**Review**

# The selective elimination of messenger RNA underlies the mitosis–meiosis switch in fission yeast

By Masayuki YAMAMOTO*1,†

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**Abstract:** The cellular programs for meiosis and mitosis must be strictly distinguished but the mechanisms controlling the entry to meiosis remain largely elusive in higher organisms. In contrast, recent analyses in yeast have shed new light on the mechanisms underlying the mitosis–meiosis switch. In this review, the current understanding of these mechanisms in the fission yeast *Schizosaccharomyces pombe* is discussed. Meiosis-inducing signals in this microbe emanating from environmental conditions including the nutrient status converge on the activity of an RRM-type RNA-binding protein, Mei2. This protein plays pivotal roles in both the induction and progression of meiosis and has now been found to govern the meiotic program in a quite unexpected manner. Fission yeast contains an RNA degradation system that selectively eliminates meiosis-specific mRNAs during the mitotic cell cycle. Mmi1, a novel RNA-binding protein of the YTH-family, is essential for this process. Mei2 tethers Mmi1 and thereby stabilizes the transcripts necessary for the progression of meiosis.

**Keywords:** meiosis, mitosis, RNA-binding protein, selective RNA degradation, exosome, polyadenylation

## Introduction

Meiosis is a specialized cellular process that forms haploid gametes from diploid germ cells. Meiosis consists of one round of DNA synthesis followed by two rounds of nuclear division and occurs in all sexually reproducing species to enable the transmission of genetic information to the next generation. Despite its biological significance, however, the molecular mechanisms that underlie meiosis remain largely elusive, particularly in higher organisms. In conjunction with a number of collaborators, this author has been involved in efforts to further elucidate the regulatory mechanisms of meiosis in the fission yeast *Schizosaccharomyces pombe*. This unicellular eukaryote has a number of properties which make it an excellent model system for the study of mitosis–meiosis switching. This review outlines the current state of play in our understanding of the molecular mechanisms underlying meiosis, focusing mainly on a recent finding that the selective elimination of meiosis-specific mRNAs plays a key role in the regulation of this process in fission yeast.

## Yeast meiosis

Certain yeast species including *S. pombe* and the budding yeast *Saccharomyces cerevisiae* can normally grow in either a haploid or diploid state. Two haploid cells conjugate to form a zygote, which then either undergoes meiosis in the absence of nutrients or grows as a diploid cell when there are no starvation conditions. Thus, yeast haploid cells behave in a similar manner to gametes in higher organisms (*i.e.* oocytes and sperm/pollen) but can also proliferate by mitosis. Furthermore, meiosis can be induced readily in yeast diploid cells by introducing a nutritional shift. These features of yeast have greatly facilitated the study of the molecular mechanisms of meiosis in this organism. The process of mating and meiosis in fission yeast is depicted schematically in Fig. 1.

As in other developmental processes, meiosis is dependent on the coordinated transcriptional regu-

*1 Professor of Graduate School of Science, University of Tokyo, Tokyo, Japan.
† Correspondence should be addressed: M. Yamamoto, Department of Biophysics and Biochemistry, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan (e-mail: yamamoto@biochem.s.u-tokyo.ac.jp).

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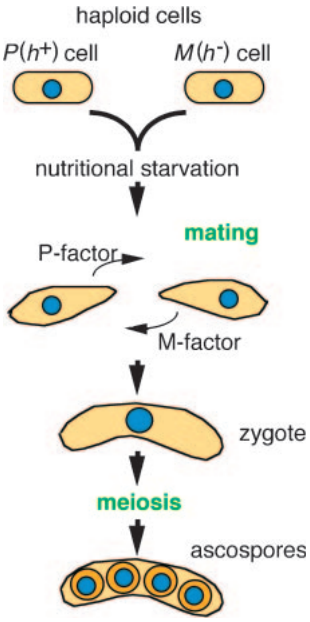


Fig. 1. Schematic illustration of the process of mating and meiosis in fission yeast. In response to nutritional starvation, haploid cells arrest vegetative growth and initiate mating. Cells of the *P* and *M* mating-types communicate each other through mating pheromones and conjugate to form a zygote, which then undergoes meiosis and generates four spores in an ascus if starved conditions are maintained. See the text for more explanation.

lation of numerous genes.[1),2)] The products of these genes may function either to modify the fundamental cell duplication machinery operating during the mitotic cell cycle, to reorganize the cellular architecture, or to generate ascus sacs containing the four haploid spores which are the eventual products of yeast meiosis. In fission yeast, meiosis occurs in zygotes or in diploid cells that are heterozygous for the mating-type genes (*mat1-P/mat1-M*) and are exposed to nutrient starvation, particularly in the case of nitrogen. These cells transiently arrest in the G1 phase of the mitotic cell cycle, initiate DNA synthesis (pre-meiotic DNA synthesis), and then perform two consecutive nuclear divisions, the first and second meiotic divisions (known as meiosis I and II). Progression of the meiotic cell cycle is also strongly dependent on Cdc2, also known as cyclin-dependent kinase 1 (CDK1), which is a major regulator of the mitotic cell cycle.[3)–5)] Indeed, CDK1 was originally identified as a regulator of meiosis reinitiation during frog oogenesis.[6)] If CDK activity is suppressed during meiosis in fission yeast, diploid cells generate asci containing two diploid spores instead of four haploid spores.[7),8)] It has also been shown previously that fission yeast produces a specific inhibitor of cyclin degradation prior to meiosis II so that the CDK activity necessary for this division may be maintained.[9)] Although not the focus of the present article, we have previously reviewed the possible functions of various cell cycle regulators during meiosis.[10)]

