Ancestral eukaryotes reproduced asexually, facilitated by polyploidy: A hypothesis

Hypothesis: Insights & Perspectives

Asexual reproduction, facilitated by polyploidy, was the ancestral eukaryote condition Ancestral eukaryotes reproduced asexually, facilitated by polyploidy: A hypothesis

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Abstract

The notion that eukaryotes are ancestrally sexual has been gaining attention. This idea comes in part from the discovery of sets of "meiosis-specific genes" in the genomes of protists. The existence of these genes has persuaded many that these organisms may be engaging in sex, even though this has gone undetected. The involvement of sex in protists is supported by the view that asexual reproduction results in the accumulation of mutations that would inevitably result in the decline and extinction of such lineages. I have argued that this phenomenon can be obviated by polyploidy and here I argue that the "meiosis-specific genes" are used in other processes, including polyploidy control and homologous recombination, independent of meiosis. These phenomena account for our finding that these genes are expressed in cultures devoid of apparent cell fusion events. Hence, I also propose that asexual, and not sexual, reproduction is the ancestral condition.

1. Introduction

The last eukaryotic common ancestor (LECA), is speculated to have been an amoeboid phagocyte [1] and is in addition supposed already to have acquired the other core eukaryote-specific features including a distinct nucleus, an internal membrane system, mitochondria, chromosomes with telomeres, a cytoskeleton, sterol synthesis and meiotic sex [2] [3, 4]. The order and timing of these acquisitions is of course not at all certain and how, and even whether, meiotic sex is ancestral continues to be the subject of much debate. This is despite the contention that "That the LECA was sexual is no longer a matter of speculation/debate as evidence of sex, and of genes exclusively involved in meiosis, has been found in all of the major eukaryotic radiations" [5]. Many extant protists have been assumed to reproduce asexually. However, it has been pointed out that asexual animals are rare and evolutionarily short-lived [6], and the idea that an organism could reproduce in a persistently asexually manner has been questioned on strong theoretical grounds. It is argued that without recombination afforded by sex, mutations would accumulate in the lineage until it was no longer fit, and it would face extinction [7]. This idea became known as "Muller's ratchet" [8], and indeed, evidence for this phenomenon has been reported in bacterial populations [9] and in viruses [10]. Similarly, according to a hypothesis known as Kondrashov's hatchet [11] sex is essential if the rate of deleterious mutations exceeds one per genome per generation, so asexuals should rapidly become extinct. Many microorganisms seemed to have evolved different strategies to reduce their mutation rate so to avoid mutational meltdown. *Paramecium tetraurelia* is famed for its extremely low mutation rate, which is accomplished in part by replacing the active macronuclear genome with the replicatively silent micronucleus about every 75 generations [12], but recently the fairy-ring fungus *Marasmius oreades* was found to have a mutation rate

an order of magnitude lower than this ciliate, at 3.79 x10^{-12} per generation [13]. These very low mutation rates mean that these organisms escape or are less prone to Muller's ratchet, but also that they evolve at a very low rate. Could it be that in a stable environment, organisms such as fungi covering large areas are not under selective pressure to evolve rapidly?

Parasites, however, are in a perpetual evolutionary race with their hosts, and according to the Red Queen hypothesis, must evolve constantly. Indeed, it is noticeable that parasitic protists (*Leishmania*, *Trypanosoma* and perhaps *Giardia*) tend to be sexual. Many have argued that sexual reproduction is essential for organisms to compete, and that organisms that normally reproduce asexually must occasionally use sex to generate the recombination necessary to permit diversity and evolution. This has become known as "facultative sex", and there is recent evidence for its operation in the protistan parasite *Trypanosoma* [14, 15].

