Review

Regulation of entry into gametogenesis

Folkert J. van Werven and Angelika Amon*

David H. Koch Institute for Integrative Cancer Research and Howard Hughes Medical Institute, Massachusetts Institute of Technology, Cambridge, MA 02139, USA

Gametogenesis is a fundamental aspect of sexual reproduction in eukaryotes. In the unicellular fungi Saccharomyces cerevisiae (budding yeast) and Schizosaccharomyces pombe (fission yeast), where this developmental programme has been extensively studied, entry into gametogenesis requires the convergence of multiple signals on the promoter of a master regulator. Starvation signals and cellular mating-type information promote the transcription of cell fate inducers, which in turn initiate a transcriptional cascade that propels a unique type of cell division, meiosis, and gamete morphogenesis. Here, we will provide an overview of how entry into gametogenesis is initiated in budding and fission yeast and discuss potential conserved features in the germ cell development of higher eukaryotes.

Keywords: entry into gametogenesis; meiosis; germ cell; sporulation; master regulator; yeast

1. INTRODUCTION

A fundamental aspect of cell-fate decisions is to convert temporary changes in gene expression induced by external signals into a stable phenotype. A crucial and highly coordinated cell-fate decision is the production of gametes (sex cells) from diploid progenitor cells in a process known as gametogenesis. An essential aspect of gametogenesis is the specialized cell division, meiosis, during which the diploid genome of the progenitor cell is reduced to the haploid state by two consecutive chromosome segregations to produce gametes. Thus, the two key aspects of gametogenesis are the expression (i) of genes that transform the canonical mitotic cell division programme into the specialized meiotic division pattern and (ii) of a morphogenesis programme that produces gametes.

Given that proper production of gametes is critical for sustaining a sexually reproducing species, it is important to understand how this cell fate is established. In this review, we will discuss how gametogenesis is induced. First, we will review how entry into the gametogenesis, known as sporulation, is controlled in budding and diploid cells. This process is initiated by the production of Mating-type information, respiratory state and nutritional information all converge at a single promoter, which cells form long-branched chains of elongated cells also known as pseudohyphae. Both haploid and diploid cells can form pseudohyphae, and this cell type is employed to forage for new food sources by enabling invasion and survival in host tissues. Last but not least, nitrogen starvation and the lack of a fermentable carbon source trigger gametogenesis, known as sporulation in yeast. This developmental choice is restricted to \( MAT^a/MAT^o \) diploid cells that are respiratory competent (have functional mitochondria). In what follows, we will discuss the signals that lead to this developmental decision.

3. SPORULATION IS GOVERNED BY Ime1

Mating-type information, respiratory state and nutritional information all converge at a single promoter, the \( IME1 \) promoter. \( IME1 \) (Inducer of meiosis 1) is the master regulator of gametogenesis and encodes a transcription factor that activates the expression of the so-called early meiotic genes (figure 1) [1]. Ime1 induces the expression of genes important for pre-meiotic DNA replication, genes essential for meiosis-specific chromosome remodelling and genes encoding factors essential for homologous recombination [2–4]. Ime1 also sets in motion the subsequent events of the sporulation programme. Among the genes whose expression, albeit indirectly, depends on Ime1 is \( NDT80 \), which encodes a transcription factor. Ndt80, through an autoregulatory positive feedback loop, induces a wave of transcripts called the ‘middle genes’ (figure 1) [3,5]. Once \( NDT80 \)-dependent genes are induced, cells are irreversibly committed to meiosis because this group of genes bring about and regulate the processes necessary for entry into and progression through the two meiotic chromosome segregation phases [6]. After the induction of middle genes a third
wave of transcripts called the ‘late genes’ is initiated, which control spore formation.

