Regulated repression, and not activation, governs the cell fate promoter controlling yeast meiosis

Janis Tam¹ and Folkert J. van Werven¹,²

¹ Cell Fate and Gene Regulation Laboratory, The Francis Crick Institute, 1 Midland Road, London, NW1 1AT, UK

² Corresponding author: folkert.vanwerven@crick.ac.uk
Abstract:
Intrinsic signals and cues from the external environment drive cell fate decisions. In budding yeast, the decision to enter meiosis is controlled by nutrient and mating-type signals that regulate expression of the master transcription factor for meiotic entry, IME1. How nutrient signals control IME1 expression remains poorly understood. Here we show that IME1 transcription is regulated by multiple sequence-specific transcription factors that mediate association of Tup1-Cyc8 co-repressor to its promoter. We find that at least eight transcription factors bind the IME1 promoter when nutrients are ample. Remarkably, association of these transcription factors is highly regulated by different nutrient cues. Mutant cells lacking three transcription factors (Sok2/Phd1/Yap6) displayed reduced Tup1-Cyc8 association, increased IME1 expression and earlier onset of meiosis. Our data demonstrate that the promoter of a master regulator is primed for rapid activation while repression by multiple transcription factors mediating Tup1-Cyc8 recruitment dictates the fate decision to enter meiosis.

Key words:
IME1, Tup1, Cyc8, transcription, promoter, meiosis, cell-fate, gametogenesis, sporulation, repression, co-repressor, PKA, TORC1, Sok2, Yap6 and Phd1
Introduction:

The choice of whether to differentiate into another cell type is directed by multiple cell intrinsic and extrinsic environmental factors. These cues signal to master regulatory genes, which in turn control the initiation of cell differentiation programs. As a result, multiple signals are transformed into a binary decision: whether or not to undergo cell differentiation. How signalling cues coordinate a cell fate outcome has important implications for the understanding of development and diseases such as cancer.

Diploid budding yeast cells undergo an irreversible differentiation program called gametogenesis or sporulation during which a diploid cell gives rise to four haploid spores. The yeast gametogenesis program is characterized by one round of DNA replication and recombination, two consecutive chromatin segregation events called meiosis followed by spore formation \(^1\). As a result, an ascus with four haploid spores is produced. In yeast, the decision to enter meiosis is controlled by a master regulatory transcription factor named inducer of meiosis 1, \textit{IME1} \(^2,3\). In the absence of \textit{IME1}, cells cannot enter meiosis and produce gametes. Thus, understanding how \textit{IME1} is regulated is key to understanding how the decision to enter meiosis or gametogenesis is made.

Transcriptional control mechanisms regulate \textit{IME1} expression. The \textit{IME1} gene has an unusually large promoter (over 2.2 kilobases) that integrates multiple signals to control \textit{IME1} expression \(^4\). Nutrient and mating type signals ensure that \textit{IME1} is only expressed in the appropriate nutrient environment and in the correct cell type. \textit{IME1} is expressed in cells harbouring opposite mating-type loci (\textit{MATa} and \textit{MATα}) \(^5,6\). In
cells with a single mating type (MATa or MATα), the transcription factor Rme1 is expressed and induces transcription of the long noncoding RNA (lncRNA) IRT1, which in turn transcribes through the IME1 promoter and thereby represses IME1 expression. In MATα/α diploid cells, a second lncRNA upstream of IRT1 named IRT2 interferes with IRT1 transcription forming a positive feedback loop by which Ime1 promotes its own expression.

In addition to mating-type control, environmental cues also play a critical role in regulating IME1 expression. In order to induce IME1 transcription, diploid cells must be starved for glucose and nitrogen, and cells need to be respiring. The glucose and nitrogen signals integrate at the IME1 promoter. Several sequence elements in the IME1 promoter are important for control of IME1 transcription. For example, a distinct sequence element mediates IME1 repression by glucose signalling, while other parts of the promoter respond to nitrogen availability. Notably, the transcription factor Sok2 controls IME1 promoter activity via the glucose responding element. In addition, multiple other transcription factors contribute to regulation of IME1 transcription. Over 50 transcription factors have a conserved consensus site in the IME1 promoter. A genome-wide reporter screen revealed that about 30 transcription factors may directly or indirectly control IME1 transcription. How different transcription factors and functional elements of the IME1 promoter interact to control IME1 expression and thus regulate the decision to enter meiosis is not well understood.

The nutrient control of IME1 expression is mediated by multiple signalling pathways including PKA, TOR complex 1 (TORC1), AMP-activated protein kinase (AMPK) and...
mitogen-activated protein kinase (MAPK)\textsuperscript{15-17}. Inhibiting two signalling pathways, PKA and TORC1, is sufficient to induce IME\textit{1} expression in cells exposed to a nutrient rich environment where IME\textit{1} expression is normally repressed\textsuperscript{16}. Thus PKA and TORC1 signalling is essential for controlling IME\textit{1} expression and hence the decision to enter meiosis (Figure 1A). Previously, we showed that Tup1 represses the IME\textit{1} promoter under nutrient rich conditions. Tup1 is part of the Tup1-Cyc8 co-repressor complex, which is involved in repression of more than 300 gene promoters in yeast\textsuperscript{18-20}. During starvation, however, Tup1 disassociates from the IME\textit{1} promoter and IME\textit{1} transcription is concomitantly induced. Tup1 binding to the IME\textit{1} promoter is controlled by PKA and TORC1\textsuperscript{16}. When both signalling pathways are inhibited Tup1 dissociates from the IME\textit{1} promoter. Thus understanding how Tup1-Cyc8 binds to the IME\textit{1} promoter may reveal how nutrient signalling controls IME\textit{1} expression and consequently how cells make the decision to enter meiosis.

Here we set out to investigate how the Tup1-Cyc8 co-repressor complex regulates IME\textit{1} transcription. In short, we found that regulated repression by multiple sequence specific transcription factors mediating the association of Tup1-Cyc8 with the IME\textit{1} promoter is the means by which IME\textit{1} transcription is controlled. Our data further indicate that nutrient cues highly regulate the association of Tup1-Cyc8 interacting transcription factors with the IME\textit{1} promoter, which is key to regulating IME\textit{1} expression and thus the cell fate decision to enter meiosis in yeast. Our work provides a framework to understand how nutrient signals integrate at a cell fate promoter and thereby control a developmental decision in yeast.

\textbf{Results:}
To gain insight into how Tup1 association with the *IME1* promoter is controlled, we first examined whether the region of the promoter where Tup1 binds contains key regulatory elements for *IME1* transcription. Previously, we found that Tup1 associates between 800 and 1400 base pairs (bp) upstream of the *IME1* translation start site. If the region of the *IME1* promoter where Tup1 binds is also important for *IME1* activation, then deleting that part of the promoter should affect the onset of meiosis. We generated six truncation mutants with a 200 bp interval in the *IME1* promoter and examined the ability of these mutants to undergo meiosis (Figure 1B). The largest truncation mutant that underwent meiosis with comparable kinetics as wild-type cells harboured 1400 bp of the *IME1* promoter (*pIME1*(-1400-2315Δ)) indicating that this region harbours the regulatory elements required for complete activation of the *IME1* promoter (Figure 1B). In addition, we found that meiosis in *pIME1*(-800-2315Δ) was completely impaired, whereas *pIME1*(-1200-2315Δ) had a much milder effect on meiosis. The result suggests that a region between -800 and -1200 bp harbours regulatory elements essential for *IME1* promoter function. We also made smaller truncations in the promoter and found that the region between -800 and -850 bp contains regulatory elements important for *IME1* activation because a large fraction of *pIME1*(-850-2315Δ) cells underwent meiosis (Supplementary Figure 1). In conclusion, the region important for Tup1 binding to the *IME1* promoter is also required for transcription of *IME1*.

Tup1 forms a complex with Cyc8. The Tup1-Cyc8 co-repressor complex is conserved and plays various roles in regulating gene transcription. Like Tup1, Cyc8 has also been implicated in regulation of *IME1* expression. To investigate how Cyc8 regulates *IME1* expression, we first determined Cyc8 binding with the
**IME1** promoter under nutrient rich conditions. We found that Cyc8 peaked in the same region as Tup1 in the **IME1** promoter (Figure 1C and 1D). These data indicate that Tup1 is in a complex with Cyc8 at the **IME1** promoter.

Various models for Tup1-Cyc8 mediated repression of target gene promoters have been described. It has been proposed that Tup1-Cyc8 primarily regulates promoters by masking transcription factors from recruiting co-activators. Thus, Tup1-Cyc8 interacts with transcription factors with activation potential at promoters. We hypothesized that if Tup1-Cyc8 represses the **IME1** promoter by shielding co-activators, then transcriptional activators should be at the promoter under repressive conditions. To test this, we measured under nutrient rich repressive conditions the association of a known transcriptional activator of **IME1**, Pog1. We found that Pog1, like Tup1-Cyc8, is indeed enriched at the **IME1** promoter under repressive conditions (Figure 1D). We conclude that Tup1-Cyc8 complex associates with the **IME1** promoter, and possibly masks transcriptional activators such as Pog1.