For a single-celled organism such as the LECA and contemporary protists such as amoebae, the necessity for sex incurs many inconveniences and complications [16]. If, as is commonly presumed, the driving force of a living organism is to pass on genes to the next generation, then sexual reproduction is immediately disadvantageous, because each partner is only able to pass on approximately half of its genome. Also finding a biologically compatible partner may be an impossible task, given the low density of life forms in the early days of LECA;even in modern soils, amoebae may well struggle to find a partner. Slime moulds including *Dictyostelium discoideum* overcome this challenge by secreting mating factors to gather conspecifics, and even this is risky because of cheats such as *Dictyostelium caveatum,* which uses the same system to lure amoebae for consumption [17]. A large advantage of asexual reproduction is that the individual can pass all of its genome to the next generation; however, it also means that all progeny are identical. This may be a disadvantage: for example, if a virus or bacterium is encountered that is able to infect the population, the entire population may be destroyed.

An article [18] was recently published in this journal with the provocative title "All eukaryotes are sexual, unless proven otherwise: Many so-called asexuals present meiotic machinery and might be able to have sex". Here I argue that this contention is based on the premise that "meiosis-specific genes" (MSGs) are expressed only during meiosis. I conclude that this is not true, and that MSGs are expressed in many situations outside meiosis. These are listed in table 1. Consequently, it is not safe to conclude that because an organism possesses MSGs, or even is proven to express them, that it is necessarily sexual. I also conclude that the ancestral condition was, after all, a simple asexual lifestyle, and I argue that, for many protists, an asexual lifestyle is sustainable, advantageous and even inevitable.

2. Nullification of Muller's ratchet and Kondrashov's hatchet by polyploidy

The large free-living amoebae, such as the *Amoeba proteus* group, have been studied for more than a century and a half, with no indication of sex, despite such close inspection [19]. There is a similar lack of data on sexual processes in other well-studied amoebae, including *Naegleria* [20] and *Acanthamoeba* [21]. That some protists exist with no or very little indication of a sexual or parasexual cycle poses the question of how, despite Muller's ratchet and Kondrashov's hatchet, this is possible. I have postulated that amoebae can replicate asexually without running into mutational crises by virtue of their being polyploid [16], and this argument has also been suggested in the case of plastid genomes [22]. According to this notion, mutations are minimised by reversion through homologous recombination (HR). Having multiple copies of the same chromosome allows these cells to reverse mutations by base-pair comparison of the mutant chromosome with the more numerous wild type copies. Evidence suggesting that HR reverses mutations has come from the study of plant plastids [22]. In the haloarchaeon *Haloferax volcanii*, the low mutation rate of the pyrE gene was also suggested to be due to the presence of polyploidy, which might enable repair of mutated chromosomes, making use of the presence

of wild-type copies [23]. The low intracellular genetic diversity is suggested to result from gene conversion. *Epulopiscium sp*. type B is an exceptionally large, highly polyploid bacterium [24]. It has been suggested that cyclic polyploidy may act to minimize the deleterious effects of asexuality [25], and in the *Amoeba proteus* group [19]. While others suggest that polyploidy dilutes the effect of deleterious mutations while permitting fixation of advantageous mutations [26], yet others have argued that polyploidy increases the mutation rate to an optimum [27]. On balance it seems most likely that polyploidy reduces mutations through correction by HR [16, 22]. This hypothesis may be tested in the case of *Acanthamoeba* by using the RNAi technique shown to be effective in this system [28]to knockdown the expression of genes suspected of inducing polyploidy (see below) and measuring the subsequent mutation rate.

3. Evidence for cryptic sexual processes in some protists

Some protistans that were previously thought to be asexual have turned out, on closer inspection, to undertake sexual or parasexual processes. Trypanosomes are reported to produce gametes [15] and *Leishmania* can even mate across species barriers, forming hybrids [29]. While the myxogastrid slime moulds have long been known to perform meiosis and gamete fusion, evidence for this in other members of the amoebozoa is reported in a testate amoeba [30] and in *Cochliopodium* [31]. Some have argued that sexuality is a feature of all protists and this has been lost in some lineages [32]. The fact that so many protists, so long viewed as being asexual, turn out to be facultatively sexual has naturally encouraged many to wonder whether sex is actually more widespread. The discovery of MSGs in the genomes of organisms previously held to be asexual [33] has persuaded many that most, if not all, protists are cryptically sexual. We can expect to see the list of organisms that are now held to be sexual solely on the basis of their

being discovered to possess MSGs to increase as genomes become sequenced. An additional confounding factor in the unambiguous determination of the mode of reproduction from an organism's genome is the fact that some organisms perform something very much like sex but without meiosis. The human pathogenic fungus *Candida albicans* can reproduce in a parasexual manner without meiosis by the fusion of compatible haploid cells to form a diploid, which then undergoes recombination and chromosome loss until the haploid state is approximately reached [34].

