Chromosome Research (2007) 15:523–537
DOI: 10.1007/s10577-007-1151-0

Molecular mechanisms underlying the mitosis–meiosis decision

Yuriko Harigaya & Masayuki Yamamoto*
Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, 113-0033, Japan; Tel: +81-35-8414386; Fax: +81-35-802-2042; E-mail: yamamoto@biochem.s.u-tokyo.ac.jp
**Correspondence*

Key words: cell cycle regulation, fission yeast, mRNA elimination, nutrition, pheromone signaling, RNA-binding protein

Abstract

Most eukaryotic cells possess genetic potential to perform meiosis, but the vast majority of them never initiate it. The entry to meiosis is strictly regulated by developmental and environmental conditions, which vary significantly from species to species. Molecular mechanisms underlying the mitosis–meiosis decision are unclear in most organisms, except for a few model systems including fission yeast *Schizosaccharomyces pombe*. Nutrient limitation is a cue to the entry into meiosis in this microbe. Signals from nutrients converge on the activity of Mei2 protein, which plays pivotal roles in both induction and progression of meiosis. Here we outline the current knowledge of how a set of environmental stimuli eventually activates Mei2, and discuss how Mei2 governs the meiotic program molecularly, especially focusing on a recent finding that Mei2 antagonizes selective elimination of meiotic messenger RNAs.

Introduction

Meiosis is a process for forming haploid gametes from diploid germ cells, which is essential for sexually reproducing species to transmit genetic information to the next generation. Meiosis drives a specialized cell cycle that consists of one round of DNA synthesis followed by two successive rounds of M phase. This procedure, which halves the genetic material, seems to be highly conserved in eukaryotes. However, cues that cause cells to enter the meiotic pathway appear to vary greatly among species. In multicellular organisms, extrinsic cues from surrounding cells control the differentiation of germline stem cells that will enter the meiotic cell cycle. In lower eukaryotes such as budding yeast *Saccharomyces cerevisiae* or fission yeast *Schizosaccharomyces pombe*, a reduction of available nutrition in the environment triggers the entry into the meiotic cell cycle. In either case, the decision to enter the meiotic cell cycle is tightly regulated in order to prevent execution of the meiotic program in inappropriate developmental contexts. Here, we discuss controls to commit cells to meiosis in fission yeast, which is an excellent model system for study of the mitosis–meiosis decision. Our discussion focuses on the regulatory pathways underlying the two types of cell cycle programs.

Meiotic differentiation includes complex sequential events. Yeast meiosis may be conventionally divided into two parts: (1) exit from the mitotic cell cycle and (2) induction of the alternate, meiotic cell cycle program, in which mitotic events may occur in a peculiar manner and order. The former process in fission yeast has been characterized as a multilayered network of positive and negative factors, which are

regulated at various levels of gene expression from transcription initiation to protein modification and degradation (Yamamoto 1996, 2004, Yamamoto *et al*. 1997). The network also involves many feedback loops to intensify the commitment to meiosis, which may ensure that mitosis and meiosis become mutually exclusive. In contrast, the latter process has been less characterized, although it is generally assumed to be achieved by a highly coordinated transcriptional induction of numerous genes that are specifically required for meiosis (Mata *et al*. 2002). The meiosis-specific gene products are likely to be required (1) to modify the basic cell duplication machinery that operates in the mitotic cell cycle, so that DNA synthesis followed by two successive nuclear divisions will be ensured and (2) to reorganize the cell morphology, which results in the formation of asci containing four haploid spores, the counterparts of gametes in higher eukaryotes. In the next four sections we will describe the developmental fates available for fission yeast cells and overview the current knowledge of cell cycle regulation and gene expression during mitosis and meiosis in this microbe. We will then look at key factors that constitute the central regulatory module for meiosis in fission yeast, namely the Pat1–Mei2 system, focusing on how the exit from the mitotic cell cycle and the initiation of the meiotic program are governed by this system. Finally, we will address how the environmental cues convey a signal to the key factors and regulate the exit from the mitotic cell cycle.

Developmental fates of fission yeast

Under conditions rich in nutrition, fission yeast cells proliferate through mitotic cell cycles, mainly as haploids, which carry one of the two mating types denoted as h^+ (*P*) and h^- (*M*). As in higher eukaryotes, the mitotic cell cycle can be divided into four phases, namely G_1, S, G_2, and M. Mitotic cells assume one of the three alternative fates according to the environmental conditions: They may (1) continue to progress through the mitotic cell cycle, (2) enter into the quiescent stationary phase, or (3) differentiate into the conjugation/meiosis pathway. Fission yeast cells can enter the stationary phase from either mitotic G_1 or G_2. Different environmental stimuli impose different effects on the mitotic cell cycle. Glucose starvation, for example, primarily arrests proliferating cells in G_2 and lead them to the stationary phase (Costello *et al*. 1986). Under nitrogen starvation, in contrast, haploid cells arrest in G_1 and enter the stationary phase, usually after performing two or three rounds of rapid mitotic cycle. If cells of the opposite mating type are neighboring under nitrogen starvation, haploid cells mate to form zygotes, which subsequently undergo meiosis and generate four haploid spores. Importantly, only haploid cells in G_1 phase can initiate the conjugation process. Zygotes can grow as diploids if they are transferred to a rich medium immediately after conjugation. These diploid cells undergo meiosis and form spores when they exhaust the available nutrients. Whether they are zygotes or diploids, meiotic cells follow essentially the same pathway. They arrest transiently in G_1, initiate one round of DNA synthesis, and perform two consecutive nuclear divisions, called first and second meiotic divisions (alternatively, meiosis I and meiosis II). Importantly also, only G_1-arrested diploid cells can enter the meiotic pathway.

Cell cycle regulation during mitosis

During the mitotic cell cycle, the onset of S phase and that of M phase are under tight regulation, in order to ensure that these two phases are coupled in the correct order (reviewed in MacNeill & Nurse 1997, Moser & Russell 2000). The major player in this control is Cdc2, the cyclin-dependent kinase (CDK), whose activity is low in G_1 phase, moderate during S phase and G_2 phase, and high during M phase. Cdc2 is associated with cyclin Cig2 at the G_1/S transition (Bueno & Russell 1993, Connolly & Beach 1994, Obara-Ishihara & Okayama 1994). Deletion of *cig2* delays entry to S phase, but does not completely block the transition, because mitotic cyclin Cdc13 can eventually substitute the function of Cig2 (Fisher & Nurse 1996). The entry to S phase also requires the function of Cdc10, which constitutes a transcription factor complex called DSC1 (Lowndes *et al*. 1992). The DSC1 complex is thought to contain Cdc10, Res1, Res2, and Rep2 to mediate G_1/S specific transcription through both stimulatory and repressive functions (Tanaka *et al*. 1992: Caligiuri & Beach 1993, Miyamoto *et al*. 1994,

Zhu *et al*. 1994, Nakashima *et al*. 1995, Baum *et al*. 1997, Whitehall *et al*. 1999). The DSC1 targets include genes essential for DNA replication, such as *cdc18* encoding a fission yeast homolog of conserved replication factor Cdc6, *cdt1* encoding another conserved replication factor, and *cdc22* encoding ribonucleotide reductase (Kelly *et al*. 1993, Fernandez-Sarabia *et al*. 1993, Hofmann & Beach 1994, White *et al*. 2001). The onset of mitosis is driven by the Cdc2–Cdc13 complex, which is regulated positively by the Cdc25 phosphatase and negatively by the Wee1 and Mik1 kinase (Lundgren *et al*. 1991, Millar et al. 1991). E3 ubiquitin ligase APC/C (anaphase-promoting complex/cyclosome) associated with its activator Slp1 (APC/C^{Slp1}) is required for Cdc13 degradation upon exit from M phase, whereas APC/C associated with another activator Ste9 (APC/C^{Ste9}) and the CDK inhibitor (CKI) Rum1 are responsible for suppressing Cdc2 activity during G_1 phase (Correa-Bordes & Nurse 1995, Yamaguchi *et al*. 1997, Kitamura *et al*. 1998, Blanco *et al*. 2000).

An important concept in the yeast cell cycle is 'Start', which is a point (or an interval) during G_1 at which the cell becomes committed to the mitotic cell cycle. Prior to passing Start, cells have the potential to take the three alternative developmental programs mentioned above. At Start, cells carefully monitor their own size and nutritional conditions and determine their fate. The transition at G_2/M is another control point in the mitotic cell cycle, where again both size and nutritional controls are in operation. Cell cycle regulators relevant to the switch between the mitotic and meiotic cell cycles are shown schematically in Figure 1. Regulation by TOR complex TORC1, included in this figure, will be discussed at the end of this review.