## Signaling pathways involved in the initiation of meiosis

Signal cascades emanating from environmental stimuli and leading to the initiation of meiosis have now been elucidated in fission yeast. Four signal transduction pathways have been shown to be important for the induction of meiosis and respond, respectively, to the carbon source, the nitrogen source, the mating pheromone, and stress stimuli (Fig. 2). A shift from a glucose-based rich medium to a poor carbon source results in a lowered intracellular cAMP level in fission yeast, followed by the down-regulation of cAMP-dependent protein kinase (PKA). This lowered PKA activity stimulates transcription of the *ste11* gene, which encodes an HMG-type transcription factor pivotal for sexual development.[11)–16)]

Nitrogen starvation also contributes to the transcriptional activation of *ste11*, in this case *via* the TOR (target of rapamycin) protein kinase pathway.[17),18)] Recent studies have shown that TOR kinase plays an important role when various eukaryotic cells recognize and then adapt to different external conditions.[19)] Fission yeast has two TOR kinases, Tor1 and Tor2. Tor1 is not essential for vegetative growth, but is required for sexual development and growth under stressed conditions.[20),21)] In contrast, Tor2 is indispensable for growth and it is of interest that a loss of its function induces sexual development on nutrient medium *via* the up-regulation of most nitrogen starvation-responsive genes.[17),18),22),23)] Tor2 constitutes TOR complex 1 (TORC1), which is the counterpart of TORC1 in mammals and budding yeast.[17),18),24)] Thus, in fission yeast, the TORC1 pathway appears to mediate signals from nitrogen sources. Further details of TOR functions in fission yeast can be found in a number of recent reviews.[25)–27)]

The MAP kinase cascade response to mating pheromones should be active during both mating and meiosis in fission yeast. Fission yeast *P* ($h^+$) cells produce the mating pheromone P-factor, which is a simple peptide of 23 amino acids,[28)] whereas *M* ($h^-$) cells produce M-factor, a farnesylated peptide of 9

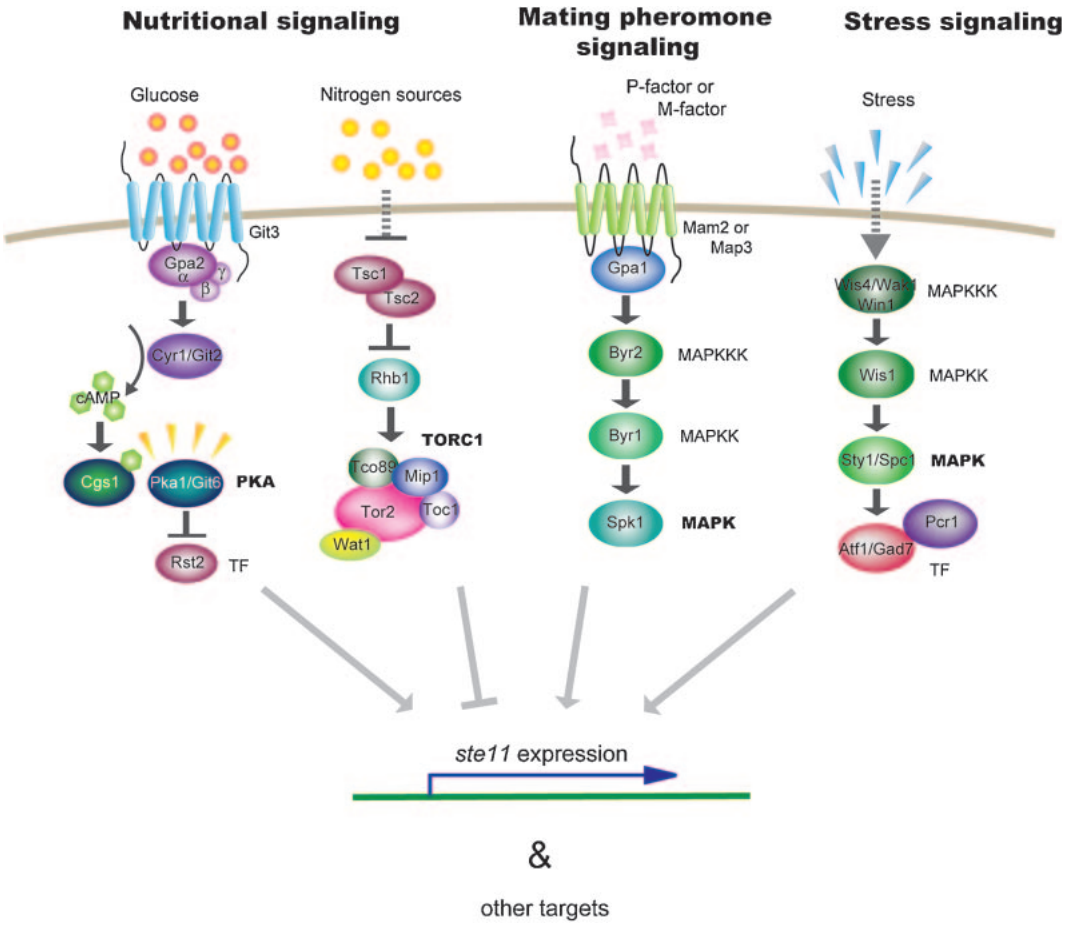


Fig. 2. Signal transduction pathways that regulate the initiation of meiosis in fission yeast. Four pathways that regulate expression of the *ste11* gene, which encodes a transcription factor crucial for sexual development, are schematically depicted. From left to right: The cAMP pathway response to glucose; the TORC1 pathway response to nitrogen sources; the MAP kinase pathway response to mating pheromones; and the MAP kinase pathway response to environmental stresses. The former two pathways down-regulate, whereas the latter two up-regulate, *ste11* expression. Reproduced from Ref. 25 with some modifications.

