In the beginning: the initiation of meiosis

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Summary
The most-critical point of reproductive development in all sexually reproducing species is the transition from mitotic to meiotic cell cycle. Studies in unicellular fungi have indicated that the decision to enter meiosis must be made before the beginning of the premeiotic S phase. Recent data from the mouse[1] suggest that this timing of meiosis initiation is a universal feature shared also by multicellular eukaryotes. In contrast, the signaling cascade that leads to meiosis initiation shows great diversity among species. *BioEssays* 29:511–514, 2007.

Introduction

It is generally believed that meiosis arose as a modification of the mitotic cell cycle.[2] Indeed, in many meiotic processes, we can identify a mitotic (somatic) core as well as meiosis-specific additions. A good example is meiotic recombination where, in addition to proteins that also function in somatic DNA repair, there are several meiosis-specific players that make meiotic recombination a process very different from the somatic DNA repair.[3,4]

While identifying meiosis-specific components has been very successful, elucidating the regulatory mechanisms that switch them on for meiosis and keep them turned off during the mitotic cell cycle has been much more difficult. Mutations in most meiosis-specific genes, even if they disrupt key meiotic processes, do not prevent initiation of meiosis or direct premeiotic cells to undergo mitosis instead of meiosis. On the contrary, most of these mutants do initiate meiosis and, in some cases, even progress through a complete, albeit defective, meiotic division.

Three questions are key for understanding the meiosis initiation process. (1) What are the cues that instruct premeiotic cells to switch from the mitotic cell cycle to meiosis? (2) What is the molecular nature of the cell cycle switch? (3) When during the cell cycle does this switch take place? The last question is especially critical because, to find components of the mitosis/meiosis switch mechanism, one has to know when to look.

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Much has been learned about the initiation of meiosis in yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*. Most importantly, it was discovered that the decision to initiate meiosis in both species is made before the onset of premeiotic S phase.[5–7] Given how much we already know from yeast, one could think that elucidating meiosis initiation in multicellular eukaryotes would be anticlimactic. However, this could not be further from the truth. Meiosis in multicellular organisms is preceded by a long developmental pathway that first differentiates germ cells, then divides them into cells destined to become meiocytes and somatic nursing cells (e.g. the Sertoli cells in mammals and anther tapetum in plants), and finally differentiates premeiotic cells (Figure 1). Consequently, meiosis initiation takes place in the context of the multicellular nature of these organisms. Mechanisms that initiate meiosis must integrate developmental cues, which may differ among species, with the universal cell cycle control machinery. Indeed, we are discovering that signals initiating meiosis are specific for certain groups of organisms. However, cellular responses to these cues may be more uniform since the timing of meiosis initiation is evolutionarily conserved, as shown by the recent mouse data.[1]

Lessons from yeast

In *S. cerevisiae* and *S. pombe*, nutrient conditions provide the cues for the switch from mitotic to meiotic cell cycle.[5,8] Interestingly, the nutritional signals significantly differ between the two species.[8] Accordingly, the signaling networks triggering meiosis differ as well.

In budding yeast, meiosis-triggering nutritional signals induce expression of the *Initiator of Meiosis 1* (*IME1*) gene encoding a meiosis-specific transcription factor responsible for activating a slew of approximately 300 meiotic genes.[9,10] One of these genes is *IME2*, which encodes a meiosis-specific Ser/Thr protein kinase. Ime2 phosphorylates and targets for degradation Sic1, an inhibitor of the cyclin-dependent kinase (CDK) Cdc28.[11,12] Activity of Cdc28, in turn, promotes entry into the meiotic cell cycle.

The initiation of meiosis in *S. pombe* differs from *S. cerevisiae* in several aspects. First, unlike *S. cerevisiae*, a fission yeast spends most of its life cycle as a haploid. The haploid cells must conjugate prior to meiosis and they do so in response to the nutrient starvation conditions. Second, *S. pombe* does not have sequence homologs of Ime1 or Ime2. The function of Ime1 is replaced by Ste11, a transcription

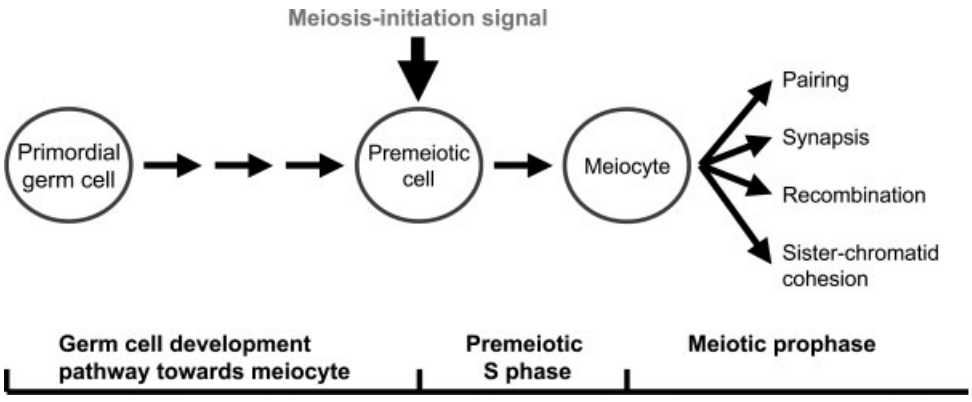

Figure 1. The sequence of events leading to meiosis in multicellular eukaryotes.