Cell cycle regulation during meiosis

It has been suggested that Cdc2 kinase plays essential roles in driving the meiotic cell cycle. Genetic analyses have established the absolute requirement of the *cdc2* gene for the meiotic G_1/S transition and the second meiotic division (Iino *et al*. 1995). Cdc2 cooperates with cyclin Cig2 to launch premeiotic S phase (Borgne *et al*. 2002). Cyclin Cdc13 and the CDK activator Cdc25 are required for both the first and second meiotic divisions (Iino *et al*. 1995) (Figure 1). During premeiotic S phase, transcription of two groups of genes are simultaneously activated: (1) a group that is also expressed in the mitotic G_1/S interval and required for DNA synthesis (e.g., *cdc18*, *cdt1*, and *cdc22*) and (2) a group that is specific to meiosis, including the *rec* genes (e.g., *rec8* and *rec11*) involved in meiotic recombination (Fox & Smith 1998, Watanabe *et al*. 2001, Mata *et al*. 2002). Genes of both groups appear to be regulated by the DSC1 complex, which is responsible for the transcriptional activation of the former class of genes in mitotic cells (White *et al*. 2001, Cunliffe *et al*. 2004). It is proposed that a meiotic form of DSC1, containing Cdc10, Res2, and a meiosis-specific component Rep1, is responsible for the meiotic pattern of gene expression (Sugiyama *et al*. 1994). The meiosis-specific S phase transcription may lead to a peculiar mode of DNA synthesis coupled with incorporation of a meiosis-specific cohesin complex (Watanabe *et al*. 2001) and recombination at a high frequency, eventually followed by two consecutive nuclear divisions.

Classical genetic analyses have identified meiotic mutants that arrest at a specific point in the meiotic cell cycle (reviewed in Yamamoto *et al*. 1997). The *mei2*, *mei3*, and *mei4* mutants arrest with a single nucleus in the cell, whereas the *mes1* mutants arrest with two nuclei, indicating cell cycle arrest before the second meiotic division. The *mei2* and *mei3* mutants fail to proceed to premeiotic S phase, whereas the *mei4* mutant arrests before the first meiotic division after completing DNA synthesis. One possible function of these gene products is to act on cell cycle machineries that are utilized also in the mitotic cell cycle. Indeed, emerging evidence supports this idea with regard to *mei4* and *mes1*. *mei4* encodes a transcription factor that is required for transcriptional activation of *cdc25* during meiosis, in addition to numerous other target genes (Iino *et al*. 1995, Horie *et al*. 1998, Mata *et al*. 2002) (Figure 1). Lowered Cdc25 activity presumably explains the cell cycle arrest in the *mei4* mutant, because artificial expression of *cdc25* restores meiotic divisions in the *mei4Δ* cells (H. Murakami, personal communication). Mes1 binds and inhibits the APC/C activator Slp1, and hence secures the Cdc2–Cdc13 MPF activity to carry out the second meiotic division (Izawa *et al*. 2005). Meanwhile, *mei3* is required to activate the *mei2* gene product, Mei2, which is the

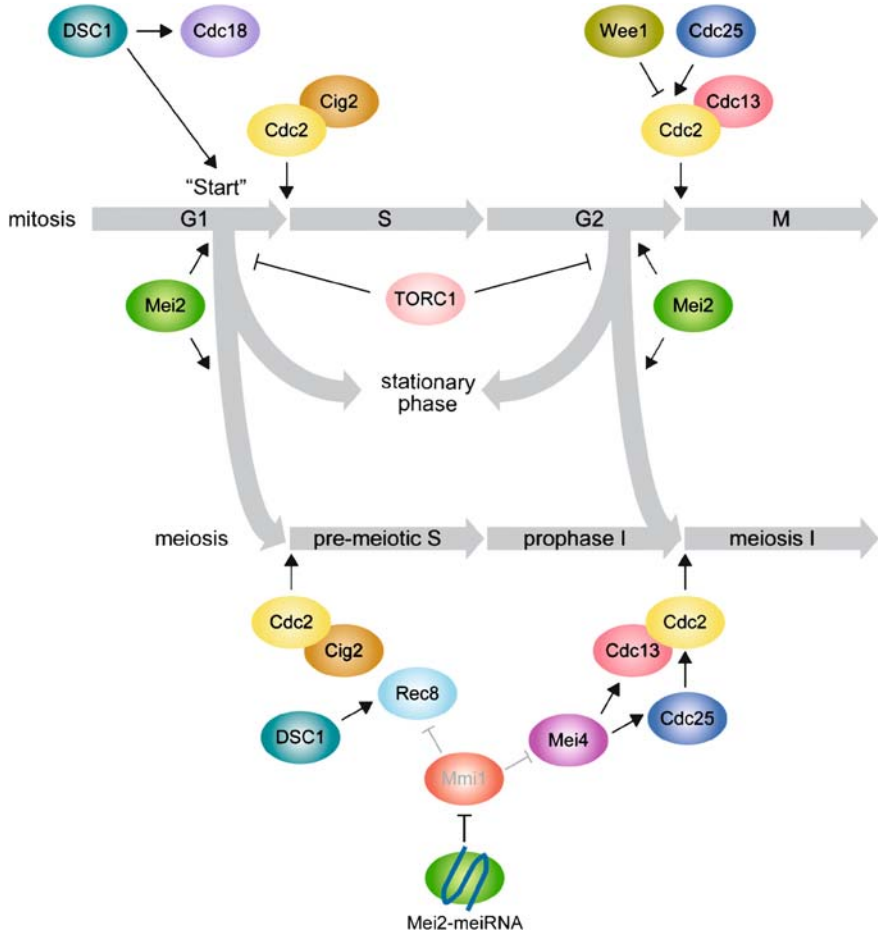

Figure 1. A simplified network of cell cycle regulators operating at the mitosis–meiosis decision in fission yeast. The upper line represents the mitotic cycle, and the lower, the meiotic pathway up to meiosis I. An arrow indicates each cell-cycle window but its length is arbitrary and does not necessarily represent the time span of the window. With regard to the cell cycle switch, active TOR complex TORC1 suppresses exit from the mitotic cell cycle (discussed in the text), whereas Mei2 functions to promote it. Upon exit from the mitotic cycle, Mei2 plays an essential role for execution of the alternate meiotic cell cycle program. To initiate premeiotic S phase, the DSC1 complex promotes transcription of two groups of genes: (1) genes required for DNA replication per se, and (2) genes that function specifically for meiotic replication, such as *rec8*. A subpopulation of the Mei2 protein binds to meiRNA and form a single nuclear dot, which sequesters Mmi1. Mmi1 eliminates a subset of meiosis-specific transcripts (e.g., *mei4* and *rec8*) during the mitotic cell cycle, to suppress the incidence of untimely meiosis (more details in Figure 2). The sequestration of Mmi1 by the Mei2 dot is apparently essential for the promotion of meiosis I. Mei2 normally induces meiosis only from G_1 phase of the mitotic cell cycle, but under artificial conditions it can induce meiosis from G2 phase.

master regulator of meiosis in fission yeast (see below). The molecular function of Mei2 has remained largely unknown until recently, and will be discussed extensively in this review.

Expression and selective elimination of meiotic mRNAs

The cell cycle switch from mitosis to meiosis is accompanied by a striking change in gene expression profiles. Many genes required for the progression of meiosis are up-regulated by a series of transcription factors (Mata *et al.* 2002). In addition, our recent study has revealed a new mode of regulation that contributes to the differential gene expression between mitosis and meiosis (Harigaya *et al.* 2006), as outlined below.

This new regulation operates during the mitotic cell cycle to suppress meiotic gene expression. A hint of the presence of this kind of regulation came from an observation that some meiosis-specific transcripts

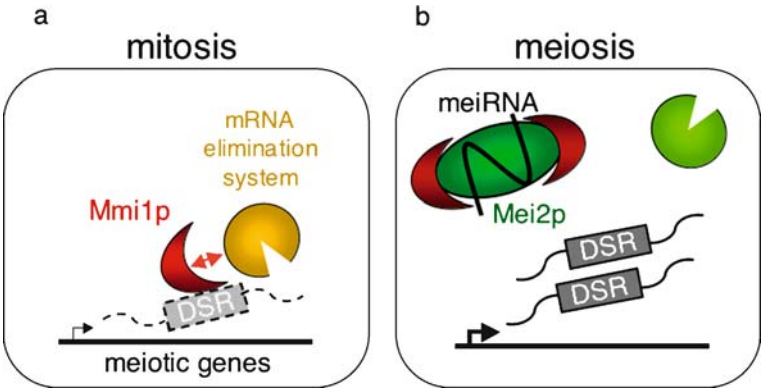

Figure 2. Selective elimination of meiosis-specific mRNAs in mitotic cells. (a) A number of meiosis-specific transcripts in fission yeast carry a region designated DSR, which renders them eliminated if they are expressed during the mitotic cell cycle. Mmi1, a YTH-family RNA-binding protein localized in the nucleus, binds to the DSR and promotes elimination of the transcript, probably cooperating with RNA-degrading exosome machinery. (b) During meiotic prophase, Mei2, which has affinity for Mmi1, forms a dot structure in the nucleus together with meiRNA and sequesters Mmi1 to this dot, so that meiosis-specific transcripts become free from Mmi1-dependent mRNA elimination and remain stably. This figure is reproduced from the Supplemental Figure S1 of our previous publication (Harigaya *et al.* 2006).