amino acids.[29] These pheromones bind and then activate seven-transmembrane receptors on the surface of opposite mating-type cells, which are coupled with a G protein alpha subunit Gpa1.[30–32] Activated Gpa1 stimulates a MAP kinase cascade, composed of MAPKKK Byr2, MAPKK Byr1 and MAPK Spk1. This cascade enhances the expression of *ste11* and induces the expression of additional genes required for mating and meiosis.[33–35]

Nutrient starvation activates the stress-responsive MAPK pathway (MAPKKKs Wis4/Wak1 and Win1, MAPKK Wis1, and MAPK Sty1/Spc1), which also responds to temperature and osmolarity stresses.[36–40] A target of Sty1/Spc1 MAP kinase is a bZIP protein Atf1/Gad7, which constitutes a transcription-activating complex[41,42] and forms a heterodimer with its partner Pcr1.[43,44] The *wis1*, *sty1*, *atf1* and *pcr1* genes are required for proper G1 arrest following nitrogen starvation and for the full activation of *ste11*.[41–46] It remains unknown however whether the Atf1/Gad7-Pcr1 complex directly regulates the *ste11* gene. Interestingly, this complex has been shown to play an important role in chromatin remodeling.[47–49]

## The Pat1–Mei2 system: a mitosis–meiosis switch in fission yeast

The independent identification of the *pat1* mutation (also known as *ran1*) by Nurse and by our own laboratory provided a new avenue to analyze the molecular mechanisms underlying the regulation of meiosis in fission yeast.[50,51] Yeast cells carrying the *pat1-114* mutation isolated in our laboratory, showed temperature-sensitive growth and performed

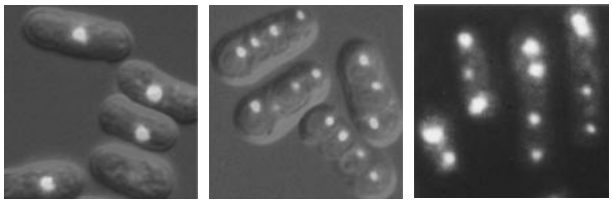

Fig. 3. Ectopic meiosis induced artificially by either inactivation of Pat1 kinase or dephosphorylation of Mei2. Even haploid cells execute meiotic divisions and produce spores, in which chromosomes are distributed unevenly, under these conditions. Left panel: haploid cells in the mitotic cell cycle, shown as a control. Middle panel: *pat1-114* cells incubated at the restrictive temperature. Right panel: cells expressing an unphosphorylatable form of Mei2 (Mei2-SATA). Bright regions represent DNA stained with a fluorescent dye. Phase-contrast images are overlaid in the left and middle panels.

ectopic meiosis and sporulation at high temperature regardless of the nutritional conditions or ploidy state (Fig. 3). Hence, Pat1 was considered to be a factor that normally prevents fission yeast cells from entering meiosis under conditions suitable for vegetative growth.[52),53)]

The *pat1* gene was subsequently found to encode a Ser/Thr protein kinase,[54)] the major target of which is Mei2, an RRM-type RNA binding protein crucial for meiotic entry.[55),56)] Pat1 phosphorylates Mei2 on residues Ser438 and Thr527, and expression of a mutant Mei2 with an alanine substitution in these two positions (Mei2-SATA) causes ectopic meiosis, as does the inactivation of Pat1 kinase (Fig. 3).[57)] These findings indicated that the unphosphorylated form of Mei2 is a critical factor during the meiotic entry in fission yeast.

Two kinds of mechanisms by which phosphorylation by Pat1 suppresses Mei2 activity have been uncovered. In the first of these, phosphorylation reduces the stability of Mei2, increasing its susceptibility to proteasome-dependent destruction.[58)] In the second pathway, phosphorylated Mei2 shows increased affinity for Rad24, the major 14-3-3 protein in fission yeast, and this binding inhibits the association of Mei2 with its partner RNAs[59)] (see also below).

### Pat1 kinase undergoes inactivation under physiological conditions

Fission yeast cells initiate meiosis physiologically when two prerequisites are met. One is the heterozygosity of the mating-type loci, *i.e.* cells should carry both *mat1-P* and *mat1-M*. The other is a condition of nutrient deficiency, most notably a depletion of nitrogen with a reduction of glucose. When these conditions are satisfied, the gene

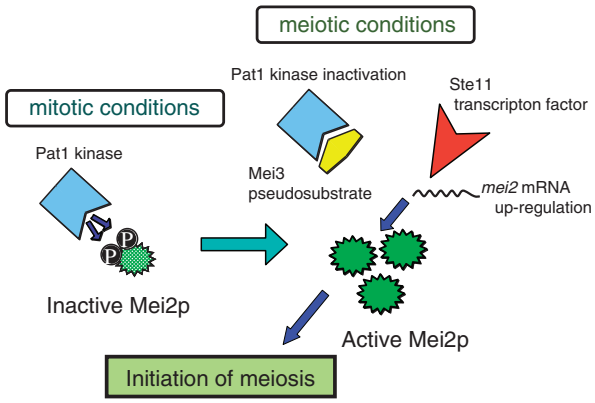


Fig. 4. Outline of the regulation of meiotic initiation by Pat1 kinase and its substrate Mei2. During vegetative growth, Pat1 kinase is functional and inactivates Mei2 by phosphorylation. In diploid cells exposed to nutritional starvation, an inhibitor of Pat1 kinase, Mei3, is produced and blocks the kinase activity of Pat1. This, together with enhanced transcription of the *mei2* gene under starved conditions, results in the accumulation of the unphosphorylated form of Mei2, which then turns on meiosis.

products of *mat1-P* and *mat1-M* cooperatively induce expression of the *mei3* gene, which encodes an inhibitor (pseudo-substrate) of Pat1 kinase.[60)–63)] This process underpins why meiosis occurs only in diploid cells that are heterozygous for the *P* and *M* mating-type genes and are exposed to nutritional starvation. Nitrogen starvation also facilitates meiotic entry by enhancing the expression of *ste11*, the product of which in turn activates the transcription of *mei2*.[12)] The control of meiotic initiation by the Pat1–Mei2 system is schematically shown in Fig. 4.