Various transcription factors have been implicated in controlling in **IME1** expression. To further examine whether transcriptional activators are readily present at the **IME1** promoter, we measured **IME1** expression after depletion of Tup1 or Cyc8. We reasoned that if Tup1-Cyc8 represses the **IME1** promoter by restraining activating transcription factors, then depletion of Tup1 or Cyc8 should allow activators present to concomitantly induce **IME1** transcription. We used the auxin inducible degron (AID) system (**TUP1-AID** and **CYC8-AID**) and treated cells with indole-3-acetic acid (IAA) to achieve rapid protein depletion in cells. Rapid and sustained depletion of Tup1 and Cyc8 was achieved within 30 minutes after IAA treatment (Figure 1E).
Strikingly, *IME1* transcript levels strongly increased concurrently, and were comparable to those in wild-type cells entering meiosis when *IME1* expression is typically at its peak (Figure 1F). These data show that Tup1-Cyc8 repressor complex is pivotal for repressing the *IME1* promoter under nutrient rich conditions. Our data further suggest that the default state of the *IME1* promoter is active due to the presence of transcriptional activators, which are restrained by Tup1-Cyc8 under repressive conditions.

Although the transcriptional activator Pog1 is already bound in repressive conditions the at *IME1* promoter, it is possible that other transcriptional activators factors associate with the *IME1* promoter after Tup1-Cyc8 dissociates. This may results in a delay between Tup1-Cyc8 depletion and activation of *IME1* transcription. We therefore decided to monitor *IME1* expression by single molecule RNA fluorescence in situ hybridization (smFISH). This technique can detect individual transcripts in single cells 27. We found that as soon as Tup1 was depleted, *IME1* transcripts were detected in single cells (Figure 1G, 1H, 1I, and Supplementary Figure 2A). An increase in *IME1* transcripts was detected 10 minutes after IAA treatment when Tup1 was partially depleted (Figure 1G). After 15 min of inducing Tup1 depletion, 5.3 *IME1* transcripts were detected per cell on average, and 12% of the cells (Tup1-AID+IAA) had more than 10 *IME1* transcripts compared to 2% in control cells (Figure 1H and 1I). At 30 minutes after IAA treatment, more than 55% of cells expressed more than 10 *IME1* transcripts. It is worth noting that the AID-tag fused to Tup1 had some effect on *IME1* expression in the absence of IAA as *IME1* transcript levels were increased by five-fold in *TUP1-AID* compared to wild-type cells (Figure 1H, 1I, and Supplementary Figure 2B and 2C). We conclude that there is little to no temporal
delay between Tup1-Cyc8 depletion and IME1 expression suggesting that transcriptional activators are bound or readily available for recruitment to the IME1 promoter.

The Tup1-Cyc8 complex also controls gene promoters by altering chromatin states. Specifically, Tup1-Cyc8 directly interacts with class I and II histone deacetylases, which in turn confer repression through deacetylation of nucleosomes. For example, repression of the FLO1 promoter is achieved by Tup1-Cyc8 mediated recruitment of Hda1 and Rpd3. In hda1Δ rpd3Δ cells significant derepression of the FLO1 gene can be observed. To examine whether histone deacetylases mediate Tup1-Cyc8 repression at the IME1 promoter, we generated single and double mutants of known Tup1-Cyc8 interacting histone deacetylases (Rpd3, Hda1, Hos1, and Hos2) and measured IME1 expression levels by smFISH (Figure 1J and Supplementary Figure 2C). Deletion of individual HDACs (rpd3Δ, hda1Δ, hos1Δ, and hos2Δ) did not increase expression of IME1. About 10% of rpd3Δhda1Δ cells expressed four or more IME1 transcripts, a marginal increase when compared to Tup1 depleted cells (Supplementary Figure 2C). Two other double mutants (rpd3Δhos1Δ and rpd3Δhos2Δ) displayed no detectable increase in IME1 expression. We conclude that HDACs that are known to interact with Tup1-Cyc8 play only a marginal role in repressing the IME1 promoter.

The Tup1-Cyc8 interacts with DNA sequence specific transcription factors to form co-repressor complexes at promoters. These transcription factors facilitate Tup1-Cyc8 association with promoters and thereby mediate repression of target genes. Our observation that Tup1-Cyc8 is the key repressor of the IME1 promoter...
prompted us to examine which transcription factors recruit Tup1-Cyc8 to the *IME1* promoter and how they control *IME1* transcription. First, we assembled a list of transcription factors previously reported to interact with either Tup1 or Cyc8. In addition, we examined the region of the *IME1* promoter (-600 to -1200 bp) where Tup1-Cyc8 binds, and scanned for sequence motifs among transcription factors known to interact with Tup1-Cyc8 (Figure 2A, Supplementary Figure 3A and 3B). We identified 13 candidate transcription factors that were known or implicated to interact with Tup1-Cyc8 and have binding sites in the *IME1* promoter (Figure 2A and Supplementary Figure 3A). We also included the transcription factor Sok2 in our analyses because it has been proposed to interact with Tup1-Cyc8 and Sok2 is known to directly repress *IME1* transcription. After the curation of the list of transcription factors, we subsequently measured the binding of under nutrient rich conditions by epitope tagging each transcription factor and performing ChIP. Remarkably, eight transcription factors displayed enrichment at the *IME1* promoter (Figure 2B). As expected, a known regulator of the *IME1* promoter, Sok2, was strongly enriched. In addition, Phd1 (a paralog of Sok2) and Yap6 also displayed strong enrichment (Figure 2B). The transcription factor Sut1, which is known to interact with Cyc8, was also enriched. The transcription factors Mot3, Sko1, Nrg1, and Nrg2 displayed a milder enrichment, but their binding was above background levels. For all transcription factors that displayed enrichment, we also assessed their binding to other parts of the *IME1* promoter (Figure 2C). Transcription factors exclusively co-localised with Tup1-Cyc8 in the same region of the IME1 promoter around 1000 bp upstream of the *IME1* start codon. Thus, at least eight transcription factors that are known or have been implicated to interact with Tup1-Cyc8 associate with the *IME1* promoter.
Next, we examined whether the candidate transcription factors are responsible for recruiting Tup1 to the *IME1* promoter. We reasoned that candidate transcription factors should associate independently of Tup1-Cyc8 to the *IME1* promoter, while the binding of transcription factors and Tup1-Cyc8 should depend on the presence of specific sequence motifs (Figure 3A). First, we depleted Tup1 (*TUP1*-AID+IAA), and measured binding for a subset of the bound transcription factors (Supplementary Figure 4A). Except for Sko1, the binding of the transcription factors to the *IME1* promoter was not affected by Tup1 depletion (Figure 3B). Thus, multiple transcription factors known to interact with Tup1-Cyc8 associate with the *IME1* promoter independently of Tup1-Cyc8. Second, we examined whether the candidate transcription factors contribute to Tup1-Cyc8 recruitment. We mutated putative binding sites of the transcription factors that showed binding to the *IME1* promoter. To do so, we generated a construct containing the full-length promoter, followed by sfGFP and the *IME1* gene (*pIME1-WT*). Subsequently, we mutated 103 nucleotides distributed across a region of 400 bp in the *IME1* promoter (*pIME1-bsΔ*) (Figure 3C and Supplementary Figure 4B). We integrated the constructs in the *TRP1* locus in cells harbouring a deletion of the endogenous *IME1* gene and promoter sequence. Tup1 association with the *IME1* promoter was nearly completely lost in *pIME1-bsΔ* cells (Figure 3C). As expected, control cells (*pIME1-WT*) did not show a decrease in Tup1 binding. Finally, we assessed how *IME1* expression and the onset of meiosis is affected in *pIME1-bsΔ* cells. Surprisingly, *IME1* expression in *pIME1-bsΔ* cells was reduced, suggesting the regulatory elements essential for Tup1-Cyc8 recruitment are also important for *IME1* activation (Figure 3D). In conclusion, DNA sequence motifs
of transcription factors bound to the IME1 promoter are required for Tup1-Cyc8 association with the IME1 promoter.

So far, our analyses of the IME1 promoter showed that multiple transcription factors and multiple binding sites are essential for Tup1-Cyc8 recruitment. Next, we examined how the different transcription factors control IME1 expression and mediate Tup1-Cyc8 recruitment under nutrient rich conditions. First, we assessed how the paralogs Sok2 and Phd1 control IME1 expression. sok2Δ cells displayed a negligible increase in IME1 expression (average transcripts per cell: 0.6 for sok2Δ versus 0.3 for control) (Figure 4A and 4B). In the sok2Δphd1Δ double mutant, IME1 expression was marginally increased (average transcripts per cell: 2.2) and about 5% of cells displayed more than 10 transcripts per cell suggesting that Sok2 and Phd1 play redundant roles in tightly repressing the IME1 promoter (Figure 4A and 4B). We also measured IME1 expression in yap6Δ cells or in combination with the sok2Δphd1Δ mutation. IME1 repression was not affected in cells containing yap6Δ, but in the sok2Δphd1Δyap6Δ triple deletion mutant IME1 expression was increased (average transcripts per cell: 2.8 for sok2Δphd1Δyap6Δ versus 2.2 for sok2Δphd1Δ) (Figure 4A and B). About 8% of sok2Δphd1Δyap6Δ cells expressed more than 10 IME1 transcripts per cell. It is worth noting that IME1 transcript levels in sok2Δphd1Δyap6Δ cells were much lower than in cells depleted for Tup1 (Figure 1F and 1H) - suggesting that additional transcription factors contribute to IME1 repression.