failed to accumulate in mitotic cells even when they were transcribed from a constitutive promoter. These transcripts include *mei4* mRNA, which encodes a key transcription factor (Horie *et al.* 1998), and *rec8* mRNA, which encodes a subunit of the meiotic cohesin complex (Watanabe & Nurse 1999). They carry a *cis*-acting region termed DSR (determinant of selective removal), which is responsible for the block of their accumulation in mitotic cells (Harigaya *et al.* 2006). Mmi1, a YTH-family protein (Stoilov *et al.* 2002), binds DSR RNA and causes removal of DSR-containing transcripts. Moreover, Rrp6, a fission yeast ortholog of a conserved nuclear-restricted component of the exosome (Houseley *et al.* 2006), seems to be involved in this removal, suggesting the possibility that DSR-containing transcripts are selectively degraded in the nucleus of growing cells (Harigaya *et al.* 2006) (Figure 2a). Importantly, inactivation of *mmi1* leads to a severe growth defect due to untimely expression of the *mei4* gene and genes stimulated by Mei4. This indicates that the DSR-dependent elimination of meiosis-specific transcripts is not a mere safeguard mechanism that would operate when their promoter happens to be accidentally activated; Rather, dividing cells should keep this system constantly in operation in order to curtail expression of meiotic genes. Microarray analyses have shown that at least a dozen meiosis-specific transcripts are subject to elimination by the DSR–Mmi1 system (Harigaya *et al.* 2006). It seems unlikely, however, that the DSR–Mmi1 system targets all the genes up-regulated during meiosis. For example, *rec12*, which encodes an ortholog of the conserved meiotic DSB inducer Spo11 (Keeney *et al.* 1997), does not appear to be regulated by this system (our unpublished results).

Fission yeast cells may use another mechanism to prevent expression and function of meiotic genes during proliferation. It has been found that a fraction of intron-containing transcripts remain unspliced until a specific period during meiosis, among which are *crs1* and *rem1* mRNAs, both encoding meiosis-specific cyclin (Averbeck *et al.* 2005). Analyses of gene chimeras have indicated that splicing of *crs1* and *rem1* mRNAs is prevented in mitotically growing cells through sequences located outside the coding region (Averbeck *et al.* 2005). During meiosis, the inhibition of *rem1* splicing appears to be overcome by the function of Mei4 (Malapeira *et al.* 2005).

The Pat1–Mei2 system: central regulator for fission yeast meiosis

Discovery of the *pat1* mutation (initially also called *ran1*) opened the door to molecular understanding of the meiotic entry in fission yeast (Beach *et al.* 1985, Iino & Yamamoto 1985, Nurse 1985). Cells carrying the *pat1-114* mutation show temperature-sensitive growth and perform ectopic meiosis and sporulation at the restrictive temperature, regardless of nutritional

conditions and the ploidy of the cells, indicating that Pat1 is a factor that prevents cells from entering meiosis when they should grow mitotically.

The *pat1* gene encodes a Ser/Thr protein kinase (McLeod & Beach 1988), which phosphorylates Mei2, an RRM-type RNA-binding protein critical for the entry to meiosis, on residues Ser438 and Thr527 (Watanabe *et al*. 1997). Even weak expression of mutant Mei2 carrying alanine in these two positions ($Mei2^{SATA}$) results in ectopic meiosis and sporulation, as does inactivation of Pat1 kinase (Watanabe *et al*. 1997). This indicates that Mei2 is a critical target of Pat1 and a pivotal factor for the entry to meiosis.

How is the Mei2 activity suppressed through phosphorylation by Pat1 kinase? As one possible mechanism, this phosphorylation confers a shorter half-life to Mei2, due to increased susceptibility of it to proteasome-dependent destruction involving E2 Ubc2 and E3 Ubr1 (Kitamura *et al*. 2001). As another possibility, phosphorylated Mei2 shows increased affinity for Rad24, the major 14–3–3 protein in fission yeast. The binding of Rad24 to phosphorylated Mei2 inhibits association of Mei2 with meiRNA (Sato *et al*. 2002), which is a non-coding RNA that cooperates with Mei2 to propel meiotic progression (Watanabe & Yamamoto 1994). Deletion of *rad24* accelerates commitment to meiosis, but this mutation by itself does not result in such a conspicuous meiosis-inducing phenotype as shown by the *pat1-114* or $mei2^{SATA}$ mutation. Thus, it seems that there are yet-unidentified functions of Mei2 that are critical to inducing meiosis and are inhibited by the phosphorylation by Pat1.

How could Mei2 provoke and govern the meiotic program?

Activation of Mei2 can confer a cell cycle switch from mitosis to meiosis. Therefore, understanding the molecular function of Mei2 is a key to fission yeast meiosis, but this has not been an easy task. In addition to its function as a meiotic inducer, Mei2 is known to play essential roles in the induction of premeiotic S phase and first meiotic division. This has been deduced from the following observations. (1) Cells devoid of the *mei2* gene can cease growth in response to nutritional starvation but cannot initiate premeiotic DNA synthesis. (2) In a temperature-sensitive mutant of *mei2* (*mei2–33*), the meiotic cell cycle is arrested before the initiation of first meiotic division after completing premeiotic DNA synthesis (Watanabe & Yamamoto 1994). An early study has shown that transcription of *mei2* is up-regulated by Ste11, an HMG-type transcription factor, upon nutrient deprivation (Sugimoto *et al*. 1991). Mei2 has an intrinsic feature to shuttle between the nucleus and the cytoplasm (Sato *et al*. 2001). Upon entry to meiosis, a subpopulation of this protein forms a peculiar single dot in the nucleus, which we hereafter call the Mei2 dot (Watanabe *et al*. 1997, Yamashita *et al*. 1998). The Mei2 dot is located on the chromosomal locus of the *sme2* gene, from which meiRNA is transcribed (Shimada *et al*. 2003). In *sme2Δ* cells, which lack meiRNA, the Mei2 dot formation is impaired, and the meiotic cell cycle is blocked prior to the initiation of meiosis I (Watanabe *et al*. 1997, Yamashita *et al*. 1998). Thus, meiRNA performs a function required specifically for meiosis I, in cooperation with Mei2. Given that *mei4* mRNA is undetectable in *sme2Δ* cells (A. Yamashita and M. Yamamoto, unpublished), the meiotic cell cycle arrest in this mutant appears to be attributable at least in part to the absence of Mei4 function.

In summary, Mei2 is apparently involved in multiple steps of sexual differentiation: (1) the withdrawal from the mitotic division cycle; (2) the start of premeiotic S phase; and (3) the initiation of meiosis I. It has been speculated that the molecular activity of Mei2 could be linked to the cell cycle regulators and gene expression machinery, either directly or indirectly. Indeed, our recent study has demonstrated that one function of Mei2 is to act on a regulatory factor for meiotic gene expression. We will discuss this in the next section.

Mei2 turns off the DSR–Mmi1 system

Our study has revealed a direct link between Mei2 and the DSR–Mmi1 system for elimination of meiosis-specific transcripts (Harigaya *et al*. 2006). This was recognized originally through localization analysis of Mmi1. During vegetative growth, Mmi1 was localized to multiple foci in the nucleus, but it converged to a single dot that overlapped with the Mei2 dot during meiotic prophase. The concentration of Mmi1 did not occur in the absence of the Mei2 dot in *mei2Δ* or *sme2Δ* cells. These observations have

lead us to speculate that Mei2 may turn off the DSR–Mmi1 system by sequestering Mmi1 to the dot, and thereby ensure stable expression of meiosis-specific transcripts (Figure 2b). Consistent with this idea, the arrest prior to meiosis I caused by loss of the Mei2 dot can be rescued by a reduction in the Mmi1 activity, presumably because it allows accumulation of DSR-containing transcripts, *mei4* mRNA among others (Harigaya *et al*. 2006).

Shutdown of the DSR–Mmi1 system by the Mei2 dot can fully account for the function of Mei2 necessary to initiate meiosis I. However, it does not appear to be the only task that Mei2 executes during meiosis. This is because inactivation of Mmi1 does not lead to the conspicuous meiosis-inducing phenotype of the *mei2*^{SATA} mutant. Rather, loss of *mmi1* causes a severe growth defect that seems likely to be a perturbation of the mitotic cell cycle by untimely function of meiotic genes (our unpublished results). This may also indicate that Mei2-dependent inactivation of the DSR–Mmi1 system should be under strict temporal control so that expression of meiotic genes may never precede the withdrawal from the mitotic cycle. There may be a mechanism that signals Mei2, after the exit from the mitotic cycle, to launch the next action to carry forward the meiotic program by promoting expression of DSR-containing transcripts.