### Characteristics of the Mei2 protein

Given the above observations, it became clear that elucidating the molecular function of Mei2 would be critically important in furthering our understanding of meiotic regulation in fission yeast. Hence, we have analyzed and clarified various features of Mei2 in a number of previous studies from our laboratory. Mei2 is essential for the induction of both pre-meiotic DNA synthesis and the first meiotic division,[56)] and shuttles between the nucleus and the cytoplasm.[64)] Mei2 also forms an atypical single dot in the nucleus during meiotic prophase, which we have denoted the Mei2 dot. This structure contains the Mei2 protein and its partner RNA of some 0.5 kb in length termed meiRNA and encoded by the *sme2* gene.[57),65)] The Mei2 dot is also associated with the *sme2* locus on chromosome 2 in fission yeast.[66)] In *sme2Δ* cells, which form no Mei2 dot, meiotic progression arrests prior to the initiation

of meiosis I.[57),65)] From these observations, we speculated that Mei2 might be involved in multiple steps of sexual differentiation, such as withdrawal from the mitotic cell cycle, the initiation of pre-meiotic DNA synthesis, or the promotion of meiosis I. Although we were unable to confirm these possibilities in a number of trials, we uncovered a crucial function of Mei2 during our analyses of meiotic gene expression. This fortuitous and unexpected finding is described in the next two sections.

### The selective elimination of meiotic mRNAs

When fission yeast cells undergo meiosis, the expression of hundreds of genes is newly induced or significantly up-regulated by the function of specific transcription factors including Ste11.[2)] However, our recent analyses of meiosis-specific genes have indicated that, in addition to transcriptional regulation, a new mode of regulation is likely to operate to differentiate mitosis and meiosis in fission yeast. We refer to this as selective elimination of mRNAs,[67)] a process which functions to suppress meiotic gene expression during the mitotic cell cycle.

We previously observed a curious phenomenon in fission yeast in which some meiosis-specific mRNAs did not accumulate in mitotic cells even when they were artificially transcribed from a constitutive promoter. These included transcripts for *mei4*, which encodes a key transcription factor necessary for meiosis I,[68)] *rec8*, which encodes a subunit of the meiosis-specific cohesin complex,[69)] *ssm4*, which encodes a homologue of the dynactin component Glued,[70)] and *spo5*, which encodes an RRM-type RNA-binding protein required for meiosis II.[71)] We subsequently found that these mRNAs each harbor a region that blocks their accumulation in mitotic cells, and denoted this element the DSR (determinant of selective removal).[67)]

Our search for factors required for the selective elimination of meiosis-specific mRNAs yielded Mmi1, an RNA-binding protein of the poorly characterized YTH-family[72)] that can bind to the DSR.[67)] Disruption of *mmi1* results in a severe growth defect, and hence we constructed temperature-sensitive (*ts*) alleles of this gene. The *mmi1-ts* mutants accumulate meiosis-specific mRNAs at high temperature, even in rich medium.[67)] Because deletion of *mei4* considerably alleviates growth retardation of these *ts* mutants, it appeared that the ectopic expression of *mei4* and/or genes stimulated by Mei4 could severely affect vegetative cell growth. Hence, the DSR/Mmi1-dependent elimination of meiosis-specific transcripts is not a system that works only in extreme circumstances but is an intrinsic process that facilitates robust vegetative growth, during which the transcription of meiotic genes is not completely suppressed. Almost 20 meiosis-specific transcripts have now been shown to undergo elimination *via* the DSR–Mmi1 system (Ref. 67 and our unpublished data), and this number is more than likely to increase in the future.

### The Mei2 dot blocks the function of the DSR–Mmi1 system

Unexpectedly, a direct link between the DSR–Mmi1 system and Mei2 was identified in our laboratory. Localization analysis of Mmi1 revealed that this protein is present in multiple nuclear foci during vegetative growth. Interestingly, these foci converge to a single dot in meiotic prophase, which we found to overlap with the Mei2 dot. The conversion of Mmi1 to a single dot did not occur however in the absence of Mei2 or meiRNA.[67)] These observations led us to speculate that the Mei2 dot may sequester Mmi1 and thus inhibit its function and ensure the stable expression of meiosis-specific transcripts (Fig. 5). Consistent with this idea, we observed that Mei2 and Mmi1 can physically interact. Furthermore, we found that the cell growth arrest prior to meiosis I caused by the loss of the Mei2 dot in fission yeast could be suppressed by an

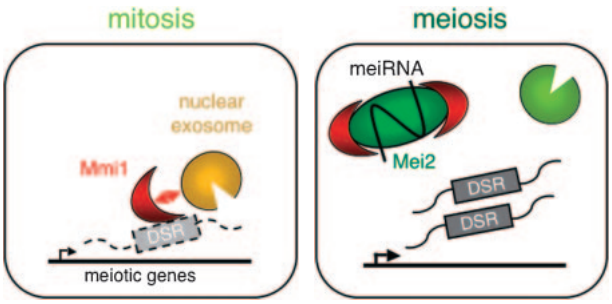


Fig. 5. Function of the Mei2 dot as the mitosis–meiosis switch. Left panel: a number of meiosis-specific transcripts in fission yeast harbor a region designated the DSR. The DSR renders the selective elimination of these mRNAs if they are expressed during the mitotic cell cycle. Mmi1, a YTH-family RNA-binding protein localized in the nucleus, binds to the DSR and promotes the elimination of the corresponding transcript in cooperation with the RNA-degradation machinery exosome. Right panel: to promote meiosis, Mei2, which has an affinity for Mmi1, forms a dot structure in the nucleus together with meiRNA. Mmi1 is thereby sequestered to this structure so that meiosis-specific transcripts are shielded from Mmi1-dependent mRNA elimination and can function stably. Reproduced from Ref. 67 with some modifications.

artificial reduction of Mmi1 activity.[67] Hence, the key molecular function of the Mei2 dot in fission yeast is most likely to be the shutdown of the DSR–Mmi1 system during meiotic prophase.

