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The Evolution of Meiosis From Mitosis

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> . . . if there is one event in the whole evolutionary sequence at which my own mind lets my awe still overcome my instinct to analyse, and where I might concede that there may be a difficulty in seeing a Darwinian gradualism hold sway throughout almost all, it is this event—the initiation of meiosis.
>
> W. J. Hamilton (1999, p. 419)

THE origins of meiosis in early eukaryotic history have never been satisfactorily explained. Since the reduction-division process in meiosis is essential for sexual life cycles, discussion of the origins of meiosis has been closely tied to debates about the evolutionary value of sex itself and the selective pressures for its maintenance. Yet the cytological events involved in the origins of meiosis are as puzzling as the question of selective pressures. While meiosis almost certainly evolved from mitosis, it has not one but four novel steps: the pairing of homologous chromosomes, the occurrence of extensive recombination between non-sister chromatids during pairing, the suppression of sister-chromatid separation during the first meiotic division, and the absence of chromosome replication during the second meiotic division. This complexity presents a challenge to any Darwinian explanation of meiotic origins. While the simultaneous creation of these new features in one step seems impossible, their step-by-step acquisition via selection of separate mutations seems highly problematic, given that the entire sequence is required for reliable production of haploid chromosome sets. Both Maynard Smith (1978) and Hamilton (1999) regarded the origins of meiosis as one of the most difficult evolutionary problems.

In this *Perspectives* article, we present a hypothesis of the origins of meiosis that encompasses both the cytological novelties and the selective forces that might have favored them. We first present the reasons for thinking that the initial step involved a key innovation, that of extensive homolog pairing (synapsis), and then discuss how the other three distinctive properties can be accounted for. We next ask what selective pressures might have favored the acquisition of homolog synapsis. The conclusion is surprising: the initial function of chromosome pairing was to *limit*, not enhance, recombination. Finally, we review the evidence that much of the molecular machinery required for the initial forms of homolog pairing probably existed in proto-eukaryote unicellular forms prior to the evolution of meiosis and therefore could have been readily "recruited" for the new role. Some experimental tests of the hypothesis are proposed.

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IDENTIFYING A KEY STEP IN THE EVOLUTION OF MEIOSIS FROM MITOSIS

In the evolution of the eukaryotes, it can be assumed that the earliest eukaryotic species were single-cell haploid forms, possessing just a single set of chromosomes, and that they propagated by mitosis. While many of the simplest contemporary eukaryotes, namely protists and fungi, exhibit the mitotic propagation of both haploid and diploid states, diploidy is almost certainly a derived state. In principle, the very first diploid cells could have first arisen either by cell fusion or by endomitosis. Hurst and Nurse (1991) have argued that the first diploids probably arose via rare endomitotic errors rather than by cell fusion. Yet, since non-sexual cell and nuclear fusions can occur independently of sex ("parasexuality"), either route to early diploid states is possible. In this view, the formation of occasional diploid cells predated regular sexual life cycles in eukaryotes.

The origins of mitosis itself in the first eukaryotes are, of course, of high interest. The fact that mitosis is a universal eukaryotic property suggests that it arose at the base of the eukaryotic tree. A key point is that there

TABLE 1

Comparison of mitotic and meiotic stages

Mitotic stage	Result	Meiotic stage	Result
S phase	Chromatid duplication	S phase, I	Chromatid duplication; DNA breaks introduced
Prophase	Chromosome condensation	Prophase, I	Chromosome condensation; *homolog pairing, recombination*
Metaphase	Chromosome alignment in center of spindle body	Metaphase, I	Alignment of homologs in center of spindle body
Anaphase	Centromere splitting; chromatids separated	Anaphase, I	Separation of homologs with independent assortment; *centromere splitting suppressed*
Telophase	Chromatid decondensation; two daughter nuclei with mother-cell ploidy, single-chromatid chromosomes	Telophase, I	Partial or complete chromatid decondensation; two haploid nuclei with replicated chromatids
		Prophase, II	*No S phase*; chromosome condensation
		Metaphase, II	Alignment of replicated chromatids
		Anaphase, II	Centromere splitting; separation of chromatids
		Telophase, II	Chromatid decondensation; four haploid nuclei, single-chromatid chromosomes

The four novel properties of meiosis are indicated by italics.

are prokaryotic homologs of all the key molecules employed in eukaryotic mitosis (see reviews by Hirano 2005 and Erickson 2007). These include the actins, required for daughter cell separation in eukaryotes; the tubulins, required in eukaryotes for the mitotic spindle and movement of chromosomes; and the molecules required for chromosome condensation and sister-chromatid cohesion, members of the so-called *s*tructural *m*aintenance of *c*hromosomes (SMC) family. The prokaryotic members of the tubulin family are the FtsZ genes, which were first discovered in *Escherichia coli* but later found in many prokaryotic species, while similarly, the homologs of the SMC proteins are found throughout the eubacterial and archaebacterial kingdoms. It is not difficult to imagine that members of the actin-related, FtsZ, and SMC gene families could have been evolutionarily recruited for use in the first primitive forms of mitosis; the latter must have involved a switch from membrane-based to spindle-based attachment points for segregating sister chromosomes.