Mei2 and growth inhibition

Transient G_1 arrest occurs at the very beginning of both *pat1*-driven and *mei2*^{SATA}-driven meiosis. Although *mei2Δ* diploids can arrest in G_1 in response to nutritional starvation, the kinetics are delayed (Watanabe & Yamamoto 1994). Loss of *mei2* function completely blocks *pat1*-driven meiosis. Furthermore, the *pat1-114 mei2Δ* double mutant can continue to grow at the temperature restrictive for *pat1-114*. All these observations indicate a function of active Mei2 to inhibit growth and cause transient arrest in G_1 phase at the entry to the meiotic cell cycle. The molecular mechanism by which unphosphorylated Mei2 induces transient G_1 arrest remains elusive. It is possible that a reduction in the level of *rep2* mRNA in response to the expression of *mei2*^{SATA} may contribute to the cell cycle arrest in G_1, given that deletion of *rep2* leads to the accumulation of cells in G_1 (Nakashima *et al*. 1995, Watanabe *et al*. 1997). The link between Mei2 and the Cdc2 activity, whose down-regulation is essential for imposing cell cycle arrest, has not been pursued thoroughly but it may be indirect (see below).

Mei2 and meiotic G_1/S transition

The function of Mei2 is absolutely necessary for the meiotic G_1/S transition (or 'Start' of the meiotic cell cycle), since *mei2Δ* diploid cells starved of nitrogen are unable to increase the DNA content after arresting at G_1 (Watanabe & Yamamoto 1994). The molecular mechanism by which Mei2 promotes DNA synthesis also remains elusive. Given that Mei2^{SATA} induces a transcriptional switch between *rep1* and *rep2* (Watanabe *et al*. 1997), and that transcription of *rep1*, the putative meiotic counterpart of *rep2*, is essential for premeiotic S phase (Sugiyama *et al*. 1994), it is possible that Mei2 may promote initiation of premeiotic S phase by enhancing *rep1* expression. It should be noted, however, that *rep1* mRNA can be expressed in *mei2Δ* diploid cells in response to nitrogen starvation (Sugiyama *et al*. 1994). Nevertheless, it may be presumed that the protein level and/or the activity of Rep1 may be reduced in the absence of *mei2* and cannot support premeiotic DNA synthesis. Cdc2 is essential for premeiotic DNA synthesis and is thought to form a complex with cyclin Cig2 during this S phase. It also remains an interesting question whether and how Mei2 acts on the S phase CDK–cyclin complex to initiate premeiotic DNA synthesis.

Implications from the G_2-exit meiosis

The meiotic program naturally starts from G_1 because activation of Mei2 is restricted to this phase of the cell cycle. However, in certain settings, meiosis appears to be induced from other cell cycle windows than G_1 (Watanabe *et al*. 2001). In *rum1Δ* cells, which have a very short G_1 phase, meiosis can be induced from the G_2 window in three different ways to activate Mei2 artificially: (1) by inactivation of Pat1 kinase, (2) by ectopically expressing *mei3* (see below), and (3) by ectopically expressing *mei2*^{SATA}. It seems from these results that unphosphorylated Mei2 induces quiescence of cell proliferation rather than cell cycle arrest specific to G_1 phase

(Figure 1). After the inhibition of cell proliferation, Mei2 appears to function to induce premeiotic DNA synthesis if cells are arrested in G_1. However, if they are arrested in G_2, Mei2 appears to induce meiosis from G_2, which undergoes no DNA synthesis but only two rounds of nuclear division. If meiosis is induced from G_2, DSC1-regulated genes required for both premitotic and premeiotic S phase, such as *cdc18* and *cdc22*, fail to be expressed. Curiously, however, DSC1-regulated genes required only for premeiotic S phase, such as *rec8*, are expressed substantially, though the timing and the level of their expression may somewhat change (Watanabe *et al*. 2001). It may be too tempting to speculate that Mei2 can recognize in which window of the cell cycle the cells are and can start a meiotic program adjusted to the window. Alternatively, once the *mei2*-dependent growth inhibition is established, the state of the CDK activity and/or other cell cycle-specific features, such as the licensing state of replication origins, may autonomously set the start point of the meiotic program.

Signal pathways linking extracellular cues to the commitment to meiosis

Here we address which signaling cascades emanate from environmental stimuli to the activation of Mei2. This section provides an overview, and more detailed aspects will be discussed in the next four sections. The initiation of meiosis is regulated by two major signal transduction pathways in fission yeast, one responding to the nutritional conditions (Figure 3a) and the other to the mating pheromones (Figure 3b). Nutrient starvation results in transcriptional activation of the *ste11* gene, through down-regulation of the activity of cAMP-dependent protein kinase (PKA) (Maeda *et al*. 1990, Sugimoto *et al*. 1991, Mochizuki & Yamamoto 1992). Ste11, an HMG-type transcription factor, activates a number of genes required for mating and meiosis, including genes encoding mating pheromones and their receptors, mating-type genes (*mat1-Pc*, *mat1-Pi*, *mat1-Mc*, and *mat1-Mi*), and *mei2* (Sugimoto *et al*. 1991). Binding of the pheromone to its receptor results in activation of a MAPK cascade, which leads to the induction of additional genes required for the mating process. Pheromone communication and subsequent MAPK activation occur not only in haploid but also in diploid cells. In zygotes and diploid cells, h^+-specific Mat1-Pi and h^--specific Mat1-Mc cooperatively induce the *mei3* gene, which encodes an inhibitor of Pat1 kinase (McLeod & Beach 1988, Li & McLeod 1996, Van Heeckeren *et al*. 1998). The binding of Mei3 is thought to confer full inactivation of Pat1, thereby liberating Mei2 from the inhibitory phosphorylation (Watanabe *et al*. 1997). In cells under physiological conditions, complete inactivation of Pat1 is strictly dependent on expression of Mei3,

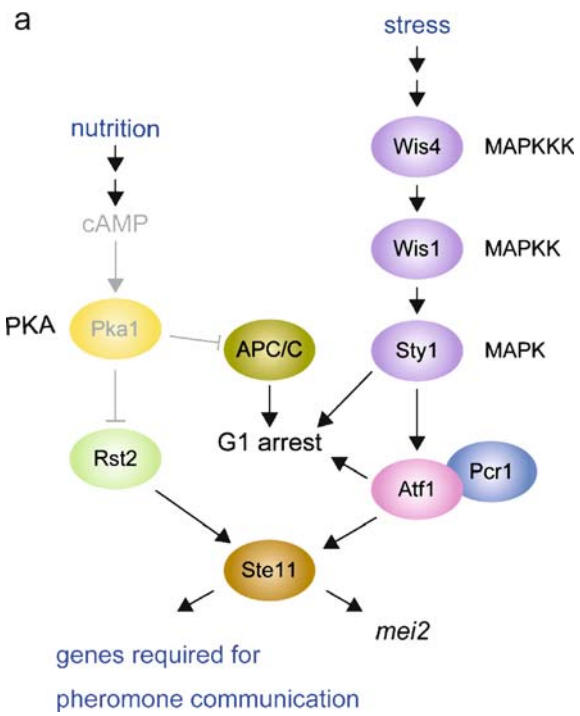

Figure 3. Signal transduction pathways that regulate the initiation of meiosis. (a) The cAMP–PKA pathway and the stress-responsive MAPK pathway mediate signals from external nutrition and stress, respectively, which ensure cell cycle arrest in G_1 and transcriptional activation of the *ste11* gene under starved conditions. Ste11 activates a number of genes required for pheromone communication and induction of meiosis. (b) Binding of either *P* or *M* pheromone to its receptor leads to activation of the Byr2–Byr1–Spk1 pathway. Ste11 is again targeted by Spk1 to activate the pheromone-induced transcriptional program and to promote G_1 arrest, both of which are prerequisites for the mating and meiotic process. Successful conjugation results in production of both h^+-specific Mat1-Pi and h^--specific Mat1-Mc in a single cell, which cooperatively induce transcription of *mei3*. Mei3 inhibits Pat1 kinase, and hence liberates Mei2 from the inhibitory phosphorylation. For simplification, many factors and interactions known to be involved in this process are omitted in this illustration.