4. The "Meiosis Toolkit" is a false concept: MSGs are mostly HR genes that also operate in mitosis and in other processes.

The notion of a "meiosis toolkit" that could be utilized to reveal under-cover sex "scandals" [35] has gained popularity since it was proposed for *Giardia* [33], not least because evidence for sex in *Giardia* was discovered soon after [36]. The idea was to create a list of MSGs and to search for them in genomes to infer the presence of meiosis and so a sexual reproductive life cycle. Here I point out that MSGs are not specific to meiosis, and so their presence cannot be taken as evidence of meiosis. Instances of MSGs being expressed in situations other than meiosis are tabulated in Table 1. For most MSGs there is clear evidence for their also being involved in other processes, most notably mitosis, in which they carry out similar roles. These roles are mainly in HR or in support of HR, a process used in many other cellular activities such as telomere maintenance and DNA repair (Table 1).

We have shown [21] that although we could find homologs of the core meiosis-specific genes in the *Acanthamoeba* genome, these genes are expressed to various extents in cultured *Acanthamoeba*, showing no cell fusion or other events that would support the existence of

meiosis. These *Acanthamoeba* MSGs are also suggested to have functions distinct from meiosis.

HR occurs during meiosis [37], and the genes expressed there now constitute the MSG set that has become for some the hallmark for sexual reproduction; however, HR also takes place in mitotic vertebrate cells, especially during S and G2 phases [38], where it is important in repairing damaged DNA and in gene editing through CRISPR-Cas9 [37]. HR in mitosis is essential for the maintenance of genomic stability, and it suppresses tumorigenesis in metazoans [39]. There are differences in HR between meiosis and mitosis: in meiosis it occurs between homologous chromosomes [40], but in mitosis it is restricted to sister chromatids [41].

5. HR is found in diverse cellular processes and so are MSGs

HR is common across biology from bacteria, in which it mediates double stranded break repair, to sophisticated recombination events in immunological disguise. HR is perhaps best known in the context of meiosis, but many of the same MSG genes are also involved in HR during mitosis (Table 1). The maintenance of telomeres involves HR, and again some of the MSGs are known to be expressed here too. A similar set of genes also performs DNA repair in somatic vertebrate cells [42]. Additionally, it is evident that some of the genes involved in meiosis also have roles in DNA replication stress relief (DRSR) pathways, promoting the stability of replication forks [37]. In *Entamoeba* HR occurs during stress and encystation [43], processes that also involve MSGs [44]. However, it is at the cyst-forming stage in the life cycle of some sexual protists that evidence for meiotic processes is also found. The bloodstream stage of *Trypanosoma brucei* uses HR to generate antigenic variation in its Variant Surface Glycoprotein (VSG) genes, which affords it a protective barrier against the host immune system [45]. The bacterium *Helicobacter pylori* uses HR to avoid the human immune response by antigenic variation [46]. The widespread occurrence of HR involves many of the same genes that operate in meiosis, so

they cannot be said to be meiosis-specific and their existence in the genomes of organisms cannot be used as proof of the existence of meiosis.

6. Some MSGs encode structural proteins used in the Synaptonemal Complex.

Almost all eukaryotes capable of meiosis form some sort of synaptonemal complex (SC) [47], a protein-rich structure, often visible by electron microscopy, that stabilizes the connection between the pairing chromosomes in a zipper-like fashion. Perhaps the genes encoding SC or analogues would offer a better marker for sex, because organisms across many protistan phylogenies are known to produce morphologically identifiable SCs [48]. There is, however, a significant problem, since many of these genes are not highly conserved, and there are great differences in the structures of SCs throughout the eukaryotes [49]. For example, the fission yeast *Schizosaccharomyces pombe* possesses distinctly different structures known as "Linear Elements" (LinEs), which perform the function of SC found in the budding yeast *Saccharomyces cerevisiae* [49].