### Molecular mechanisms underlying selective elimination

The DSR–Mmi1 system appears to be the first example of a mechanism that selectively removes unnecessary mRNA species in order to maintain a specific cellular status. The underlying molecular mechanisms will thus be of great interest. Mmi1p consists of 488 residues and has no obvious domain features other than a putative RNA-binding motif. This suggests that this protein is unlikely to degrade RNA by itself. Indeed, we have further found that Rrp6, a fission yeast ortholog of a conserved nucleus-specific subunit of the exosome,[73] physically interacts with Mmi1, and that the exosome, a multi-subunit protein complex with nuclease activity,[74),75] is indispensable for selective mRNA degradation.[67),76] In addition, screens for factors that might interact with Mmi1 have identified a number of components of polyadenylation. Significantly, we have now shown that the polyadenylation of target mRNAs and the subsequent recruitment of a poly(A)-binding protein Pab2 are crucial for their degradation.[76] As a summary of these observations, a proposed model is shown in Fig. 6. A remarkable feature of this putative model is that the polyadenylation required for selective elimination is carried out by the conventional poly(A) polymerase and not by the TRAMP complex which targets poor quality RNAs for degradation.[77)–79]

As the YTH-family RNA-binding proteins have not been well characterized to date, little is known about how Mmi1 recognizes DSR sequences. The DSR sequences thus far identified through functional analyses are quite heterogeneous in size and length and show no obvious consensus sites or predictable structures. However, our recent analyses have now clarified that despite that lack of any extensive homology, the DSR regions are enriched with specific hexanucleotide repeat motifs which are responsible for their function (A. Yamashita, H. Tanaka, M.Y. unpublished results). These results may assist with future analysis of RNA-protein recognition by the YTH-family of proteins.

### Conclusions and future prospects

The Mei2 protein was postulated to be the master regulator of fission yeast meiosis in early studies but its molecular function remained enigmatic for more than two decades. Our recent analyses have revealed however that fission yeast contains a system to selectively remove unnecessary meiosis-specific transcripts in mitotically growing cells. Our finding of this new regulatory system opened up a new avenue to elucidate the molecular functions of Mei2. As described herein, the evidence indicates that Mei2 plays a key role in sequestering and inactivating

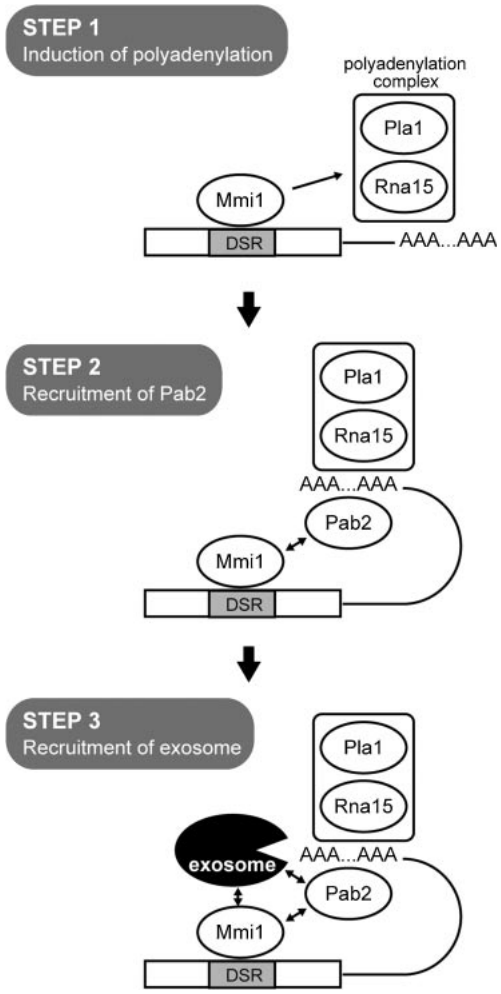


Fig. 6. Possible steps leading to the elimination of a DSR-containing mRNA. Step 1, Mmi1p bound to the DSR sequence interacts with a conventional polyadenylation complex and promotes a polyadenylation reaction of a somewhat unusual nature. Step 2, the poly(A)-binding protein Pab2p is recruited to the produced poly (A) tail. The affinity of Mmi1p for Pab2p is likely to facilitate this recruitment. Step 3, Pab2p, probably in collaboration with Mmi1p, recruits a nuclear exosome to the target transcript, which then digests it from the 3' end. Reproduced from Ref. 76.

Mmi1, an RNA-binding protein crucial for the removal of hazardous meiosis-specific mRNAs during the mitotic cell cycle.

The functions of Mei2, elucidated *via* analysis of the Mei2 dot, can fully account for its requirement during meiosis I. However, circumstantial evidence also suggests that Mei2 may execute additional tasks during meiosis. For example, although ectopic expression of the active form of Mei2, Mei2-SATA, results in a full round of meiosis, the inactivation of Mmi1 does not induce meiosis. This loss of Mmi1 function causes a growth defect only which appears to be induced by the untimely activation of certain meiotic genes. In addition, although it has been shown that Mei2 is required for pre-meiotic DNA synthesis, meiRNA, which is an essential component of the Mei2 dot, is not essential for this process.[56] Importantly also, the mechanism by which Mei2 is involved in the promotion of DNA synthesis remains completely unknown. A number of critical molecular functions of Mei2 thus remain to be clarified and this will be essential for a fuller understanding of meiosis.