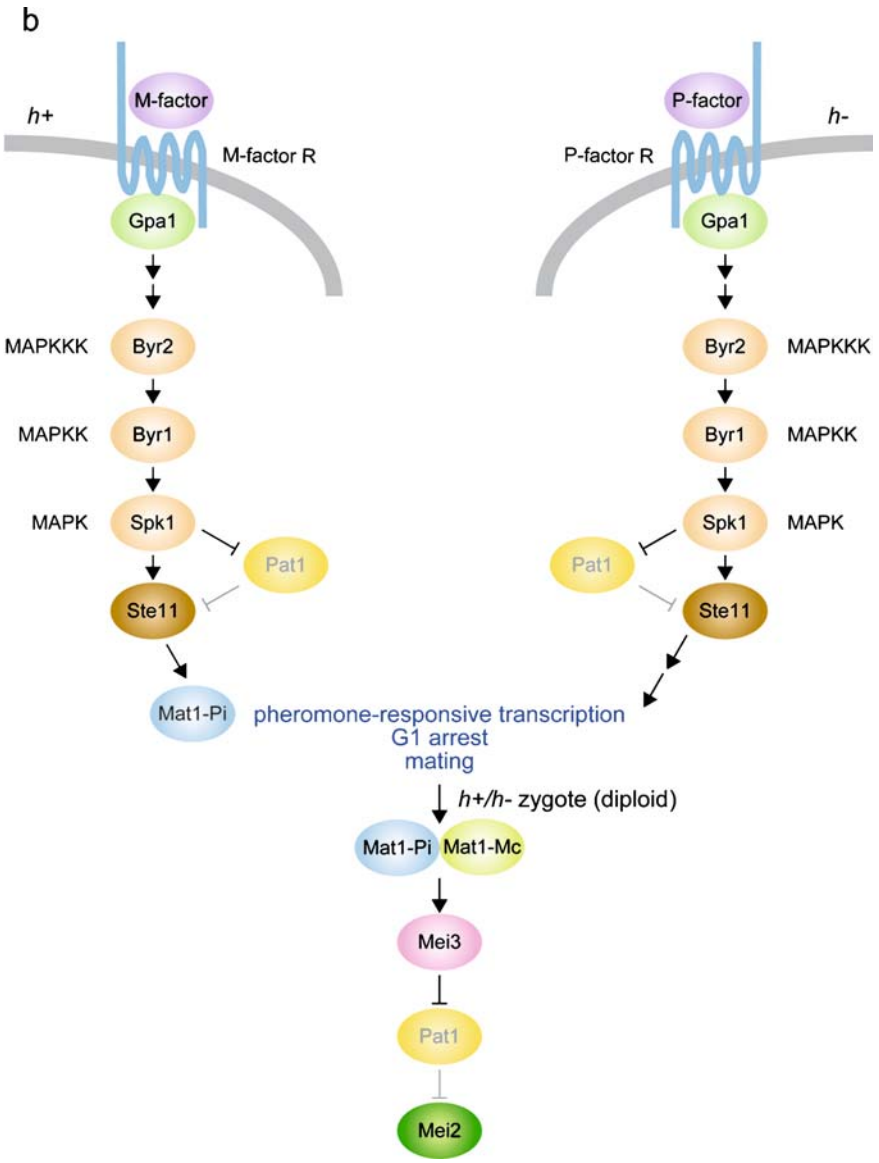

Figure 3. continued

which ensures that meiosis is induced only in diploid cells heterozygous for *P* and *M* mating-type genes.

Nutritional starvation induces G_1 arrest and *ste11* transcription

The sexual differentiation process is triggered by reduced availability of nutrients in fission yeast, as in many microorganisms. Starvation, especially of nitrogen, promotes (1) arrest in the pre-Start G_1 phase, which is a prerequisite for sexual differentiation, and (2) transcriptional activation of the *ste11* gene, which in turn activates transcription of a number of genes required for mating and meiosis (Figure 3a).

Starvation-induced pre-Start arrest requires down-regulation of the CDK activity that drives the G_1/S transition. It is necessary to inhibit both the Cdc2–Cig2 complex, which triggers timely S phase, and the Cdc2–Cdc13 complex, which can take over Cig2 function in its absence (Fisher & Nurse 1996).

Cig2 cyclin is thought to be a primary target for degradation in response to nutritional starvation (discussed in Nielsen 2004). Degradation of Cig2 delays G_1/S transition, which leads to accumulation of Rum1. Rum1 directly inhibits the Cdc2–Cdc13 complex and promotes degradation of both Cig2 and Cdc13 via APC/C^{ste9} (Correa-Bordes & Nurse 1995). Two pathways, the cAMP–PKA pathway and the stress-responsive MAPK pathway, have been shown to link nutritional conditions with CDK regulation.

Nutrient starvation results in a reduced level of intracellular cAMP and cAMP-dependent protein kinase (PKA) activity (Maeda *et al*. 1990, Mochizuki & Yamamoto 1992). A reduction in the PKA activity relieves inhibitory phosphorylation of APC/C (Yamashita *et al*. 1996). This facilitates degradation of both the Cig2 and the Cdc13 cyclin.

Starvation of nutrients also leads to activation of the stress-responsive MAPK pathway (Wis4/Wak1 and Win1 MAPKKKs, Wis1 MAPKK, and Sty1/Spc1 MAPK) (Samejima *et al*. 1997, 1998, Shieh *et al*. 1997, 1998, Shiozaki *et al*. 1997). A target of Sty1/Spc1 is a bZIP protein Atf1/Gad7, which constitutes a transcription-activating complex (Shiozaki & Russell 1996, Wilkinson *et al*. 1996). The *wis1*, *sty1,* and *atf1* genes are required for proper G_1 arrest following nitrogen starvation (Warbrick & Fantes 1991, Takeda *et al*. 1995, Kanoh *et al*. 1996, Shiozaki & Russell 1996, Wilkinson *et al*. 1996). While the mechanism by which this stress pathway induces pre-Start G_1 arrest is not yet known, the following scenario has been proposed based on the fact that nitrogen starvation speeds up mitosis, affecting G_2/M transition through the stress pathway: Starvation-induced rapid mitosis results in smaller size of the newborn G_1 cells; To attain the critical size for entry to S phase, the length of the G_1 window is prolonged; Consequently, Rum1 protein accumulates in excess and down-regulates the CDK activity completely (Nielsen 2004). Consistent with this hypothesis, the *atf1Δ* strain can arrest at G_1 if it carries the *wee1-50* mutation, which causes precocious G_2/M transition (Kanoh *et al*. 1996).

Starvation-induced *ste11* expression is also mediated by the cAMP–PKA and the stress-responsive MAPK pathways. Rst2, a zinc-finger transcription factor, is a substrate of PKA and is inactive when phosphorylated by PKA (Kunitomo *et al*. 2000, Higuchi *et al*. 2002). The unphosphorylated form of Rst2 stimulates transcription of the *ste11* gene. Atf1/Gad7 and its binding partner Pcr1 are also apparently involved in the regulation of *ste11*: Deletion of either *atf1* or *pcr1* reduces the level of *ste11* expression considerably (Kanoh *et al*. 1996, Watanabe & Yamamoto 1996). It remains to be determined whether the Atf1/Gad7-Pcr1 complex directly regulates the *ste11* gene.

Pheromone signaling

The mating type of a fission yeast cell is determined by the DNA sequence at the *mat1* locus. If it is *P* (*mat1-P*), the cell assumes the h^+ mating type, and if it is *M* (*mat1-M*), the cell assumes the h^- mating type. Both *mat1-P* and *mat1-M* consist of two divergently transcribed units, termed *mat1-Pc* and *mat1-Pi*, and *mat1-Mc* and *mat1-Mi* (Kelly *et al*. 1988). Whereas *mat1-Mc* and *mat1-Pc* are expressed in both mitotic and meiotic cells, though less intensively in the former, *mat1-Mi* and *mat1-Pi* are expressed only when the pheromone signaling is on (Kelly *et al*. 1988, Nielsen *et al*. 1992, Willer *et al*. 1995).

Ste11 activates transcription of genes required for pheromone communication (Sugimoto *et al*. 1991). The expression and the activity of Ste11 itself are apparently affected by both nitrogen starvation and pheromone signaling. This gives a somewhat complex aspect to overall gene regulation by Ste11, and certain genes targeted by Ste11 are known to be induced at various levels depending on the strength of these two signals (Aono *et al*. 1994, Sugiyama *et al*. 1994, Petersen *et al*. 1995). Ste11 enhances expression of *mat1-Pc* in h^+ cells and *mat1-Mc* in h^- cells. These genes promote the production of mating pheromones and their receptors in a mating type-specific manner. The pheromone signal is mediated by Gpa1, a receptor-coupled G-protein, and a downstream MAPK module consisting of Byr2 (MAPKKK), Byr1 (MAPKK), and Spk1 (MAPK) (Obara *et al*. 1991, Gotoh *et al*. 1993, Neiman *et al*. 1993). Substrates phosphorylated by Spk1 are thought to cause the physiological effects of pheromone stimulation.

Pheromone-induced transcription and G_1 arrest

Many genes, including those targeted by Ste11, DSC1, or Mmi1, are transcriptionally up-regulated

by pheromone stimulation (Chikashige *et al*. 2006, Harigaya *et al*. 2006, Mata & Bahler 2006). However, connections between the pheromone pathway and the regulators of gene expression are mostly unclear, except for the relation between Spk1 and Ste11. It has been recently demonstrated that Ste11 is a target of MAP kinase Spk1 (Kjaerulff *et al*. 2005), but the physiological significance of this relation remains unknown.

Interestingly, transcription of some pheromone-dependent genes, such as *mat1-Mi* and *fus1*, takes place only in G_1 cells (Stern & Nurse 1998). This may explain at least in part why cells do not conjugate when arrested in G_2. A related observation is that nitrogen starvation fails to induce *mei3* expression in *cdc25-22* or *rum1Δ* diploid cells, which have extended G_2 phase, suggesting a mechanism that restricts transcription of *mei3* to G_1 cells (Watanabe *et al*. 2001). This appears to ensure the start of the meiotic program from the G_1 window of the cell cycle. What then causes restriction of the pheromone-dependent transcription to G_1? The restriction could be due to cell cycle-specific down-regulation of either the pheromone pathway or the downstream transcriptional apparatus. At least, however, nitrogen starvation-responsive activation of transcription by Ste11 seems to be independent of the cell cycle, because induction of Ste11 target genes, such as *mei2*, occurs normally in the *nuc2–663* mutant, which is defective in G_1 arrest (Kumada *et al*. 1995).