The evolution of meiosis, however, poses problems of a different order. The crucial but reasonable deduction, based on both cytology and genetics, is that meiosis evolved from mitosis (Cavalier-Smith 1981; Simchen and Hugerat 1993). While the various similarities between the two forms of cell division argue for a close evolutionary relationship between them, the greater complexity of meiosis indicates that it is the derived process. Furthermore, while mitosis is universal in eukaryotic species, meiosis is merely ubiquitous, consistent with its loss in some eukaryotic lineages. Comparative evidence suggests that meiosis appeared early in eukaryotic cell history (Ramesh *et al.* 2005; Schurko and Logsdon 2008), and its high degree of similarity in different taxonomic groups suggests that it arose only once (Hamilton 1999; Ramesh *et al.* 2005).

As noted above and summarized in Table 1, the cytological events specific to meiosis are the following: (1) the acquisition of homolog pairing (and its concomitant, homolog separation), (2) the occurrence of efficient intergenic recombination between homologs during pairing, (3) the suppression of sister-chromatid separation in the first division, and (4) the absence of S phase at the start of the second division.

Most of the attention of evolutionary geneticists has focused on the second step—extensive genetic recombination during pairing—and its significance as a generator of genetic diversity (Fisher 1930; Muller 1932; Maynard Smith 1978; Crow 1988). Yet, while genetic recombination is a key feature of meiosis, it is not unique to this process. Recombinational capacity is found throughout the prokaryotes and therefore must considerably predate eukaryotes and, therefore, meiosis (Levin 1988; Cavalier-Smith 2002; Marcon and Moens 2005). Accordingly, the original proto-eukaryote cells must also have possessed the enzymatic machinery for recombination. In particular, a crucial set of molecules for genetic recombination, the *recA* family of proteins, is utilized for recombination in both prokaryotes and eukaryotes (Aboussekhra *et al.* 1992; Shinohara *et al.* 1992).

Furthermore, within eukaryotes, genetic recombination is not restricted to meiosis. Diploid somatic cells of fungi, plants, and animals undergo chromosomal crossing over, the phenomenon known as "mitotic recombination." There are, however, three significant contrasts between meiotic and mitotic recombination. First, mitotic recombination between homologs takes place at a very much lower frequency than in meiosis. Second, while crossing over between sister chromatids in mitotic cells is fairly frequent (as seen with physical labeling techniques), meiosis is structured to promote crossing over between non-sister chromatids. Third, as found in yeast cells, mitotic recombination is mediated efficiently by either of two recA homologs, rad51 and Dmc1, while meiotic exchange between homologs requires Dmc1 specifically (reviewed in Neale and Keeney 2006). If mitosis preceded meiosis in evolution, it seems equally likely that mitotic recombination preceded meiotic recombination.

In thinking about the origins of meiosis, a point of interest is that meiosis as it exists is not the simplest conceivable process for producing haploid cells from diploid cells. In principle, premeiotic DNA replication would not be necessary. The unreplicated chromosomes would simply pair with each other with or without recombination and would move to opposite poles to produce just two haploid nuclei. The whole process would be accomplished in one division, not two. This hypothetical sequence of events, "one-step meiosis" (Cavalier-Smith 1981; Archetti 2004), differs from the normal "two-step" meiosis in involving (1) the active suppression of DNA synthesis and (2) the pairing of homologous but unreplicated chromosomes prior to metaphase. Although one-step meiosis would achieve the same results as actual meiosis, it is hard to imagine how both properties could have arisen readily and simultaneously from mitosis.

In contrast, consider meiosis as it actually occurs. It begins with an S phase, which may differ in certain features from the normal mitotic S phase (Stern and Hotta 1977), yielding chromosomes that each consists of a pair of sister chromatids. This is followed by pairing of homologous chromosomes along their entire length (synapsis), a state that is visibly obvious in most eukaryotic species as the chromosomes condense. In this phase, homologous non-sister chromatids recombine with each other, sometimes only once, but more often at several sites along their length. After recombination, the chromosomes condense further and the paired homologs become aligned on the metaphase plate. There are already two kinetochores to which the chromatids are attached but, in contrast to mitosis, the kinetochores do not split in this first meiotic division: the homologs simply separate to opposite poles. This absence of kinetochore fission in the first meiotic division (MI) reflects a difference in the molecular mechanics of centromere–microtubule attachment, a consequence of the geometry of sister-chromatid placement when homologs are paired. In contrast to the "bi-orientation" of sister chromatids to opposite poles in mitosis, both sister chromatids of each paired chromosome in metaphase I are attached to spindle fibers running to the same pole ("mono-orientation") (reviewed in Hauf and Watanabe 2004).