Our data demonstrate that Sok2, Phd1 and Yap6 associate with the IME1 promoter and contribute to IME1 repression in nutrient rich conditions. Yet, IME1 expression
was reduced in cells with DNA sequence motifs mutated \((pIME1-bs\Delta)\). One explanation is that the mutated binding sites in \(pIME1-bs\Delta\) important for Tup1-Cyc8 recruitment also facilitate binding for transcriptional activators. Another possibility is that transcription factors important for Tup1-Cyc8 recruitment are also required for \(IME1\) activation. To discriminate between both possibilities, we generated a construct that contained binding sites for Sok2, Phd1, and Yap6 \((pIME1-spy)\), while the other transcription factor binding sites remained mutated (Figure 4C and Supplementary Figure 5). By combining \(pIME1-spy\) with \(sok2\Delta phd1\Delta yap6\Delta\), we could then determine whether Sok2, Phd1, and Yap6 are important for \(IME1\) activation or repression. Tup1 binding was restored in cells harbouring \(pIME1-spy\) (Figure 4C). Furthermore, Yap6, Sok2, and Phd1 were enriched at the \(IME1\) promoter in \(pIME1-spy\) cells but their binding was reduced compared to the wild-type promoter - suggesting that there are additional binding sites (Figure 4C). Next, we measured \(IME1\) expression in wild-type and \(sok2\Delta phd1\Delta yap6\Delta\) mutant cells harbouring \(pIME1-spy\) (Figure 4D). We found that \(IME1\) expression was further derepressed in cells harbouring both \(pIME1-spy\) and \(sok2\Delta phd1\Delta yap6\Delta\) compared to cells expressing the three transcription factors (Figure 4D and 4E). About 17% of cells harbouring \(pIME1-spy\) and \(sok2\Delta phd1\Delta yap6\Delta\) expressed more than 10 \(IME1\) transcripts per cell. As expected, the \(sok2\Delta phd1\Delta yap6\Delta\) had only a mild effect on \(IME1\) levels in cells expressing \(pIME1-WT\) or \(pIME1-bs\). We conclude that Sok2, Phd1 and Yap6 are important for repression of the \(IME1\) promoter, and play little role in \(IME1\) activation.

The Tup1-Cyc8 complex dissociates from the \(IME1\) promoter in cells exposed to nutrient starvation \(^{16}\). We hypothesized that transcription factors interacting with
Tup1-Cyc8 at the IME1 promoter control Tup1-Cyc8 dissociation during IME1 activation. For example, one possibility is that the physical interaction between Tup1-Cyc8 and transcription factors is altered during activation IME1. Alternatively, during activation of the IME1 promoter transcription factors dissociate together with Tup1-Cyc8. To examine this, we measured the binding of the eight transcription factors during activation of the IME1 promoter. In order to induce IME1 expression and meiotic entry in a synchronous manner in all cells, we typically grow cells in rich medium conditions containing glucose until saturation, then shift and grow cells in pre-sporulation medium containing acetate. Cellular respiration is required for meiosis, and growth in medium with acetate but lacking glucose ensures that cells are respiring and not subject to repressive glucose signalling to the IME1 promoter.

Subsequently, cells are starved by shifting them to sporulation medium (0.3% acetate), which induces IME1 transcription and drives meiotic entry. First, we measured the binding of Tup1 and Pog1 at the IME1 promoter prior to induction of IME1 (0 hours in SPO), and during meiotic entry when IME1 is induced (4 hours in SPO) (Figure 5A). Both Pog1 and Tup1 were enriched at 0 hours in SPO prior to IME1 induction. As expected, during meiotic entry (4 hours in SPO) Tup1 dissociated from the IME1 promoter completely while Pog1 binding was maintained albeit to a reduced level (Figure 5A). Next, we determined how binding of the different transcription factors with the IME1 promoter was regulated. We found that all eight transcription factors, were enriched at the IME1 promoter prior to induction of IME1 (Figure 5A). Strikingly, five transcription factors showed no binding to the IME1 promoter upon entry into meiosis, while three transcription factors (Yap6, Phd1, and Nrg1) displayed marginal enrichment (4 hours in SPO). These data suggest that Tup1-Cyc8 dissociates from the IME1 promoter due to the loss of binding of multiple
transcription factors. In conclusion, activation of *IME1* transcription correlates with the dissociation of transcription factors important for Tup1-Cyc8 recruitment, while a transcriptional activator remains present at the *IME1* promoter.

We showed that Sok2, Phd1 and Yap6 were strongly enriched at the *IME1* promoter under nutrient rich conditions and prior to meiotic entry (Figure 2B, Figure 5A). In addition, in *sok2Δphd1Δyap6Δ* cells, *IME1* expression was partially de-repressed when nutrients were ample (Figure 4A and 4B). These observations indicate that Sok2, Phd1 and Yap6 are important transcription factors for *IME1* repression. Next, we investigated how the three transcription factors control Tup1-Cyc8 recruitment in different nutrient conditions. Specifically, we examined Tup1 binding to the *IME1* promoter in the absence of the three transcription factors in cells grown in rich medium containing glucose (YPD) and in acetate-containing medium prior to meiotic entry. We found that Tup1 binding to the *IME1* promoter was not affected in rich medium containing glucose in *sok2, phd1*, and *yap6* single/double/triple deletion mutants, which is in line with *IME1* expression data described in (Figure 5B, 4A, and 4B). This suggests that other transcription factors contribute to *IME1* repression via Tup1-Cyc8 when glucose is used by cells as the carbon source. In contrast, prior to meiotic entry (0h in SPO) Tup1 binding was significantly reduced in *sok2*Δ and *sok2Δphd1Δ* cells, but not in *yap6Δ* and *phd1Δ* cells (Figure 5B). Strikingly, Tup1 association with the *IME1* promoter was reduced to nearly background levels in *sok2Δyap6Δ* and *sok2Δphd1Δyap6Δ* cells at 0 hours in SPO. *IME1* expression inversely correlated with Tup1-Cyc8 recruitment to the *IME1* promoter (Figure 5C). For example, *sok2Δyap6Δ* cells displayed significant de-repression of *IME1* expression prior to entry into meiosis compared to WT control cells. Likewise, cells
harbouring sok2Δphd1Δ or sok2Δphd1Δyap6Δ also displayed de-repression of IME1 expression at 0 hours in SPO (Figure 5C). Finally, we examined how Sok2, Phd1, and Yap6 mediated Tup1-Cyc8 recruitment to IME1 promoter is important for meiosis. In general, the onset of meiosis in the different mutants correlated well with IME1 expression levels (Figure 5D). Cells harbouring the sok2Δ or sok2Δphd1Δ underwent meiosis much faster than wild-type cells. For example after 3 hours in SPO, 50% of sok2Δ cells completed at least one meiotic division, while this took more than 6 hours in wild-type cells. There was little effect on the onset of meiosis in the yap6Δ or yap6Δphd1Δ mutants. In sok2Δyap6Δ and sok2Δphd1Δyap6Δ cells the kinetics of meiosis was slightly faster than sok2Δphd1Δ cells. Approximately 50% of cells underwent meiotic divisions within 2 hours in SPO for sok2Δphd1Δyap6Δ cells compared to 3 hours for sok2Δphd1Δ cells.

During meiotic entry (4 hours in SPO), Yap6, Phd1 and Nrg1 showed some enrichment at the IME1 promoter suggesting that they could contribute to activation of IME1 transcription. Therefore, we also analysed how the onset of meiosis is affected in sok2Δphd1Δyap6Δnrg1Δ cells (Supplementary Figure 6). We found no difference in meiosis between sok2Δphd1Δyap6Δnrg1Δ and sok2Δphd1Δyap6Δ cells suggesting that these transcription factors do not contribute to activation of IME1 transcription in cells induced to enter meiosis synchronously. Taken together, we conclude that Sok2, Phd1, and Yap6 direct Tup1-Cyc8 association to the IME1 promoter to ensure timely expression of IME1 in cells grown in acetate containing medium. Our data suggest that the IME1 promoter is regulated by multiple Tup1-Cyc8 co-repressor complexes.
How do nutrient signals regulate the association and dissociation of Tup1-Cyc8 recruiting transcription factors with the *IME1* promoter? While it is known that nutrients mediate PKA and TORC1 signalling pathways and thereby control *IME1* expression, little is known about the mechanisms that mediate PKA and TORC1 signalling at the *IME1* promoter. Regulated localization or abundance of transcription factors are prevalent mechanisms for controlling gene expression via PKA and TORC1. Among the transcription factors that associate with the *IME1* promoter, Sok2 protein abundance is regulated by glucose signalling via the Ras/PKA pathway. To gain insight into how Tup1-Cyc8 recruiting transcription factors (Sok2, Phd1, and Yap6) dissociate from the *IME1* promoter, we sought to investigate whether their abundance or localization is altered during entry into meiosis. First, we examined protein expression levels in exponentially grown cells (E), cells grown to saturation (S), and in cells just prior to and during meiotic entry (0h and 4h SPO). The levels of Sok2, Phd1 and Yap6 as well as Cyc8 were reduced in cells grown to saturation compared to exponentially growing cells (Figure 6A). However, in cells grown until saturation, Sok2, Phd1 and Yap6 were bound to the *IME1* promoter (Supplementary Figure 7). Interestingly, reduced Cyc8 levels mirrored the reduced binding of Cyc8 at the *IME1* promoter (Figure 6A and Supplementary Figure 7). Importantly, Sok2, Phd1 and Yap6 abundance was not altered in cells between the 0 and 4 hour time points (in SPO) when *IME1* expression is induced. These data indicate that cellular changes in Sok2, Phd1 and Yap6 abundance are unlikely to explain the dissociation of these transcription factors from the *IME1* promoter during entry into meiosis. To determine whether these transcription factors are evicted from the *IME1* promoter due to nuclear export, we determined the cellular localization for each transcription factor. We fused
mNeongreen to Sok2, Phd1, Yap6, Tup1 and Cyc8 (Supplementary Figure 8A). As expected, Sok2, Phd1, and Yap6 were concentrated in the nucleus. Neither the nuclear-to-cytoplasmic ratios nor total protein abundance in the nucleus were significantly altered in cells prior to (0h in SPO) and during entry into meiosis (4h in SPO) (Figure 6B and Supplementary Figure 8B). Furthermore, the overall localization of Tup1 and Cyc8 was also not altered during meiotic entry (Figure 6B and Supplementary Figure 8B). We conclude that cellular changes in protein abundance or localization cannot solely account for the dissociation of Tup1-Cyc8 and transcription factors from the *IME1* promoter during entry in meiosis.

