

Review

Primitive forms of meiosis: The possible evolution of meiosis*

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ABSTRACT: Meiosis is a basic process of most eukaryotes, as it forms with conjugation the basis of sexual reproduction. As sex seems to be present in the vast majority of eukaryotes, the origin of meiosis is presently unknown. Protists having optional or alternative sexual and asexual cycles seem to be the best targets for research on the evolution of meiosis. While the budding yeast *Saccharomyces cerevisiae* shows an elaborate and well-known meiotic process, the fission yeast *Schizosaccharomyces pombe*, has a much simpler meiosis, which may show some of the most primitive features of meiotic mechanisms. The present availability of whole genome sequences of many bacteria and some protists is revealing that eukaryotic sexual reproduction has recruited some prokaryotic processes for its own development. Some of these processes are analyzed and the basic role of chromosome linearity and telomere constitution in the development of meiosis is underlined.

Introduction

Every of us is so acquainted with the facts of sexual reproduction among living creatures that the only asexual cases that we are used to see is the grafting of plants. In fact, sexual reproduction has had a big success during evolution of living organisms (Maynard Smith, 1978; Bell, 1982). This success is due to the ability of sexual reproduction to recombine the genomes of individuals and in this way makes selection more efficient (Rice and Chippindale, 2001; reviewed in Solari, 1999). Despite its successful history, sex is costly and demands more

organic resources than asexual reproduction (Siller, 2001, and references therein). Under some special environments, sexual reproduction may go back to asexual as in one class of the phylum Rotifera (Mark Welch and Meselson, 2001). However, these exceptional taxa do not cast significant shadows over the overwhelming presence of sexual reproduction among eukaryotes. Furthermore, it is exceedingly difficult to find “genuine” asexual eukaryotes –that is, organisms which do not show sex in any step of their evolutionary history–. Vestiges of ancient sexual mechanisms have been found, for instance, in the assumed asexual fungus *Candida albicans*, which shows mating types and a diploid condition (Hull *et al.*, 2000), and in the amoeba *Corythion delamarei*, which shows isogamic conjugation (Iudina and Sukhanova, 2000). The most spectacular and sophisticated instances of sexual reproduction among protists are those of the budding yeast *Saccharomyces cerevisiae* (reviewed in Rose, 1996) and the malaria plasmodium *Plasmodium falciparum* (Gardner, 1999). Both have now their genomes fully sequenced.

* This paper is dedicated to the memory of Prof. Dr. Osvaldo A. Reig.

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Is it therefore impossible to look back for ancient forms of sex among primitive eukaryotes? In order to answer that question, it is useful to sketch a “universal phylogenetic tree”, that is, a tentative representation of the whole evolution of present day organisms.

1. A tentative universal phylogenetic tree

This matter is under a state of flux and it is mainly hypothetical. Despite these limitations, a tentative universal phylogenetic tree allows the observation of possible evolutionary clues and degrees of relatedness

among phyla. The presently dominant view establishes three “domains”, each one embracing several kingdoms (Olsen and Woese, 1997): **Bacteria**, **Archaea** and **Eukarya** (Fig. 1).

Thus, according to this evolutionary tree, the assumed symbiosis of bacteria with archaea to give rise to organisms with “primitive mitochondria” is probably ancestral to the origin of all presently living eukaryotes (Philippe *et al.*, 2000). That is to say that anaerobic protists like species of *Giardia*, *Trichomonas* and *Entamoeba*, are not “primitive” because of their lack of mitochondria (the so-called Archaezoa hypothesis). In fact,