When the two sets of chromosomes produced by MI are enclosed within nuclei, they are already replicated. So these nuclei are in effect the equivalent of the G_2 state of the mitotic cycle. The absence of replication in the second meiotic division (MII) presumably follows from the same mechanism that prevents further rounds of replication in the G_2 phase of cells preparing for mitosis, namely the absence of binding of one or more of the "licensing factors" (*e.g.*, the Mcm 2–7 proteins) at replication origins through their removal during S phase (reviewed in Blow and Dutta 2005). Although the precise mechanism is not known, it seems likely that sister-chromatid separation at the centromeres generates a signal that begins the process of "replication licensing." In the absence of that molecular transition, at the end of meiosis I, the chromatids cannot undergo a new round of replication.

Whatever the mechanism is that inhibits a second S phase, prophase of meiosis II consists simply of chromosome condensation. It, in turn, is followed by metaphase II and then by anaphase II, in which the sister chromatids are separated and segregated to opposite poles, yielding two haploid nuclei with single (nonreplicated) chromatids. The separation of sister chromatids in meiosis II involves molecular players and processes similar to those involved in sister-chromatid separation in mitosis (reviewed in Rivera and Losada 2006). Altogether, the second division produces a tetrad of products from each initial meiotic I nucleus, and each of these final daughter nuclei possesses one (haploid) unreplicated genome.

Thus, and perhaps counterintuitively, the evolution of two-step meiosis requires fewer new events than the seemingly simpler one-step process. Indeed, it actually necessitates only one, namely the synapsis of homologous chromosomes, each consisting of two sister chromatids, with the rest of the sequence following in the known pattern of mitosis for replicated chromosomes. Archetti (2004) has produced an argument, which is based on considerations of selection pressures, as to why the simpler hypothetical path of one-step meiosis is such a rarity, if it exists at all. In contrast, our argument is based on the known facts of cytology and molecular biology.

Our key proposition, therefore, is that the origin of meiosis involved the evolution of stable genomewide synapsis, lasting into metaphase, and the insertion of this step into the mitotic cycle. Such pairing at first might appear to be a striking novelty. Yet, widespread pairing of homologs in somatic (nonmeiotic) cells has been found both in Drosophila (McKee 2004) and in yeast (Burgess

et al. 1999). Such somatic pairing differs from meiotic synapsis in three respects: (1) it is not as extensive (McKee 2004); (2) it does not lead to the levels of genetic recombination seen in meiosis; and (3) it terminates in either interphase or prophase, allowing each chromosome to proceed to the metaphase plate independently of its homolog. Nevertheless, if such homolog pairing in mitotic cells is an ancestral eukaryotic property, then the origins of meiotic synapsis need have involved only its temporal extension into metaphase and more intimate or extensive apposition of homologs, especially at the kinetochores. Meiotic synapsis would thus be a modification of an already existing property, not a wholly novel one. As argued above, the absence of sister-chromatid separation at the end of meiosis I would reflect the altered geometry of microtubule attachment, when homolog kinetochores are paired, while the absence of S phase in meiosis II would be a consequence of the absence of sister-chromatid separation. The remaining distinctive feature of meiosis, namely high recombination levels during chromosome pairing, can be seen as a property that evolved later (see below).

Our proposal that a key innovation converted a mitotic cycle into a meiotic one is not the first suggestion of its kind. Cavalier-Smith (2002) argued that suppression of kinetochore splitting in MI was the key innovation in meiosis. This event, however, comes *after* homolog pairing, which clearly is a novelty. Furthermore, as noted above, the absence of kinetochore splitting directly reflects the difference in sister-chromatid orientation with respect to the poles between MI and MII (Hauf and Watanabe 2004). This, in turn, reflects the inherent structural-geometric differences in microtubule attachment between paired and unpaired chromosomes at the level of individual chromosomes (Paliulis and Nicklas 2000). In Table 2, we compare the stages of mitosis and meiosis in terms of our hypothesis.

TABLE 2

Relationship of key meiotic stages to mitotic stages

Meiotic stage	Relationship to mitosis	Modification	Novelty
S phase, meiosis I	Comparable to mitotic S phase[a]	X	
Prophase I	Homolog pairing		X
Metaphase II	Comparable to mitotic metaphase[b]	X	
Anaphase I	Comparable to mitotic anaphase[b]	X	
Prophase II (no S phase)	Comparable to mitotic G_2 state[c]	X	
Metaphase II	Essentially mitotic metaphase[d]	X	
Anaphase II	Essentially mitotic anaphase[d]	X	
Telophase II	Essentially mitotic telophase[d]	X	

[a] DNA breaks introduced during replication; almost certainly part of later meiotic evolution.