Successful completion of sporulation also requires that genes responsible for vegetative growth are repressed. During vegetative growth, *S. cerevisiae* proliferates by budding. During sporulation, budding is repressed and pre-meiotic S phase and the two meiotic divisions occur within the confines of the mother cell. Sporewalls are then produced around the four meiotic products and the mother cell remnants become the ascus sack that surrounds the four spores. During vegetative growth, budding and the initiation of DNA replication are induced by cyclin-dependent kinase (CDK) Cdc28 complexed with G1 cyclins (Cln1, 2 or 3). Their expression needs to be inhibited to prevent budding. Nutritional starvation represses *CLN* expression, thus inhibiting budding. However, DNA replication also relies on Cln–CDKs. How can pre-meiotic DNA replication occur in the absence of Cln–CDK function? The sporulation-specific protein kinase Ime2 takes over Cln–CDK’s role in promoting DNA replication [7]. *IME2* transcription is induced by Ime1 [8]. Thus, Ime1 ensures that pre-meiotic DNA replication occurs in the absence of Cln–CDK activity and it sets in motion the programme that culminates in the halving of the genome and the production of spores. Understanding what governs *IME1* gene expression thus lies at the heart of understanding the basis of gamete formation in budding yeast.

4. The *IME1* Promoter Functions as a Signal Integrator

Initiation of the sporulation programme requires the following events:

- glucose must be absent from the growth medium,
- nitrogen must be absent from the growth medium,
- Cln–CDKs must be repressed,
- cell must use a non-fermentable carbon source, and thus be respiratory competent, and
- cells must be of the *MATα/MATα* mating type.

These signals converge at the *IME1* promoter (figure 1). This promoter is over 2 kilobases in length, which makes it one of the largest and most regulated promoters in *S. cerevisiae*. The importance of *IME1* transcriptional regulation is revealed when *IME1* expression is mis-regulated. The nutritional control of *IME1* can be partially suppressed by over-expressing *IME1*, leading to sporulation in the presence of ample nutrients [9]. Haploid cells can also be induced to sporulate by the over-expression of *IME1*. This causes a meiotic catastrophe and cell death [10]. Together, these observations illustrate the importance of controlling *IME1* expression. In what follows, we will describe our current state of knowledge of how the various signals described above affect *IME1* expression. To sum up, a number of important players have been identified, but many of the details of regulation need to be worked out.
5. GLUCOSE REPRESSIONS IME1 EXPRESSION VIA MULTIPLE MECHANISMS

In the presence of glucose, budding yeast displays an almost exclusively fermentative metabolism, which results in the excretion of ethanol and low respiratory activity. The Ras/protein kinase A (PKA) signalling pathway is activated by glucose. The Ras guanosine triphosphatase (GTPase), anchored in the plasma membrane, is activated by glucose by an unknown mechanism. Ras–GTP activates adenylate cyclase (CYR1) to produce cyclic adenosine monophosphate (cAMP). cAMP binds to the inhibitory subunit of PKA called Bcy1, and induces its dissociation from PKA (Tpk1, 2 and 3 in budding yeast). High PKA levels promote vegetative growth and repress pseudohyphal growth, quiescence and sporulation. The importance of low PKA pathway activity for entry into sporulation has been revealed by mutations that affect PKA pathway activity. A constitutively active allele of RAS2, RAS2Val19, prevents sporulation in nutrient-poor conditions [11]. Similarly, inactivation of BCY1 results in constitutively active PKA and an inability to sporulate [11]. Conversely, deletion of one of the two RAS genes in yeast, RAS2, allows sporulation in rich medium [11]. Loss-of-function mutations in CYR1 induce sporulation even in the presence of high glucose and nitrogen [11].

PKA has multiple targets, among which are several transcription factors. For example, two stress-related transcription factors called Msn2 and Msn4 are kept inactive by PKA [12]. When PKA activity drops, Msn2 is dephosphorylated and translocates into the nucleus where it activates transcription of genes that harbour a stress-response element (STRE) in their promoters [13,14]. The STRE, which is also present within a 32 basepairs (bp) region (called IREu) of the IME1 promoter, can bind Msn2 and Msn4 in vitro [15]. In vivo this region of the IME1 promoter responds to MSN2 and MSN4 activity [15]. Whether Msn2 and Msn4 are the only transcriptional regulators of IME1 that are inhibited by the PKA pathway remains to be determined. It is however clear that PKA not only inhibits IME1 transcriptional activators, but also activates repressors of IME1 expression. The transcriptional repressor Sok2 is phosphorylated by PKA to repress IME1 transcription in the presence of glucose [16,17]. The binding site of Sok2 in the IME1 promoter overlaps with that of Msn2/4, and it has been suggested that they compete in the regulation of IME1 expression [16]. A third target of PKA that regulates IME1 expression is Rim15. This protein kinase, also regulated by target of rapamycin (TOR, discussed further below), is involved in the control of gene regulation during stationary phase and activates IME1 expression by an unknown mechanism [18,19]. Rim15 phosphorylates the Igo1 and Igo2 proteins, which prevent specific mRNAs from degradation during stationary phase [20]. Perhaps Rim15 indirectly controls IME1 mRNA stability. Rim15 also mediates the interaction between Ime1 and Ume6 (discussed later) to activate early meiotic gene expression [18,21].

