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Meiosis and the Paradox of Sex in Nature

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1. Introduction

The paradox of sex is still enigmatic and regarded as a major unresolved problem in evolutionary biology [1]. Sex is here understood as a process of organisms in which the genomes of two nuclei are brought together in a common cytoplasm to produce progeny which may then contain reassorted portions of the parental genomes. In eukaryotes, sex involves meiosis-mixis-cycles and is tied to reproduction. The prevalence of sexual reproduction in eukaryotes is striking because of the obvious high costs of sex [2]: first, recombination during meiosis breaks up beneficial gene combinations; associated with these processes are the risks of errors and mismatches during pairing of homologous chromosomes, plus the time needed for meiosis. The second cost is mixis, which requires two parental individuals for conducting fertilization, merging of cells and genomes. A couple of secondary costs are associated to mixis: mate searching, mate finding, costs of sexual selection, competition for mating partners, and finally physical contacts. The alternative, asexual reproduction without meiosis-mixis cycles, potentially avoids these costs; [3,4]. Paradoxically, rather few eukaryotes conduct asexual reproduction: less than 1% of species of animals and seed plants and 10% of ferns reproduce via asexuality, in fungi about 20%; only in protists obligate asexuality occurs more regularly in many phyla [5]. And, when eukaryotes shift to asexuality, they do not abandon sex completely, but often just modify the sexual meiosis-mixis cycle in various ways [6-8].

Meiosis, in fact, is the key process of sexual reproduction in eukaryotes, as it is the only shared and conserved feature of sex in eukaryotes. Meiosis originated early in eukaryotes, perhaps together with mitosis [9]. The cost of sex, thus, primarily applies to eukaryotes. Prokaryotes have less complex chromosomes, no meiosis, and thus they do not have a comparable cost. Mixis and syngamy, the second part of sexual reproduction, vary a lot in different eukaryote groups. Basically, two parental individuals are needed to conduct mixis, karyogamy and syngamy. Many evolutionary biologists put a lot of emphasis on the cost of “males”, as male individuals do not produce offspring; asexual animals are thus expected to produce higher

quantities of offspring than sexual ones. In animals, where separate sexes are the rule (ie. male and female individuals), the cost of males is regarded as a major problem [3,4]. However, many groups of eukaryotes do not produce “males” – most land plants are hermaphrodites in the sense that they have male and female organs on the same individual, either within one flower as in angiosperms (hermaphroditism) or in different flower-like structures on the same individual as in most gymnosperms, or on the prothallium of many ferns (monoecy). In hermaphrodites, the cost of sex is significantly reduced to the cost of producing male organs [10]. Many protists and algae even reproduce via isogamy and do not develop gender differentiation. Many fungi have dozens of different mating types, that is, genetically different hyphens, which equals to dozens of “genders”. There is no general cost of males in eukaryotes, and the group-specific “cost of males” is a side aspect in the paradox of sex discussion.

Meiosis, in contrast, is the shared feature for eukaryotic sex. In fact, the problem of maintenance of sex can be largely referred to the question: “What is meiosis good for?” About 20 major hypotheses have been proposed to explain the paradox of sex, and three main theories attempt to explain the function of meiosis (see [11], for detailed review): 1) Meiotic sex is the mechanism for creating recombination and thus new gene combinations in the offspring, which would increase the evolutionary and adaptive potential of genetically variable offspring; 2) Meiosis is a restoration tool for promoting the integrity of nuclear DNA, by repairing DNA double strand breaks (DSB), by eliminating deleterious mutations, and by repair of epigenetic damage; 3) Meiosis is a phylogenetically conserved feature which cannot be eliminated because of the ancestral fixation of meiosis-mixis-cycles. None of these theories provide an all-inclusive answer for the paradox of sex [11-12]. These theories are not a priori exclusive, but may have combinational values.

Traditionally, sex has been seen as advantageous due to the effects of recombination during meiosis, a process which generates genetic variance in the offspring. Genetic variation can provide an adaptive benefit in changing environments [13-14]. Further, recombination exposes deleterious mutations to purifying selection. Selection against less fit mutants could help to purge deleterious mutations from the genome [15-16]. However, it was already recognized in the 1980s that these benefits do not sufficiently explain the high costs of obligate and regular sexual reproduction [17]. Selection can act against recombination, and recombination does not necessarily result in beneficial new gene combinations and traits [1]. Recombination is further an investment into an uncertain future. Many evolutionary biologists have pointed out that sex must be foresighted to make beneficial new gene combinations. But, evolution is blind and cannot have foresight. Individuals conducting sex gain no immediate advantage, and there is no benefit to the mating partners bearing all the costs of mate searching and recognition as well as the risks of sex. Some animals, such as many insects and fishes, even die directly after sexual reproduction. The selective forces to act on variable offspring do not provide benefits to the parents. That is, creating variation in the offspring is only a group advantage, but no individual advantage, which weakens the efficiency of selection for maintenance of sex [11].