[b] With the difference that it is replicated chromosomes that are first aligned at metaphase, then separated at anaphase.

[c] In that the chromosomes are "unlicensed" and hence refractory to replication.

[d] The mechanics are the same; the only difference is in the number of chromosomes (one-half) relative to mitosis.

SELECTION PRESSURES TO FOSTER HOMOLOGOUS CHROMOSOME PAIRING

The conclusion that meioisis originated with the insertion of homolog synapsis into the mitotic cycle immediately raises two questions. The first concerns the nature of the selective pressures for this new chromosomal behavior and the second concerns the molecular requirements for this novel cytological feature. In this section, we discuss the possible selective pressures; in the molecular side of the scenario, we approach the molecular aspects.

A cardinal feature of contemporary meiosis is its association with high levels of intergenic recombination. The selective benefits are twofold: such recombination helps reduce unfavorable gene combinations and promotes new favorable ones. Correspondingly, most thinking about the evolution of meiosis has focused on the selection pressures to foster the elimination of harmful gene combinations and to promote beneficial ones (Fisher 1930; Muller 1932; Maynard Smith 1978; Crow, 1988). From this standpoint, any selection for homolog synapsis would actually have involved selection for improved efficiency of genetic recombination mediated by such pairing.

Nevertheless, the view that the benefits of intergenic recombination were a prime selective force for the origins of meiosis has always been problematical. Although the arguments are often constructed in terms of the immediate benefits to offspring (see review by Ghiselin 1988), the explanation implicitly invokes an element of group selection with respect to future benefits for the population. Yet natural selection cannot operate with foresight. Hence, whatever initial benefits chromosome pairing in proto-eukaryotes may have conveyed, they would have had to have been more immediate than the promotion of intergenic recombination.

An alternative view is that the initial benefit of meiosis was enhanced repair of DNA damage via recombination (Bernstein 1977; Bernstein *et al.* 1988). The need for efficient DNA repair is a basic and ancient requirement of living cells, as shown by its ubiquity among prokaryotic cells, and originally served to protect early cells from incoming solar UV irradiation and other DNA-damaging agents, as well as desiccation. Furthermore, recombination of homologous sequences provides an efficient mode of DNA repair. In *E. coli* cells, for example, inactivation of either of the key recombination functions, *recA* or the *recBC* enzyme, greatly increases lethality

upon exposure to UV irradiation, despite the presence of other DNA repair systems (Clark 1971; Smith 2004).

The argument for DNA repair as the primary (initial) benefit of meiosis implies that the existing forms of DNA repair were borderline insufficient for the needs of the earliest eukaryotic cells. Prokaryotes, however, are endowed with a rich assortment of DNA repair capacities, including inducible recombinational repair (Levin 1988; Cavalier-Smith 2002; Marcon and Moens 2005), and the existence of abundant prokaryotic life in the harsh conditions of Archean seas (Knoll 2003), well before eukaryotic cells existed, suggests that DNA repair capacities must have sufficed to cope with the kinds of DNA damage associated with that environment. Especially in light of cellular capacities to upregulate recombinational repair and the highly efficient repair of double-strand breaks (DSBs) utilizing sister chromatids in mitotic cells (Argueso *et al.* 2008), the argument that meiosis was necessary for extra repair capacity does not seem compelling.

If, however, the two standard hypotheses about selection pressures for meiosis are inadequate, then another explanation is needed. If the deduction that homolog synapsis was the key initial event in the origin of meiosis, one has to ask just what such pairing yields. The answer is "accurate alignment" and that may be the key to the puzzle: accurate alignment should promote not only recombination but also recombination between fully matched long sequences. We propose, in effect, that homolog synapsis was selected because it promotes fidelity of recombination, thus reducing the chances of ectopic pairing and consequent ectopic recombination. Genomewide homolog pairing would help to ensure that only identical regions (not diverged homologous ones at different chromosomal locations) would recombine. As in the DNA repair hypothesis, the selective benefits would be immediate but the proposed advantage would be radically different: instead of the restoration of wild-type DNA sequences following damage (as in the DNA repair hypothesis), the selective benefit of the new process would be the prevention of recombination-generated damage.