The PKA pathway is not the only regulator of IME1 expression in response to glucose. Snf1, the AMP kinase of budding yeast, is activated in response to low glucose levels (reviewed in Hedbarcker & Carlson [22]). Snf1 regulates the expression of genes involved in alternative carbon source utilization, respiration, glycogen accumulation and thermotolerance. One transcriptional repressor inhibited by Snf1 phosphorylation is Mig1 [23,24]. In the presence of high glucose, Mig1 together with the co-repressor complex, Tup1–Ssn6, inhibits transcription of genes required for energy production, such as alternative carbon source utilization and respiration [23,25]. Interestingly, IME1 is also a target of this transcription repressor complex (figure 1). In the presence of glucose, Tup1–Ssn6 represses IME1 expression by binding at two sites in the IME1 promoter [26].

Whether PKA and Snf1 are the only mediators of carbon source signals at the IME1 promoter remains to be determined. Some studies have implicated the IME1 promoter to be responsive in the presence of a non-fermentable carbon source [15,27], but the mechanisms that underlie this regulation remain to be elucidated.

6. TOR CONTROLS IME1 EXPRESSION

Nitrogen starvation is required for IME1 activation. How the nitrogen starvation signal is conveyed to the IME1 promoter is not well understood. A highly conserved signalling pathway known as the TOR pathway has been implicated in regulating cell growth in response to nutritional signals, among them nitrogen. In budding yeast, the TOR pathway promotes cell growth by stimulating multiple processes including transcription, translation and nutrient uptake. TOR is a protein kinase and budding yeast harbours two TOR genes, TOR1 and TOR2. Tor kinases form two distinct complexes. TOR-complex 1 (TORC1) is primarily involved in nutrient signalling and TOR-complex 2 (TORC2) regulates the actin cytoskeleton (reviewed in Wullschleger et al. [28]). When TOR signalling is inhibited, a starvation response ensues. TOR signalling also appears to inhibit sporulation. Treatment of cells with rapamycin, a chemical inhibitor of TORC1, leads to the induction of IME1 and sporulation regardless of the nutritional condition (figure 1) [29,30].

How TORC1 is activated by nutrient signals such as the presence of a nitrogen source is not understood, but effectors of the pathway have been identified. Among them are several transcription factors that regulate stress-response genes or genes regulated by nutritional starvation. The nitrogen catabolite repression transcription factors, also known as GATA factors, Gln3 and Gat1 are sequestered outside the nucleus by cytoplasmic protein Ure2, but upon nitrogen starvation Gln3 and Gat1 translocate into the nucleus [31–33]. During nitrogen starvation, pools of the phosphatases Sit4 and PP2A, which associate with the TORC1 target Tap42 in nutritional-rich growth conditions, are released from Tap42, which activates the phosphatases [34]. This is thought to stimulate dephosphorylation and dissociation of Ure2 from Gln3 and Gln3 translocation into the nucleus [31,35]. Msn2 and 4 are also regulated by TOR, and so is the transcription factor Sfp1 that regulates ribosome biogenesis genes [31,36]. Finally, under nutrient-poor conditions, TOR promotes the accumulation of the Rtg1 and Rtg3 transcription factors in the nucleus [32,37].
Are any of the TOR effectors IME1 regulators? Potential binding sites for Gln3/Gat1 and Rtg1/3 are found in the IME1 promoter. Msn2/4 is known to be recruited to the IME1 promoter. It is tempting to speculate that all of these or a subset of these transcription factors modulate IME1 expression in response to TOR signalling.

7. Cln–CDKs inhibit IME1
Cln–CDKs not only promote budding and hence vegetative growth, but they also actively interfere with entry into the sporulation programme. Cln–CDKs inhibit IME1 expression. Over-expression of Cln1, Cln2 or Cln3 in nutrient-starved conditions prevents sporulation and stimulates budding [38]. Furthermore, Cln overexpression represses IME1 transcription and causes translocation of the remaining Ime1 protein from the nucleus into the cytoplasm [38]. In addition, the transcription repressor Xbp1, which is induced during sporulation, reduces the transcription of CLN1 to stimulate sporulation [39]. The mutually antagonistic relationship between G1 cyclins and IME1 is a key aspect of the decision between vegetative growth and sporulation.