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## References

1) Kassir, Y., Adir, N., Boger-Nadjar, E., Raviv, N.G., Rubin-Bejerano, I., Sagee, S. *et al.* (2003) Transcriptional regulation of meiosis in budding yeast. Int. Rev. Cytol. **224**, 111–171.
2) Mata, J., Lyne, R., Burns, G. and Bahler, J. (2002) The transcriptional program of meiosis and sporulation in fission yeast. Nat. Genet. **32**, 143–147.
3) Nurse, P. and Bissett, Y. (1981) Gene required in G1 for commitment to cell cycle and in G2 for control of mitosis in fission yeast. Nature **292**, 558–560.
4) Iino, Y., Hiramine, Y. and Yamamoto, M. (1995) The role of *cdc2* and other genes in meiosis in *Schizosaccharomyces pombe*. Genetics **140**, 1235–1245.
5) Borgne, A., Murakami, H., Ayte, J. and Nurse, P. (2002) The G1/S cyclin Cig2p during meiosis in fission yeast. Mol. Biol. Cell **13**, 2080–2090.
6) Masui, Y. and Markert, C.L. (1971) Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. J. Exp. Zool. **177**, 129–145.
7) Nakaseko, Y., Niwa, O. and Yanagida, M. (1984) A meiotic mutant of the fission yeast *Schizosaccharomyces pombe* that produces mature asci containing two diploid spores. J. Bacteriol. **157**, 334–336.
8) Grallert, B. and Sipiczki, M. (1990) Dissociation of meiotic and mitotic roles of the fission yeast *cdc2* gene. Mol. Gen. Genet. **222**, 473–475.
9) Izawa, D., Goto, M., Yamashita, A., Yamano, H. and Yamamoto, M. (2005) Fission yeast Mes1p ensures the onset of meiosis II by blocking degradation of cyclin Cdc13p. Nature **434**, 529–533.
10) Harigaya, Y. and Yamamoto, M. (2007) Molecular mechanisms underlying the mitosis–meiosis decision. Chromosome Res. **15**, 523–537.
11) Maeda, T., Mochizuki, N. and 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.
12) Sugimoto, A., Iino, Y., Maeda, T., Watanabe, Y. and 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.
13) Mochizuki, N. and Yamamoto, M. (1992) Reduction in the intracellular cAMP level triggers initiation of sexual development in fission yeast. Mol. Gen. Genet. **233**, 17–24.
14) Hoffman, C.S. and Winston, F. (1990) Isolation and characterization of mutants constitutive for expression of the *fbp1* gene of *Schizosaccharomyces pombe*. Genetics **124**, 807–816.
15) Kunitomo, H., Higuchi, T., Iino, Y. and 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.
16) Higuchi, T., Watanabe, Y. and 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.
17) Alvarez, B. and Moreno, S. (2006) Fission yeast Tor2 promotes cell growth and represses cell differentiation. J. Cell Sci. **119**, 4475–4485.
18) Matsuo, T., Otsubo, Y., Urano, J., Tamanoi, F. and 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.
19) Wullschleger, S., Loewith, R. and Hall, M.N. (2006) TOR signaling in growth and metabolism. Cell **124**, 471–484.
20) Kawai, M., Nakashima, A., Ueno, M., Ushimaru, T., Aiba, K., Doi, H. *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.
21) Weisman, R. and 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.
22) Uritani, M., Hidaka, H., Hotta, Y., Ueno, M.,

Ushimaru, T. and Toda, T. (2006) Fission yeast Tor2 links nitrogen signals to cell proliferation and acts downstream of the Rheb GTPase. Genes Cells **11**, 1367–1379.

23) Weisman, R., Roitburg, I., Schonbrun, M., Harari, R. and Kupiec, M. (2007) Opposite effects of tor1 and tor2 on nitrogen starvation responses in fission yeast. Genetics **175**, 1153–1162.

24) Hayashi, T., Hatanaka, M., Nagao, K., Nakaseko, Y., Kanoh, J., Kokubu, A. *et al.* (2007) Rapamycin sensitivity of the *Schizosaccharomyces pombe tor2* mutant and organization of two highly phosphorylated TOR complexes by specific and common subunits. Genes Cells **12**, 1357–1370.

25) Otsubo, Y. and Yamamoto, M. (2010) TOR and sexual development in fission yeast. *In* The Enzymes. Structure, function and regulation of TOR complexes from yeast to mammals Part A (eds. Hall, M.N. and Tamanoi, F.). Vol. 27, Elsevier, London, pp. 229–250.

26) Kanoh, J. and Yanagida, M. (2010) Structure of TOR complexes in fission yeast. *In* The Enzymes. Structure, function and regulation of TOR complexes from yeast to mammals Part A (eds. Hall, M.N. and Tamanoi, F.). Vol. 27, Elsevier, London, pp. 271–284.

27) Weisman, R. (2010) Fission yeast TOR and rapamycin. *In* The Enzymes. Structure, function and regulation of TOR complexes from yeast to mammals Part A (eds. Hall, M.N. and Tamanoi, F.). Vol. 27, Elsevier, London, pp. 251–269.

28) Imai, Y. and Yamamoto, M. (1994) The fission yeast mating pheromone P-factor: its molecular structure, gene structure, and ability to induce gene expression and G1 arrest in the mating partner. Genes Dev. **8**, 328–338.

29) Davey, J. (1992) Mating pheromones of the fission yeast *Schizosaccharomyces pombe*: purification and structural characterization of M-factor and isolation and analysis of two genes encoding the pheromone. EMBO J. **11**, 951–960.

30) Kitamura, K. and Shimoda, C. (1991) The *Schizosaccharomyces pombe mam2* gene encodes a putative pheromone receptor which has a significant homology with the *Saccharomyces cerevisiae* Ste2 protein. EMBO J. **10**, 3743–3751.