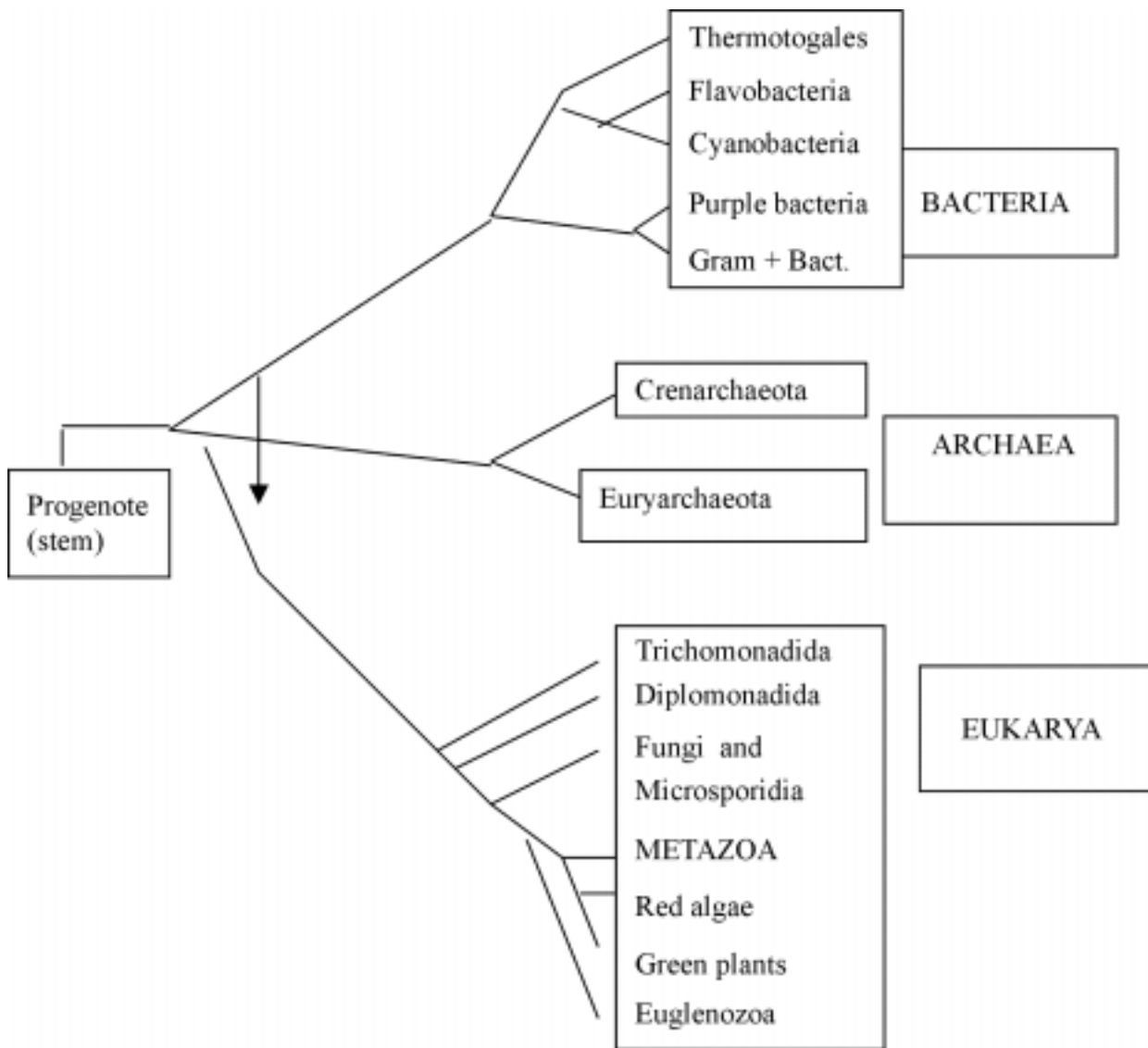


FIGURE 1. Hypothetical “universal phylogenetic tree” of presently living organisms (based on Philippe *et al.*, 2000 and Margulis, 1996, with modifications). Distances are fully arbitrary. The three great “domains” and their major subdivisions are remarked. The arrow shows the possible symbiosis between Bacteria and Archaea, which is possibly related with Eukarya (probably in an indirect way, Philippe *et al.*, 2000).

the genome of such species has shown vestiges of ancient mitochondria (Philippe *et al.*, 2000), and their “primitive” status should stand on other criteria.

From our present viewpoint it is remarkable that while sexual reproduction (SR) is overwhelmingly present among eukaryotes, it is fully absent among Archea and Bacteria.

However, some of the *components* of SR are present among the latter. Thus, molecular mechanisms of gene recombination, genome packing mechanisms, mechanisms for the separation of parental and replicated genomes, and mechanisms for the association of genomes with membranes, are some of the mechanisms that are needed in SR and that are already present among Archea and Bacteria. On the other hand, both Archea and Bacteria lack microtubules, a nuclear envelope, synaptonemal complexes and many other features proper of eukaryotes, which are also necessary for mitosis, meiosis and SR.

2. Meiotic components that are present in prokaryotes

Among prokaryotes there is neither true syngamy (conjugation) nor meiosis: there is no sexual reproduction as seen in eukaryotes. However, some proteins and even some structures that occur during SR are present in prokaryotes. In some way it happens as if an “opportunistic” evolution has recruited these proteins and these structures for the sake of a new objective: SR in Eukarya.

One of the most primitive of these components is the molecular apparatus for gene recombination. The first recognized recombinase, RecA, was isolated from the bacterium *E. coli* (Clark and Margulis, 1965). In all studied eukaryotes there are protein homologues of RecA—in the human species these homologues are mainly the proteins RAD51 and DMC1—. All these protein homologues conserve a significant part of the aminoacid sequences of the bacterial RecA, but from the functional viewpoint there are striking differences. Gene recombination is an essential process in SR in eukaryotes, and RAD51 and DMC1 are necessary for this process. On the other hand, bacterial RecA is essential for prokaryotes because of its role in processive synthesis of DNA, that is, the resumption of DNA synthesis at points blocked by DNA damage (Courcelle *et al.*, 2001) and its role in the sporadic bacterial gene recombination is non-essential.

In a similar way, the bacterial proteins MutLS and MutH are essential among prokaryotes for the correction of mismatch errors in DNA bases after DNA repli-

cation, and they have protein homologues among eukaryotes. For instance, in the human species the proteins MLH1, MSH4 and several others are components of a system of DNA repair which deals with mismatched bases. However, these same proteins have acquired an additional function during eukaryotic meiosis: during advanced phases of meiotic prophase they are essential for the resolution of crossovers and mismatch repair at heteroduplex DNA (Kirkpatrick, 1999).

A large group of proteins related to the structural maintenance of cromatin (SMCPs) is also found both in Eukarya (much more developed) and in prokaryotes. In the latter, as for instance in *Bacillus subtilis*, SMCPs are represented by only one or two proteins (Strunnikov, 1998). Among eukaryotes they are represented by more than 20 proteins, some of which are essential for meiosis (Cobbe and Heck, 2000), especially the protein Rec8 (Davis and Smith, 2001; Pasierbek *et al.*, 2001).

A more dubious component might perhaps be related to the mating types and their specific receptors found in the budding yeast (Betz and Duntze, 1979). Although these proteins may serve a function similar to that of the conjugation types in bacteria (F+, F-, Hayes, 1968), the latter are completely different and they even correspond to a different mating mechanism, which in bacteria is dependent on fimbriae or “pili”.

The above cited instances of similar proteins shared by eukaryotes and prokaryotes, cannot blur the large differences among these two groups. Some of the differences relevant to this discussion are (Drlica and Bendich, 2000; see also a temptative comparison between bacterial nucleoids and eucaryotic nuclei in Bendich and Drlica, 2000):

- a) prokaryotes lack nuclear envelope, nuclear pores and laminae
- b) prokaryotes lack true, eukaryotic chromosomes, and lack the particular molecular features of eukaryotic telomeres
- c) prokaryotes lack microtubules and a cytoskeleton akin to that of eukaryotes

These differences are related to the absence of a true mitotic process among prokaryotes (Lewis, 2001), that is, a regular process by which genomes (chromosome complements) are *equally* distributed in daughter cells. Although bacterial cells may have several genomes—and even large ones (Bendich and Drlica, 2000)—usually these cells harbor variable numbers of genomes (nucleoids), that is they are “polygenomic”. This feature is not similar to polyploidy (presence of exact multiples of the haploid chromosomal complement) of eukaryotes, but it is similar to polyplasmly among mito-

chondria (several or many DNA copies of the mitDNA). Actually, the circular molecules of mitDNA recombine very rarely, and the several genomes inside a single bacterial cell do have the same behavior.

Archaea have histone-like proteins (which are simpler than eukaryotic histones, Zlatanova, 1997), nucleosome-like particles (NLS), introns within genes, promoter sequences like TATA boxes, transcription factors and other features similar to those of eukaryotes. However, Archaea also lack the essential elements needed for mitosis: nuclear envelope, chromosomes with telomeres and a cytoskeleton.

