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The meiotic–mitotic initiation switch in budding yeast maintains its function robustly against sensitive parameter perturbations

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ABSTRACT

Experiments show that the meiotic–mitotic initiation switch in budding yeast functions robustly during the early hours of meiosis initiation. In this study, we explain these experimental observations first by understanding how this switching occurs during the early hours of meiosis by studying the temporal variation of this switch at the gene expression level. Then, we investigate the effects on this meiotic–mitotic switching from the perturbations of the most sensitive parameters in budding yeast meiosis initiation network. We use a mathematical model of meiosis initiation in budding yeast for this task and find the most sensitive group of parameters that influence the expressions of meiosis and mitosis initiators at all stages of the meiotic–mitotic switch. The results indicate that the transition region of the switch, where a double negative feedback loop between meiosis (Ime2) and mitosis (Cdk1/Cln3) initiators plays a major role, shows lower robustness. Feedback loops are frequently observed serving as a major robust adaption mechanism in many biological networks. Consequences of this less robust region appear in the transition region of the resulting switches. Most importantly, despite the differences observed in the transition region, we find that the meiotic–mitotic switch robustly maintains its main function of transition from meiosis to mitosis when the nutrients are re-supplied, against the perturbations in the sensitive parameters.

1. Introduction

For millennia, organisms have evolved to use available nutrients optimally for their survival and they are designed to function robustly with the inevitable environmental perturbations. As an example, meiosis decided budding yeast diploid cells try to use the available nutrients optimally by switching back to mitosis initiation if the nutrients are re-supplied from starvation initiation until they are committed to meiosis cell division (Honigberg and Esposito, 1994; Simchen, 2009; Simchen et al., 1972; Winter, 2012). Typically, meiosis and mitosis initiations (meiotic–mitotic switch) function robustly even with the environmental perturbations such as temperature and pressure changes which affect the parameter values. How do budding yeast cells maintain the above mentioned functions at the gene expression level? In this paper, we use our previously published mathematical model on budding yeast meiosis initiation (Wannige et al., 2014) to study how budding yeast cells function robustly at the gene expression level for the optimal usage of available nutrients.

Entries into meiosis and mitosis cell division pathways are tightly regulated to ensure that cells enter these pathways only under correct conditions of appropriate cell size and in the presence of appropriate initiation signal (Morgan, 2006). Most of these decision making points at the cell cycle are controlled by bistable switches of the main regulatory proteins (Verdugo et al., 2013). In budding yeast, the meiotic–mitotic initiation bistable switch between main meiosis initiator (Ime2) and mitosis initiator (Cdk1/Cln3) underlies the entry into either meiosis or mitosis cell division (Gurevich et al., 2010; Wannige et al., 2014). In budding yeast diploid cells, meiosis and mitosis initiate at opposite nutritional conditions. Meiosis initiates in grown diploid cells under nutrient depletion and mitosis initiates under rich nutrients (Esposito and Esposito, 1974; Gupta, 2009; Morgan, 2006). Ime1 and Ime2 are the main meiosis initiator proteins. Ime1 transmits the nutrient depletion signal and activates Ime2, which then activates the further downstream meiosis specific

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genes (Smith and Mitchell, 1989). Cyclin activated CDKs (cyclin dependent kinases) are the regulators of the mitosis cell division (Graña and Reddy, 1995; Morgan, 2006). Cdk1/Cln3 is the most upstream activator of the mitotic network, and works as the activator of the further downstream cyclins (Morgan, 2006). The meiotic–mitotic initiation bistable switch of Ime2 and Cdk1/Cln3 observed during the maximum expression time of the Ime2 protein explains the initial meiosis or mitosis entry decision (Wannige et al., 2014). However, budding yeast diploid cells choose either meiosis or mitosis initiation robustly according to the available nutrients during all the early hours of meiosis initiation; meiosis decided cells jump to mitosis initiation if they are re-supplied with nutrients from starvation initiation until they reach the meiotic commitment point despite the external perturbations (Simchen, 2009; Winter, 2012).

Robustness of the biochemical switches at pathway entry points is essential for healthy functioning despite the abundant internal and external perturbations. This robustness in complex cellular networks is typically maintained by: (1) the feedback regulation mechanisms, *i.e.*, positive or negative feedbacks; (2) modularity through hierarchically organized subunits; (3) redundancy by having alternative mechanisms; and (4) decoupling the low level variations from the high level functionalities (Kitano, 2004a,b, 2007; Szallasi et al., 2006). Robustness is a broader concept than stability or homeostasis and it emerges as a systems level phenomenon that cannot be understood by studying individual components (Kitano, 2004a). A robustness analysis may first define the performance measures of the function of interest after selecting the most important major proteins related to the performance measure. Then, considering the dimensionality and the nonlinearity of the biological systems, a set of most influential, performance related parameters may be selected and perturbed to measure the performance which indicates the robustness of the selected functionality (Kitano, 2004a, 2007).