Our suggestion is directly related to the argument that recombination, particularly in multi-chromosomal cells, can have deleterious effects and is regulated tightly to minimize them (Bernstein *et al.* 1988). There is, in fact, some direct experimental evidence for this proposition. Holliday *et al.* (1976) presented an extensive analysis of DNA repair-defective mutants in a gene that they designated *rec-1* in the fungus *Ustilago maydis*. The phenotype of these mutants, however, is more complex than a simple repair deficiency. The strains exhibited (1) 20% nonviable cells, (2) elevated rates of mitotic recombination, (3) defective meiosis in crosses between differently marked strains with formation of aneuploid and nonviable meiotic products, and (4) considerable heterogeneity in diploid but not in haploid colonies. This complex phenotype is most simply interpreted as an abnormality in the regulation or control of recombination manifested in both mitosis and meiosis. The heterogeneity of diploid rec-1 strains is probably due to abnormal genetic events generated by recombination and leading to unbalanced genomes. In effect, the wild-type strain keeps recombination in check and failure to do so leads to errors in transmission of the genetic material. That recombinogenic enzymes are normally kept to low levels of activity is shown by another study. The recA homolog RAD51 in the ciliate *Tetrahymena thermophila* is normally present at a low level of activity, but upon exposure of the cells to either UV or methyl methanesulfonate (Campbell and Romero 1998), its levels increase dramatically, presumably to facilitate recombinational repair in the highly polyploid macronucleus. This finding suggests that the activities of the *recA* enzymes, rather than homolog pairing, can be the rate-limiting steps for recombination.

A further finding that supports the general proposition that recombination has to be tightly regulated, presumably to prevent deleterious defects, comes from an analysis by Lynch (2005). Plotting the results of many studies that measured recombination frequency per unit length of DNA as a function of genome size, he finds that there is an exponential decrease in genome size with an approximate slope of −1 (see Figure 2 in Lynch 2005). Such a distribution is the strong signature of a process that has to be kept in check.

If one of the hazards of excess recombination is recombining the "wrong" sequences, then the greater the nuclear concentration of partially related sequences, the greater the probability of recombinational errors following ectopic pairing should be. Indeed, chromosome aberrations produced by induced DSBs occur preferentially at repetitive sequences in the genome (Argueso *et al.* 2008). It is probable that growing genome size and complexity, a key feature of eukaryotic evolution (Cavalier-Smith 1978), would have increased the opportunities for recombination events between such paralogous (repetitive) sequences at different chromosomal locations. The consequences would include deletions, duplications, and inversions in intrachromosomal recombination and translocations and dicentric chromosomes from interchromosomal exchanges. Other things being equal, the number of defects would be expected to increase exponentially as a function of the increase in repetitive sequences throughout the genome. Such alterations would reduce the fidelity of genome transmission, and hence the fraction of viable cells in any clonal lineage.

In contrast, homolog synapsis prior to recombination should substantially reduce this burden of recombination-induced damage. It does not eliminate it, however.

Recombinational errors occur in meiosis, even between fully homologous sequences, as first shown by the deletion-duplication phenomenon of the *Bar* and *Supra-*

bar mutations in Drosophila (Sturtevant 1925). The recent demonstrations of ubiquitous copy number variation (CNV) in mice, chimpanzees, and humans (Li *et al.* 2004; Adams *et al.* 2005; Perry *et al.* 2006; Redon *et al.* 2006) has revealed just how common such recombination errors are, even with presumably full pairing of homologs in meiosis. The key point, however, is that, in the absence of accurate extensive pairing, such errors take place even more frequently. For example, the male-specific region of the Y chromosome, which has no pairing partner on the X, seems to have accumulated a huge stock of permanent duplications and palindromes, as a result of recombinational errors between its own sequences (Rozen *et al.* 2003; Skaletsky *et al.* 2003). Similarly, imperfectly paired "homeologous" sequences within a haploid strain derived from the allopolyploid species *Brassica napus* undergo far more recombination-mediated exchange between such related but nonhomologous sequences than in the parent strain (Nicholas *et al.* 2007). A reasonable inference from all such findings is that, in early eukaryotic cell evolution, any trend toward increased genome size via the addition of new repetitive sequences would have increased the frequency of recombinational errors between such sequences.

There is a second way, however, in which recombination, prior to the advent of meiosis, might have been harmful. Imagine that recombination in a diploid cell can take place at any point in the cell cycle but that resolution of recombination events is not always instantaneous. Such unresolved recombination events at the time of chromosome separation in anaphase would produce uncompleted chromosome separations, leading to either chromosome fragmentation or nondisjunction. The larger the genome size and the greater the number of chromosomes, the greater the chances of such events. It has been shown in *E. coli* that unresolved recombination events can indeed block chromosome segregation, leading to the production of filamentous cells (Ishioka *et al.* 1998). In contemporary eukaryotic cells, such events are avoided through the use of DNA damage checkpoints, which halt chromosome separations until repair is achieved. Proto-eukaryotic cells, however, might have lacked such checkpoints, just as contemporary prokaryotic cells seem to lack replication-completion checkpoints (Bendich 2007), and might have been vulnerable to such chromosome disjunction errors.

