Supporting information on the Boolean layer

A novel yeast hybrid modeling framework integrating Boolean and enzyme-constrained networks enables exploration of the interplay between signaling and metabolism

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# A detailed description of mechanisms reflected in the Boolean model of nutrient signaling

## SNF1

Snf1 activation upon glucose depletion is associated with increased phosphorylation of Thr210 mediated by the upstream kinases Tos3, Sak1 and Elm1 which are partially redundant, this activation appears to be constitutively (Hong et al., 2003; Sutherland et al., 2003). The return to the inactive state has been attributed to the Reg1-Glc7 complex where studies show that Reg1 interacts with Snf1 (Ludin et al., 1998) and targets Glc7 to the complex(Sanz et al., 2000). The model suggests that Reg1-Glc7 binds to Snf1p, mainly relevant in low glucose conditions, and Snf1p phosphorylates Reg1, Glc7 dephosphorylates Reg1. Hxk2 is then either 1) promoting binding of Reg1 to Snf1, 2) promoting phosphorylation of Reg1 or 3) interfering with dephosphorylation by Glc7. In response to high glucose, Reg1-Glc7 is dephosphorylating and thus releasing Snf1 from the complex. The dephosphorylation of Reg1 appears to increase the efficiency of Glc7 dephosphorylation as well as allowing Reg1 to be released from SNF1(Sanz et al., 2000). This complex form of regulation is a highly adaptable system with a fast response. In our model, this is implemented in a way that when glucose is absent Snf1 is phosphorylated by the upstream kinases. The SNF1 complex phosphorylates Reg1-Glc7. To account for the involvement of Hxk2 in our model we chose to implement mechanism number 2) where Hxk2p can phosphorylate Reg1 (Fernández-García et al., 2012). Phosphorylated Reg1 obstructs the activation of Snf1 and activates Glc7. In this low glucose state, SNF1 is active, Reg1 and Hxk2 are phosphorylated and Glc7 is active. When glucose is added, Hxk2 is unphosphorylated and a “high glucose signal”, in our model mediated through the PKA pathway (Barrett et al., 2012; Castermans et al., 2012)allows Glc7 to dephosphorylate Snf1. When Snf1 and Hxk2 are no longer phosphorylated Reg1 gets dephosphorylated by Glc7 and also Glc7 becomes inactive. In this high glucose condition, Snf1 and Reg1 are unphosphorylated and Glc7 is inactive. SNF1-mediates phosphorylation of the transcriptional factors Mig1, Cat8, Sip4 and Adr1 as well as directly phosphorylates and inactivates ACC1(Woods et al., 1994). Mig1 is a repressor that is active in high glucose conditions and represses genes used for alternative carbon sources, mainly SUC, MAL and GAL genes (Broach, 2012; Santangelo, 2006; Schüller, 2003; Westholm et al., 2008). In absence of glucose, Cat8 and Sip4 are activating the transcription of genes regulated by carbon source-responsive elements (CSRE) such as FBP1, PCK1 and ICL1 (Broach, 2012; Leverentz & Reece, 2006; MacPherson et al., 2006; Turcotte et al., 2010). Adr1 induces genes involved in the use of alternative carbon sources such as ADH1, ACS1 and GUT1 as well as peroxisome biogenesis and fatty acid utilization such as POX1 and PXA1(Broach, 2012; Kacherovsky et al., 2008; Smith et al., 2011; Soontorngun et al., 2012; Turcotte et al., 2010) It has been shown that PKA can inactivate the Adr1(Cherry et al., 1989).