Experiments using synthetic pheromones have verified that the mating pheromone causes cell cycle arrest in G_1 in fission yeast (Davey & Nielsen 1994, Imai & Yamamoto 1994). It is suggested that the pheromone blocks the onset of S phase by suppressing the Cdc2 kinase activity through Rum1 and APC/C (Stern & Nurse 1997, Stern & Nurse 1998). Ste11, a target of the pheromone pathway, has been shown to contribute to this pheromone-induced G_1 arrest (Kjaerulff *et al*. 2005).

How is the nutritional status recognized?

Whereas signals from the nutritional status, which are eventually transmitted to the master meiosis-regulator Mei2, have been elucidated considerably, a fundamental question still remains unanswered: How do cells 'sense' the nutrition in molecular terms? Recent studies on Tor2, one of the two TOR kinases in fission yeast, have provided an important clue (Alvarez & Moreno 2006, Uritani *et al*. 2006, Matsuo *et al*. 2007, Weisman *et al*. 2007). Loss of *tor2* function apparently causes the same cellular responses as nitrogen starvation (i.e., entry to quiescence or conjugation in haploid cells and to meiosis in diploid cells). Thus, Tor2 appears to be central to the switch between cell growth and cell differentiation in response to the availability of nitrogen, and is likely to suppress other developmental fates than proliferation under rich nutrition. It is highly likely that down-regulation of Tor2 activity is a physiological process that occurs in cells subjected to nitrogen starvation.

It has been shown that Tor2 forms a growth-controlling TOR complex (TORC1), which is conserved from budding yeast to human cells and controls ribosome biogenesis (Alvarez & Moreno 2006, Matsuo *et al*. 2007). One component of fission yeast TORC1 is the raptor ortholog Mip1, which has been shown to interact with Mei2 (Shinozaki-Yabana *et al*. 2000). Like mammalian cells, fission yeast has an ortholog of Rheb (Rhb1), which is a Ras superfamily GTPase, and orthologs of TSC1 and TSC2 (Tsc1 and Tsc2), which form a complex that acts as a GTPase activating protein (GAP) for Rheb. Rhb1 and the Tsc1–Tsc2 complex appear to function as upstream regulators of TORC1 in fission yeast (Matsumoto *et al*. 2002, van Slegtenhorst *et al*. 2004, Urano *et al*. 2005, 2007, Uritani *et al*. 2006, Matsuo *et al*. 2007, Weisman *et al*. 2007).

Unlike Tor2, Tor1 (the other TOR homolog) is nonessential for growth, but it is required for the entry to quiescence and sexual development (Kawai *et al*. 2001, Weisman & Choder 2001, Matsuo *et al*. 2003). Tor1 appears to be the major TOR kinase that forms TORC2 in fission yeast (Alvarez & Moreno 2006, Matsuo *et al*. 2007).

Conclusion and future directions

The availability of nutrients is key for the mitosis–meiosis decision in fission yeast. Signals from nutrients converge on the master meiosis regulator Mei2. Although the entire suite of molecular functions that Mei2 exerts in order to provoke and orchestrate the meiotic program has not yet been unveiled, a Mei2 function essential for the initiation

of meiosis I has now become apparent as suppression of the DSR–Mmi1 system. This system eliminates a subset of meiosis-specific transcripts during proliferation and hence blocks an untimely entry into meiosis. In future studies it will be intriguing to elucidate the remaining molecular functions of Mei2, such as that required to arrest cell proliferation or to elicit the meiotic cell cycle program following the exit from the mitotic cycle. Another important problem to be solved will be the molecular mechanism by which cells recognize nutritional conditions. Recent findings that fission yeast TORC1 apparently plays a pivotal role in this recognition seem to provide a promising prospect of solving this question.

Acknowledgements

We thank Akira Yamashita and Kayoko Tanaka for discussion during the preparation of this manuscript. We also thank Hiroshi Murakami for permission to refer to his unpublished results. Our recent studies cited in this review were supported by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan to Y.M.

References

Alvarez B, Moreno S (2006) Fission yeast Tor2 promotes cell growth and represses cell differentiation. *J Cell Sci* **119**: 4475–4485.

Aono T, Yanai H, Miki F, Davey J, Shimoda C (1994) Mating pheromone-induced expression of the *mat1-Pm* gene of *Schizosaccharomyces pombe*: identification of signalling components and characterization of upstream controlling elements. *Yeast* **10**: 757–770.

Averbeck N, Sunder S, Sample N, Wise JA, Leatherwood J (2005) Negative control contributes to an extensive program of meiotic splicing in fission yeast. *Mol Cell* **18**: 491–498.

Baum B, Wuarin J, Nurse P (1997) Control of S-phase periodic transcription in the fission yeast mitotic cycle. *EMBO J* **16**: 4676–4688.

Beach D, Rodgers L, Gould J (1985) *ran1*$^{+}$ controls the transition from mitotic division to meiosis in fission yeast. *Curr Genet* **10**: 297–311.

Blanco MA, Sanchez-Diaz A, de Prada JM, Moreno S (2000) APC$^{ste9/srw1}$ promotes degradation of mitotic cyclins in G_1 and is inhibited by cdc2 phosphorylation. *EMBO J* **19**: 3945–3955.

Borgne A, Murakami H, Ayte J, Nurse P (2002) The G_1/S cyclin Cig2p during meiosis in fission yeast. *Mol Biol Cell* **13**: 2080–2090.

Bueno A, Russell P (1993) Two fission yeast B-type cyclins, Cig2 and Cdc13, have different functions in mitosis. *Mol Cell Biol* **13**: 2286–2297.

Caligiuri M, Beach D (1993) Sct1 functions in partnership with Cdc10 in a transcription complex that activates cell cycle START and inhibits differentiation. *Cell* **72**: 607–619.

Chikashige Y, Tsutsumi C, Yamane M, Okamasa K, Haraguchi T, Hiraoka Y (2006) Meiotic proteins bqt1 and bqt2 tether telomeres to form the bouquet arrangement of chromosomes. *Cell* **125**: 59–69.

Connolly T, Beach D (1994) Interaction between the Cig1 and Cig2 B-type cyclins in the fission yeast cell cycle. *Mol Cell Biol* **14**: 768–776.

Correa-Bordes J, Nurse P (1995) p25^{rum1} orders S phase and mitosis by acting as an inhibitor of the p34^{cdc2} mitotic kinase. *Cell* **83**: 1001–1009.

Costello G, Rodgers L, Beach D (1986) Fission yeast enters the stationary phase G0 state from either mitotic G_1 or G_2. *Curr Genet* **11**: 119–125.

Cunliffe L, White S, McInerny CJ (2004) DSC1-MCB regulation of meiotic transcription in *Schizosaccharomyces pombe*. *Mol Genet Genomics* **271**: 60–71.

Davey J, Nielsen O (1994) Mutations in *cyr1* and *pat1* reveal pheromone-induced G_1 arrest in the fission yeast *Schizosaccharomyces pombe*. *Curr Genet* **26**: 105–112.

Fernandez-Sarabia MJ, McInerny C, Harris P, Gordon C, Fantes P (1993) The cell cycle genes *cdc22*$^{+}$ and *suc22*$^{+}$ of the fission yeast *Schizosaccharomyces pombe* encode the large and small subunits of ribonucleotide reductase. *Mol Gen Genet* **238**: 241–251.

Fisher DL, Nurse P (1996) A single fission yeast mitotic cyclin B p34^{cdc2} kinase promotes both S-phase and mitosis in the absence of G_1 cyclins. *EMBO J* **15**: 850–860.

Fox ME, Smith GR (1998) Control of meiotic recombination in *Schizosaccharomyces pombe*. *Prog Nucleic Acid Res Mol Biol* **61**: 345–378.

Gotoh Y, Nishida E, Shimanuki M, Toda T, Imai Y, Yamamoto M (1993) *Schizosaccharomyces pombe* Spk1 is a tyrosine-phosphorylated protein functionally related to *Xenopus* mitogen-activated protein kinase. *Mol Cell Biol* **13**: 6427–6434.

Harigaya Y, Tanaka H, Yamanaka S, *et al.* (2006) Selective elimination of messenger RNA prevents an incidence of untimely meiosis. *Nature* **442**: 45–50.

Higuchi T, Watanabe Y, Yamamoto M (2002) Protein kinase A regulates sexual development and gluconeogenesis through phosphorylation of the Zn finger transcriptional activator Rst2p in fission yeast. *Mol Cell Biol* **22**: 1–11.

Hofmann JF, Beach D (1994) *cdt1* is an essential target of the Cdc10/Sct1 transcription factor: requirement for DNA replication and inhibition of mitosis. *EMBO J* **13**: 425–434.

Horie S, Watanabe Y, Tanaka K, *et al.* (1998) The *Schizosaccharomyces pombe mei4*$^{+}$ gene encodes a meiosis-specific transcription factor containing a forkhead DNA-binding domain. *Mol Cell Biol* **18**: 2118–2129.

Houseley J, LaCava J, Tollervey D (2006) RNA-quality control by the exosome. *Nat Rev Mol Cell Biol* **7**: 529–539.