3. Mitosis as a necessary basis for meiosis occurrence

Meiosis in present day organisms requires the activity of a significant number (hundreds) of genes, many of which are also needed for mitosis. Some of the “meiotic” genes are the same or simple variants of “mitotic” ones, but other are additional to the mitotic gene pool. The relationship between mitosis and meiosis is obvious from the microscopical observations, as meiosis needs the mitotic apparatus for genome separation. A growing pool of molecular data is adding more evidence on this relationship. In fact, it is difficult to think on the evolution of meiosis without a fully functional mitosis. From a theoretical viewpoint, population geneticists think that meiosis is derived from, and more recent than, as regards to mitosis (Gessler and Xu, 1999). Furthermore, experiments have shown that an incipient meiosis can revert to mitosis, while the contrary has never been observed.

If mitosis is ancestral to meiosis, the question may be asked on which of the mitotic mechanisms are essential and which are non-essential for meiosis. As regards to this matter, we can analyze which are the simpler and presumably more primitive forms of mitosis. Mitosis can be classified into two large classes:

- a) “closed” mitosis (also called pleuromitosis), as present in many protists
- b) “open” mitosis as typical of most metazoan and higher organisms.

Within each of these two classes, there are several sub-types, according to the kind of microtubular apparatus, the degree of chromosome condensation and other variables (Heath, 1980). Closed mitosis, because its apparent simplicity and its presence in presumably primitive protists, is generally considered as the primitive type of mitosis (Heath, 1980). In closed mitoses the nuclear envelope is present throughout the mitotic cycle and the final genome partition is accomplished

with a mid-constriction of this envelope which gives rise to two daughter nuclei. Another presumably primitive feature is the lack of a higher degree of chromatin packing to form definite chromosomes. Both of these features (closed and lack of chromatin condensation) are shown in the mitosis of protists as the budding yeast (*S. cerevisiae*) and very typically in trypanosomes (Kinetoplastida) (Fig. 2).

These protists with “closed” mitoses are obvious targets for the search of primitive meiotic forms, especially those protists that have sporadic SR – those in which sex is only an alternative, perhaps an infrequent

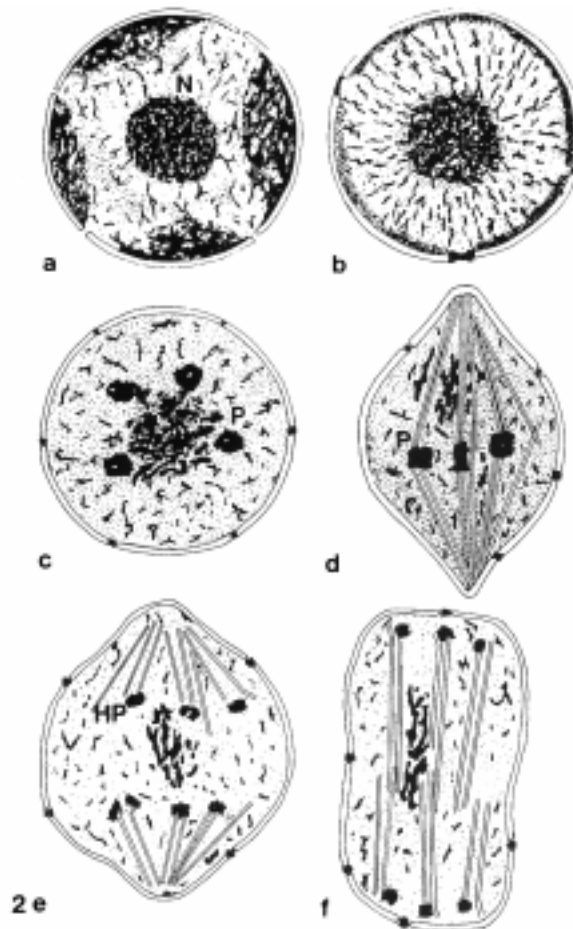


FIGURE 2. Mitosis in the trypanosome *T. cruzi*, from interphase (a) to anaphase (f). N: nucleolus; P: kinetochores, double (dense plaques); HP: half-plaques or single kinetochores, moving towards the poles. (Solari, 1995, from Biocell, with permission).

one- as regards to asexual reproduction. Even when these conditions are fulfilled, primitive meiosis is not warranted: the budding yeast *S. cerevisiae* has an extremely sophisticated meiosis, similar in many ways to that of higher eukaryotes.

On the other hand, a simple unicellular fungus, phylogenetically distant from the common yeast, *Schizosaccharomyces pombe*, shows a meiotic process significantly simpler than the classical type and may be used to analyze the evolution of meiosis.

4. The sophisticated meiosis of the common (budding) yeast *S. cerevisiae*

The common yeast has two life cycles: a) an asexual (clonal) one, and b) an alternation of haploid / diploid phases with SR (meiosis and conjugation). Thus, yeast can be kept in culture either as haploid or diploid forms, both dividing by mitosis. When diploid forms are transferred to nitrogen- and carbohydrate-poor media, meiosis (sporulation) is induced. Haploid sporocytes may replicate by mitosis, but if they contain opposite mating types (MATa and MAT α) they can enter conjugation and give rise to a diploid form (Fig. 3).

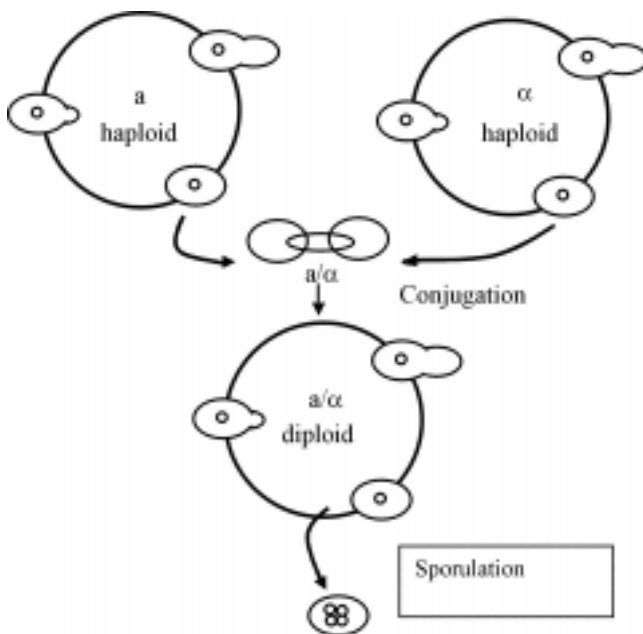


FIGURE 3. Life cycle of the budding yeast *Saccharomyces cerevisiae*.