A further difficulty is that some genes that are involved in the production of stable SCs in sexual cells at meiosis may also perform duties unconnected with meiosis but rather in similar situations during strand stabilization during HR, while others are less ambiguously known and have specified roles in SC formation and function. Within the amoebozoa, well-developed SCs are known to occur in the myxomycete *Didymium iridis* [50]; however, in that study no gene was identified as being an SC component in the stage-specific transcriptome, whereas the MSGs were [51]. Sexual conjugation is well known in the ciliate *Tetrahymena,* and the MSGs are characterised. By contrast, no SC homologs could be found, and there is an apparent lack of morphological SCs [52]. It is suggested that *Tetrahymena* (and other ciliates) rely on a Mus81-dependent class II crossover pathway rather than on the more usual class I crossover associated with SC.

7. Sex is advantageous for metazoans and for protistan parasites, but not for the LECA and not for free-living protists.

Protists are reproductively unique, since each protist is a germ cell. This makes the choice between reproductive methods very different from that of metazoans. There is likely to be conflict between the immediate benefit of passing on all genes by the individuals during asexual reproduction versus the long-term benefit to the lineage afforded by sexual reproduction. An obligate parasitic lifestyle facilitates the union of conspecific parasites because the host provides both nutrition and a meeting place. It is noticeable that the protists for which there is good evidence for sex tend to be parasites.

The 'red queen hypothesis' suggests that sexual reproduction will be necessary to outpace parasitism [53], and so non-obligate parasites such as free-living amoebae may not be obligatorily sexual. However, it has often been pointed out that *all* organisms are subject to parasitism, and free-living amoebae are no exception: even if they are not under selection pressure for novel genetic variation to counter their ever-changing host populations, they still need to counter the presumably ever-changing intracellular challenge from bacteria and viruses.

Like many aspects of sex, it could be argued that the early eukaryotes were not ready for it, and sexual reproduction was only beneficial to multicellular organisms, which could make the best use of it. One difficulty in the delivery of benefits of sexual reproduction in early eukaryotes is the age-old problem of meeting a suitable mate. The populations of the first eukaryotes were likely to have existed in dilute marine suspension. These first cells would have been unlikely to find conspecifics with which to fuse/mate, so an asexual lifestyle may have been the only one feasible.

8. The search for true meiosis-specific genes

If we cannot use SC or LinE genes as unambiguous markers for meiosis in protists because they are not conserved enough, we should look elsewhere. Perhaps a more promising source of absolutely meiosis-specific genes may be those that regulate the process, but since these are kinases and phosphatases, it may be difficult to establish the identity of true homologs, especially in the distantly related protists, about which less is known. This difficulty is exemplified by the MEK1/Mre4 kinase gene, which is pivotal in regulating meiosis in budding yeast [54]; this kinase is structurally similar to myosin light chain kinase genes in sharing an N-terminal forkhead-associated domain followed by a CAM kinase family serine threonine kinase domain. Differentiation between these two kinase families is not practical, because of a lack of characterization.

Meiosis involves the fusion of haploid gametes and their nuclei, so a sexual protist would be expected to express proteins that facilitate the fusion of the membranes surrounding the cell and the nucleus HAP2/GCS1 and GEX1 [4], and indeed a paralogue of both exists in the *Acanthamoeba* genome, and is expressed in exponentially dividing *Acanthamoeba* [21]. Heterothallic strains of *Dictyostelium discoideum* have been found to express two HAP2/GCS1 homologs (HgrA and HgrB). While 2 out of 3 mating-types require both for sexual cell fusion, the third one does not [55]. However, the expression of neither gene strongly correlated with gamete formation in *Dictyostelium*. The *Drosophila* homolog is expressed during gametogenesis but here HAP2/GCS1 may have a role in acrosome function rather than directly in gamete fusion [56]. It is possible that the HAP2/GCS1 homolog in *Acanthamoeba* is required for the fusion of plasma membranes within an individual amoeba during events such macrophagocytosis.