8. Respiration is required for entry into the sporulation programme
Cells can only sporulate in the absence of a fermentable carbon source, but a non-fermentable carbon source must also be present to provide the energy necessary to undergo the meiotic divisions and to form four spores. Utilization of a non-fermentable carbon source requires that yeast cells respire to produce energy. It is thus perhaps not surprising that cells with impaired respiratory abilities fail to form spores [27,29]. However, IME1 expression is completely repressed in respiratory-deficient mutants raising the possibility that additional control mechanisms rather than simple lack of energy are responsible for inhibiting IME1 expression when respiration is impaired (figure 1) [27,29]. The Rim101 transcription repressor, which is involved in pH response and cell wall construction, has been implicated in IME1 regulation [40,41]. Rim101 regulates the transcription of two transcriptional repressors, NRG1 and SMP1 [42]. Deletion of SMP1 can partly suppress the sporulation defects of a rim101 mutant [42]. In respiration-deficient cells, RIM101 expression is strongly reduced and could at least in part account for why IME1 is not transcribed in these cells [29]. Respiration also appears to be critical for induction of sporulation in fission yeast (see below) suggesting that communication between mitochondria and the nucleus is a fundamental aspect of gametogenesis. Understanding the relationship between gene expression and respiratory state will therefore be critical to determine how entry into gametogenesis is controlled.

9. Rme1 represses IME1 expression in haploid cells
Haploid cells are of either the MATa or the MATα mating type and must not express IME1, as this is sufficient to induce sporulation. In both mating types, the DNA-binding protein Rme1 (repressor of IME1) prevents IME1 expression (figure 1). The IME1 promoter harbours two Rme1-binding sites and it is thought that binding of Rme1 to these sites inhibits IME1 expression [43–45]. Indeed, deletion of these RME1 binding sites will cause haploid cells to sporulate [44]. The mediator complex subunits Rgr1 and Sin4, global regulators of transcription and involved in chromatin organization, are required for the repression of IME1 by Rme1, suggesting that chromatin plays an important role in the repression mechanism [46–48].

Encoded at the MAT locus are DNA-binding proteins, a1 at the MATα locus and α2 at the MATa locus. When expressed together, the two proteins form a transcriptional repressor complex, a1–α2 that binds to the RME1 promoter and inhibits RME1 expression. Thus, RME1 inhibition of IME1 expression is alleviated in MATα/MATα diploid cells and the sporulation programme can be set in motion provided that appropriate nutritional cues are present.

10. OTHER FACTORS CONTROLLING ENTRY INTO SPORULATION
IME1 is not only regulated at the level of transcription, but multiple post-transcriptional mechanisms also ensure that Ime1 protein is produced in the right cell type and in response to the appropriate cues. There is some evidence to indicate that IME1 RNA, protein stability, protein localization and activity are also regulated.

Entry into the sporulation programme in some but not all genetic backgrounds depends on an N6-methyl-adenosine mRNA methyltransferase called Ime4 [49]. Substrates of Ime4 are the IME1, IME2 and IME4 mRNAs [50]. We do not yet know how Ime4 methylation affects these transcripts, but in the absence of IME4 entry into the sporulation programme is delayed or does not occur at all depending on the strain background [40,49,51]. IME4 transcription is regulated in a manner highly similar to that of IME1. The IME4 promoter responds to the same nutritional cues as the IME1 promoter [49]. IME4 is also only expressed in diploid cells, and repressed in haploids. However, this inhibition in haploids is not brought about by RME1, but by an unusual mechanism involving the expression of an antisense RNA. In haploids, an antisense transcript (recently named RME2; [52]) is initiated from the 3′ end of IME4 to repress IME4 transcription [51]. In diploid cells, this antisense transcript is repressed by the a1–α2 repressor [51].