Thus, SR is not essential for the budding yeast. However, meiosis in this organism is extremely complex and it is quite similar to that of higher eukaryotes (reviewed in Dresser, 2000). Some of the special features of meiosis and the genome in the budding yeast are:

- a) Cell divisions are “closed” (primitive type).
- b) It shows 16 pairs (diploid form) of well-defined chromosomes, which have different size going from 1.9 Mb to 0.22 Mb of DNA. The whole genome is 14 Mb and it is completely sequenced.
- c) It has about 6,000 genes, and most of them are represented in higher eukaryotes.
- d) It shows a very high rate of meiotic gene recombination per DNA unit: 0.3-1.5 cM per Kb (Dresser, 2000), which is about 1,000 times that of human meiosis.
- e) Meiotic recombination in yeast shows *positive interference*, as in higher eukaryotes.
- f) This organism **shows synaptonemal complexes (SCs)** during meiosis.
- g) Meiosis initiation is dependent on the occurrence of double strand breaks (DSBs) in DNA, which are induced by the protein SPO11.
- h) Yeast has several proteins of the SMCP group: cohesins Sccp1/Mcd1, Smc3, Rec8p and other which are present during mitosis and meiosis, as well as other related proteins.
- i) There are telomeres similar in molecular organization to those of higher eukaryotes.
- j) There is "polarization" or "bouquet" of chromosome ends during early meiotic stages.

Yeast meiosis has been extensively studied (reviews in Dresser, 2000, and in Zickler and Kleckner, 1999) and forms a research area by itself: it is the best studied one from the molecular viewpoint, and it has provided a degree of detail that is not available in other organisms. In particular, the study of the SC proteins and the recombination mechanisms in yeast have been very fruitful. For instance, the central region of SCs in yeast has been shown to result from the self-assembly of the protein Zip1 (Dong and Roeder, 2000), and the lateral elements of the SC contain the proteins Red1, Hop1 and Mek1, which seem to become associated in the cited order, but that require also proteins of the SCMP group as Smc3 and Scc1p/Mcd1p (Dresser, 2000).

In the budding yeast the proteins directly related to recombination and homologous to the bacterial RecA are at least four: Rad51, Dmc1, Rad55 and Rad57. However, many other proteins are needed for the completion of the recombinational process (Zickler and Kleckner, 1999).

In summary, meiosis in the budding yeast is very similar to that of metazoans and higher eukaryotes. One of the most conserved features of meiosis in eukaryotes is the building up of a protein apparatus specific of meiotic prophase, the "synaptonemal complex" (SC), which is formed by each pair of homologous chromosomes (Fig. 4).

The SC was first described by M.J. Moses (1956) in crayfish spermatocytes, and it was found in many eukaryotic species by other authors. In a recent and extensive review on meiosis (Zickler and Kleckner, 1999) only three species are classified as lacking SCs. As several thousands of eukaryotic species have been searched for its meiotic behavior, it may be said that the SC is practically present throughout the eukaryotic taxa.

5. The primitive meiosis of the unicellular fungus *Schizosaccharomyces pombe*

S. pombe –or fission yeast- is phylogenetically distant from the budding yeast and shows many cytologi-

cal and genomic differences with the common yeast. *S. pombe* is usually in a haploid condition instead of the usual diploidy of *S. cerevisiae*. Furthermore, *S. pombe* has three well-defined chromosomes at mitosis ($n=3$, haploid) because of the presence of chromatin condensation during mitosis- which is also closed, as in other protists-. Meiosis in *S. pombe* occurs immediately after the fusion of two haploid cells ("postcigotic" meiosis) and has fundamental features differing from those of most eukaryotes: the most obvious is the absence of synaptonemal complexes (it is one of the three exceptional species as regards to this feature). Some of the more relevant features of meiosis in this protist are:

- The absence of synaptonemal complexes
- The absence of crossing-over interference
- The presence of a centrosome equivalent, the *spindle pole body* (SPB) attached to one pole of the nuclear envelope, and which is essential for meiosis
- During meiotic prophase, the nucleus undergoes a series of "oscillatory movements" that carry the nucleus to alternate cellular ends