The presence of nucleus fusion protein GEX1 has been assumed to be a marker for meiosis [4]. However, nuclear fusion has been observed in living *Balamuthia,* a large relative of

Acanthamoeba [57], and *Acanthamoeba* are frequently multinucleated [58]; this, together with the possibility that GEX1 is involved in both nuclear fission and fusion, makes GEX1 an unlikely specific marker gene for meiosis.

9. MSGs regulate ploidy levels

Several observations implicate MSG genes in the regulation of ploidy levels (Figure 1). Activation of MSGs was associated with depolyploidization in human tumour cells [59], and the forced expression of MSGs in the pathogenic fungus *Cryptococcus neoformans* by transfection with a promotor of meiosis, resulted in ploidy reduction [60]. Two MSGs, DMC1 and REC8/RAD21, are known to be expressed throughout the *Cryptococcus* disease progression in mice. In vegetative tissue of the cotton plant (*Gossypium hirsutum*) a SPO11 isoform (*GhSPO11-3*) is expressed, the silencing of which results in endoreduplication failure and ploidy reduction [61]. In the soil amoeba *Acanthamoeba*, we have found that MSGs including SPO11 and REC8/RAD21 are constitutively expressed in culture with no indication of meiosis [21]. This is in agreement with the polyploid nature of this amoeba and suggests that the purpose of MSGs in *Acanthamoeba* may be to regulate the observed polyploid cycles and to maintain homologous recombination. This hypothesis could be tested by using RNAi to knockdown the expression of these MSG and measuring subsequent ploidy levels.

10. As you were: Meiosis evolved from mitosis incorporating HR mechanisms

Before the current movement to suggest that all eukaryotes are sexual it was widely held that meiosis evolved from mitosis [1, 62], and this from-simplicity-to-complexity argument is attractively simple. Meiosis is a very complicated phenomenon, and it is natural to assume that

the LECA would have been as simple a cell as possible. From an ancient amoeba's (LECAs) perspective sexual reproduction would come at a prohibitive cost [16]. In view of the finding that MSGs are not in fact meiosis-specific, we can assume that sex is not ubiquitous in the protists, and that many (or most) reproduce asexually. If sex is now recognized as not being ubiquitous, then it seems most probable that it also was not at the time of the LECA, and it follows therefore that asexuality is the ancestral condition.

11. Why this is important

Despite being one of biology's earliest topics of discussion, the purpose or advantage of sexual reproduction is still incompletely understood. There are many deadly diseases such as malaria, Chagas disease, amoebic dysentery and trypanosomiasis that are caused by protists. Some of the drugs that have been effective are now failing because of drug resistance, and so new drugs are urgently needed. This is a well-known challenge; however, as these cells, like ours, are eukaryotes, so drug targets that might usefully discriminate between host and parasite are ever harder to identify. Any difference between the human host and these troublesome protists in the crucial process of reproduction is therefore an attractive target [63]. The genes of the SC or equivalent may harbour such targets because they seem to be lineage-specific and variable. Similarly, genes involved in HR processes in asexual protistan parasites may also be different enough from their human counterparts to constitute targets for drug design.

12. Conclusions and outlook

The notion that sexual reproduction may be inferred by the possession of MSGs is wrong because these genes are not specific to meiosis. Most of these genes are involved in HR, a process shared with mitosis and which also operates in non-dividing cells during telomere