Iino Y, Yamamoto M (1985) Mutants of *Shizosaccharomyces pombe* which sporulate in the haploid state. *Mol Gen Genet* **198**: 416.

Iino Y, Hiramine Y, Yamamoto M (1995) The role of *cdc2* and other genes in meiosis in *Schizosaccharomyces pombe*. *Genetics* **140**: 1235–1245.

Imai Y, Yamamoto M (1994) The fission yeast mating pheromone P-factor: its molecular structure, gene structure, and ability to induce gene expression and G_1 arrest in the mating partner. *Genes Dev* **8**: 328–338.

Izawa D, Goto M, Yamashita A, Yamano H, Yamamoto M (2005) Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p. *Nature* **434**: 529–533.

Kanoh J, Watanabe Y, Ohsugi M, Iino Y, Yamamoto M (1996) *Schizosaccharomyces pombe gad7*$^+$ encodes a phosphoprotein with a bZIP domain, which is required for proper G_1 arrest and gene expression under nitrogen starvation. *Genes Cells* **1**: 391–408.

Kawai M, Nakashima A, Ueno M, *et al*. (2001) Fission yeast tor1 functions in response to various stresses including nitrogen starvation, high osmolarity, and high temperature. *Curr Genet* **39**: 166–174.

Keeney S, Giroux CN, Kleckner N (1997) Meiosis-specific DNA double-strand breaks are catalyzed by Spo11, a member of a widely conserved protein family. *Cell* **88**: 375–384.

Kelly M, Burke J, Smith M, Klar A, Beach D (1988) Four mating-type genes control sexual differentiation in the fission yeast. *EMBO J* **7**: 1537–1547.

Kelly TJ, Martin GS, Forsburg SL, Stephen RJ, Russo A, Nurse P (1993) The fission yeast *cdc18*$^+$ gene product couples S phase to START and mitosis. *Cell* **74**: 371–382.

Kitamura K, Maekawa H, Shimoda C (1998) Fission yeast Ste9, a homolog of Hct1/Cdh1 and Fizzy-related, is a novel negative regulator of cell cycle progression during G_1-phase. *Mol Biol Cell* **9**: 1065–1080.

Kitamura K, Katayama S, Dhut S, *et al*. (2001) Phosphorylation of Mei2 and Ste11 by Pat1 kinase inhibits sexual differentiation via ubiquitin proteolysis and 14-3-3 protein in fission yeast. *Dev Cell* **1**: 389–399.

Kjaerulff S, Lautrup-Larsen I, Truelsen S, Pedersen M, Nielsen O (2005) Constitutive activation of the fission yeast pheromone-responsive pathway induces ectopic meiosis and reveals ste11 as a mitogen-activated protein kinase target. *Mol Cell Biol* **25**: 2045–2059.

Kumada K, Su S, Yanagida M, Toda T (1995) Fission yeast TPR-family protein nuc2 is required for G_1-arrest upon nitrogen starvation and is an inhibitor of septum formation. *J Cell Sci* **108** (Pt 3): 895–905.

Kunitomo H, Higuchi T, Iino Y, Yamamoto M (2000) A zinc-finger protein, Rst2p, regulates transcription of the fission yeast *ste11*$^+$ gene, which encodes a pivotal transcription factor for sexual development. *Mol Biol Cell* **11**: 3205–3217.

Li P, McLeod M (1996) Molecular mimicry in development: identification of *ste11*$^+$ as a substrate and *mei3*$^+$ as a pseudosubstrate inhibitor of *ran1*$^+$ kinase. *Cell* **87**: 869–880.

Lowndes NF, McInerny CJ, Johnson AL, Fantes PA, Johnston LH (1992) Control of DNA synthesis genes in fission yeast by the cell-cycle gene *cdc10*$^+$. *Nature* **355**: 449–453.

Lundgren K, Walworth N, Booher R, Dembski M, Kirschner M, Beach D (1991) mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* **64**: 1111–1122.

MacNeill SA, Nurse P (1997) Cell cycle control in fission yeast. In: Pringle J, Broach J, Jones E, eds. *The Molecular and Cellular Biology of the Yeast Saccahromyces*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Maeda T, Mochizuki N, Yamamoto M (1990) Adenylyl cyclase is dispensable for vegetative cell growth in the fission yeast *Schizosaccharomyces pombe*. *Proc Natl Acad Sci USA* **87**: 7814–7818.

Malapeira J, Moldon A, Hidalgo E, Smith GR, Nurse P, Ayte J (2005) A meiosis-specific cyclin regulated by splicing is required for proper progression through meiosis. *Mol Cell Biol* **25**: 6330–6337.

Mata J, Bahler J (2006) Global roles of Ste11p, cell type, and pheromone in the control of gene expression during early sexual differentiation in fission yeast. *Proc Natl Acad Sci USA* **103**: 15517–15522.

Mata J, Lyne R, Burns G, Bahler J (2002) The transcriptional program of meiosis and sporulation in fission yeast. *Nat Genet* **32**: 143–147.

Matsumoto S, Bandyopadhyay A, Kwiatkowski DJ, Maitra U, Matsumoto T (2002) Role of the Tsc1-Tsc2 complex in signaling and transport across the cell membrane in the fission yeast *Schizosaccharomyces pombe*. *Genetics* **161**: 1053–1063.

Matsuo T, Kubo Y, Watanabe Y, Yamamoto M (2003) *Schizosaccharomyces pombe* AGC family kinase Gad8p forms a conserved signaling module with TOR and PDK1-like kinases. *EMBO J* **22**: 3073–3083.

Matsuo T, Otsubo Y, Urano J, Tamanoi F, Yamamoto M (2007) Loss of the TOR kinase Tor2 mimics nitrogen starvation and activates the sexual development pathway in fission yeast. *Mol Cell Biol* **27**: 3154–3164.

McLeod M, Beach D (1988) A specific inhibitor of the *ran1*$^+$ protein kinase regulates entry into meiosis in *Schizosaccharomyces pombe*. *Nature* **332**: 509–514.

Millar JB, McGowan CH, Lenaers G, Jones R, Russell P (1991) p80^{cdc25} mitotic inducer is the tyrosine phosphatase that activates p34^{cdc2} kinase in fission yeast. *EMBO J* **10**: 4301–4309.

Miyamoto M, Tanaka K, Okayama H (1994) *res2*$^+$, a new member of the *cdc10*$^+$*/SWI4* family, controls the 'start' of mitotic and meiotic cycles in fission yeast. *EMBO J* **13**: 1873–1880.

Mochizuki N, Yamamoto M (1992) Reduction in the intracellular cAMP level triggers initiation of sexual development in fission yeast. *Mol Gen Genet* **233**: 17–24.

Moser BA, Russell P (2000) Cell cycle regulation in *Schizosaccharomyces pombe*. *Curr Opin Microbiol* **3**: 631–636.

Nakashima N, Tanaka K, Sturm S, Okayama H (1995) Fission yeast Rep2 is a putative transcriptional activator subunit for the cell cycle 'start' function of Res2-Cdc10. *EMBO J* **14**: 4794–4802.

Neiman AM, Stevenson BJ, Xu HP, *et al*. (1993) Functional homology of protein kinases required for sexual differentiation in *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* suggests a conserved signal transduction module in eukaryotic organisms. *Mol Biol Cell* **4**: 107–120.

Nielsen O (2004) Mating-type control and differentiation. In: Egel R, ed. The Moleular Biology of Schizosaccharomyces pombe. Berlin, Heidelberg: Springer, pp. 281–296.

Nielsen O, Davey J, Egel R (1992) The ras1 function of *Schizosaccharomyces pombe* mediates pheromone-induced transcription. *EMBO J* **11**: 1391–1395.

Nurse P (1985) Mutants of the *Shizosaccharomyces pombe* which alter the shift between cell proliferation and sporulation. *Mol Gen Genet* **198**: 497.

Obara T, Nakafuku M, Yamamoto M, Kaziro Y (1991) Isolation and characterization of a gene encoding a G-protein α subunit from *Schizosaccharomyces pombe*: involvement in mating and sporulation pathways. *Proc Natl Acad Sci USA* **88**: 5877–5881.

Obara-Ishihara T, Okayama H (1994) A B-type cyclin negatively regulates conjugation via interacting with cell cycle 'start' genes in fission yeast. *EMBO J* **13**: 1863–1872.

Petersen J, Weilguny D, Egel R, Nielsen O (1995) Characterization of *fus1* of *Schizosaccharomyces pombe*: a developmentally controlled function needed for conjugation. *Mol Cell Biol* **15**: 3697–3707.

Samejima I, Mackie S, Fantes PA (1997) Multiple modes of activation of the stress-responsive MAP kinase pathway in fission yeast. *EMBO J* **16**: 6162–6170.

Samejima I, Mackie S, Warbrick E, Weisman R, Fantes PA (1998) The fission yeast mitotic regulator *win1*$^{+}$ encodes an MAP kinase kinase kinase that phosphorylates and activates Wis1 MAP kinase kinase in response to high osmolarity. *Mol Biol Cell* **9**: 2325–2335.