factor, which is produced in response to environmental conditions and initiates the sexual cycle.[8,13] The entry to meiosis itself is regulated by two proteins, a protein kinase Pat1 and an RNA-binding protein Mei2.[5,13] During mitotic growth, Pat1 blocks meiosis by phosphorylating Mei2. In response to meiosis-inducing conditions, the repression of Mei2 by Pat1 is released.[5] Although the details are still not entirely clear, evidence suggests that Mei2 acts as a master regulator of a complex system that sequesters meiotic transcripts during the vegetative growth.[14] To initiate meiosis, Mei2 turns off this regulatory system so that meiosis-specific RNAs are no longer intercepted.

Even though the mechanisms of meiosis initiation in the two yeasts share little if any similarity, the decision to enter meiosis is in both species made at the beginning or before premeiotic S phase.[5–7] This timing reflects the fact that processes taking place during premeiotic S are essential for successful meiosis. It is not yet clear which of the S phase processes decide about its meiotic character. Installation of the meiotic sister-chromatid cohesin Rec8 during S is certainly one of them.[6] However, events that take place during premeiotic S may also be essential for other meiotic processes, such as recombination.[7,15,16]

Meiosis initiation in the mouse

Several recent papers from the laboratories of David Page at MIT and Peter Koopman at the University of Queensland shed light on the process of meiosis initiation in mammals. These findings, together with the earlier observations from yeast, begin to uncover the patterns of regulation of meiosis shared by all eukaryotes.

The context of meiosis initiation is very different in multicellular organisms than in yeast. Unlike yeast, mammals are sexually dimorphic with females and males initiating meiosis at different stages of development.[1,17,18] In the ovaries, germ cells enter meiosis at the end of the first two weeks of embryonic development, where oocytes progress through meiotic prophase to arrest at diplotene. The rest of meiosis takes place after the animal reaches puberty. In contrast, in the testis, spermatocytes initiate meiosis throughout the life of the animal in synchronous waves starting at about a week after birth. Consequently, the signal for initiating meiosis must be regulated differently in males and females.

Research in fungi, as detailed above, revealed that meiosis-initiating signals are not conserved even within a single kingdom. So what could be the meiosis-initiating signal in mammals, which are even more evolutionarily distant? The answer, at least in the mouse, is retinoic acid (RA), a derivative of vitamin A. RA is produced in the cells of the mesonephros (the excretory organ of the embryo) during embryonic development and from there enters the gonads.[19] It has the ability to induce meiosis in both sexes.[19,20] The level of RA in the gonads is regulated by the *Cyp26b1* gene encoding a P450 cytochrome enzyme that degrades RA.[19,20] A high level of RA and no *Cyp26b1* expression are characteristic of meiotic ovaries. However, in young ovaries before the meiosis initiation and in embryonic testis, expression of *Cyp26b1* prevents RA accumulation.[19,20] If CYP26B1 is inactivated in embryonic testis, meiosis is initiated prematurely.[19] Females lacking functional CYP26B1 also show an earlier than normal meiosis initiation. Consequently, the role of *Cyp26b1* is both preventing meiosis in testis during embryonic development and preventing premature meiosis in ovaries.

Research in the Page laboratory identified *Stimulated by Retinoic Acid 8* (*Stra8*), a vertebrate-only gene required for the transition to meiosis in both sexes.[1,20,21] *Stra8* expression is specifically induced in the germ cells of both sexes by RA signaling and prevented by CYP26B1-mediated RA degradation.[20] This important discovery opened the doors to elucidating the meiosis-initiation pathway downstream from RA.

At what stage of the cell cycle is meiosis initiated in mammals?

A new paper by Andrew Baltus and co-workers from the Page laboratory[1] focuses on the key question of when the

commitment to enter meiosis is made in the mouse. Baltus et al.[1] generated a knockout mouse lacking the *Stra8* function. Predictably, both male and female animals homozygous for the mutation produced no gametes and were infertile. In *Stra8*-deficient ovaries, germ cell development proceeds normally until embryonic day 13.5 and the germ cells assume the typical premeiotic morphology with patches of condensed chromatin at the periphery of the nucleus. But then, while germ cells in wild-type siblings commence the events of meiotic prophase, *Stra8*-deficient germ cells stall at the premeiotic state and, eventually, deteriorate. A similar situation exists in the testis, where spermatogenesis begins shortly after birth.