maintenance, in encystation of protists and in other processes. This being the case, it follows that the ancestral state of eukaryotes is asexual, as was presumed by many until the 'toolkit' hypothesis appeared. The statement of Goodenough & Heitman "That the LECA was sexual is no longer a matter of speculation/debate as evidence of sex, and of genes exclusively involved in meiosis, has been found in all of the major eukaryotic radiations" [5] is incorrect, because the genes assumed to be meiosis-specific are not, in fact, specific to meiosis. Similar statements made by others on the same basis are also incorrect, for the same reason [4, 18, 25, 64] amongst others. While there are many amoebozoans (mainly mycetozoa) for which there is convincing evidence for meiosis and sex, there are others (such as *Acanthamoeba*) for which there is no evidence, except the possession of the misnamed MSGs. Absence of evidence does not constitute evidence of absence, but *Acanthamoeba* does not appear to possess synaptonemal complex genes, nor is there any report of synaptonemal complex formation or cell fusion in this well-studied amoeba. We have studied hours of time lapse video microscopy searching for the fusion events that are to be expected of a population undergoing cryptic sex, yet we have found no evidence of this, despite demonstrating expression of the 'MSGs' in amoebae from the same cultures [21]. Organisms falsely accused of sex 'scandals' on the basis of having the equipment [35] should now be exonerated! Finally, perhaps it is time to rename 'Meiosis-Specific Genes' to 'Meiosis-Associated Genes'.

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Conflict of Interest

The author declares no conflict of interest

Keywords

Meiosis, mitosis, Meiosis specific genes, Homologous recombination, Synaptonemal complex

References

Box 1 Glossary

1. Depolyploidization: The reduction of the polyploid genome down to the initial level (haploid or diploid). This usually occurs at mitosis through the reduction of the number of chromosomes. The term may also be used to describe the process whereby a polyploid (usually a plant) species becomes a non-polyploid species.

2. Endoreplication: Has been defined as “any type of cell cycle leading to endopolyploidy” [65]. Endoreplication can occur as continuous S and G phases or as starting but not completing mitosis. This is known as endomitosis.

3. Homologous Recombination: The recombination of DNA in which nucleotides or contiguous groups of nucleotides are exchanged between two similar DNA strands.

4. Parasexual Reproduction: Nonsexual mechanisms of reproduction which produce recombination without the involvement of meiosis or gametes development.

5. Polyploidy: Normally the prefix “poly” means more than one, but in this instance, it makes sense to take the word as meaning more than diploid, or more than twice the haploid state. Polyploidy is therefore having more than two copies of the genome in the same cell. Polyploidy may result from the replication of the genome without cell division.

6. LECA, The Last Eukaryotic Common Ancestor: A theoretical population of cells that constituted the ancestral state that gave rise to all extant eukaryotes.

7. Meiosis-specific genes (MSGs): A group of genes that are proposed to be expressed only in meiosis and that can therefore be used as a hallmark for the process of meiosis wherever their expression is discovered. (In this article I contend that this is a false premise.)

8. Red Queen hypothesis: In evolutionary biology this hypothesis suggests that organisms must constantly adapt by evolution to survive against organisms (usually parasites) that are also constantly changing. The name derives from observation from the Red Queen character to Alice “Now, here, you see, it takes all the running you can do, to keep in the same place” in Lewis Carroll's Through the Looking-Glass.

9. Synaptonemal complex: This is a zipper-like protein complex that holds together homologous chromosomes during the process of meiosis. It was first identified by electron microscopy as a ladder-like structure organized as two lateral elements, which are attached to the homologous chromosomes in a series of loops.

Figure 1 Ploidy cycles in an amoeba (from supergroup amoebozoa). **A.** The trophozoite amoeba is polyploid (blue bars represent 1 genome copy). The amoeba may divide mitotically to produce two daughter cells (**B**), each with half the original genome copy number, but there may be additional DNA loss in the *Amoeba proteus* group [19]. When an amoeba (**A**) form a viable cyst (**D**) MSG are upregulated causing a decrease in ploidy in *Entamoeba* [44]. If after depolyploidization a nascent cyst receives a fatal mutation (red diagonal bar) then it is non-viable, thus removing this mutant gene from the population.