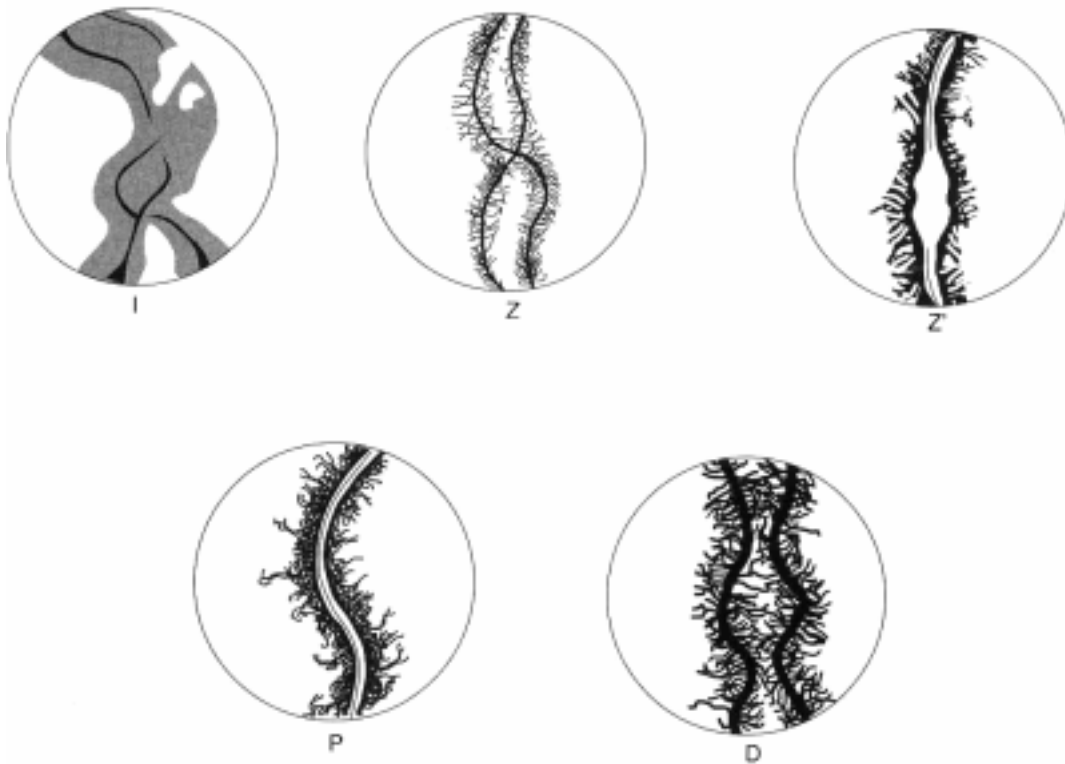


FIGURE 4. Schematic drawing of the typical stages of meiotic prophase. I: leptotene stage with the appearance of partial chromosomal axes; Z and Z', early and late zygotene stages with the pairing of the axes and the formation of SCs; P, pachytene stage with fully formed SC; D: diplotene stage with the axes separating from each other (from Solari, 1999).

- e) The prophase nucleus has a “horsetail” shape, as a result of the oscillatory movements
- f) During these movements, the prophase nucleus always is guided by the SPB attached to the thinned pole nucleus
- g) The three chromosomes have well-defined telomeres associated to the nuclear envelope, that change in location at the beginning of meiosis: they move towards the SPB and become adjacent to it
- h) The centromeres of the three chromosomes are adjacent to the SPB during mitosis, but move away from it and towards the nuclear center at the beginning of the sex cycle.
- i) Instead of having SCs, the meiotic nucleus develops about 24 “linear elements” (Bahler *et al.*, 1993) –a number much higher than that of chromosomes– which are segments, and that have a total length of about 34 μm (Bahler *et al.*, 1993).
- j) The number of crossovers per meiosis is about 45, which is lower than that of the budding yeast
- k) Normal chromosome segregation depends heavily on the protein Rec8, which is a cohesin that has homologues among many eukaryotes.

6. Primitive structural features of meiosis in *S. pombe*

As previously cited, *S. pombe* lacks SCs during meiosis. That this absence of SCs could be an evolutionary regression from a typical meiosis seems to be highly improbable: this is a unicellular organism with a closed, primitive mitosis, and its usual life cycle is asexual. Furthermore, the genome sequence of *S. pombe* is well known, and it does not contain a coding sequence similar to that of Zip1 (of the central region of the SC). *S. pombe* lacks also any homologues of the proteins of the lateral elements of SCs in the budding yeast, RED1, HOP1 and MER2 (Egel, 2000).

On the other hand, *S. pombe* shows “linear elements” which are similar to sketchy segments of chromosomal axes, and that become approximately parallel to each other during meiotic prophase, after the “oscillatory movements” of the nucleus. It may be assumed that these linear elements represent a primitive way to align homologous chromosomes and maintain attached their sister chromatids, that is, these linear elements would be “protoaxes” in an organism that is in an evolutionary stage previous to the formation of full SCs-. In fact, *S. pombe* has the relevant protein Rec8, which is essential for the formation of the “linear elements” during meiotic prophase, and which is widely conserved in eukaryotes.

7. A primitive mechanism of chromosome pairing: telomere convergence and alignment through the oscillatory movements in *S. pombe*

The lack of an SC that could keep each pair of homologous chromosomes aligned in register during the time necessary for developing and resolving the recombinational events (crossovers) and assuring a regular meiotic segregation have led to a new mechanistic model that is based in the observations that telomere convergence and the oscillatory nuclear movements are needed for recombination in *S. pombe* (Yamamoto and Hiraoka, 2001) (Fig. 5).

The oscillatory movements seem to be directed by the SPB that is attached to the thinned pole of the prophase nucleus, and the whole nucleus seems to be pulled from this pole, and it gets a “horsetail” shape (Chikashige *et al.*, 1994) (Fig. 5). If these nuclear movements are inhibited, crossovers are drastically reduced in number and the nuclear shape remains round, as when antimicrotubular agents are added to the culture medium or when the heavy chain of dynein is abolished (Yamamoto and Hiraoka, 2001). These movements also

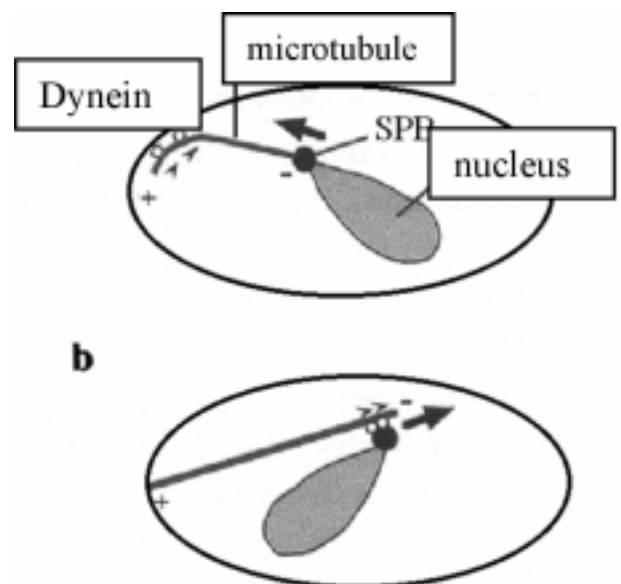


FIGURE 5. The oscillatory movements of the prophase nucleus of *S. pombe*. The two sequential states of the nucleus show the opposite directions (arrows) of the moving nucleus. The movements are led by the SPB, which interacts with cytoplasmic microtubuli (redrawn from Yamamoto and Hiraoka, 2001).

depend on the presence of a normal SPB (equivalent to a ciliary basal body), besides cytoplasmic microtubules (with a negative end adjacent to the SPB) and functional dynein in the cytoplasm.

A second and essential factor for crossover preservation is the relocation of telomeres (Fig. 6). During mitosis and in interphase telomeres are attached to the inner side of the nuclear envelope, but far away from the SPB (Fig. 6).