These theoretical problems were largely recognized by evolutionary biologists already in the 1980s [17]. Several modifications have been proposed, but the key problem of recombination-based models remains: the benefit of recombination is context-dependent, but in many

eukaryotic organisms, sex is not at all context-dependent but obligatory. Recombination thus might be rather not the cause but a consequence of sex [12]. In this book chapter I will expand my recently proposed combinational theory for maintenance of sex [12] which suggests a combinational effect of DNA restoration mechanisms during meiosis as the major function of sex. DNA restoration is beneficial in any ecological context, and it provides a benefit for each offspring generation. However, this function of meiosis must be understood in a context of evolutionary history, eukaryotic metabolism and its inherent chemistry of life, in particular redox chemical reactions. First I will review some basic features of oxidative stress during aerobic respiration and photosynthesis, and the DNA damages caused by ROS. Second, I will review the current knowledge on evolution of meiosis as an homologous recombinational DNA repair mechanism. Third, I will discuss the evolution and function of meiosis proteins. Fourth, a hypothesis on the function of segregation and reductional division at meiosis will be discussed. Finally I will provide some suggestions for further studies to obtain more support for this hypothesis.

2. The origin of eukaryotic life, oxidative stress and DNA damage

Oxidative stress and DNA damage. Carol and Harris Bernstein [18-20] and coworkers were the first to propose a consistent hypothesis that crossing over at meiosis might have evolved as a repair mechanism of oxidative double strand DNA damage. Their ideas stemmed from observations that, in fact, meiosis is not at all optimized to create new gene combinations, as Holliday junctions can be resolved with and without cross-over. The frequencies of non-crossovers were shown to be higher than those of cross-overs, but only the latter create new gene combinations in flanking regions, which is most important for recombination. The idea emerged that meiosis could be a repair mechanism of DNA double strand breaks [18], for which a homologous chromosome is needed as template. Later, this idea was linked up to oxidative DNA damage [19-20]. However, since many other DNA repair mechanisms exist, and since permanent diploidy would also serve for DNA repair, the theory was not broadly accepted by evolutionary biologists [11]. It is useful to discuss this theory in view of evolutionary history, which was first attempted by Lynn Margulis [21].

The origin of the eukaryotic cell via endosymbiosis [21] is meanwhile a well-established theory; the inclusion of prokaryotic endosymbionts, which later became cell organelles, i.e. the mitochondria and, in plants, the plastids, were key innovations for eukaryotic metabolism as they allowed for aerobic respiration and photosynthesis, respectively. However, both aerobic respiration and photosynthesis are major sources of reactive oxygen species (ROS). Triplet oxygen, $^3\text{O}_2$, is not highly reactive but this is not the case for its radicals, which are formed by accidental one-electron transfers and its electronically excited state singlet oxygen [22], $^1\text{O}_2$. Especially the hydroxyl radical (OH^\bullet) and, to a lesser extent, the superoxide anion radical ($\text{O}_2^{\bullet-}$) cause many kinds of damages. By contrast, hydrogen peroxide (H_2O_2) is more stable, but also an oxidizing agent, and can be reduced to OH^\bullet in the Fenton reaction in the presence of transition metal catalysts, such as iron or copper:



Photosynthesis is an old mechanism, invented by cyanobacteria, and probably evolved between 3.8 and 3.5 billion years ago. The basic chemical machinery of photosynthesis likely evolved out of defense mechanisms against UV-induced photochemical radicals, in a time before the development of a protective ozone layer. Early cyanobacteria probably used hydrogen sulfide (H_2S) as an electron source for an anoxygenic photosynthesis, which is only driven by photosystem I (PSI). As H_2S resources were depleted, mutants arose which combined PSI with PSII activity, which provided sufficient energy to facilitate the utilization of water as an electron source [23-24]. Another hypothesis explains the evolution of photosynthesis from the presence of hydrogen peroxide, H_2O_2 , which was formed by UV irradiation (due to the lacking ozone layer); hydrogen peroxide can also serve as an electron source for photosynthesis but provides only two electrons per molecule [25]. Modern photosynthesis uses the energy of light to oxidize water (H_2O) as an electron source to generate chemical energy in the form of ATP; this energy is used to reduce carbon dioxide (CO_2) to sugars. The oxygenic photosynthesis, as we know it today, enabled organisms to produce organic compounds like sugars from atmospheric CO_2 much more efficiently. Oxygen was released into the atmosphere and hydrosphere as a gaseous waste product, simplified as:



The rise of oxygen concentrations in the atmosphere was initially slow, probably because oxygen was initially bound by metals, mainly iron, available in rocks and minerals. In the presence of oxygen, soluble ferrous iron (Fe^{2+}) is oxidized to insoluble rust (ferric iron, Fe^{3+}). Only between 2.2 and 2 billion years ago, the earth's atmosphere started to enrich in molecular oxygen released from the oxygenic photosynthesis of cyanobacteria; around 1.8 billion years ago the O_2 in the atmosphere reached 5-18% of the present level [26-27]. Around 1 billion years ago, the ozone layer formed from the release of oxygen in the atmosphere, protecting organisms from UV light. From this time onwards, the threats from UV irradiation for life have been reduced, but the ability to use oxygen and to cope with its high reactivity became a major evolutionary constraint for life on earth.

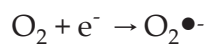
In modern green plants, photosynthesis during the light period is the major source of free oxygen radicals. Normally, the electron flow from the excited photosystem I is directed to NADP, which is reduced to NADPH. It then enters the Calvin cycle and reduces the final electron acceptor, CO_2 . Transfer of excitation energy from excited chlorophylls to oxygen in the light-harvesting complexes leads to the formation of $^1\text{O}_2$ and O_2^\bullet , which can be further converted to H_2O_2 and OH^\bullet . The production of ROS is enhanced by strong light and also by deceleration of the Calvin cycle [28]. In the dark period, most oxygen radicals are produced by mitochondria [29].

Aerobic respiration basically is an oxidative breakdown of organic molecules for gaining energy in the form of ATP equivalents, and, for this purpose, is a magnitude more efficient than anaerobic respiration. The reduction of oxygen provides the largest free energy release per electron transfer among all elements of the periodic system [27]. Anaerobic respiration uses sulfur, methane or hydrogen as electron acceptors. These sources were probably depleted

in the early history of life, and may have been only locally abundant (e.g. sulfur may be locally concentrated around volcanoes, fumaroles etc.). By contrast, aerobic respiration can use dispersed atmospheric oxygen as the final electron acceptor. Oxygen respiration may have evolved in facultatively aerobic / anaerobic organisms, such as alpha-proteobacteria [30]. This second major invention was a major precondition for the origins of eukaryotes, because it allowed for an improved energy gain from food and substantial increase of growth and body size [27]. However, the price for this metabolic innovation was coping with oxygen radicals in the vital organelles *inside* the cells.



This transfer of four electrons to oxygen is controlled by the mitochondrial electron-transport chain but accidentally one-electron transfer may occur:



The mitochondrial electron-transport chain is perhaps the most important source of reactive oxygen species in animal cells [22, 31]. $O_2^{\bullet-}$ is transformed into H_2O_2 by enzymes such as superoxide dismutase (SOD). H_2O_2 is relatively stable, but membrane-permeable and can diffuse into the cytosol. When H_2O_2 is not removed by the antioxidant defense systems, such as ascorbic acid, hydroxyl radicals (OH^{\bullet}) can be generated through the metal-catalyzed Fenton reaction (see above). Moreover, oxygen may not only generate oxygen radicals, but also reactive nitrogen species (reviewed by [22]).

The electron transfers during respiration and photosynthesis basically occur in the membranes of mitochondria and plastids, respectively. The more stable H_2O_2 can potentially penetrate into the nucleus. In summary, aerobic respiration and the presence of oxygen in the cell created a new and continuous source of free radicals adding a new major *endogenous* threat of DNA damage. In anoxic periods, DNA damage was mostly caused exogenously by photosensitization of light exposed tissues. An excited photo-reactive compound can excite molecular oxygen into singlet 1O_2 , which is much more easily reduced to $O_2^{\bullet-}$ than 3O_2 [32]. As previously described, superoxide anion radical can lead to the formation of hydroxyl radicals in the presence of catalytic transition metals. This new endogenous source of ROS, however, endangered the cell itself and is countered by an elaborate antioxidant defense system comprised of ascorbic acid, glutathione, and a complex enzyme machinery guaranteeing the regeneration of these reducing agents in attempts to maintain a redox homeostasis, which is vital for cell survival.