31) Tanaka, K., Davey, J., Imai, Y. and Yamamoto, M. (1993) *Schizosaccharomyces pombe map3*$^+$ encodes the putative M-factor receptor. Mol. Cell. Biol. **13**, 80–88.

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

33) Gotoh, Y., Nishida, E., Shimanuki, M., Toda, T., Imai, Y. and 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.

34) Neiman, A.M., Stevenson, B.J., Xu, H.P., Sprague, G.F. Jr., Herskowitz, I., Wigler, M. *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.

35) Kjaerulff, S., Lautrup-Larsen, I., Truelsen, S., Pedersen, M. and 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.

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

37) Shieh, J.C., Wilkinson, M.G., Buck, V., Morgan, B.A., Makino, K. and Millar, J.B. (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.

38) Shiozaki, K., Shiozaki, M. and 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.

39) Shieh, J.C., Wilkinson, M.G. and Millar, J.B. (1998) The Win1 mitotic regulator is a component of the fission yeast stress-activated Sty1 MAPK pathway. Mol. Biol. Cell **9**, 311–322.

40) Samejima, I., Mackie, S., Warbrick, E., Weisman, R. and Fantes, P.A. (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.

41) Shiozaki, K. and 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.

42) Wilkinson, M.G., Samuels, M., Takeda, T., Toone, W.M., Shieh, J.C., Toda, 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.

43) Watanabe, Y. and 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.

44) Kanoh, J., Watanabe, Y., Ohsugi, M., Iino, Y. and Yamamoto, M. (1996) *Schizosaccharomyces pombe gad7*$^+$ encodes a phosphoprotein with a bZIP domain, which is required for proper G1 arrest and gene expression under nitrogen starvation. Genes Cells **1**, 391–408.

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

46) Takeda, T., Toda, T., Kominami, K., Kohnosu, A., Yanagida, M. and Jones, N. (1995) *Schizosaccharomyces pombe atf1*$^+$ encodes a transcription factor required for sexual development and entry into stationary phase. EMBO J. **14**, 6193–6208.
47) Wahls, W.P. and Smith, G.R. (1994) A heteromeric protein that binds to a meiotic homologous recombination hot spot: correlation of binding and hot spot activity. Genes Dev. **8**, 1693–1702.
48) Kon, N., Krawchuk, M.D., Warren, B.G., Smith, G.R. and Wahls, W.P. (1997) Transcription factor Mts1/Mts2 (Atf1/Pcr1, Gad7/Pcr1) activates the M26 meiotic recombination hotspot in *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **94**, 13765–13770.
49) Hirota, K., Hasemi, T., Yamada, T., Mizuno, K.I., Hoffman, C.S., Shibata, T. *et al.* (2004) Fission yeast global repressors regulate the specificity of chromatin alteration in response to distinct environmental stresses. Nucleic Acids Res. **32**, 855–862.
50) Iino, Y. and Yamamoto, M. (1985) Mutants of *Schizosaccharomyces pombe* which sporulate in the haploid state. Mol. Cell. Biol. **198**, 416–421.
51) Nurse, P. (1985) Mutants of the fission yeast *Schizosaccharomyces pombe* which alter the shift between cell proliferation and sporulation. Mol. Gen. Genet. **198**, 497–502.
52) Beach, D., Rodgers, L. and Gould, J. (1985) *ran1*$^+$ controls the transition from mitotic division to meiosis in fission yeast. Curr. Genet. **10**, 297–311.
53) Iino, Y. and Yamamoto, M. (1985) Negative control for the initiation of meiosis in *Schizosaccharomyces pombe*. Proc. Natl. Acad. Sci. USA **82**, 2447–2451.
54) McLeod, M. and Beach, D. (1986) Homology between the *ran1*$^+$ gene of fission yeast and protein kinases. EMBO J. **5**, 3665–3671.
55) Watanabe, Y., Iino, Y., Furuhata, K., Shimoda, C. and Yamamoto, M. (1988) The *S. pombe mei2* gene encoding a crucial molecule for commitment to meiosis is under the regulation of cAMP. EMBO J. **7**, 761–767.
56) Watanabe, Y. and 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.
57) Watanabe, Y., Shinozaki-Yabana, S., Chikashige, Y., Hiraoka, Y. and Yamamoto, M. (1997) Phosphorylation of RNA-binding protein controls cell cycle switch from mitotic to meiotic in fission yeast. Nature **386**, 187–190.
58) Kitamura, K., Katayama, S., Dhut, S., Sato, M., Watanabe, Y., Yamamoto, M. *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.
59) Sato, M., Watanabe, Y., Akiyoshi, Y. and 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.
60) McLeod, M. and Beach, D. (1988) A specific inhibitor of the *ran1*$^+$ protein kinase regulates entry into meiosis in *Schizosaccharomyces pombe*. Nature **332**, 509–514.
61) Willer, M., Hoffmann, L., Styrkarsdottir, U., Egel, R., Davey, J. and Nielsen, O. (1995) Two-step activation of meiosis by the *mat1* locus in *Schizosaccharomyces pombe*. Mol. Cell. Biol. **15**, 4964–4970.
62) Li, P. and 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.
63) Van Heeckeren, W.J., Dorris, D.R. and Struhl, K. (1998) The mating-type proteins of fission yeast induce meiosis by directly activating *mei3* transcription. Mol. Cell. Biol. **18**, 7317–7326.
64) Sato, M., Shinozaki-Yabana, S., Yamashita, A., Watanabe, Y. and Yamamoto, M. (2001) The fission yeast meiotic regulator Mei2p undergoes nucleocytoplasmic shuttling. FEBS Lett. **499**, 251–255.
65) Yamashita, A., Watanabe, Y., Nukina, N. and Yamamoto, M. (1998) RNA-assisted nuclear transport of the meiotic regulator Mei2p in fission yeast. Cell **95**, 115–123.
66) Shimada, T., Yamashita, A. and 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.
67) Harigaya, Y., Tanaka, H., Yamanaka, S., Tanaka, K., Watanabe, Y., Tsutsumi, C. *et al.* (2006) Selective elimination of messenger RNA prevents an incidence of untimely meiosis. Nature **442**, 45–50.
68) Horie, S., Watanabe, Y., Tanaka, K., Nishiwaki, S., Fujioka, H., Abe, H. *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.
69) Watanabe, Y. and Nurse, P. (1999) Cohesin Rec8 is required for reductional chromosome segregation at meiosis. Nature **400**, 461–464.
70) Yamashita, A., Watanabe, Y. and Yamamoto, M. (1997) Microtubule-associated coiled-coil protein Ssm4 is involved in the meiotic development in fission yeast. Genes Cells **2**, 155–166.
71) Kasama, T., Shigehisa, A., Hirata, A., Saito, T.T., Tougan, T., Okuzaki, D. *et al.* (2006) Spo5/Mug12, a putative meiosis-specific RNA-binding protein, is essential for meiotic progression and forms Mei2 dot-like nuclear foci. Eukaryot. Cell **5**, 1301–1313.
72) Stoilov, P., Rafalska, I. and Stamm, S. (2002) YTH: a new domain in nuclear proteins. Trends Biochem. Sci. **27**, 495–497.
73) Houseley, J., LaCava, J. and Tollervey, D. (2006) RNA-quality control by the exosome. Nat. Rev.