At the beginning of the sexual process the telomeres migrate towards the SPB and are kept adjacent to it during meiotic prophase. This convergence of telomeres towards the SPB is induced by the pheromones that activate conjugation, although the telomeric migration can be experimentally dissociated from conjugation (Yamamoto and Hiraoka, 2001). The telomeric protein Taz1 is necessary for telomere migration. Both mutants

devoid of Taz1 and mutants *kms1* (that cannot form a normal SPB) have a greatly diminished recombination rate of the normal type (between homologues) but they also show enhanced levels of *ectopic* recombination (non homologous) (Yamamoto and Hiraoka, 2001).

From these observations it is concluded that the efficiency of pairing between homologues, or homologous regions, is dependent on both the telomere relocation and the oscillatory nuclear movements. Thus, the hypothetical mechanism is that the grouping together of telomeres results in a gross level of alignment between homologous chromosomes, and then the repeated oscillatory movements provide an increasing probability that homologous DNA segments can get together and interact to begin the recombination processes. In fact, it is easy to imagine that two threads bound by their four ends to the same point, and subjected to alternating pullings from this region (the one having the SPB) will increase progressively their coming into an almost full register of their homologous regions.

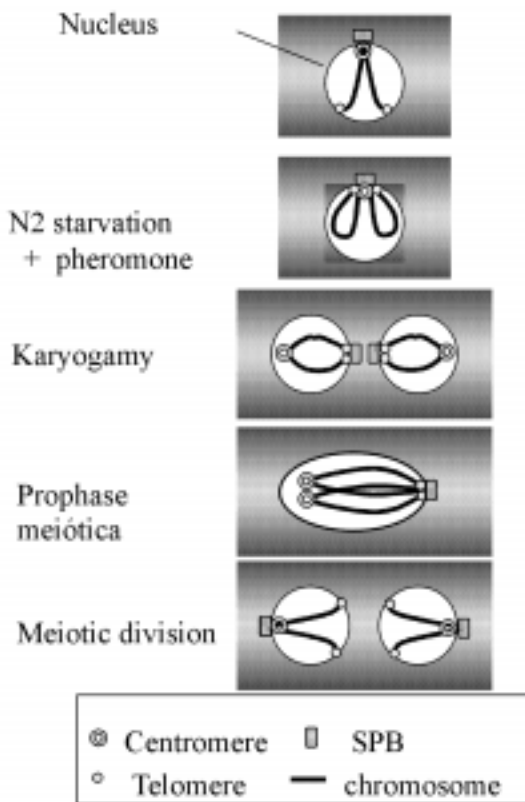


FIGURE 6. Schematic sequence of the changes in location of telomeres and centromeres from interphase to the completion of meiosis in *S. pombe*. From the beginning of conjugation and during meiotic prophase the telomeres move towards the SPB. (re-drawn from Yamamoto and Hiraoka, 2001).

8. The primitive function of cohesin Rec8 in meiotic segregation

The protein Rec8 belongs to the already cited group called SMCPs, and it is a variant homologue (paralogue) to the protein Scc1, which is a subunit of the *mitotic cohesin* that keeps together the sister chromatids up to their separation at mitotic anaphase. Despite this homology, Rec8 is specific of meiotic cells, and it was originally identified in mutants of *S. pombe* showing reduced recombination (Ponticelli and Smith, 1989). Furthermore, Rec8 is necessary for homologue pairing and for sister chromatid cohesion during meiosis (Cobbe and Heck, 2000). As a member of the SMCP group, that is present in Archaea, Bacteria and Eukarya -although much more abundant in the latter- it is remarkable that Rec8 has no homologue among prokaryotes -and even there is no homologue of the mitotic Sccp1- (Jones and Sgouros, 2001). However, among eukaryotes Rec8 has homologues and is conserved from *S. pombe* to the human species (Parisi *et al.*, 1999). The protein Rec8 also belongs to the "family" Rad21, which is formed by mitotic cohesins and presumably also contribute to the repair of DSBs of DNA. However, Rad 21 is independent from Rec8, and the latter only appears in the premeiotic stage of meiocytes. The function of Rec8 has been extensively studied in *S. pombe* (Watanabe and Nurse, 1999). The Rec8 gene has 5 exons coding a protein of 561 aa, and this protein becomes phosphorylated in advanced stages of meiotic prophase.

The human Rec8 analog (Rad21) has 631 aa and the N terminus is 57% homologous to the Rec8 protein from *S. pombe* (Watanabe and Nurse, 1999). In *S. pombe*, Rec8 has the following features:

1. It is necessary for the appearance of the "linear elements" of meiotic prophase
2. It is necessary for the maintenance of normal levels of meiotic recombination
3. It is localized in the chromatin, especially in the centromeric one, in "horsetail" nuclei
4. It is required for normal segregation of homologues during metaphase I
5. It is required for the maintenance of the usual number of DSBs at early meiotic prophase
6. It is preserved at the centromeres at metaphase I, and is completely absent at metaphase II.

According to these observations, it has been assumed that Rec8 organizes a rudiment of a discontinuous, chromosomal axis that keeps the interchromatid cohesion -especially at the centromeres- and that recruits other proteins needed for DSB formation and recombination. In the budding yeast the functions of Rec8 are similar (Klein *et al.*, 1999): it is localized in the lateral elements of SCs (and precedes their formation), and it is necessary for sister chromatid cohesion and for a normal recombination rate during meiosis. Therefore, it is assumed that the "protoaxes" (rudimentary axes) made by Rec8 recruit other proteins, including those that form the elements of the SC, and this view is supported by the fact that Rec8 null mutants do not form SC in *S. cerevisiae* (Klein *et al.* 1999). Furthermore, the absence of Rec8 results in the change of segregation into equational division at Metaphase I in *S. pombe* (Davis and Smith, 2001).

Can these assumptions be extended to metazoan eukaryotes? There are a number of facts supporting this generalization. The functions of Rec8 seem to be con-

served, in some taxa replaced by related proteins as STAG3. In the nematode *C. elegans* the Rec8 homolog protein has very similar functions and it is needed for chromosome pairing and normal segregation (Pasierbek *et al.*, 2001). Among mammals, the Rec8-related STAG3 has identical functions (Prieto *et al.*, 2001). In summary, it can be concluded that this type of "meiotic cohesins" is primitive and conserved, and that during the evolution of meiosis these cohesins have been co-opted by the more evolved taxa that have SCs.