Oxygen radicals and DNA damage. Free radicals react readily with DNA, and the majority of oxidative damages occur in nuclear DNA [19]. Under the presence of oxygen, peroxyl radicals are formed by addition of molecular oxygen to DNA base or sugar radicals, which in turn may undergo complex reactions. For instance, thymine may react with OH^{\bullet} at the C5–C6 double bond to form thymine glycol [33]. Thymine glycol is known to be a quite frequent source of DNA damage and blocks DNA replication and transcription [19]. This is just one of the many reactions and products of free radicals with DNA, which have been reviewed comprehensively elsewhere [22,33,34]. Hydroxyl radicals can cause not only single, but tandem lesions of purine

bases [35]. An important feature of free radicals is that single initiation events have the potential to generate multiple reactions and multiple peroxide molecules by complex chain reactions [22]. Most importantly, free radicals may destroy the DNA 2-deoxyribose sugar. The highly reactive DNA sugar radicals may lead to the formation of altered sugars which may consequently lead to strand breaks at the sugar-phosphate backbone of DNA [33]. Free radicals are also involved in the formation of DNA-protein crosslinks, in which one DNA radical and one protein radical are involved [33].

As previously mentioned, various mechanisms have evolved in eukaryotes to deal with the toxicity of oxygen radicals. For instance, the superoxide dismutase enzymes (SODs) remove superoxide by catalyzing a redox reaction to form H_2O_2 and O_2 . Catalases are the most important H_2O_2 -removal systems in various eukaryotic organisms. Halliwell [22] gives a survey of the various antioxidant mechanisms in plants and animals; however, these systems are not 100% perfect. Moreover, reactive oxygen species may also have beneficial effects, such as defense mechanisms against pathogens, and regulation of cellular processes by influencing phosphorylation [22]. In plants, reactive oxygen species produced by photosynthesis may even be involved in regulation of gene expression and cell to cell signaling [36]. For these reasons, reactive oxygen species are never completely scavenged, but even may be produced on purpose [37]. When the balance of reactive oxygen species and antioxidants becomes shifted towards an excess of ROS, then increasing oxidative stress may lead to a damaging downward loop process, finally resulting in damage of DNA. Excessive DNA damage either halts the cell cycle to initiate repair, or ends in apoptosis [22].

With aerobic respiration and photosynthesis, eukaryotes were forced into the “oxygen paradox:” a highly efficient metabolism, but producing a continuous, internal source of damaging agents inside cells. Oxygen respiration was crucial for the evolution of high energy gain, and therefore complexity and multicellularity of eukaryotes. For its benefits, it is reasonable that oxygen respiration was maintained by selection in eukaryotes despite its detrimental side-effects. Because of the benefits of oxygen respiration, natural selection should rather favor improved repair mechanism of oxidative damages than abandoning oxygen respiration. The same principal may hold true for photosynthesis. Therefore, it is likely that enzymatic DNA repair mechanisms inherited from prokaryotes have been maintained and improved in eukaryotes. Eukaryotic cells have to repair DNA continuously and multiple DNA repair mechanism are known [34].

3. Homologous recombinational DNA repair of oxidative damage

Recombinational repair. It is well established that recombinational repair of DNA is the most efficient and accurate mechanism for repairing DNA double strand damages [38-40]. Many repair mechanisms deal with single strand-breaks that represent the most common type of DNA lesions. Single strand damages use the other strand as a template for repair. However, if a replication fork encounters a single strand break in its template, then a double-strand break might be the consequence [34]. For the evolution of meiosis, the repair mechanisms of double

strand breaks are of major interest. Double strand breaks can be repaired by non-homologous repair such as end-joining as well, but this mechanism is prone to error and a source of insertions, because ligation of free ends often leaves flaps of DNA strands [34,38,41]. Homologous recombinational repair, by contrast, requires a second, homologous, undamaged chromosome. This can be either a sister chromatid or a chromatid from another homologous chromosome.