Gene / Protein	Function in meiosis	Function in mitosis	Other functions
HAP2/GCS1	Fusion of gametes [4, 66]	-	Possible acrosome activation [56]
GEX1	Nuclear fusion [4]	Nuclear fission?	-
SPO11	DSB initiating meiosis [67]	-	Expressed constitutively in vegetative & reproductive tissue in cotton where it controls ploidy [61]. Expressed in some somatic tissues in mice and humans [68].
MRE11	Exonuclease DSB end processing. [6, 69]	Exonuclease DSB end processing.[69]	Telomeric silencing [6]. HR in DRSR [37]
REC8/RAD21	Helps hold sister chromatids together [70]	Helps hold sister chromatids together [71]	Centrosome integrity [72]. Expressed in the fungal pathogen *Cryptococcus*

			neoformans during ploidy reduction [60]
HED1	Inhibits RAD51 prevents RAD54 associating with the presynaptic complex [73]	Down-regulates mitotic recombination machinery [74]	-
HOP1/MAD2/ Hormad1	HOP1. Homologous alignment. Forms stable complexes with linear duplex DNA[75]	MAD2. Mitotic checkpoint [76]	MAD2. Inhibits DNA damage repair systems [77]
PCH2/TRIP13	Meiotic crossover formation checkpoint [78]	Mitotic DNA damage checkpoint [79]	Regulation of nematode lifespan [80]
DMC1	HR. Strand invasion in homology search [81]	Expressed in mitotic rice cells. HR [82]Also in vertebrate HR [83]	Expressed in the fungal pathogen *Cryptococcus neoformans* during ploidy reduction [60]
MND1	HR [84]	DSB repair [85]	Combines with HOP2 and RAD51 in telomere maintenance [86]
HOP2	HR [84]	HR [85]	HR mediated telomere maintenance [86]
MEK1 kinase (RAD53)	Down-regulates Rad51 during yeast meiosis through HED1 [73] & Histone H3 [87]	-	-
MSH2	Mismatch repair [88]	Mismatch repair [89] [90]	-
MSH3	Mismatch repair, corrects insertion/deletion mispairs	Mismatch repair [89, 90]	-
MSH4	HR, Halliday junction resolution [42]	-	Expressed in many non-germ line cells [91] binds to the von Hippel-Lindau Tumour Suppressor-binding Protein 1
MSH5	HR, Halliday junction resolution [42]	Halliday junction resolution [92, 93]	Radiation-induced apoptosis [93]. DNA damage response [94]
MLH1	Mismatch repair. Resolution of COs [95]	Mismatch repair [96]	Mismatch repair in DNA repair [42]
MLH3	Mismatch repair. Resolution of COs [95]	Mismatch repair [96]	Mismatch repair in DNA repair [42]
MUS81	Resolution of meiotic COs associated with induced DSBs	Resolution of mitotic COs associated with induced DSBs [97]	Rescue of stalled replication forks and tumour suppression[98]. HR in DRSR [37]
RAD50	Creation of DSB and removal of SPO11 [6]	HR [6]	Telomeric silencing [6]

RAD51	Role in yeast [99]. HR in plants [100].	DSB repair [101]. DNA damage checkpoint [102]. HR in plants [100].	HR-mediated telomere maintenance [86]. VSG recombination in Trypanosomes [45]. Homologous DNA pairing in *Leishmania* [103]. HR in DRSR [37]
RAD52	DSB repair pathway through SSA of long stretches of homologous sequences flanking the DSB site. [104]	DSB repair pathway through SSA of long stretches of homologous sequences flanking the DSB site. [104]	DRSR [105]
RAD54	Works with RAD51 in HR [106]	Required for mitotic diploid-specific recombination and repair in yeast [107]	Enhances accessibility of DNA to other proteins and HR [108]. HR in DRSR [37]
RPA	Prevents premature association of RAD54 and HED1 with ssDNA [109]	Prevents premature association of RAD54 and HED1 with ssDNA [109]	ssDNA binding activity replaced by RAD51 in DNA repair [42]
PMS1	Mismatch repair [110]	Mismatch repair [89]	-

Table 1 "Meiosis-specific genes" are not specific to meiosis. The reported functions of the genes in meiosis, in mitosis and their other functions are tabulated. HR, homologous recombination. CO, crossover. SSA, single-strand annealing. DSB, double-stranded breaks. DRSR, DNA replicative stress relief.

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