Ime1 stability and protein localization are also regulated. Little is known about these controls, but the protein kinase Ime2 is required for destabilizing Ime1 protein once cells have entered the sporulation programme [53]. Subcellular localization of Ime1 appears to be governed by nutritional cues. When TOR signalling is re-activated by switching cells to a preferred nitrogen source, Ime1 translocates from the nucleus into the cytoplasm [54]. Finally, Ime1 collaborates with another transcriptional regulator, Ume6, to regulate early gene expression during sporulation.
In vegetatively growing cells, Ume6 acts as repressor by recruiting the Sin3 co-repressor and Rpd3 histone deacetylase complex to the promoters of early meiotic genes [21,55]. Upon nitrogen starvation, Ume6 transforms into an activator, and is essential for the induction of early meiotic genes [56]. Through a direct interaction Ume6 recruits Ime1 to early gene promoters, upon which Ume6 is degraded by ubiquitin-mediated protein degradation to activate gene expression [57]. The switch from activator to repressor function of Ume6 is under nutritional control and involves the kinase activities of PKA and Rim15 [21,57].

Ime1 is the master regulator of entry into gametogenesis in budding yeast. Understanding the regulatory mechanisms which ensure that the transcription factor is active in the right cell type and responds to certain extracellular cues is essential, and much needs to be learned about the details of this control. Is initiation of gametogenesis wired similarly in other eukaryotes in the sense that multiple signals converge on a single factor, which then triggers gametogenesis or not? In what follows, we will summarize how entry into gametogenesis is controlled in the two yeasts.

**11. INITIATION OF SPORULATION IN SCHIZOSACCHAROMYCES POMBE**

*Schizosaccharomyces pombe* and *S. cerevisiae* diverged approximately 330–420 million years ago. The two ascomycete fungi are thus evolutionarily highly divergent from each other [58]. Despite this divergence, many of the signals and pathways shown to be important for entry into the sporulation programme in budding yeast are also operative in fission yeast. The vegetative state in *S. pombe* is haploid. Fission yeast cells do mate to form diploids, but this state is transient. Concomitant with the mating programme, fission yeast cells initiate the sporulation programme to undergo meiosis, once a diploid has been produced, and form four haploid spores. Thus, in fission yeast, a concerted mating—sporulation programme is initiated when nutrients are limiting. In response to nitrogen starvation, haploid fission yeast cells arrest in G1 and cells of opposite mating type, $h^+$ and $h^-$, undergo conjugation. The resulting zygote undergoes meiosis and forms four haploid spores.

The proteins that regulate the initiation to gamete formation are not conserved between budding and fission yeast. Ime1 is not present in *S. pombe*, but analogously to budding yeast a master transcription factor, Ste11, determines whether cells enter the sporulation programme. As conjugation is tightly coupled with sporulation, it is not surprising that Ste11 also regulates mating.

**12. STE11: MASTER REGULATOR OF SEXUAL DIFFERENTIATION AND SPORULATION IN SCHIZOSACCHAROMYCES POMBE**

As in the case of *IME1* in budding yeast, multiple external signals converge on the *ste11*+ promoter (figure 2). Entry into gametogenesis in *S. pombe* requires a nitrogen starvation response, and glucose has an inhibitory effect on the expression of genes important for meiosis and sporulation. These nutritional signals control *ste11*+ expression. When *ste11*+ is expressed from a constitutive promoter, cells mate and sporulate even in the presence of ample nitrogen [59]. When activated, *ste11*+ induces the transcription of genes required for mating and the early stages of sporulation. Furthermore, *ste11*+ also indirectly conveys mating-type control into sporulation. Finally, as in budding yeast, respiratory activity is also essential for sporulation [29]. However, mating does not appear to be affected indicating that this signal does not operate on the...
ste11+ promoter. In what follows, we will describe how ste11+ expression is controlled by various nutrient sensing pathways.

13. THE PROTEIN KINASE A PATHWAY REPresses ste11+ TRANSCRIPTION

Glucose also regulates the PKA pathway in fission yeast. Mutations that lead to constitutively active PKA activity or addition of cAMP to the growth medium prevent the initiation of sporulation. Conversely, low levels of PKA activity promote sexual development and subsequent sporulation irrespective of nutritional conditions. ste11+ transcription mirrors this response to PKA pathway activity. Loss of PKA activity brought about by deleting pak1+ (encoding the catalytic subunit of PKA) or cyr1+ (encoding adenylate cyclase) results in constitutive expression of ste11+, whereas a deletion of the gene encoding the inhibitory subunit of PKA, cgs1+, represses ste11+ transcription [60,61].