9. The function of the archaeal topoisomerase homolog SPO11 in recombination initiation

The title of a recent paper by M. Lichten (2001): "Meiotic recombination: Breaking the genome to save it is illustrative". In fact, the central feature of meiosis is recombination, which essentially is the breakage of maternal and paternal chromosomes in order to reconstruct new chromosomes with fragments of these two sets, that will form the gametic genomes that will contribute each half of the genome for the next generation. For years the possible existence of enzymes able to break the DNA molecules at meiosis was suspected. DNA molecules are structured in such a way that they are very easy to be cut and resealed, as the restriction endonucleases and ligases do in experimental gene recombination (see review in Solari, 1999). In 1997 the action of a very primitive enzyme, SPO11, was demonstrated in meiotic cells (Keeney *et al.*, 1997). This enzyme bears a significant similarity to one subunit A of the topoisomerase VI of the archaean (prokaryote) *Sulfolobus shibatae*, a hyperthermophilic archeobacterium (Bergerat *et al.*, 1997). This subunit is a dimer able to cut one molecule of DNA while the enzymatic complex moves another DNA molecule through the gap between the two fragments (Fig. 7).

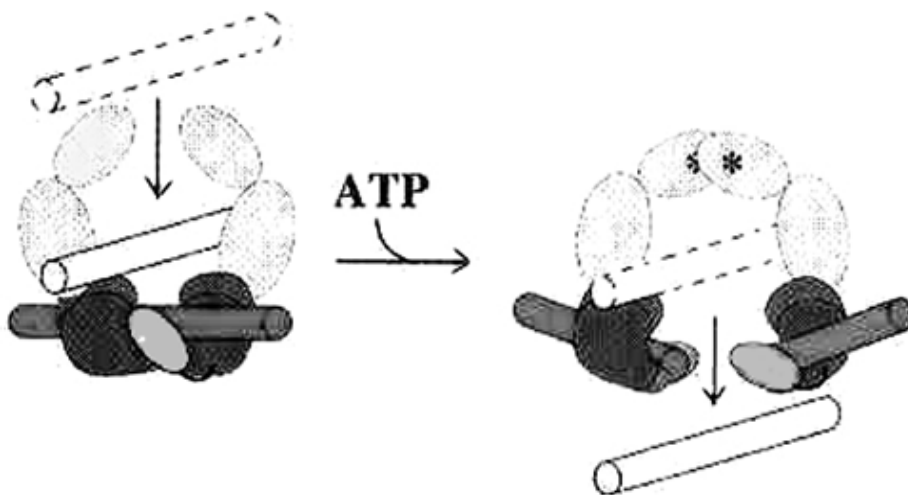


FIGURE 7. Possible mechanism of the A subunit (shaded) of the Topo VI in the archaean *Methanococcus sp.* (redrawn from Nichols *et al.*, 1999). The shaded cylinder represents the DNA molecule that is broken, while the white cylinder is the moved DNA molecule.

Essentially, SPO11 is synthesized and begins to act at leptotene or zygotene in the budding yeast and in other eukaryotes, resulting in a number of double-strand breaks (DSBs) in DNA. In the breakage sites there are protein complexes, as SPO11 acts in conjunction with the products of other 8 genes (Keeney *et al.*, 1997). The 5' ends of the broken chains are initially bound to a tyrosil residue of the enzyme, which is then withdrawn and allows a chain resection in the 5' to 3' direction. These chains are then joined to protein complexes containing Rad51 and DMC1 (as already cited, these are eukaryotic homologs of RecA). Thus, antibodies against Rad51 and DMC1 show many "foci" (fluorescent signals) inside leptotene and zygotene nuclei, which diminish in number and become more restricted in location as meiotic prophase advances.

The action of SPO11 triggers sequential steps of meiotic recombination, and it can be mimicked by ionizing radiation, which results in random DSBs. However, the action of SPO11 seems to be selective for some DNA sequences containing a 50 bp segment with a center rich in polyA (Blumental-Perry *et al.*, 2000). Only an absolute minor number of the original "foci" can finally develop into a crossover, through a series of steps in which Rad51 takes an active part.

While in many eukaryotes SPO11 is necessary for chromosome pairing, in some other SPO11 is not required either for synapsis or for SC formation, as in the nematode *C. elegans*. Among mammals, the action of

SPO11 seems to be as essential as in lower eukaryotes (Romanienko and Camerini-Otero, 2000). Thus, it can be concluded that the functions of SPO11 are archaic in origin, but its use among eukaryotes has become diversified among taxa.

10. The adaptive advantages of modern meiosis: functions of the SC

Almost all living eukaryotes have SCs. In mammals, these complexes allow for a considerable time (days or weeks) of tight and generally specific pairing of maternal and paternal chromosomes. Meiotic recombination is highly regulated, and the number of crossovers is several orders of magnitude less than in the budding yeast (in Man it is about 1 cM per Mb DNA). Although there are very little comparative studies among protists and mammals, the frequencies of illegitimate (ectopic) recombination should be lower than in protists.

However, the major feature distinguishing meiosis with SCs from primitive meiosis as in *S. pombe*, is the presence of **chiasmatic interference** in the former. This means the inhibition of another recombinational event in the immediacy of a formerly occurring one. Interference keeps this inhibition for a threshold distance, beyond which it is relaxed and further crossovers may occur. This feature is visible in all eukaryotic organisms bearing SCs, and it makes a striking difference with the random distribution of crossovers as in *S. pombe* (Fig. 8).