There is evidence that signalling kinases can act locally at gene promoters. For example, Tpk1 and Tpk2 kinases of PKA can associate with specific gene promoters and regulate transcription locally 45. We reasoned that our measurements of transcription factor abundance and localization may be masked if PKA and TORC1 signalling is heterogeneous within cells. Therefore, perhaps inhibiting PKA and TORC1 altogether could reveal how both signalling pathways regulate transcription factors important for repressing the *IME1* promoter. To inhibit PKA we used the *tpk1*-as allele previously described, while we used rapamycin to inhibit TORC1 16. Strikingly, Sok2, Phd1 and Yap6 protein levels were reduced between five to ten-fold when PKA was inhibited (*tpk1*-as + NMPP1) (Figure 6C). Inhibiting TORC1 activity with rapamycin also lowered Yap6 and Phd1, but not Sok2 levels. We conclude that inhibiting PKA and TORC1 affects the abundance of transcription factors important for repressing the *IME1* promoter, and coincides with Tup1 disassociation and activation of *IME1* transcription as described previously 16. Our data suggest that the
association of transcription factors with the IME1 promoter may be regulated locally by PKA and TORC1 signalling.

Our data indicate that at least eight transcription factors that are known or implicated to interact with Tup1 or Cyc8, associate with the IME1 promoter (Figure 2B). Motif analyses revealed that there are 52 corresponding binding sites present in the region of the IME1 promoter where Tup1-Cyc8 associates (Supplementary Figure 3). In addition, there are multiple binding motifs for almost every transcription factor present suggesting that multiple copies of each transcription factors bind to the IME1 promoter (Supplementary Figure 3A). Why is there need for so many binding sites in the IME1 promoter? One possibility is that the transcription factors are important for facilitating Tup1-Cyc8 recruitment under different nutrient conditions. With this logic, the repression of the IME1 promoter can be maintained under various nutrient conditions, and will only be fully activated when all the nutrient signalling requirements are met. In agreement with this model, IME1 expression was only marginally increased in sok2Δ phd1Δ yap6Δ cells grown in the presence of ample nutrients with glucose as the carbon source (YPD), while the IME1 promoter was nearly completely de-repressed in sok2Δ phd1Δ yap6Δ cells grown in acetate-containing medium (Figure 4A, 4B, 5B and 5C). To examine how the different transcription factors respond to nutrient signalling at the IME1 promoter more systematically, we measured their association under different nutrient conditions (Figure 7A). We grew cells until the pre-sporulation stage, and subsequently shifted cells to sporulation medium (SPO) (1), SPO plus 2% glucose (2), YP (3), and YP plus 2% glucose (YPD) (4). First, we measured Tup1 association with the IME1 promoter. We found that in SPO plus glucose, Tup1 binding to the IME1 promoter
was partially restored (Figure 7B). The association of Tup1 with the \textit{IME1} promoter was further increased in YP and was the highest in YPD growth medium. The transcriptional activator of \textit{IME1}, Pog1, was enriched in all four nutrient conditions, but at higher levels in YP and YPD (Figure 7B). Interestingly, transcription factors important for Tup1-Cyc8 recruitment to \textit{IME1} promoter responded to nutrient signals in distinct ways (Figure 7C). For example, Yap6, Sok2, Sko1 and Nrg1 did not associate with the \textit{IME1} promoter in response to glucose, but their binding was restored due to nutrient cues present in YP. Phd1 binding partially recovered in the presence of glucose, and showed the strongest enrichment in cells exposed to YP and YPD. The carbon source, glucose, but not YP, induced association of Mot3 and Nrg2 with the \textit{IME1} promoter, while Sut1 association with the \textit{IME1} promoter was restored in YPD only.

Given that Sok2, Phd1, and Yap6 were strongly enriched in cells exposed to YP medium (Figure 7C), we hypothesized that Tup1-Cyc8 association with the \textit{IME1} promoter is affected in \textit{sok2Δphd1Δyap6Δ} cells in YP, but not in SPO containing glucose. We therefore examined how Tup1-Cyc8 association with \textit{IME1} promoter was affected in \textit{sok2Δphd1Δyap6Δ} cells under different nutrient conditions. Indeed, in \textit{sok2Δphd1Δyap6Δ} cells, Tup1 binding was detected in SPO plus glucose, but not in YP medium (Figure 7D). These data indicate that Sok2, Phd1, and Yap6 are important for mediating Tup1-Cyc8 association in YP, while other transcription factors are required for glucose signalling to the \textit{IME1} promoter (Figure 7E). In summary, our analyses revealed that the association of one set of transcription factors (i.e. Mot3 and Nrg2) with the \textit{IME1} promoter is induced by glucose signalling, while another set of transcription factors (i.e. Yap6, Sok2, Phd1, Sko1, and Nrg1)
was recruited to the *IME1* promoter in cells exposed to YP. Thus, only when all the required nutrient signalling pathways are repressed, all transcription factors interacting with Tup1-Cyc8 dissociate from the *IME1* promoter allowing activation of *IME1* transcription. Taken together, we propose that transcription factors important for Tup1-Cyc8 recruitment to the *IME1* promoter respond to different environmental cues to ensure tight control of *IME1* expression and thereby regulate the fate decision to enter meiosis in yeast.

**Discussion:**

Our study of the *IME1* promoter sheds new light on how the fate decision to enter meiosis is regulated. We report that the Tup1-Cyc8 complex together with multiple sequence-specific transcription factors constitute the essential components that control repression of the *IME1* promoter. The decision to enter meiosis and produce gametes is remarkably simple in yeast: environmental signals regulate the association and disassociation of transcription factors that recruit Tup1-Cyc8 to the *IME1* promoter. We propose that multiple transcription factors ensures repression of *IME1* expression under various environmental conditions and thus establishes tight control of entry into meiosis in yeast.

**Model for Tup1-Cyc8 mediated regulation of the *IME1* promoter**

Repression of *IME1* transcription, and not activation, is highly regulated. Depletion of either Tup1 or Cyc8 completely de-repressed *IME1* expression (Figure 1). Remarkably, there was little or no delay between depletion of Tup1 and *IME1* transcription in the presence of ample nutrients. From these two observations, we can infer two important features of the *IME1* promoter. First, the transcriptional
activators are bound to the *IME1* promoter or readily available prior to activation of *IME1* transcription. Second, transcriptional activators do not require nutrient or environmental signalling to activate the *IME1* promoter. Indeed, we found that the activator Pog1 is bound to the *IME1* promoter prior to activation. Pog1 does not have clear DNA binding domain, and thus Pog1 likely interacts with other proteins to associate with the *IME1* promoter. A *pog1* mutant has only a mild effect on *IME1* expression indicating that there must be other transcriptional activators controlling *IME1* transcription\(^7\). Several transcriptional activators have been implicated in regulating *IME1* transcription that have not been linked with Tup1-Cyc8\(^12\).

How does Tup1-Cyc8 control the *IME1* promoter? The Tup1-Cyc8 regulates transcription of a subset of promoters in yeast\(^18,46\). Several mechanisms have been described for Tup1-Cyc8 mediated gene repression\(^21,23,24\). For example, Tup1-Cyc8 interacts with HDACs, which in turn facilitate repression of promoters by deacetylating nucleosomes. However, we find that deletion mutants of HDACs have little effect on *IME1* expression and do not mimic *IME1* expression levels detected in Tup1 or Cyc8 depleted cells. Our data are largely consistent with a model in which Tup1-Cyc8 masks or shields activating transcription factors from recruiting co-activators at promoters\(^25\). In line with Tup1-Cyc8 depletion experiments described in this work, *IME1* was among the genes that showed increased transcription upon rapid depletion of Tup1-Cyc8 from nucleus\(^25\). Furthermore, we show that the transcription activator Pog1 is bound to the *IME1* promoter prior to activation, and remains bound during activation of *IME1* transcription.
Transcription factors that interact with Tup1-Cyc8 can converted into transcriptional activators in the absence of Tup1-Cyc8. We showed that multiple (at least eight) transcription factors that are known to interact with Tup1-Cyc8 associate with the IME1 promoter. However, our data suggest these transcription factors are important for facilitating Tup1-Cyc8 binding but play little role in IME1 transcriptional activation. Multiple lines of evidence indicate that Tup1-Cyc8 acts predominantly as a co-repressor at the IME1 promoter. First, almost all transcription factors involved in Tup1-Cyc8 recruitment dissociate from the IME1 promoter upon activation of IME1 transcription (Figure 5A, SPO 4h). Second, deleting multiple transcription factors led to activation, not repression of IME1 transcription. For example, cells lacking four of the transcription factors that showed the strongest enrichment with the IME1 promoter (sok2Δphd1Δyap6Δnrg1Δ) displayed an earlier onset of meiosis (Supplementary Figure 6). We cannot exclude that the Tup1-Cyc8 recruiting transcription factors can function as transcriptional activators in some conditions. Indeed, Yap6 and Sok2 have both been implicated as activators of transcription at some promoters. In the context of the IME1 promoter, however, transcription factors mediating Tup1-Cyc8 recruitment and transcriptional activators are likely not the same. Each transcription factor likely has a designated function in either repression or activation of IME1 transcription. We propose that multiple transcription factors ensure that Tup1-Cyc8 co-repressor is bound to the IME1 promoter. The Tup1-Cyc8 co-repressor complexes, in turn, mask transcriptional activators (which are different from Tup1-Cyc8 recruiting transcription factors) and prevent them from recruiting co-activators to induce IME1 transcription.
The regulatory logic of employing multiple sequence specific transcription factors to repress the IME1 promoter

Why are multiple transcription factors required for recruiting Tup1-Cyc8 to the IME1 promoter? Expression of IME1 only occurs when the cells are starved for nitrogen and glucose. Under other environmental conditions, the IME1 promoter must be repressed to prevent cells from inappropriately entering meiosis and form gametes. We propose that multiple transcription factors ensure IME1 repression under various environmental conditions. First, we found that distinct sets of transcription factors associate with the IME1 promoter in different nutrient environments (Figure 7).