PKA regulates the transcription factor Rst2. Phosphorylation of Rst2 by PKA causes this zinc finger transcription factor to localize to the cytoplasm where it is kept inactive [62,63] (figure 2). When glucose levels are low, Rst2 translocates into the nucleus and binds a stress-response DNA element in the ste11+ promoter to stimulate transcription [63]. Interestingly, Rst2 has a similar type of zinc finger motif as budding yeast Msn2, which activates IME1 (see previous section), indicating that activation of IME1 and ste11+ by the PKA pathway is a conserved feature of spore development in the two fungi [62].

14. TOR SIGNALLING REPresses ste11+

As in budding yeast, TOR signalling controls sporulation. Inactivation of TOR signalling through the use of a temperature-sensitive tor2+ allele showed that loss of TOR signalling causes a G1 arrest, and initiates sexual differentiation and subsequent sporulation irrespective of nutritional conditions [64,65] (figure 2). Accordingly, tor2+ inactivation also results in transcription of ste11+. Although it is clear that TOR signalling is a key regulator of gametogenesis, it is not known how TOR signalling causes the repression of ste11+. Unlike in budding yeast, transcriptional regulators controlled by TOR activity await identification. It should be noted that TOR signalling not only regulates ste11+ transcription, but also Ste11 activity. Tor2 interacts with Ste11 and Mei2, a factor critical for entry into sporulation described below, to inhibit their cellular function [65]. Furthermore, PKA and TOR act together to regulate Ste11 localization and control Ste11-dependent transcription [66]. Thus, TOR signalling affects ste11+ in multiple ways to ensure that mating and sporulation do not occur under nutrient-rich conditions.

15. RESPIRATION IS REQUIRED FOR SPORULATION IN SCHIZOSACCHAROMYCES POMBE

Unlike S. cerevisiae, S. pombe was initially described as a 'petite negative' strain, requiring mitochondrial activity for survival [67]. Subsequently, fission yeast cells lacking mitochondrial DNA, which causes respiration failure, have been isolated, but these mutants require a nuclear mutation for survival [68]. These second-site suppressor mutations allowed researchers to ask whether respiration was required for mating and/or sporulation. Defects in respiration do not appear to interfere with mating, but respiration defective fission yeast cells fail to form spores [29]. We do not yet know which aspect of gametogenesis requires respiration, but it is clear that in fission yeast, it is not an indirect consequence of a lack of energy as could be argued in budding yeast because S. pombe cells can sporulate in the presence of a fermentable carbon source. Thus, energy generation should occur even in the absence of respiration. Understanding the respiratory requirement for sporulation initiation may thus be best examined in fission yeast.

16. MATING-TYPE CONTROL OF SPORULATION: Pat1 KINASE PREVENTS HAPLOID SPORULATION IN SCHIZOSACCHAROMYCES POMBE

Haploid fission yeast cells, like haploid budding yeast cells, do not initiate meiosis and sporulation. In both species, sporulation is repressed in cells harbouring only one of the two mating types. The molecular mechanism of repression of sporulation in haploid fission yeast cells differs from the mechanism in S. cerevisiae, perhaps because mating and sporulation are coupled.

In haploid cells of S. pombe, entry into the sporulation programme is repressed by a protein kinase called Pat1 [69,70] (figure 2). Pat1 represses the activity of two early sporulation proteins. First, Pat1 phosphorylates Ste11. This causes binding to the 14-3-3 protein Rad24, and inhibition of Ste11’s transcriptional activity [69,70]. Secondly, Pat1 phosphorylates an RNA-binding protein known as Mei2 that is essential for the early stages of sporulation including the initiation of pre-meiotic DNA synthesis [71,72] (figure 2). Phosphorylation of Mei2 by Pat1 targets the protein for ubiquitin-mediated degradation [70]. A Mei2 mutant refractory to Pat1 phosphorylation causes haploid cells to undergo a fatal meiosis [72].