Mol. Cell Biol. **7**, 529–539.
74) Mitchell, P., Petfalski, E., Shevchenko, A., Mann, M. and Tollervey, D. (1997) The exosome: a conserved eukaryotic RNA processing complex containing multiple 3′→5′ exoribonucleases. Cell **91**, 457–466.
75) Allmang, C., Petfalski, E., Podtelejnikov, A., Mann, M., Tollervey, D. and Mitchell, P. (1999) The yeast exosome and human PM-Scl are related complexes of 3′ → 5′ exonucleases. Genes Dev. **13**, 2148–2158.
76) Yamanaka, S., Yamashita, A., Harigaya, Y., Iwata, R. and Yamamoto, M. (2010) Importance of polyadenylation in the selective elimination of meiotic mRNAs in growing *S. pombe* cells. EMBO J. **29**, 2173–2181.
77) LaCava, J., Houseley, J., Saveanu, C., Petfalski, E., Thompson, E., Jacquier, A. *et al.* (2005) RNA degradation by the exosome is promoted by a nuclear polyadenylation complex. Cell **121**, 713–724.
78) Vanacova, S., Wolf, J., Martin, G., Blank, D., Dettwiler, S., Friedlein, A. *et al.* (2005) A new yeast poly(A) polymerase complex involved in RNA quality control. PLoS Biol. **3**, e189.
79) Wyers, F., Rougemaille, M., Badis, G., Rousselle, J.C., Dufour, M.E., Boulay, J. *et al.* (2005) Cryptic pol II transcripts are degraded by a nuclear quality control pathway involving a new poly(A) polymerase. Cell **121**, 725–737.

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## Profile

Masayuki Yamamoto was born in 1947. He graduated from the University of Tokyo, Faculty of Science in 1970. He performed his graduate study at the University of Tokyo under the supervision of Prof. Hisao Uchida and obtained a Ph.D. degree in 1975. He then joined Prof. Masayasu Nomura's group at the University of Wisconsin-Madison as a postdoctoral fellow, and was engaged in analysis of *Escherichia coli* genes encoding ribosomal proteins, ribosomal RNAs, transfer RNAs, and RNA polymerase subunits.

He came back from the United States in 1978 and became a Research Associate at the Institute of Medical Science, University of Tokyo. He then moved to Kyoto University, Faculty of Science in 1979 as a Research Associate. After coming back to Japan, he started to handle fission yeast *Schizosaccharomyces pombe* as his major experimental material, aiming to establish a recombinant DNA system in this eukaryotic microorganism. As a first step, he isolated new mutants and cloned marker genes which might facilitate future gene manipulation in fission yeast, while he was in Kyoto for two years.

In 1981, he obtained a Lectureship in the Laboratory of Molecular Genetics, Institute of Medical Science, University of Tokyo, which was one of the specialized laboratories constructed to promote recombinant DNA research in Japan, conforming to the recombinant DNA research guideline just legislated. There he continued to develop fission yeast as a certified host-vector system for recombinant DNA studies, and also initiated to analyze molecular mechanisms underlying the cell cycle switch from mitosis to meiosis, as he believed that fission yeast would be one of the most suitable organisms for this type of exploration. He has been investigating the switching mechanisms ever since, which constitute the major topic of the review presented in this issue.

Dr. Yamamoto was promoted to an Associate Professor at the Institute of Medical Science, University of Tokyo in 1985, and moved to the School of Science, University of Tokyo in 1989 as a Professor (Dept. of Biophysics and Biochemistry). He keeps this Professorship till now. He headed the Molecular Genetics Research Laboratory, University of Tokyo from 1997 to 2007, and served as a Dean of the School of Science, University of Tokyo from 2007 to 2009. He is currently a Director of the Life Science Network, University of Tokyo, which is a university-wide organization comprising some 1,600 researchers. He was also an Adjunct Professor of Division of Cell Proliferation, National Institute for Basic Biology in Okazaki from 1992 to 1998, and an Adjunct Professor of Division of Cytoplasmic Genetics, National Institute of Genetics in Mishima from 2004 to 2007. He served as a President of the Molecular Biology Society of Japan from 2003 to 2005, and has been a Member of the Science Council of Japan since 2008.