Second, deleting three transcription factors (Sok2, Phd1, and Yap6) led to very mild IME1 expression in rich medium containing glucose, but IME1 was almost fully expressed in cells grown in an acetate containing medium (Figure 4A, 4B and 5C). Thus, additional transcription factors facilitate Tup1-Cyc8 association with the IME1 promoter in rich medium containing glucose.

How nutrient signalling controls the association of transcription factors directing Tup1-Cyc8 to the IME1 promoter is key to dissecting how meiotic entry is regulated. We previously showed that inhibiting PKA and TORC1 is sufficient to drive entry into meiosis despite that cells were exposed to a nutrient rich medium. Here we showed that inhibiting PKA and TORC1 lowers the abundances of transcription factors important for Tup1-Cyc8 recruitment to the IME1 promoter (Sok2, Phd1 and Yap6) (Figure 6C), however the mechanisms remains unclear. One possibility is that PKA or TORC1 phosphorylation controls the stability of Sok2, Phd1 and Yap6. Indeed, Sok2 is a direct substrate of PKA. It is also possible that nutrient or stress induced phosphorylation regulates the interaction between transcription
factors and Tup1-Cyc8 \(^{39,50}\). In addition, nutrient signalling may regulate Tup1-Cyc8 itself. Several studies have shown that sumoylation modulates the function of Tup1-Cyc8 \(^{51,52}\). Our data further suggest that PKA and TORC1 locally regulates the \(IME1\) promoter. We observed no change in cellular localization and only a minor change in abundance during meiotic entry, while chemical inhibition of PKA and TORC1 resulted in much lower levels of Sok2, Phd1 and Yap6 (Figure 6). Perhaps, PKA and TORC1 associate with \(IME1\) promoter directly. Future work will pinpoint the mechanism by which nutrient signalling pathways control the transcription factors regulating Tup1-Cyc8 binding with the \(IME1\) promoter.

**Concluding remarks**

Our observation that regulated repression by multiple transcription factors controls a cell fate decision in yeast shows similarities with how multicellular organisms undertake developmental decisions. In \textit{Drosophila}, plants, and mammals, transcriptional repressors of the Groucho family (structurally related to Tup1) are important for regulating of various developmental processes such as body patterning and determination of organ identity \(^{53-55}\). Like Tup1-Cyc8, the association of Groucho repressor with promoters relies on sequence specific transcription factors, and Groucho repressor integrates multiple signals to control gene expression and cell fate outcomes. Regulation of the \(IME1\) promoter also demonstrates features of enhancer-directed transcriptional control of cell-fate master regulators in mammalian cells \(^{56-58}\). Like the \(IME1\) promoter, an array of transcription factors associate and control the activity of enhancers. In addition, developmentally controlled enhancers are typically regulated by multiple upstream signalling pathways and are often
primed for activation. Our findings in yeast may provide insights to better understand how signal integration controls master regulatory genes and developmental decisions in all eukaryotic cells.

Acknowledgements:

We are grateful to Andrew Wu and Fabien Moretto for their critical reading of the manuscript, and to Andreas Doncic for providing the mNeongreen tagging cassette. This work was supported by the Francis Crick Institute (FC001203), which receives its core funding from Cancer Research UK (FC001203), the UK Medical Research Council (FC001203), and the Wellcome Trust (FC001203).

Declaration of Interests:

The authors declare no competing interests.

Supplementary Information:

Materials and Methods
Supplementary References
Supplementary Figure legends
Supplementary Figures 1-8
Supplementary Tables 1 and 2
References:

1. Neiman, A.M. Sporulation in the budding yeast Saccharomyces cerevisiae. *Genetics* **189**, 737-765 (2011).
2. Kassir, Y., Granot, D. & Simchen, G. IME1, a positive regulator gene of meiosis in S. cerevisiae. *Cell* **52**, 853-862 (1988).
3. Nachman, I., Regev, A. & Ramanathan, S. Dissecting timing variability in yeast meiosis. *Cell* **131**, 544-556 (2007).
4. van Werven, F.J. & Amon, A. Regulation of entry into gametogenesis. *Philos Trans R Soc Lond B Biol Sci* **366**, 3521-3531 (2011).
5. Covitz, P.A. & Mitchell, A.P. Repression by the yeast meiotic inhibitor RME1. *Genes Dev* **7**, 1598-1608 (1993).
6. Mitchell, A.P. & Herskowitz, I. Activation of meiosis and sporulation by repression of the RME1 product in yeast. *Nature* **319**, 738-742 (1986).
7. van Werven, F.J. *et al.* Transcription of two long noncoding RNAs mediates mating-type control of gametogenesis in budding yeast. *Cell* **150**, 1170-1181 (2012).
8. Moretto, F., Wood, N.E., Kelly, G., Doncic, A. & van Werven, F.J. A regulatory circuit of two lncRNAs and a master regulator directs cell fate in yeast. *Nat Commun* **9**, 780 (2018).
9. Jambhekar, A. & Amon, A. Control of meiosis by respiration. *Curr Biol* **18**, 969-975 (2008).
10. Sagee, S. *et al.* Multiple and distinct activation and repression sequences mediate the regulated transcription of IME1, a transcriptional activator of meiosis-specific genes in Saccharomyces cerevisiae. *Mol Cell Biol* **18**, 1985-1995 (1998).
11. Shenhar, G. & Kassir, Y. A positive regulator of mitosis, Sok2, functions as a negative regulator of meiosis in Saccharomyces cerevisiae. *Mol Cell Biol* **21**, 1603-1612 (2001).
12. Kahana, S. *et al.* Functional dissection of IME1 transcription using quantitative promoter-reporter screening. *Genetics* **186**, 829-841 (2010).
13. Zhao, H. *et al.* A Role for the Respiratory Chain in Regulating Meiosis Initiation in Saccharomyces cerevisiae. *Genetics* **208**, 1181-1194 (2018).
14. Kunoh, T., Kaneko, Y. & Harashima, S. YHP1 encodes a new homeoprotein that binds to the IME1 promoter in Saccharomyces cerevisiae. *Yeast* **16**, 439-449 (2000).
15. Kahana-Edwin, S., Stark, M. & Kassir, Y. Multiple MAPK cascades regulate the transcription of IME1, the master transcriptional activator of meiosis in Saccharomyces cerevisiae. *PloS One* **8**, e78920 (2013).
16. Weidberg, H., Moretto, F., Spedale, G., Amon, A. & van Werven, F.J. Nutrient Control of Yeast Gametogenesis Is Mediated by TORC1, PKA and Energy Availability. *PLoS Genet* **12**, e1006075 (2016).
17. Honigberg, S.M. & Lee, R.H. Snf1 kinase connects nutritional pathways controlling meiosis in Saccharomyces cerevisiae. *Mol Cell Biol* **18**, 4548-4555 (1998).
18. DeRisi, J.L., Iyer, V.R. & Brown, P.O. Exploring the metabolic and genetic control of gene expression on a genomic scale. *Science* **278**, 680-686 (1997).
19. Keleher, C.A., Redd, M.J., Schultz, J., Carlson, M. & Johnson, A.D. Ssn6-Tup1 is a general repressor of transcription in yeast. *Cell* **68**, 709-719 (1992).
20. Tzamarias, D. & Struhl, K. Functional dissection of the yeast Cyc8-Tup1 transcriptional corepressor complex. *Nature* **369**, 758-761 (1994).
21. Smith, R.L. & Johnson, A.D. Turning genes off by Ssn6-Tup1: a conserved system of transcriptional repression in eukaryotes. *Trends Biochem Sci* **25**, 325-330 (2000).
22. Mizuno, T. *et al.* The Tup1-Ssn6 general repressor is involved in repression of IME1 encoding a transcriptional activator of meiosis in Saccharomyces cerevisiae. *Curr Genet* **33**, 239-247 (1998).
23. Parnell, E.J. & Stillman, D.J. Shields up: the Tup1-Cyc8 repressor complex blocks coactivator recruitment. *Genes Dev* **25**, 2429-2435 (2011).
24. Malave, T.M. & Dent, S.Y. Transcriptional repression by Tup1-Ssn6. Biochim Cell Biol 84, 437-443 (2006).
25. Wong, K.H. & Struhl, K. The Cyc8-Tup1 complex inhibits transcription primarily by masking the activation domain of the recruiting protein. Genes Dev 25, 2525-2539 (2011).
26. Nishimura, K., Fukagawa, T., Takisawa, H., Kakimoto, T. & Kanemaki, M. An auxin-based degron system for the rapid depletion of proteins in nonplant cells. Nat Methods 6, 917-922 (2009).
27. Raj, A., van den Bogaard, P., Rifkin, S.A., van Oudenaarden, A. & Tyagi, S. Imaging individual mRNA molecules using multiple singly labeled probes. Nat Methods 5, 877-879 (2008).
28. Rizzo, J.M., Mieczkowski, P.A. & Buck, M.J. Tup1 stabilizes promoter nucleosome positioning and occupancy at transcriptionally plastic genes. Nucleic Acids Res 39, 8803-8819 (2011).
29. Dave, J.K., Trumbly, R.J. & Dent, S.Y. Histone-dependent association of Tup1-Ssn6 with repressed genes in vivo. Mol Cell Biol 22, 693-703 (2002).
30. Edmondson, D.G., Smith, M.M. & Roth, S.Y. Repression domain of the yeast global repressor Tup1 interacts directly with histones H3 and H4. Genes Dev 10, 1247-1259 (1996).
31. Watson, A.D. et al. Ssn6-Tup1 interacts with class I histone deacetylases required for repression. Genes Dev 14, 2737-2744 (2000).
32. Dave, J.K., Edmondson, D.G., Coco, C.B. & Dent, S.Y. Tup1-Ssn6 interacts with multiple class I histone deacetylases in vivo. J Biol Chem 278, 50158-50162 (2003).
33. Wu, J., Suka, N., Carlson, M. & Grunstein, M. Tup1 utilizes histone H3/H2B-specific HDA1 deacetylase to repress gene activity in yeast. Mol Cell 7, 117-126 (2001).
34. Fleming, A.B., Beggs, S., Church, M., Tsukihashi, Y. & Penning, S. The yeast Cyc8-Tup1 complex cooperates with Hda1p and Rpd3p histone deacetylases to robustly repress transcription of the subtelomeric FLO1 gene. Biochim Biophys Acta 1839, 1242-1255 (2014).
35. Hanlon, S.E., Rizzo, J.M., Tatomer, D.C., Lieb, J.D. & Buck, M.J. The stress response factors Yap6, Cin5, Phd1, and Skn7 direct targeting of the conserved ENA1 gene in Saccharomyces cerevisiae: bZIP protein Sko1p confers HOG dependent osmotic regulation. Mol Cell Biol 19, e19060 (2011).
36. Park, S.H., Koh, S.S., Chun, J.H., Hwang, H.J. & Kang, H.S. Nrg1 is a transcriptional repressor for glucose repression of STA1 gene expression in Saccharomyces cerevisiae. Mol Cell Biol 19, 2044-2050 (1999).
37. Proft, M. & Serrano, R. Repressors and upstream repressing sequences of the stress-regulated ENA1 gene in Saccharomyces cerevisiae: bZIP protein Sko1p confers HOG-dependent osmotic regulation. Mol Cell Biol 19, 537-546 (1999).
38. Regnacq, M., Alimardani, P., El Moudni, B. & Berges, T. SUT1p interaction with Cyc8p(Ssn6p) relieves hypoxic genes from Cyc8p-Tup1p repression in Saccharomyces cerevisiae. Mol Microbiol 40, 1085-1096 (2001).
39. Treitel, M.A. & Carlson, M. Repression by Ssn6-TUP1 is directed by MIG1, a repressor/activator protein. Proc Natl Acad Sci U S A 92, 3132-3136 (1995).
40. Ozcan, S. & Johnston, M. Three different regulatory mechanisms enable yeast hexose transporter (HXT) genes to be induced by different levels of glucose. Mol Cell Biol 15, 1564-1572 (1995).
41. Berkey, C.D., Vyas, V.K. & Carlson, M. Nrg1 and nrg2 transcriptional repressors are differently regulated in response to carbon source. Eukaryot Cell 3, 311-317 (2004).
42. Loewith, R. & Hall, M.N. Target of rapamycin (TOR) in nutrient signaling and growth control. Genetics 189, 1177-1201 (2011).
43. Conrad, M. et al. Nutrient sensing and signaling in the yeast Saccharomyces cerevisiae. FEMS Microbiol Rev 38, 254-299 (2014).
44. Ward, M.P., Gimeno, C.J., Fink, G.R. & Garrett, S. SOK2 may regulate cyclic AMP-dependent protein kinase-stimulated growth and pseudohyphal development by repressing transcription. Mol Cell Biol 15, 6854-6863 (1995).
45. Pokholok, D.K., Zeitlinger, J., Hannett, N.M., Reynolds, D.B. & Young, R.A. Activated signal transduction kinases frequently occupy target genes. *Science* **313**, 533-536 (2006).
46. Green, S.R. & Johnson, A.D. Promoter-dependent roles for the Srb10 cyclin-dependent kinase and the Hdal deacetylase in Tup1-mediated repression in *Saccharomyces cerevisiae*. *Mol Biol Cell* **15**, 4191-4202 (2004).
47. Wasserstrom, L., Dunkler, A., Walther, A. & Wendland, J. The APSES protein Sok2 is a positive regulator of sporulation in *Ashbya gossypii*. *Mol Microbiol* **106**, 949-960 (2017).
48. Rodrigues-Pousada, C. *et al.* Yeast AP-1 like transcription factors (Yap) and stress response: a current overview. *Microb Cell* **6**, 267-285 (2019).
49. Raithatha, S., Su, T.C., Lourenco, P., Goto, S. & Sadowski, I. Cdk8 regulates stability of the transcription factor Phd1 to control pseudohyphal differentiation of *Saccharomyces cerevisiae*. *Mol Cell Biol* **32**, 664-674 (2012).
50. Proft, M. & Struhl, K. Hog1 kinase converts the Sko1-Cyc8-Tup1 repressor complex into an activator that recruits SAGA and SWI/SNF in response to osmotic stress. *Mol Cell* **9**, 1307-1317 (2002).
51. Ng, C.H. *et al.* Sumoylation controls the timing of Tup1-mediated transcriptional deactivation. *Nat Commun* **6**, 6610 (2015).
52. Oeser, M.L. *et al.* Dynamic Sumoylation of a Conserved Transcription Corepressor Prevents Persistent Inclusion Formation during Hyperosmotic Stress. *PLoS Genet* **12**, e1005809 (2016).
53. Buscarlet, M. & Stifani, S. The 'Marx' of Groucho on development and disease. *Trends Cell Biol* **17**, 353-361 (2007).
54. Liu, Z. & Karmarkar, V. Groucho/Tup1 family co-repressors in plant development. *Trends Plant Sci* **13**, 137-144 (2008).
55. Turki-Judeh, W. & Courey, A.J. Groucho: a corepressor with instructive roles in development. *Curr Top Dev Biol* **98**, 65-96 (2012).
56. Jin, F., Li, Y., Ren, B. & Natarajan, R. Enhancers: multi-dimensional signal integrators. *Transcription* **2**, 226-230 (2011).
57. Pott, S. & Lieb, J.D. What are super-enhancers? *Nat Genet* **47**, 8-12 (2015).
58. Rickels, R. & Shilatifard, A. Enhancer Logic and Mechanics in Development and Disease. *Trends Cell Biol* **28**, 608-630 (2018).
59. Spitz, F. & Furlong, E.E. Transcription factors: from enhancer binding to developmental control. *Nat Rev Genet* **13**, 613-626 (2012).
Figure legends:

Figure 1. Tup1-Cyc8 repressor complex prevents activation of the *IME1* promoter independent of HDACs

(A) PKA and TORC1 integrate at the *IME1* promoter to control the association of the Tup1. Tup1, in turn, controls *IME1* repression. (B) The effect of truncations in the *IME1* promoter on the onset of meiosis. For the analyses we used diploid cells harbouring one copy of *IME1* deleted (control, FW4128), while different promoter deletion mutants were generated at the wild-type *IME1* locus (*pIME1*(-1600-2315Δ), FW3946; *pIME1*(-1400-2315Δ), FW3947; *pIME1*(-1200-2315Δ), FW3948; *pIME1*(-1000-2315Δ), FW3949; *pIME1*(-800-2315Δ), FW3950; *pIME1*(-600-2315Δ), FW3951). Control and mutant cells were grown to saturation in rich medium (YPD), grown for an additional 16 hours in pre-sporulation medium (SPO), and subsequently cells were shifted to sporulation medium. Samples were taken at the indicated time points, fixed, and DAPI masses were counted for the indicated time points to determine the percentage of cells that underwent meiosis (MI+MII). Cell harbouring two, three or four DAPI masses were classified as meiosis. (C) Cyc8, the interacting partner of Tup1, associates with the *IME1* promoter in the same region as Tup1. Diploid cells harbouring Cyc8 tagged with the V5 epitope (FW6381) were grown in rich medium (YPD) to the exponential phase. Cells were cross-linked, and chromatin extracts were prepared. Subsequently, Cyc8 bound DNA fragments were isolated by immunoprecipitation, reverse cross-linked, and quantified by qPCR. Eight different primer pairs were used to determine where Cyc8 binds in the *IME1* promoter. The signals were normalized over the silent mating locus *HMR*, which was not bound by Cyc8. (D) Similar as C except that the binding of Cyc8 (Cyc8-V5, FW6381), Pog1 (Pog1-V5, FW968), and wild type (control, FW1511) cells are displayed. The region
around -1000 bp upstream of the AUG start codon of IME1 was analysed for Cyc8 and Pog1 binding. The mean signals and SEM are displayed. (E) Diploid cells harbouring Tup1 or Cyc8 fused to auxin induced degron (AID) (TUP1-AID, FW5057 and CYC8-AID, FW6371) were grown till the exponential growth phase. Subsequently, cells were treated with IAA or DMSO for 0, 30 or 60 minutes, and samples for fixed with trichloroacetic acid. Protein extracts were prepared to determine Tup1 and Cyc8 protein levels by Western blot. As a control, Hxk1 levels were determined with anti-Hxk1 antibodies. (F) Similar as E except that IME1 mRNA expression was determined by qPCR of IME1 in cells TUP1-AID and CYC8-AID treated with IAA or DMSO. RNA was isolated, reverse transcribed, and quantified by qPCR. Signals were normalized by ACT1. Mean and SEM are displayed. (G) IME1 expression in single cells upon Tup1 depletion. Tup1 protein levels (TUP1-AID, FW5057) detected by Western blot as described in E, except that levels at 0, 5, 10, 15 and 30 min after IAA. (H) Distribution of IME1 transcript levels in single cells (TUP1-AID) treated with IAA or DMSO as described in G. Single molecule RNA fluorescent in situ hybridization (smFISH) was used detect IME1 expression. Cells were fixed, and hybridized with IME1 (AF594) and ACT1 (Cy5) probes. Only cells positive for ACT1 were used for the analyses. The mean number of transcripts per cell for each time point (black line) is displayed. At least 50 cells were used for the analysis. Unpaired parametric two-tailed Welch’s t-test with 95% confidence was used. Non-significant (ns) and P-values (** = ≤ 0.01, *** = ≤ 0.001) are indicated. (I) Same data as in H, except that the single cells data for IME1 were binned according to expression levels. (J) Scheme of Tup1-Cyc8 interacting with histone deacetylases (HDACs) (left). IME1 expression in diploid cells harbouring rpd3Δ, hda1Δ, hos1Δ, or hos2Δ single deletion mutants (FW8102, FW8426, FW8430, and FW8103) or
rpd3Δhda1Δ, rpd3Δhos1Δ and rpd3Δhos2Δ double deletion mutants (FW8457, FW8428, and FW8171) in single cells as determined by smFISH (right). The mean number of transcripts per cell for each time point (black line) is displayed. At least 100 cells were used for the analysis.