Diploid cells in early (proto-) eukaryotes would thus have faced a dilemma. They would have required efficient recombinational repair for survival but would have needed to avoid the potential concomitants of such repair, namely recombinational errors between nonidentical sequences or unresolved recombinational events at the time of mitosis. What sort of events or process could have helped these cells to navigate between the Scylla of unrepaired DNA and the Charybdis of recombinationally induced errors?

Any process that both promotes accurate DNA sequence alignment and restricts recombination to a distinct period prior to the separation of chromosomes would help to resolve this dilemma. This is precisely what meiotic pairing of homologs achieves. Such pairing should promote accurate homology searches, thereby reducing the number of additions or deletions that a more random DNA search procedure would generate. At the same time, concentration of recombination events to a period that precedes chromosome segregation, as occurs in homolog synapsis, would promote the maintenance of genomic integrity through the reduction of chromosomal disjunctional events and hence the fidelity of genome transmission.

To sum up, we propose that the selection pressures for homolog synapsis and the origins of meiosis were to improve recombinational accuracy and to restrict it to a safe interval, while retaining its short-term (repair) benefits. A cell lineage that had evolved this capability for diploid cells would be less error-prone in transmitting its genetic material.

Subsequent optimizing mutations could have included those that enhanced recombination enzyme activities during the chromosome pairing period and reduced them outside this interval, as seen in normal mitotic cells. By our hypothesis, the reduction-division process, restoring the haploid state, would have occurred automatically. In effect, the proposed initial sequence of events need not have involved the union of sex cells but instead a "parasexual" process, as discussed below.

THE MOLECULAR SIDE OF THE SCENARIO

Even if the puzzle of meiotic origins is largely reduced to explaining the evolution of stable post-prophase homolog synapsis, the precise molecular foundations of that process remain obscure. The molecular and cytological complexity of the pairing process in present-day species (Kleckner 2006) at first seems to preclude the origination of synapsis via one or two mutational steps, although the evolution of meiosis-specific rec8 cohesins from a preexisting cohesin (Parisi *et al.* 1999) was undoubtedly a crucial element. Other cytogenetic features such as synaptonemal complexes and the requirement for recombination to promote normal chromosome disjunction could well have evolved subsequently.

Initially, pairing in simple diploid cells, perhaps containing just one or two homolog pairs, might have involved fewer components and steps. In principle, the molecular evolution of a new cohesin molecule that specifically promoted homolog pairing might have provided the crucial trigger for meiosis. In contemporary yeast cells, the cohesin protein rec8 is maintained specifically at centromeres and the adjoining regions during normal synapsis of homologs and is essential for synapsis; its absence leads to the loss of reduction division and the occurrence of sister-chromatid separa-

tion (equational division) in MI (Watanabe and Nurse 1999; Hauf and Watanabe 2004).

Alternatively, it is possible that homolog synapsis was initially produced by elevated rates of chromosome breaking and joining, mediated by homologous sequence annealing, and promoted by existing cohesins. Although synapsis of homologs does not require DSBs in all contemporary organisms (Joyce and Kim 2007) and might not have been involved in the earliest forms of synapsis, in proto-eukaryotes with a small number of chromosomes, such recombination induction might, in principle, have sufficed to initiate homolog pairing. Whatever the trigger for the origins of synapsis, the resulting opportunity for repair and recombination might have permitted these lineages to repress non-damage-induced recombinational repair at other times, thus concentrating such repair in one discrete period.

Although the origins of homolog synapsis can never be known with certainty, it is striking how much of the molecular machinery that it brings into play is conserved between prokaryotes and eukaryotes and between mitosis and meiosis. In particular, the involvement of recA-family recombination enzymes and their enrichment in present-day eukaryotes at the sites of "recombination nodules" during meiosis (Bishop 1994; Tarsounas *et al.* 1999) is evidence of the evolutionary continuity between prokaryotic and eukaryotic recombination. The molecular evolution of Dmc1 was clearly a key step in promoting interhomolog recombination, but as a member of the recA gene family, its origins are not problematical. Strikingly, a number of the SMC family proteins, in particular the condensins and the cohesins, play similar roles in controlling sister-chromatid behavior in both meiosis and mitosis (reviewed in Haering and Nasmyth 2003). Finally, as noted earlier, the molecular machinery for centromere splitting is shared between mitosis and meiosis II. These molecules include a serine/threonine phosphatase, PP2A, and one of its substrates, the kinetichore-associated protein Shugoshin (reviewed in Rivera and Losado 2006).