How does Mei2 promote entry into the sporulation programme? Mei2 regulates an RNA degradation system known as the determinant of selective removal (DSR)–Mmi1 system that selectively removes spore-specific transcripts [73] (figure 2). Mmi1 is an RNA-binding protein of the YTH21-B homology (YTH) family and binds to early and middle meiotic transcripts that contain an element in their 3′ UTRs. Subsequently, Mmi1 interacts with Pab1, a polyA-binding protein, and the exosome, a protein complex involved in RNA degradation, to degrade meiotic mRNAs [73–75]. Mei2 inhibits the DSR–Mmi1 by binding to Mmi1. The interaction between the two proteins can be seen as a dot-like structure in the nucleus [73]. The polyadenylated meiRNA, encoded by the sme2+ locus, is also found in this focus and interacts with Mei2 and is required for Mei2–Mmi1 nuclear localization [71,73,76]. However, the role of meiRNA in the Mei2–Mmi1 complex is not understood.
In diploid cells, Pat1 must be inactivated for Mei2 to bind to and inhibit the DSR–Mmi1. Ste11 brings about this inhibition, albeit indirectly (figure 2). As part of Ste11’s role in mating, the transcription factor induces expression of the mat1-locus-induced genes, mat1-Mc/mat1-Mm (in h− cells) and mat1-Pc/mat1-Pm (in h+ cells) [59]. In diploid cells, mat1-Pc and mat1-Mc are induced first to generate a pheromone signal and to activate transcription of mat1-Pm and mat1-Mm, which are required for sporulation [77]. The Mat1-Pm, a homeodomain protein, and Mat1-Mc, a high mobility group (HMG) box protein, synergistically activate the transcription of the mei3+ gene [77–79] (figure 2). Mei3 inhibits Pat1 kinase activity. The protein acts as a pseudosubstrate preventing the protein kinase from phosphorylating other substrates [69]. When mei3+ is ectopically expressed in haploid cells, they will enter a lethal meiosis [80].

The ste11+ promoter, like the IME1 promoter, integrates nutritional signals to induce the mating–sporulation programme. In contrast to IME1 however, its transcription is not controlled by mating type but, instead, Ste11 is required to establish mating-type control over sporulation. These differences in wiring probably reflect differences in lifestyle. Mating and sporulation are uncoupled in budding yeast, whereas the two events are tightly coupled in fission yeast. This coupling is facilitated by ste11+. Thus, ste11+ must be active in both haploid and diploid cells, which necessitates loss of mating-type control on ste11+ expression.

We will also highlight parallels between mouse and budding and fission yeast.

In mammals, PGCs are specified during early embryogenesis. They migrate into the gonad during day 10.5–11.5 of embryonic development. The fate of male and female PGCs diverges after their arrival in the somatic gonad. Female PGCs will differentiate into oogonia that undergo mitotic divisions to increase cell number, and then enter gametogenesis. By day 13.5 of embryonic development, chromosome condensation indicative of meiotic prophase is observed. In males, PGCs will remain arrested in G0 and only enter gametogenesis after birth.

The putative transcription factor Stra8 could be viewed as the functional counterpart of IME1 and ste11+ in mammals (figure 3) [86]. It is essential for gamete formation in males and females. Mice-lacking Stra8 do not enter meiosis and gametogenesis [87,88]. Importantly, Stra8 expression appears to function as an integrator of internal and external cues to induce gametogenesis. Cell intrinsic cues are mediated by Dazl and pluripotency factors. Stra8 is repressed by the pluripotency factor and RNA-binding protein NANOS2 and requires Dazl for expression [89]. Dazl, for Deleted in azoospermia-like, is the mouse homologue of the human Y-chromosome-encoded DAZ gene, which was first identified as deleted in patients with azoospermia [90]. Dazl is expressed in post-migratory PGCs of both sexes and, unlike human DAZ, is essential for gametogenesis in both sexes. In its absence neither female nor male PGCs show any sign of entry into the meiotic programme or gametogenesis [84]. Proteins specific for the meiotic divisions, such as REC8 and SYCP3, which are produced during the mitotic divisions preceding entry into the germ cell fate, do not associate with chromosomes in Dazl null mice, indicating that entry into the meiotic programme and hence gametogenesis does not occur [84]. Instead, germ cells of Dazl-deficient mice retain an undifferentiated PGC-like state with the expression levels of pluripotency factors such as Oct4, Nanog and Sox2 remaining high [85]. These results indicate that Dazl is essential for committing PGCs to gametogenesis. The protein sets in motion a developmental programme that downregulates pluripotency factors and induces gametogenesis in females and G0 arrest in males (figure 3). How Dazl commits PGCs to gametogenesis is not known. The protein encodes an RNA-binding protein. Perhaps, as in fission yeast, an RNA stability regulatory mechanism is important for gametogenesis in mammals.