Figure 2. Multiple Tup1-Cyc8 interacting transcription factors associate with IME1 promoter

(A) Table of transcription factors that have been implicated to interact with Tup1-Cyc8, and have motif sequences within the IME1 promoter. (B) Multiple transcription factors associate with IME1 promoter in the same region where Tup1-Cyc8 binds. Transcription factors listed in A were tagged with V5 epitope tag. Diploid cells harboring V5-tagged transcription factor (YAP6-V5, FW3833; SOK2-V5, FW5638; PHD1-V5, FW4466; SUT1-V5, FW6974; MOT3-V5, FW4383; SKO1-V5, FW4389; NRG1-V5, FW4393; NRG2-V5, FW4396; SKN7-V5, FW4399; CIN5-V5, FW7072; SFL1-V5, FW7070; MIG1-V5, FW4665; RGT1-V5, FW4386) and control cells (untagged, FW1511) were grown till exponential growth. The region around -1000 bp upstream AUG of IME1 was analysed for binding by ChIP. The signals were normalized over HMR. Mean and SEM are displayed. (C) Similar as B, except that transcription factors that showed enrichment in B were analysed for binding throughout the whole IME1 promoter. Eight primer pairs were used for the analyses.

Figure 3. Tup1-Cyc8 is recruited by transcription factors associated with the IME1 promoter

(A) Scheme displaying how transcription factors and Tup1-Cyc8 could interact at the IME1 promoter. (B) Diploid cells harbouring TUP1-AID and V5-tagged transcription factors were grown till exponential growth. The region around -1000 bp upstream AUG of IME1 was analysed for binding by ChIP. The signals were normalized over HMR. Mean and SEM are displayed.
factors (YAP6-V5, FW4214; SOK2-V5, FW4218; PHD1-V5, FW5056; MOT3-V5, FW4229; NRG1-V5, FW4230; NRG2-V5, FW5055; SKO1-V5, FW4224) were grown to exponential phase. As a control, TUP1-AID cells were also included, which also harbours a V5 tag. Cells were either treated with IAA or DMSO, and the binding of each transcription factors was determined by ChIP. Mean signals and SEM are displayed. (C) Binding of Tup1 is affected in cells lacking transcription factor binding motifs in IME1 promoter. Diploid cells with a chromosomal deletion of the IME1 locus, and an integrated plasmid that contained the full IME1 gene fused at amino terminus with sfGFP and promoter (pIME1-WT, FW5370) or the same construct with all the candidate motif sequences mutated (pIME1-bsΔ, FW5372) were used for the analysis. These cells also harboured TUP1-V5. The binding of Tup1 was determined by ChIP. Mean and SEM values are displayed. (D) Expression of Ime1 during entry in meiosis in pIME1-WT and pIME1-bsΔ. Strains described in C were grown till saturation in rich medium (YPD), grown for an additional 18 hours in pre-sporulation medium, and subsequently shifted in sporulation medium (SPO). The levels of Ime1 expression was determined by imaging and quantifying fluorescent signal generated by sfGFP-Ime1 in single cells. At least 50 number of cells were quantified. The mean signal and error bars representing the 95% confidence interval are displayed.

**Figure 4. Multiple Tup1-Cyc8 interacting transcription factors repress IME1 in nutrient rich conditions**

(A) IME1 expression in single cells harbouring: sok2Δ, phd1Δ or yap6Δ single mutants (FW3979, FW3991, FW3603); sok2Δphd1Δ, yap6Δphd1Δ and sok2Δyap6Δ double mutants (FW4710, FW4406, FW4239); sok2Δphd1Δyap6Δ triple mutant (FW4010). Cells were grown to exponential growth, were fixed, and hybridized with
IME1 (AF594) and ACT1 (Cy5) probes. Only cells positive for ACT1 were used for the analyses. The mean number of transcripts per cell for each time point (black line) is displayed. Between 190 and 300 cells were used for the analysis. Unpaired parametric two-tailed Welch’s t-test with 95% confidence was used. Non-significant (ns) and P-values (** = ≤ 0.01, *** = ≤ 0.001) are indicated. (B) Same data as in A, except that the single cells data for IME1 were binned according to expression levels. (C) Sok2, Phd1, Yap6 binding in pIME1-WT (FW8081, FW8083, FW8079), pIME1-bsΔ(FW8087, FW8089, FW8085), and pIME1-spy (FW8093, FW8095, FW8091) as determined by ChIP. Mean and SEM values are displayed. (D) IME1 expression in single cells as determined by smFISH in pIME1-WT, pIME1-bsΔ, and pIME1-spy in cells wild type for SOK2, PHD1 and YAP6 (FW5370, FW5372, FW7733) or harbouring the sok2Δphd1Δyap6Δ triple deletion mutant (FW7650, FW8420, FW8177). Unpaired parametric two-tailed Welch’s t-test with 95% confidence was used. P-values (** = ≤ 0.01, *** = ≤ 0.001) are indicated. (E) Same data as in D, except that the single cell data were binned according to IME1 expression levels.

Figure 5. Sok2, Phd1 and Yap6 are important for repressing the IME1 promoter prior entry into meiosis

(A) Transcription factors binding at the IME1 promoter prior and during entry into meiosis. Diploid cells harbouring V5 tagged transcription factors (YAP6-V5, FW3833; PHD1-V5, FW4466; NRG1-V5, FW4393; SOK2-V5, FW5638; MOT3-V5, FW4383; SKO1-V5, FW4389; NRG2-V5, FW4396; SUT1-V5, FW6974; TUP1-V5, FW3456; POG1-V5, FW968) were used for the analyses. Samples for ChIP were taken at 0 and 4 hours in SPO. Mean and SEM values are displayed. (B) Tup1 binding at the
*IME1* promoter at 0 hours in SPO (prior entry in meiosis) in wild-type cells or cells harbouring *sok2Δ, phd1Δ* or *yap6Δ* single deletion mutants (FW3979, FW3991, FW3603), and *sok2Δphd1Δ, yap6Δphd1Δ* and *sok2Δyap6Δ* double mutants (FW4710, FW4406, FW4239); *sok2Δphd1Δyap6Δ* triple mutant (FW4010). Mean and SEM values are displayed. (C) *IME1* expression in deletion mutants described in C. The signals were normalized over the *ACT1* gene. Mean and SEM values are displayed. (D) Meiosis in mutant strains described in B. Samples were taken at the indicated time points, fixed, and DAPI masses were counted for the indicated time points to determine the percentage of cells that underwent meiosis (MI+MII). Cell harbouring two, three or four DAPI masses were classified as meiosis. At least 200 cells were analysed.