In sum, it appears that most of the molecular components required for the evolution of homolog pairing and recombination between homologs were present in one form or another in the earliest premeiotic proto-eukaryotic cells.

LINKING PARASEXUAL REDUCTION DIVISION TO SEXUAL REPRODUCTION

The discussion so far has neglected one crucial element: the fact that meiosis is intimately linked to sexual reproduction. Indeed, cycles of sexual reproduction would be impossible without the reduction division that takes place in meiosis. Our hypothesis, however, links the evolutionary advent of homolog pairing to diploidization events that may have occurred independently of sex-cell fusion. Such diploidization events, followed by recombination and reduction division to regenerate haploid states, are termed "parasexual cycles." Parasexual sexual cycles were first described in fungi (Pontecorvo 1959) and fungal parasexual cycles remain the best characterized, but they are also known in the cellular slime molds and in tetraploid cancer cells where the reduction of ploidy is from tetraploidy to diploidy (Rajaraman *et al.* 2005).

We propose, therefore, that homolog synapsis and the concomitant reduction of diploid states originated in some form of parasexual cycle in the early proto-eukaryote lineage and that the functional relationship between diploidization via sex-cell union and meiosis was a subsequent evolutionary event. In this view, some form of "parameiosis" (Becker and Castro-Prado 2006)—a reduction division of some higher ploidy to a lower level without a preceding sex-cell fusion—preceded true meiosis in evolution. The possibility of such an evolutionary dissociation between early diploidization events (and their concomitant reduction/division sequels) and meiosis is consistent with the fact that, developmentally, diploidization and meiosis can be uncoupled. In many unicellular eukaryotes, haploid sex-cell fusion leads promptly to nuclear fusion, which immediately triggers meiosis, thus regenerating the haploid state. In contrast, in more complex, multicellular eukaryotes, meiosis is greatly delayed following the initial fusion of sex cells, taking place much later in the life cycle, during gametogenesis. Clearly, different signals in different organisms trigger the onset of meiosis and the particular one(s) employed reflect the organism's evolutionary history.

The idea presents a way of cutting the Gordian knot posed by the difficulty of accounting for the simultaneous origins of sex and meiosis in evolution. In effect, some form of reduction division could have preceded both true meiosis and the first systems of sex-cell union in early (unicellular) eukaryotes, as also suggested by Hurst and Nurse (1991).

TESTING THE HYPOTHESIS

There is, of course, no direct way to test the basic hypothesis presented here since the cells in which meiosis first originated existed well over 1 billion years ago and this progenitor lineage undoubtedly vanished long ago. Nevertheless, the hypothesis makes two strong experimental predictions. The first is that, if extensive homolog pairing could be induced in the prophase of diploid mitotic cells, it could trigger a meiotic-like sequence of two cell divisions. In principle, this might be achievable in transgenic yeast cells by the induction of rec8 and Dmc1 activities. A positive result would provide strong support for the hypothesis. A negative result, however, would be less informative, given the possibility that modern cells have evolved properties that make the original behavior less automatic.

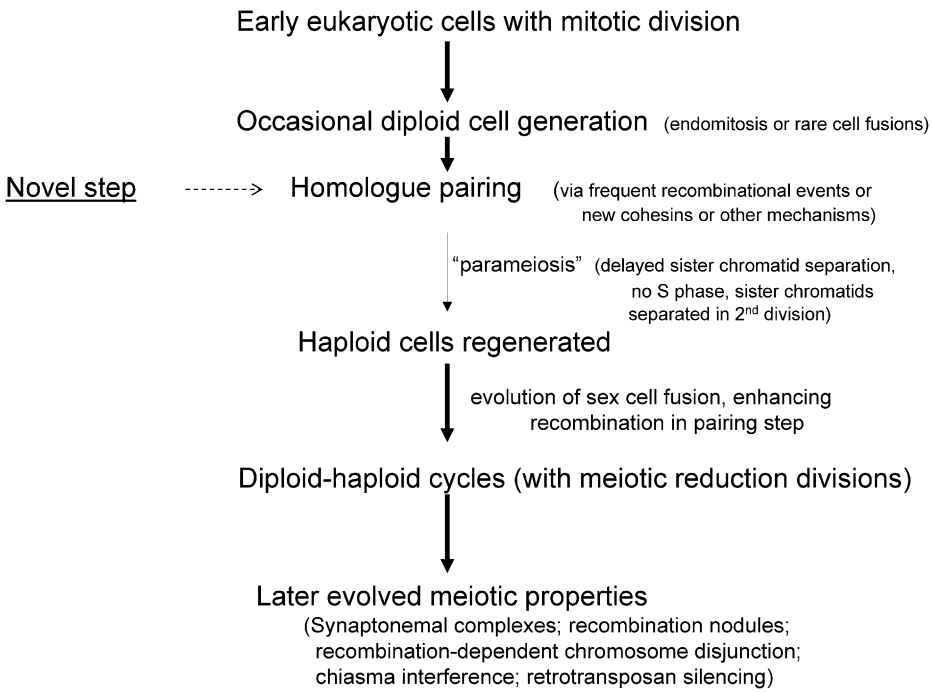

FIGURE 1.—Schematic of our hypothesis, which is shown as a time line of events in the evolution of meiosis. Thick arrows indicate long-term events (evolutionary timescale or multi-generation) while the thin arrow for the proposed parameiosis process indicates an immediate consequence and event.