In this paper, first we use our mathematical model (Wannige et al., 2014) to explain how the initial meiosis to mitosis decision making happens at the gene expression level during the early hours of meiosis initiation as seen in experiments. We then investigate the robustness of the meiosis and mitosis initiation at the gene expression level. We also study the effects on meiotic–mitotic switch by the most sensitive parameter perturbations of the meiotic network. We use local (LSA) and global sensitivity analysis (GSA) techniques to identify the most influential parameters with respect to the meiosis and mitosis initiator protein levels. We choose a set of common parameters sensitive in three main nutrient driven states (meiosis, mitosis and transition) of the switch, and simultaneously perturb this parameter set to examine the robustness of the switch behavior. The robustness analysis of the switch reveals that transition region between meiosis and mitosis, where the double negative feedback loop between meiosis and mitosis initiators plays a major role, is more susceptible for the uncertainties despite the fact that double negative feedback loops have always been identified as a mechanism of robust response for the perturbations (Alon et al., 1999; Barkal and Leibler, 1997; Kitano, 2004a). Further, this study reveals that the meiotic–mitotic switch maintains its main function of the transition from meiosis to mitosis when the nutrients are re-supplied in spite of the perturbations in the most sensitive parameters.

The organization of this paper is as follows: the second section of this paper provides a succinct background of the biology required to discuss the biological implications of the *in-silico* outputs. In Section 3, we provide a summary of the mathematical model used in this study. Section 4 provides a detailed description of the materials and methods used in this work. Section 5 provides the results and discussion of the outputs and finally we present the main conclusions in Section 6.

2. Biology of meiosis and mitosis initiation in *Saccharomyces cerevisiae*

To discuss the results of this study in context, we briefly review the biology of meiosis and mitosis initiation in budding yeast. More details are available in (Harvey Lodish et al., 1995; Kassir et al., 2003; Morgan, 2006). In this paper, protein names are written in lower case starting with an uppercase letter, and genes and mRNAs are written in upper case.

2.1. Meiosis initiation under stressful nutrient conditions

Meiosis process in diploid cells initiates only when the type, size and nutritional requirements are attained. Meiosis initiates only in grown diploid cells in nitrogen depletion medium with no glucose and acetate as the non-fermentable carbon source (Esposito and Esposito, 1974). Initiation and progression through meiotic process is regulated by a main initiator and a set of meiosis specific genes, which are expressed sequentially (Fig. 1) (Smith et al., 1990).

The protein kinase, Initiator of meiosis1 (Ime1) is mainly responsible for transferring the nutrient signal to meiosis initiation gene network (Smith et al., 1990). Stimulated by the nutrition stress signals, Ime1 activates the early meiosis specific genes (EMG), which reside at the top of the meiosis specific gene cascade consisting of four sets of genes: early meiosis specific genes (EMG), early middle meiosis specific genes (EMMG), middle meiosis specific genes (MMG), late meiosis specific genes (LMG) (Smith and Mitchell, 1989). The sequential expression of meiosis specific genes correlates with the execution of the major events of meiosis process in diploid cells such as DNA replication, nuclear division and spore formation (Fig. 1). Activated by Ime1, IME2 gene, which is a member of EMGs, transcribes the second meiosis initiator (Initiator of meiosis 2) (Smith and Mitchell, 1989). Ime2 is responsible for activating the transcription of the early middle meiosis specific genes such as NDT80 and middle meiosis specific genes. Late meiosis specific gene expression is indirectly dependent on the Ime1, Ime2 and Ndt80 (Chu et al., 1998).

Upon nitrogen depletion in the acetate medium, IME1 mRNA is transiently increased in diploid cells, resulting in a similar Ime1 protein expression pattern with a peak around 6–9 h in starvation (Shefer-Vaida et al., 1995). The transcription of IME1 is positively self-regulated to generate the required amount of Ime1 protein to activate further downstream meiosis specific genes, such as IME2. Ime1 is an unstable protein that is degraded by the 26S proteasome. The life time of Ime1 depends on Ime2 as Ime2 phosphorylates Ime1 and tags it for degradation (Guttmann-Raviv et al., 2002). Ime1 and Ime2 protein expressions are transient and sequential (Fig. 2B of Rubinstein et al., 2007).

The early meiosis specific genes are tightly regulated so that they are only expressed under correct meiotic conditions. In vegetative growth conditions, these genes are silent because of the repressions by the recruitment of the protein complex, Sin3/Rpd3 (Sin3/Rpd3 is a complex of histone deacetylase, Rpd3 and the protein Sin3) (Kadosh and Struhl, 1997), and Isw2 (a chromatin remodeling complex) (Goldmark et al., 2000) to the EMG promoters by the DNA binding protein Ume6 (Pnueli et al., 2004).

Under mitotic conditions, protein kinases, Rim11 and Rim15 are required to relieve the repression of Sin3/Rpd3 and Isw2 from Ume6 and help to recruit Ime1 to the Ume6 on EMGs (Pnueli et al., 2004). The presence of nutrients deactivates protein kinases Rim11 and Rim15 by phosphorylations. In starvation conditions, Rim11 and Rim15 phosphorylate Ume6 and Ime1, and partially remove Rpd3/Sin3 on Ume6 and replace it with Ime1 (Fig. 9 of Pnueli et al., 2004). EMG expression, including Ime2, is then activated by Ime1 in starvation conditions (Honigberg and Purnapatre, 2003; Kassir et al., 2003; Pnueli et al., 2004; Vershon