**Figure 6. PKA and TORC1 control Sok2, Phd1 and Yap6 protein abundance**

(A) Expression of Sok2, Phd1, Yap6, Tup1, and Cyc8 in cells grown to exponential phase or saturation in YPD, and 0 and 4 hours in SPO (*SOK2*-V5, FW5638; *PHD1*-V5, FW4466; *YAP6*-V5, FW3833; *TUP1*-V5, FW3456; *CYC8*-V5, FW6381). Samples for western blotting were taken at the indicated time points. Hxk1 was used as a loading control. Relative abundance is displayed for each transcription factor. (B) Nuclear to cytoplasmic ratio of Sok2-mNG (FW7475), Phd1-mNG (FW7477), Yap6-mNG (FW7473), Tup1-mNG (FW7644) and Cyc8-mNG (FW7642) prior (0 hours in SPO) and during entry into meiosis (4 hours in SPO). Each transcription factor was fused to mNeongreen (mNG). These cells also expressed a mCherry fused to SV40 nuclear localization signal (NLS) (mCherry-NLS). As a control, the signals of cells harbouring no mNG-tag (FW5199) are displayed. The black bar indicates the mean signal, and each point indicates a single cell measurement. (C) Similar as A, except
that expression of Sok2, Phd1, and Yap6 was monitored after inhibiting PKA and TORC1. We used cells harbouring the *tka1-as* allele for inhibiting PKA activity with ATP analog 1NMPP1 (*YAP6-V5*, FW5453; *SOK2-V5*, FW5454; *PHD1-V5*, FW5528). TORC1 was inhibited with rapamycin. Cells were grown to exponential growth, treated with 1NMPP, rapamycin, or both for 4 hours. Sok2, Phd1 and Yap6 protein levels are normalized over Hxk1. Relative abundance with respect to YPD (E) is displayed.

**Figure 7. Nutrient signalling triggers distinct responses of transcription factors binding with the *IME1* promoter**

(A) Scheme of experimental set up. Following growth in YPD and pre-sporulation media, cells were shifted to SPO, SPO plus 2% glucose, YP or YP plus 2% glucose for 4 hours. (B) Binding of Tup1 and Pog1 at the *IME1* promoter under distinct nutrient conditions described in A and measured by ChIP. Diploid cells harbouring V5 tagged Tup1 (FW3456) or Pog1 (FW968) were used for the analyses. Mean signals and SEM are displayed. (C) Similar to B, except that V5 tagged Yap6, Sok2, Phd1, Nrg1, Mot3, Nrg2, Sko1 and Sut1 were used for the analyses (FW3833, FW5638, FW4466, FW4393, FW4383, FW4396, FW4389, and FW6974). (D) Similar to A, except that Tup1 binding with the *IME1* promoter was determined in the *sok2Δphd1Δyap6Δ* cells (FW4010). (E) Model for how nutrient signalling controls the *IME1* promoter. Multiple Tup1-Cyc8 recruiting transcription factors associate in nutrient rich conditions. Upon entry into meiosis transcription factors and Tup1-Cyc8 disassociate, and consequently *IME1* transcription is induced.
**Figure 1**

**A**

- Ampo nutrients → PKA/TORC1 → IME1
- Starvation → PKA/TORC1 → IME1

**B**

- Graph showing % MI + MII over time (0-24 h in SPO)
- Curves for different conditions:
  - Control
  - IME1(-1000-2315Δ)
  - IME1(-1600-2315Δ)
  - IME1(-1400-2315Δ)
  - IME1(-1200-2315Δ)
  - IME1(-600-2315Δ)

**C**

ChIP Cyc8-v5

**D**

- Bar graph showing fold enrichment of Cyc8-AID
- Control vs. Cyc8-V5
- Treatment with DMSO + IAA

**E**

- Western blot of Tup1-AID, Hxk1, Cyc8-AID, Hxk1
- Treatment with +IAA, +DMSO

**F**

- Graph showing IME1/ACT1 ratio
- Conditions: DMSO, +IAA, 4h in SPO(WT)

**G**

- Western blot of Tup1-AID, Hxk1
- Treatment with +IAA in min

**H**

- Graph showing IME1 mRNA (TUP1-AID, smFISH)
  - Transcripts per cell
  - Conditions: DMSO, +IAA

**I**

- Graph showing IME1 mRNA (TUP1-AID + IAA, smFISH)
  - Number of IME1 transcripts
  - % of cells

**J**

- Diagram showing regulation of Tup1-Cyc8 by Rpd3, Hda1, Hos1, Hos2
Figure 2

A. Putative BS in -600 to -1200 Tup1-Cyc8

| Gene   | Fold Enrichment |
|--------|-----------------|
| Yap6   | 2x y            |
| Sok2   | 8x nd           |
| Phd1   | 6x y            |
| Nrg1   | 6x y            |
| Nrg2   | 6x y            |
| Cin5   | 3x y            |
| Mig1   | 2x y            |
| Sut1   | 1x y            |
| Mot3   | 19x y           |
| Sko1   | 1x y            |
| Sfl1   | 2x y            |
| Rgt1   | 2x y            |
| Skn7   | 2x y            |

B. Interacts with Tup1-Cyc8

Yap6-v5
Sok2-v5
Phd1-v5
Sut1-v5
Mot3-v5
Nrg1-v5
Nrg2-v5
Nrg1-v5
Nrg2-v5
Skn7-v5
Cin5-v5
Sfl1-v5
Mig1-v5
Rgt1-v5

C. Control (untagged)
Figure 3

A. TFs recruit Tup1-Cyc8

B. TUP1-(V5)-AID

C. plME1-WT

D. plME-WT vs. plME-bsΔ

TFs recruit Tup1-Cyc8

Tup1-Cyc8 recruits TFs

Tup1-Cyc8

TF TF TF

IME1

TF TF TF

TF TF TF

IME1

Tup1-Cyc8

control Yap6-V5 Sok2-V5 Pho1-V5 Mox3-V5 Nrg1-V5 Nrg2-V5 Sko1-V5

+IAA +DMSO

fold enrichment

0 150

pIME-WT pIME-bs Δ IME1

0 15

Tup1-V5

pIME-WT pIME-bs Δ

total sfGFP signal (AU)

0 10

SPO (h)
**Figure 4**

**A**

IME1 mRNA (smFISH) vs. WT, yapΔ, sokΔ, and phdΔ

**B**

Number of IME1 transcripts:

- 0-3
- 4-10
- 11-20
- 21 or more

**C**

pIME1-bsΔ

pIMEm-Δ: 1100 700

pIMEm-spy: 1100 700

+ 33 nt

(Sok2/Phd1/Yap6)

**D**

IME1 mRNA (smFISH) vs. WT, pIME1-bsΔ, and pIME1-spy

**E**

Number of IME1 transcripts:

- 0-3
- 4-10
- 11-20
- 21 or more

**Legend**

- pIME1-WT
- pIME1-bsΔ
- pIME1-spy

**Note**

The copyright holder for this version posted January 14, 2020. It is made available under a CC-BY-NC-ND 4.0 International license.
Figure 5

(A) 0 hours SPO and 4 hours SPO fold enrichment of Yap6-V5, Phd1-V5, Nrg1-V5, Sok2-V5, Mot3-V5, Sko1-V5, Nrg2-V5, Sut1-V5, Tup1-V5, and Pog1-V5.

(B) Tup1 ChIP in YPD and 0h SPO fold enrichment in WT, yap6Δ, sok2Δ, phd1Δ, and their combinations.

(C) YPD and SPO 0h MI + MII for WT, sok2Δ, yap6Δ, sok2Δ Δ, phd1Δ Δ, and yok6Δ Δ.

(D) Growth curves for WT, yap6Δ, sok2Δ, phd1Δ, yap6Δ Δ, sok2Δ Δ phd1Δ Δ, and yok6Δ Δ.
Figure 6

A

|          | Sok2-V5 | Phd1-V5 | Yap6-V5 | Tup1-V5 | Cyc8-V5 |
|----------|---------|---------|---------|---------|---------|
|          | YPD     | SPO     | 0h      | 4h      | YPD     | SPO     | 0h      | 4h      | YPD     | SPO     | 0h      | 4h      | YPD     | SPO     | 0h      | 4h      |
| V5-tagged protein | ![Image](image1) | ![Image](image2) | ![Image](image3) | ![Image](image4) | ![Image](image5) |
| Hxk1 relative levels: | 1 0.13 0.33 0.32 | 1 0.30 0.33 0.22 | 1 0.39 0.27 0.60 | 1 0.6 0.87 1.1 | 1 0.17 0.21 0.15 |

B

![Graph showing nuclear to cytoplasmic ratio](image6)

C

|          | Sok2-V5 | Phd1-V5 | Yap6-V5 |
|----------|---------|---------|---------|
|          | YPD     | SPO     | 0h      | 4h      | YPD     | SPO     | 0h      | 4h      | YPD     | SPO     | 0h      | 4h      |
| tpk1as rapamycin NMPP1 | - - + + | - - + + | - - + + | - - + + | - - + + | - - + + | - - + + |
| V5-tagged protein | ![Image](image7) | ![Image](image8) | ![Image](image9) | ![Image](image10) |
| Hxk1 relative levels: | 1 0.1 2.1 0.2 | 1 0.19 0.62 0.07 | 1 0.18 0.23 0.22 |
Figure 7

A

4h SPO

0h SPO

4h SPO + 2% glucose (D)

4h YP

4h YP + 2% glucose (D)

B

Tup1-v5

Pog1-v5

fold enrichment

fold enrichment

0h SPO

4h SPO

4h SPO + 2% D

4h YP

4h YP + 2% D

fold enrichment

fold enrichment

fold enrichment

fold enrichment

C

Yap6-v5

Sok2-v5

Phd1-v5

Nrg1-v5

fold enrichment

fold enrichment

fold enrichment

fold enrichment

Mot3-v5

Nrg2-v5

Sko1-v5

Sut1-v5

fold enrichment

fold enrichment

fold enrichment

fold enrichment

D

Tup1-v5

fold enrichment

sok2Δ yap6Δ phd1Δ

E

nutrient rich (YP + glucose)

partial starvation (e.g. no glucose)

starvation

PKA/TORC1

PKA/TORC1

PKA/TORC1

IME1 promoter

IME1 promoter

IME1 promoter

TC=Tup1-Cyc8

TR= repressor (e.g. Sok2)

TA= activator (e.g. Pog1)