The second prediction is that inducing high recombination activities in either diploid mitotic cells or hyperrecombination events in meiotic cells should promote more recombinational errors, with consequent declines in cell progeny viability. Furthermore, the number of such events should increase dramatically as a function of the number of chromosomes per haploid set, the ploidy level, and the number of induced recombination events per nucleus. In particular, it should be possible to engineer diploid and tetraploid yeast strains with inducible rad51 and/or Dmc1 constructs. To test this possibility, one could then induce excess activities of these genes in various stages of the mitotic cell cycle or in meiosis I. The prediction is that CNVs or aneuploid variants, having reduced fitness, should be induced and that tetraploid strains should have even more than diploids. In yeast strains genetically crippled in their DNA damage checkpoints, such excess recombination events in somatic cells should lead to additional chromosomal nondisjunction or chromosomal breakage events.

It is possible, however, that induction of recombination enzymes would be insufficient to induce extra recombination events, although the Tetrahymena results of CAMPBELL and ROMERO (1998) suggest otherwise. In this case, very mild conditions promoting a low level of chromosome breakage should be included, either by very low level nonlethal X ray or by enzymatically induced DSBs. The latter have been shown to recruit cohesin to those sites, promoting sister-chromatid pairing in diploid yeast cells (STROM *et al.* 2004). Indeed, even a few DSBs trigger enhanced genomewide sister-chromatid cohesion (STROM *et al.* 2007; UNAL *et al.* 2007). Our hypothesis predicts that such treatment should produce more CNVs and various rearrangements in polyploid yeast strains than in diploid strains. Results of this kind would support the proposition that there were strong selection pressures to limit ectopic recombination and promote the accuracy of recombination.

CONCLUSIONS

The evolutionary origins of meiosis have been a matter of intense debate for decades and are intimately connected to the controversy about the biological value of sexual reproduction itself, which dates from the 19th century (GHISELIN 1988). Yet the predominant focus in this literature has been on the nature of the putative selection pressures rather than on the actual cytological changes involved. Furthermore, much of the discussion has been about the maintenance of sex (and meiosis) rather than its origins, particularly in animals (MAYNARD SMITH 1978; HAMILTON 1999; ARCHIPOVA and MESELSON 2004), a group of organisms that arose long after meiosis originated. For the origins of meiosis, one must consider the earliest eukaryotic-like cells and their probable environment (ARCHETTI 2004; MARCON and MOENS 2005; HOLLIDAY 2006).

Here, we have argued that the origins of meiosis from mitosis initially involved only one new step, namely homolog synapsis. Two of the other unusual features of meiosis are prefigured in mitosis and would have been brought into play as consequences of the existing regulatory features of mitosis while the remaining one (extensive recombination) could have evolved later. We further propose that the selective pressures for acquiring extensive homolog pairing capacity in early eukaryotes were to localize and restrict recombination,

minimizing ectopic recombination and thus reducing duplications and deletions and larger aneuploid changes. (Extensive synapsis would also have probably simultaneously promoted genetic recombination but primarily among the "right" sequences.) A similar general conclusion from a consideration of cancer cells has been proposed by Heng (2007). Our brief comparative survey of the molecular machinery needed for the evolution of meiosis from mitosis suggests that much of it could have been recruited for use in meiosis via appropriate point mutations. Other features of meiosis, such as synaptonemal complexes and the requirement for recombination to ensure chromosome disjunction, would have been secondarily evolved properties. A schematic summary of our evolutionary scenario is shown in Figure 1.

Our hypothesis in no way contradicts the idea that meiosis serves to promote intergenic recombination, thereby providing new variation for selection to act upon. Indeed, one of us has proposed that the advantages of increased intergenic recombination were important in the early establishment of eukaryotic cells competing for niches with prokaryotic cells (Holliday 2006). We argue here, however, that this benefit of meiosis did not provide the initial selective pressure for its origins. Although our idea differs from traditional thinking about the advantages of meiosis, it is consistent with the known facts, and its central premise—that recombination has to be limited in extent to ensure the fidelity of the transmission of the genetic complement—is testable.

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