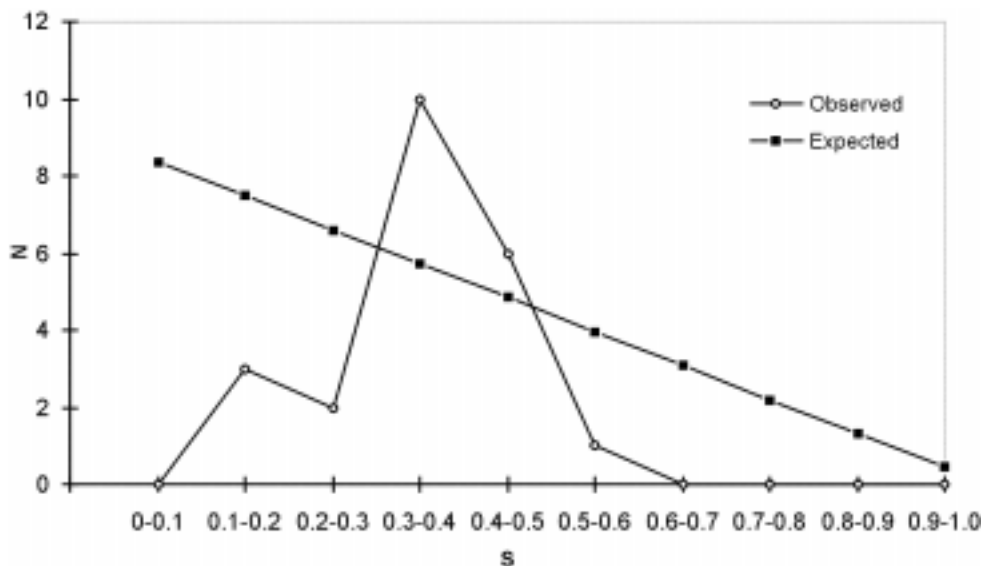


FIGURE 8. Graph of chiasmatic distribution (recombination nodules) in one avian chromosomal pair (segmented line) and a random distribution in a theoretical case without interference (crosses). N: number of chiasmata pairs. S: separation (in $\mu/10$) between each pair (Modified from Pigozzi and Solari, 1999).

The result of chiasmatic interference is that the presence of SCs allows the optimization of recombination (Gorlov and Gorlova, 2001), even though a formal proof is lacking.

Other results from the presence of SCs are the possibilities of “proofreading” errors between homologues and the possibility of introducing gene “imprinting”, but they will not be dealt with here.

11. Speculations on the origin of meiosis: the need for linear chromosomes, telomeres and distributive motor apparatuses

The vast majority of prokaryotes have main circular genomes (“chromosomes”) (Drlica and Bendich, 2000). The exceptional prokaryotes with main linear genomes are not phylogenetically near each other, for instance *Borrelia burgdorferi*, *Streptomyces sp* and *Agrobacterium tumefaciens*, and this suggests that they are isolated occurrences among bacteria. Furthermore, these cases differ from each other in the structure of their chromosomal ends: while *Borrelia* has ends with 26 bp inverted repeats –which are similar to those of vaccinia virus–, *Streptomyces sp.* use a terminal protein covalently linked to the 5′ ends (Ishikawa and Naito,

1999). Thus, these exceptional prokaryotes with linear genomes do not have true telomeres, while all studied eukaryotes have conserved telomeric structures. This fact has suggested that this difference underlies the upsurge of a new eukaryotic function: the possibility of a regular meiosis with recombination (Ishikawa and Naito, 1999). Indeed, the presence of circular (closed) genomes poses a number of problems for meiosis. If two circular homologous chromosomes undergo *one* or any other *uneven* number of crossovers, they will produce dicentric chromosomes, which should break up at meiotic division (Fig. 9). If the circular chromosomes do not undergo any crossover, they should segregate at random. In both cases, many of the meiotic products should be inviable.

Furthermore, the circularity of chromosomes raises difficulties for their pairing with homologous regions in register, and the absence of telomeres impedes a general convergence of chromosomal ends, that is, the establishment of a “bouquet”.

It is then reasonable to conclude that the existence of linear chromosomes with differentiated telomeres is previous to the evolution of meiosis. Furthermore, the telomere complexes in eukaryotes, with their telomerases –related to reverse transcriptases– and their RNA primers, might be very ancient from an evolutionary viewpoint: in fact they have reminiscences of the “RNA world” previous to DNA. It may be possible that linear chromosomes with telomeres is an ancient feature present in living eukaryotes and that intermediate forms are no longer existent. Even the more primitive protists, those assumedly asexual as South-American trypanosomes, do have functional telomeres similar to that of other eukaryotes, with TTAGGG or variant repeats.

Another universal feature of eukaryotes is the association of telomeres with the inner side of the nuclear envelope and their laminae. This association is needed for the telomeric convergence movement at the beginning of meiosis. The evolutionary origin of the nuclear envelope is unknown: there is apparently a single case of a prokaryotic nucleoid surrounded by a membrane, that of the bacterium *Gemmata obscuriglobus* (Fuerst and Webb, 1991). However, this membrane seems to differ from the nuclear envelope: there are no nuclear pores and the nucleoid does not show a chromatin structure.

Finally, the appearance and evolution of the microtubular cytoskeleton in eukaryotes remains unknown, despite the fact that this cytoskeleton is necessary both for mitosis and meiosis. The suggestions made on the possible symbiotic origin of eukaryotic features through

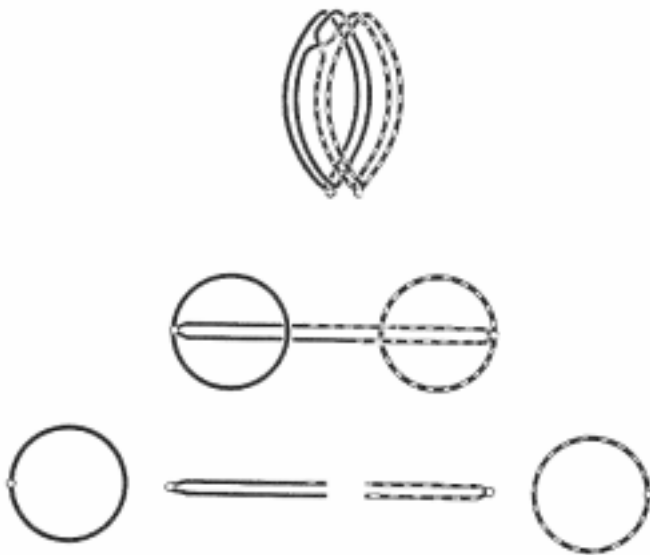


FIGURE 9. Effects of a hypothetical meiosis with circular chromosomes. The product of a single crossover (or an uneven number of crossovers) is dicentric, and it is stretched and finally breaks up at anaphase, and it is generally inviable.

the fusion of one archaean and one *Spirillum* (eubacterium) (Margulis, 1996) are only a possible beginning in this search. As noted before, Eukarya share several genomic features with Archaea, but symbiotic, primitive forms may have disappeared long time ago (Philippe *et al.*, 2000).

As a general conclusion, it may be suggested that the analysis of the origin of meiosis will probably require an extended study of many primitive protists, from

several viewpoints: structural, functional and the systematic analysis of their genomes and proteomes.

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