## PKA pathway

The protein kinase A (PKA)/cAMP pathway mainly represses genes involved in stress tolerance and post diauxic growth when glucose is available. This means that properties associated with slow, reparative growth and stationary phase are negatively regulated by glucose (Conrad et al., 2014). Intra- and extracellular glucose sensing is carried out by two distinct G-protein systems, namely the Ras pathway and the Gpr1/Gpa2 pathway (Rolland et al., 2000). Ras proteins are small monomeric GTP-binding proteins that are regulated through a cycle of GDP/GTP exchange and GTP hydrolysis. This process is regulated by Cdc25 that triggers the exchange from GDP to GTP on the one hand (Broek et al., 1987; Jones et al., 1991; Robinson et al., 1987) and by Ira1 and Ira2 that stimulate GTP hydrolysis on the other hand(K Tanaka et al., 1989, 1990; Kazuma Tanaka et al., 1990). Sensing of extracellular glucose occurs via the G-protein coupled receptor (GPCR) Gpr1 that interacts with Gpa1. Glucose availability causes a Gpr1-mediated nucleotide exchange in Gpa2 from GDP to GTP yielding its activation (Colombo et al., 1998; Kraakman et al., 1999). Activated Gpa2 as well as/together with activated Ras can stimulate cellular cAMP production via the adenylate cyclase (AC) (Kataoka et al., 1985; Rolland et al., 2000; Takashi Toda et al., 1985). GPCRs require Ras activation to activate AC which requires activity in the upper metabolism such as activity in hexose kinases (Rolland et al., 2000). It has been shown that the accumulation of F16BP is coupled to Ras activation (K. Peeters et al., 2017). RAS mutants imitate the AC mutants and the lethality of RAS deletion can be alleviated by *bcy1* mutant cells in the same fashion as AC mutants (Takashi Toda et al., 1985). In the model, this is implemented so that Cdc25 is activated by F1,6BP which is present when glucose is present, and Ira is active when no glucose is present. In this model, Ras can activate AC but Gpa2 also requires active Ras to activate AC. AC is deactivated by crosstalk with the SNF1 pathway (Nicastro et al., 2015). The protein kinase A (PKA) is a heterotetrameric protein complex consisting of two catalytic (Tpk1-3) and two regulatory (Bcy1) subunits(Matsumoto et al., 1982; T Toda et al., 1987; Takashi Toda et al., 1987). The binding of cAMP to the Bcy1 subunits causes the complex to dissociate, thus releasing the blockade of Tpk1-3 kinase activity (Conrad et al., 2014). In contrast, the kelch repeat proteins Krh1 and Krh2 stimulate the association of catalytic and regulatory subunits resulting in an increased amount of cAMP required for PKA activation. However, it was shown that active Gpa2 inhibits Krh activity (T. Peeters et al., 2006). Upon the numerous PKA targets are the phosphodiesterases Pde1 and Pde2 which conciliate a negative feedback mechanism on PKA itself by degrading cAMP (Hu et al., 2010; Ma et al., 1999; Nikawa et al., 1987; Sass et al., 1986). This is implemented in the model so that the PKA complex is defined as the catalytic subunits and the regulatory subunit is required when the PKA complex is inactive. To activate PKA, the Krh proteins have to be inactive. In the model only phosphorylated Pde breaks down cAMP. PKA also inactivates the Rim15 protein kinase by phosphorylation which is therefore not able to activate the transcription factors Msn2, Msn4 and Gis1 (Swinnen et al., 2006). This can also be achieved through crosstalk with the TOR pathway (Wanke et al., 2008). In an active state, the former two induce expression of genes containing a stress response element (STRE) in their promoter whereas the latter induces transcription of genes comprising a post diauxic shift (PDS) element in their promoter (Martínez-Pastor et al., 1996; Pedruzzi et al., 2000). PKA also directly phosphorylates cytosolic enzymes such as trehalase (Schepers et al., 2012), phosphofructokinase 2 (Dihazi et al., 2003), pyruvate kinase (Portela et al., 2002) and fructose-1,6-bisphosphatase (Rittenhouse et al., 1987).

## TOR pathway

The target of Rapamycin (Tor) kinase complex 1 (TORC1) is not directly involved in glucose sensing; however, glucose availability has been shown to highly influence the activity of TORC downstream targets (Hughes Hallett et al., 2014). The strongly conserved TORC1 pathway plays a crucial role in promoting anabolic processes and cell growth in response to nitrogen availability which is probably sensed as the level of intracellular amino acids (Broach, 2012). TORC1 comprises either Tor1 or Tor2 kinase in association with Kog1, Lst8 and Tco89 (Reinke et al., 2004) and its activity is regulated by the EGO complex consisting of Ego1, Ego2, Gtr1 and Gtr2 (Dubouloz et al., 2005). Multiple complex nitrogen-sensing mechanisms (Bar-Peled et al., 2013; Binda et al., 2009; Bonfils et al., 2012) lead to the physical interaction of EGO with TORC1 resulting in activation of the latter under nitrogen-rich conditions (Binda et al., 2009). Active TORC1 then induces several signaling branches - the Sch9 branch, the Tap42-PPase branch as well as the activation of further transcription factors such as Sfp1 (Marion et al., 2004; Urban et al., 2007; Yan et al., 2006). TORC1 phosphorylates Sch9(Urban et al., 2007) which then directly phosphorylates Rim15 (Wanke et al., 2008). Tap42 phosphorylation is catalyzed by active TORC1 (Yan et al., 2006). Phosphorylated Tap42 interacts with TORC1 and associates with the catalytic subunit of type 2A phosphatases (PP2A) like Pph21 or Sit4 and thus inhibits their phosphatase activity (Di Como & Arndt, 1996; Jiang & Broach, 1999). Dissociation of the complex occurring in the case of TORC1 inactivity results in PP2A activation (Beck & Hall, 1999). PP2A dephosphorylates its downstream targets such as Gat1, Gln3. (Beck & Hall, 1999; Kuruvilla et al., 2001). This regulation is complex and different transcription factors are regulated differently depending on TORC1 stimuli (Broach, 2012; Conrad et al., 2014; Georis et al., 2009). In this model, we chose a reduced representation where Gat1 and Gln3 are dephosphorylated by active PP2A and either of them induces nitrogen catabolite repression (NCR) genes. Rtg1 and Rtg3 promote the expression of retrograde signaling (RTG) genes whose gene products enable alpha-ketoglutarate production and further processing into glutamine and glutamate to sustain amino acid biosynthesis (Liu & Butow, 1999). However, signaling via the RTG branch requires some additional regulation which involves Rtg1, 2 and 3 and the negative regulators Mks1 and Bmh1 and 2. In nitrogen-rich conditions, TORC1 phosphorylates Mks1 that complexes with the Bmh proteins thus sequestering Rtg1 and 3 in the cytoplasm. In contrast, nitrogen depletion causes reduced phosphorylation of Mks1 mediated by PP2A, so that the former complexes with Rtg2, consequently releasing Rtg1 and 3 into the nucleus where they act as transcriptional activators (Broach, 2012; Dilova et al., 2004). Again, we chose a reduced representation where TORC1 phosphorylates Mks1 and active PP2A dephosphorylates Mks1. Rtg1,3 is phosphorylated unless dephosphorylated Mks1 and Rtg2 are present. Then Rtg1,3 becomes dephosphorylated in the model and activates RTG transcription. TORC1-mediated phosphorylation of Sfp1 results in the transcription factor's nuclear translocation where it induces ribosomal protein and ribosome biogenesis gene expression (Lempiäinen et al., 2009; Marion et al., 2004). There is also a negative feedback mechanism in which phosphorylated Sfp1 negatively regulates the phosphorylation state of Sch9 (Lempiäinen et al., 2009). However, this negative feedback is not implemented in the model.

