extracellular and intracellular changes.

In conclusion, when considering cell proliferation and sexual differentiation, we must not talk about a “switch” mechanism. It appears to be more as a balance between the processes promoting one or the other option. Also, the proliferation/growth and stress response states are continuously in balance and one does not completely negate other. Cells must respond to the tilts of the balance.

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