To understand whether the mutant germ cells arrest before entering meiosis or initiate an abnormal meiotic prophase, Baltus and colleagues examined four markers that are specific for early meiotic prophase: (1) meiotic chromosome condensation, (2) installation of the REC8 sister-chromatid cohesin, (3) installation of the axial element protein SCP3, and (4) meiotic DSB formation. In addition, they tested expression of two key recombination genes, *Spo11*, which is required for meiotic DSB formation[22,23] and *Dmc1*, which encodes a meiosis-specific recombinase involved in DSB repair.[24,25] All these tests came out negative, suggesting that *Stra8*-deficient germ cells do not enter meiosis at all.

One question then remained to be addressed: when do the mutant germ cells arrest? Using static-fluorometry, the authors discovered that *Stra8*-deficient oocytes arrest before the onset of DNA replication in the premeiotic S phase. Thus, the *Stra8*-mediated decision to enter meiosis is made before the premeiotic DNA replication. This conclusion indicates that, although the meiosis-initiating signals may be very different in different species, the timing of meiosis initiation is the same in vertebrates and fungi.

What about other species?

While conclusive data are still lacking, indirect evidence suggests that other groups of eukaryotes may share the pattern observed in yeast and mammals, i.e., meiosis is universally initiated before premeiotic S phase although the cues triggering this process vary widely.

In maize, the switch between mitotic and meiotic cell cycles is controlled by the *ameiotic1* gene[26–28] (Pawlowski et al., unpublished data). In contrast to the mouse, *am1* mutant cells that fail to enter meiosis do not always arrest but in some cases undergo mitosis instead. The *am1* gene encodes a novel protein that has no sequence homologs outside of plants (Pawlowski et al., unpublished data). Moreover, it seems that the closest homolog of *am1* in Arabidopsis, *SWITCH1* (*SWI1*)[29–31] may play a slightly different role in meiosis initiation than *am1*, supporting the notion that components of meiosis entry control mechanisms evolve faster than typical meiotic proteins. Surprisingly, *am1*, in addition to its role in meiosis initiation, also regulates progression through the early stages of meiotic prophase[27] (Pawlowski et al., unpublished data). Although it is not clear whether meiosis initiation by *am1* takes place before the start of premeiotic S phase, some evidence suggests that this may well be the case. In several *am1* mutants, female meiocytes arrest at interphase—a phenotype similar to the phenotype of the mouse *stra8* mutants. Moreover, the Arabidopsis SWI1 is expressed exclusively during premeiotic G_1 and S.[31]

Outlook

Mechanisms that trigger meiosis initiation in yeast, mammals and plants show extreme diversity, embodying the fact that meiosis is one of the steps of the organism development program, which, obviously, varies widely among species. These differences may reflect different origins of premeiotic cells: (1) in animals, germ line cells are pre-determined during early embryonic development, (2) in plants they are established from somatic cells much later during development, and (3) in yeast, any cell can undergo meiosis in specific environmental conditions. At the same time, there seems to be only one way to enter the meiotic cell cycle—through the premeiotic S phase.

Now that the main principles of the meiosis switch-controlling mechanism have been discovered in several species, new questions are mounting. First, is the decision to initiate meiosis made at one time point during the cell cycle or is a series of consecutive decisions required to switch to meiosis? The fact that premeiotic cells in the *stra8* mutant deteriorate rather than continue dividing mitotically[1] suggests that, in the mouse, meiosis initiation requires a multistep decision process. However, the situation may be different in maize where premeiotic cells that do not enter meiosis as a result *am1* mutation, may undergo mitosis instead.[26–28]

Another question is: what is special about the premeiotic S phase and in what ways does premeiotic S differ from the premitotic S? In this area, data from higher eukaryotes are paricularily scarce.

Yet another fascinating issue is the nature of the signaling pathway downstream from the species-specific meiosis initiation signals. This pathway not only links the meiosis-initiation signals with the conserved machinery regulating the S phase entry but also must control the meiotic character of the premeiotic S. Although little is known about components of this pathway, data from yeast suggest cyclin involvement.[5,11,12] Another potential target of the meiosis-initiating signals could be the PCNA (Proliferating Cell Nuclear Antigen) protein.[32] PCNA is known to regulate the cell progression though S and can interact with a large number of partners, including cyclins, proteins involved in chromatin assembly, DNA repair and cohesin installation.[32,33] With its "sequential docking" feature, which allows multiple proteins to bind to the same active sites, PCNA could have been easily co-opted during the

evolution of meiosis to interact with meiosis-specific proteins while preserving interactions with its existing mitotic partners.

While guaranteeing continued excitement for many years to come when new elements of the meiosis initiation mechanism are discovered, understanding the initiation of meiosis may also, some day, be useful in animal and plant breeding if we learn how to induce meiosis and produce gametes in vitro by manipulating the meiosis-initiation genes in somatic cells.

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