Sato M, Shinozaki-Yabana S, Yamashita A, Watanabe Y, Yamamoto M (2001) The fission yeast meiotic regulator Mei2p undergoes nucleocytoplasmic shuttling. *FEBS Lett* **499**: 251–255.

Sato M, Watanabe Y, Akiyoshi Y, Yamamoto M (2002) 14–3–3 protein interferes with the binding of RNA to the phosphorylated form of fission yeast meiotic regulator Mei2p. *Curr Biol* **12**: 141–145.

Shieh JC, Wilkinson MG, Buck V, Morgan BA, Makino K, Millar JB (1997) The Mcs4 response regulator coordinately controls the stress-activated Wak1-Wis1-Sty1 MAP kinase pathway and fission yeast cell cycle. *Genes Dev* **11**: 1008–1022.

Shieh JC, Wilkinson MG, Millar JB (1998) The Win1 mitotic regulator is a component of the fission yeast stress-activated Sty1 MAPK pathway. *Mol Biol Cell* **9**: 311–322.

Shimada T, Yamashita A, Yamamoto M (2003) The fission yeast meiotic regulator Mei2p forms a dot structure in the horse-tail nucleus in association with the *sme2* locus on chromosome II. *Mol Biol Cell* **14**: 2461–2469.

Shinozaki-Yabana S, Watanabe Y, Yamamoto M (2000) Novel WD-repeat protein Mip1p facilitates function of the meiotic regulator Mei2p in fission yeast. *Mol Cell Biol* **20**: 1234–1242.

Shiozaki K, Russell P (1996) Conjugation, meiosis, and the osmotic stress response are regulated by Spc1 kinase through Atf1 transcription factor in fission yeast. *Genes Dev* **10**: 2276–2288.

Shiozaki K, Shiozaki M, Russell P (1997) Mcs4 mitotic catastrophe suppressor regulates the fission yeast cell cycle through the Wik1-Wis1-Spc1 kinase cascade. *Mol Biol Cell* **8**: 409–419.

Stern B, Nurse P (1997) Fission yeast pheromone blocks S-phase by inhibiting the G_1 cyclin B-p34^{cdc2} kinase. *EMBO J* **16**: 534–544.

Stern B, Nurse P (1998) Cyclin B proteolysis and the cyclin-dependent kinase inhibitor rum1p are required for pheromone-induced G_1 arrest in fission yeast. *Mol Biol Cell* **9**: 1309–1321.

Stoilov P, Rafalska I, Stamm S (2002) YTH: a new domain in nuclear proteins. *Trends Biochem Sci* **27**: 495–497.

Sugimoto A, Iino Y, Maeda T, Watanabe Y, Yamamoto M (1991) *Schizosaccharomyces pombe ste11*$^{+}$ encodes a transcription factor with an HMG motif that is a critical regulator of sexual development. *Genes Dev* **5**: 1990–1999.

Sugiyama A, Tanaka K, Okazaki K, Nojima H, Okayama H (1994) A zinc finger protein controls the onset of premeiotic DNA synthesis of fission yeast in a Mei2-independent cascade. *EMBO J* **13**: 1881–1887.

Takeda T, Toda T, Kominami K, Kohnosu A, Yanagida M, Jones N (1995) *Schizosaccharomyces pombe atf1*$^{+}$ encodes a transcription factor required for sexual development and entry into stationary phase. *EMBO J* **14**: 6193–6208.

Tanaka K, Okazaki K, Okazaki N, *et al*. (1992) A new cdc gene required for S phase entry of *Schizosaccharomyces pombe* encodes a protein similar to the *cdc10*$^{+}$ and *SWI4* gene products. *EMBO J* **11**: 4923–4932.

Urano J, Comiso MJ, Guo L, *et al*. (2005) Identification of novel single amino acid changes that result in hyperactivation of the unique GTPase, Rheb, in fission yeast. *Mol Microbiol* **58**: 1074–1086.

Urano J, Sato T, Matsuo T, Otsubo Y, Yamamoto M, Tamanoi F (2007) Point mutations in TOR confer Rheb-independent growth in fission yeast and nutrient-independent mammalian TOR signaling in mammalian cells. *Proc Natl Acad Sci USA* **104**: 3514–3519.

Uritani M, Hidaka H, Hotta Y, Ueno M, Ushimaru T, Toda T (2006) Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. *Genes Cells* **11**: 1367–1379.

Van Heeckeren WJ, Dorris DR, Struhl K (1998) The mating-type proteins of fission yeast induce meiosis by directly activating *mei3* transcription. *Mol Cell Biol* **18**: 7317–7326.

van Slegtenhorst M, Carr E, Stoyanova R, Kruger WD, Henske EP (2004) *Tsc1*$^{+}$ and *tsc2*$^{+}$ regulate arginine uptake and metabolism in *Schizosaccharomyces pombe*. *J Biol Chem* **279**: 12706–12713.

Warbrick E, Fantes PA (1991) The wis1 protein kinase is a dosage-dependent regulator of mitosis in *Schizosaccharomyces pombe*. *EMBO J* **10**: 4291–4299.

Watanabe Y, Nurse P (1999) Cohesin Rec8 is required for reductional chromosome segregation at meiosis. *Nature* **400**: 461–464.

Watanabe Y, Yamamoto M (1994) *S. pombe mei2*$^{+}$ encodes an RNA-binding protein essential for premeiotic DNA synthesis and meiosis I, which cooperates with a novel RNA species meiRNA. *Cell* **78**: 487–498.

Watanabe Y, Yamamoto M (1996) *Schizosaccharomyces pombe pcr1*$^{+}$ encodes a CREB/ATF protein involved in regulation of gene expression for sexual development. *Mol Cell Biol* **16**: 704–711.

Watanabe Y, Shinozaki-Yabana S, Chikashige Y, Hiraoka Y, Yamamoto M (1997) Phosphorylation of RNA-binding protein controls cell cycle switch from mitotic to meiotic in fission yeast. *Nature* **386**: 187–190.

Watanabe Y, Yokobayashi S, Yamamoto M, Nurse P (2001) Pre-meiotic S phase is linked to reductional chromosome segregation and recombination. *Nature* **409**: 359–363.

Weisman R, Choder M (2001) The fission yeast TOR homolog, *tor1*$^+$, is required for the response to starvation and other stresses via a conserved serine. *J Biol Chem* **276**: 7027–7032.

Weisman R, Roitburg I, Schonburn M, Harari R, Kupiec M (2007) Opposite effects of Tor1 and Tor2 on nitrogen starvation responses in fission yeast. *Genetics* **175**: 1153–1162.

White S, Khaliq F, Sotiriou S, McInerny CJ (2001) The role of DSC1 components *cdc10*$^+$, *rep1*$^+$ and *rep2*$^+$ in MCB gene transcription at the mitotic G_1–S boundary in fission yeast. *Curr Genet* **40**: 251–259.

Whitehall S, Stacey P, Dawson K, Jones N (1999) Cell cycle-regulated transcription in fission yeast: Cdc10-Res protein interactions during the cell cycle and domains required for regulated transcription. *Mol Biol Cell* **10**: 3705–3715.

Wilkinson MG, Samuels M, Takeda T, *et al*. (1996) The Atf1 transcription factor is a target for the Sty1 stress-activated MAP kinase pathway in fission yeast. *Genes Dev* **10**: 2289–2301.

Willer M, Hoffmann L, Styrkarsdottir U, Egel R, Davey J, Nielsen O (1995) Two-step activation of meiosis by the mat1 locus in *Schizosaccharomyces pombe*. *Mol Cell Biol* **15**: 4964–4970.

Yamaguchi S, Murakami H, Okayama H (1997) A WD repeat protein controls the cell cycle and differentiation by negatively regulating Cdc2/B-type cyclin complexes. *Mol Biol Cell* **8**: 2475–2486.

Yamamoto M (1996) The molecular control mechanisms of meiosis in fission yeast. *Trends Biochem Sci* **21**: 18–22.

Yamamoto M (2004) Initiation of Meiosis. In: Egel R, ed. *The Molecular Biology of Schizosaccharomyces pombe*. Berlin, Heidelberg: Springer, pp 297–310.

Yamamoto M, Imai Y, Watanabe Y (1997) Mating and sporulation in *Shizosaccharomyces pombe*. In: Pringle J, Broach J, Jones E eds. *The Molecular and Cellular Biology of the Yeast Saccahromyces*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 1037–1106.

Yamashita A, Watanabe Y, Nukina N, Yamamoto M (1998) RNA-assisted nuclear transport of the meiotic regulator Mei2p in fission yeast. *Cell* **95**: 115–123.

Yamashita YM, Nakaseko Y, Samejima I, *et al*. (1996) 20S cyclosome complex formation and proteolytic activity inhibited by the cAMP/PKA pathway. *Nature* **384**: 276–279.

Zhu Y, Takeda T, Nasmyth K, Jones N (1994) *pct1*$^+$, which encodes a new DNA-binding partner of $p85^{cdc10}$, is required for meiosis in the fission yeast *Schizosaccharomyces pombe*. *Genes Dev* **8**: 885–898.