The described signaling activity may be valid for conditions in which enough glucose is available, and cells are not exposed to any stress factors. However, under glucose depletion, both branches downstream of TORC1, namely the PP2A and the Sch9 branch, show no or only little activity which is probably caused by crosstalk with the Snf1 pathway (Hughes Hallett et al., 2014). In this model, it is implemented in that way that phosphorylated Snf1 inhibits TORC1 activity as well as it phosphorylates Tap42.

## Crosstalk

To enable an efficient and fine-tuned adaption to environmental conditions such as different nutrient availabilities, interaction of the induced signaling pathways is required to integrate information. Although we integrated some of these crosstalk mechanisms we considered relevant for the model, many more pathway interactions were reported which highlights the fine-tuned regulation of integrating environmental changes. (Shashkova et al., 2015)

## Dynamics of the Boolean model indicate either a model gap or rate differences in the pathways.

When implementing the crosstalk, we found that the dynamics did not operate according to literature in contrast to the steady state result. In contrast to the literature, Adr1 was inactivated by PKA instead of Snf1(Cherry et al. 1989) and PKA acted as the main regulator of Rim15 instead of Sch9 (Ivo Pedruzzi et al. 2003). This could either indicate that these pathways may not operate on the same time scale or the complexity of the pathways is not equally known. When iterating over discrete time steps, one does not consider time but the complexity of the modeled pathways. For instance, if a pathway is well described and can be model in great detail, it takes many iterations to reach a steady state. In contrast, a poorly understood pathway may need very few iterations to reach the steady state although, in reality, signaling via the poorly understood pathway may take more steps than via the well-annotated pathway. In this work, a synchronous modeling scheme was used, meaning that at each iteration the state vectors are updated simultaneously as there is little information on the order and duration of state transitions available (Garg et al. 2008). In these cases, it is hard to say if the simulations reflect the reality, indicates that the PKA pathway is less understood than the Snf1 pathway or if the discrepancy is a result of a difference in rates between the pathways. Either way, these results highlight the lack of understanding of the dynamics of signal transduction in nutrient signaling pathways.

## Knock out of major signaling components reveals question marks about reported crosstalk mechanisms.

In the deletion experiments in the Boolean model during high nutrient availability, the simulation of Reg1 knockout showed almost the same effect on the SNF1 pathway as nutrient depletion. Only Adr1 activity was not affected which opposes the observations by Dombek et al. (1999) that described constitutive ADH2 expression in Reg1 mutant cells (Dombek et al. 1999). Since Adr1 is the main regulator of ADH2 activity, the previously discussed inhibition of Adr1 activity by active PKA (Cherry et al. 1989) may be the reason for these contradicting results and thus, the relevance of this crosstalk may be questioned.

In literature the Snf1 knockout is described to have a phenotype resembling over activation of PKA, however in our simulated deletion experiments in the Boolean model during low nutrient availability, AC was activated but the Krh proteins inhibit PKA if no glucose is present. PKA activation is a fine-tuned process that requires more complexity such as high cAMP concentrations upon Krh activation (T. Peeters et al., 2006) which could not be modeled using our Boolean approach.

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