Stra8 is also regulated by signals generated by the somatic gonad. Stra8 transcription is induced by retinoic
acid (RA), which activates the retinoic acid receptors (RARs α, β and γ) and X (RXR α, β and γ figure 3). In fact Stra8 stands for stimulated by retinoic acid gene 8. In embryonic ovaries, RA induces Stra8 expression in an anterior—posterior manner [91]. This RA response of Stra8 expression can be recapitulated in vitro. Local injection of RA is sufficient to induce Stra8 expression in cultured embryonic ovaries [88,92]. In the embryonic testes, RA levels are low and as a result Stra8 is not expressed [92]. It has been suggested that RA levels are kept low by CYP26B, an enzyme that is expressed in the somatic testes and oxidizes RA into non-active polar metabolites such as 4-oxo-RA [93]. Stra8 expression is induced in the testes later in development, at day 10 postpartum and then induces meiosis and spermatogenesis [94]. The sexually dimorphic regulation of Stra8 by extrinsic signals explains why entry into gametogenesis occurs during embryogenesis in females and postpartum in males. How Stra8 induces entry into pre-meiotic S phase and triggers gametogenesis is not yet understood. The protein localizes to the nucleus and cytoplasm and harbours transcriptional activity [86]. It will be interesting to determine whether Stra8, like IME1 and ste11+, regulates transcription of genes critical for meiotic chromosome morphogenesis and homologous recombination, one of the earliest events of gametogenesis.

18. CONCLUDING REMARKS
At first glance, the mechanisms underlying gametogenesis appear quite diverse between budding yeast, fission yeast and mammals. However, some striking similarities exist. In all three species, a master regulator is essential for the entry into gametogenesis: IME1 in budding yeast, ste11+ in fission yeast and Stra8 in mice. Cell intrinsic and extrinsic signals converge at their promoters to regulate their expression. Cell intrinsic signals such as mating-type information and respiration in the yeasts and germ cell-specific expression of Dazl in the mouse create a gametogenesis competency state on which extracellular signals act to induce gamete formation. In S. pombe and S. cerevisiae, these extracellular signals are nutritional signals that function through highly conserved pathways—the PKA and the TOR pathways—to induce sporulation. It will be interesting to determine whether the two pathways also regulate entry into gametogenesis in mammals. Downregulation of PKA activity is required during oocyte maturation [95,96], and perhaps it is also needed during entry into gametogenesis. A recent study showed that downregulation of mTORC1 is important for maintenance of the spermatogonial progenitor cells population [97]. Perhaps reduced mTOR activity is also needed for initiation of gametogenesis.

The involvement of nutrient-sensing pathways in controlling gametogenesis in mammals or higher eukaryotes, in general, is speculation. However, another signal critical for entry into the sporulation programme in both fission and budding yeast—respiration—could very well play an important role in mammalian gametogenesis. Defects in mitochondrial function correlate with female infertility in humans [98]. In addition, mutations in mitochondrial DNA affecting respiratory activity in the female mouse germline are selectively removed within four generations, suggesting that functional mitochondria are required for oogenesis [99]. Exploring the importance of the pathways governing gametogenesis identified in yeasts could—as it has done in many instances previously—provide important clues as to how gametogenesis is controlled in humans.

We are grateful to Alexi Goranov, Elcin Ünal and Yoshinori Watanabe for their critical reading of this manuscript. This work was supported by a grant GM62207 from the NIH and a Rubicon grant (825.09.004) from The Netherlands Organization for Scientific Research to F.W. A.A. is also an Investigator of the Howard Hughes Medical Institute.

REFERENCES


Papamichos-Chronakis, M., Gilgoris, T. & Tzamarias, D. 2004 The Snf1 kinase controls glucose repression in yeast by modulating interactions between the Mig1 repressor and the Cyc8-Tup1 co-repressor. *EMBO Rep.* 5, 368–372. (doi:10.1038/sj.embor.7400120)


Jiang, Y. W., Dohrmann, P. R. & Stillman, D. J. 1995 Genetic and physical interactions between yeast RGR1 and SIN4 in chromatin organization and transcriptional regulation. *Genetics* 140, 47–54.