The early eukaryotes were probably unicellular, haploid organisms. The increased internal oxidative damage introduced by cellular respiration (in plants, mainly by photosynthesis) in early eukaryotes required an elaborate mechanism for repair of double strand damage of the nuclear genome: this is only provided by homologous recombinational repair. Mitotic repair via the sister chromatid can cope with internal DNA damage as well, but this mechanism has two limitations: First, a sister chromatid is needed which is not available in the G1 and S phase of the cell cycle. DNA damage at these stages rather triggers regulated arrest mechanisms, so that replication is blocked [34]. That means that mitotic homologous recombinational repair is only available during growth, either in cell colonies or in tissues. DNA damage increases in postmitotic tissues of multicellular organisms [19], likely because homologous repair during mitosis is not available any more. Other repair mechanisms are mutagenic and lead to a slow but steady accumulation of mutations in somatic cells of multicellular organisms. The second limitation of mitotic repair is the need for resources, as each round of mitotic cell division requires DNA and protein synthesis to produce viable daughter cells. Hence, two rounds of mitosis with two synthesis phases are required to result in four daughter cells, while meiosis produces four daughter cells with just one synthesis phase. If resources are depleted, then organisms have problems to continue mitotic cell divisions because homologous recombinational repair with sister chromatids is too costly. If at the same time oxidative damage is accumulating, then the organism would have to conduct non-recombinational repair to avoid cell death. Non-recombinational repair, however, is prone to errors and mutational changes.

For a eukaryotic, unicellular organism, non-recombinational repair would not allow for continuity and integrity of the genome. Therefore, the early haploid protists had to find a way out of this dilemma: the most efficient way to survive oxidative stress under unfavorable growth conditions was to merge with another individual and to use the homologous chromosome set of the mating partner for DNA recombinational repair. Mixis provides a second, homologous chromosome – one that may also be damaged to some extent, but at sites different from damages in the other paired chromosome. This merging had a two-fold advantage: first, DNA double strand breaks can be efficiently repaired by homologous recombination with a second template; second, four daughter cells can be produced with only one synthesis phase. For a unicellular protist, mixis followed by meiosis was an efficient way to avoid cell death in unfavorable environments.

Meiosis and DNA repair. Initially, double strand breaks caused by oxidative damage could have been the primary trigger for the onset of meiosis. Carol Bernstein [20] summarized the main requirements for oxidative damage being a selective pressure for the maintenance of meiosis as a DNA repair mechanism in eukaryotes. To maintain meiosis by selection for DNA repair caused by oxidative damage, the following conditions must be fulfilled: (1) Oxidative damage

is deleterious to cell function; (2) oxidative damage accumulates in somatic cells; (3) oxidative damage should be repairable by recombinational repair during meiosis; (4) the mechanisms of recombination enzymes should be more adapted to DNA repair than to simple random genetic exchange. Current evidence supports all these predictions.

1. Oxidative damage is deleterious to cell function. Since oxygen radicals are highly reactive, they basically attack each organic molecule around, damaging membrane lipids, proteins, and DNA. The immediate consequences of DNA damage are transcription termination, interruption of replication, and reduced cell survival. Oxidative stress leads to release of transition metal ions which may catalyze free-radical reactions. Oxidative stress further increases levels of free Ca^{2+} , which may result in a permeability transition of mitochondria. It is established that excessive oxidative stress initiates cell death, i.e., apoptosis [22]. Dead cells may further release metal ions and other toxic compounds to the neighboring cells, increasing the damage.
2. Oxidative damage accumulates in somatic cells of multicellular organisms. Oxidative DNA damages in somatic cells are by far the most frequent ones in mammals. Around 130,000 endogenous damages of DNA occur per cell per day for rats, whereby 86,000 are due to oxidative damage [20]. Oxidative damage may accumulate in the following way: free radicals first cause mutations of mitochondrial (mt) DNA, which, in turn, affects structure and function of mt proteins. Defective mt-encoded proteins result consequently in defective electron transport, which increases frequencies of free radicals. Free radicals finally diffuse into the cytosol and cause damage in the whole cell, including nuclear damage. Oxidative damage occurs first in the DNA of mitochondria and chloroplasts, and later also affects nuclear DNA [42]. This downward loop process continuously increases effects of oxidative damage and is seen as a main cause of somatic degeneration and ageing in multicellular organisms. Somatic cells accumulate oxidative damage as their fate is anyway death. In the germline, oxidative stress may even be a major cause for the evolution of anisogamy and gender differentiation. In complex multicellular organisms, the female germline is usually kept in a stage with inactive pro-mitochondria to protect from oxidative damage of aerobic respiration; the mitochondria are therefore inherited maternally. ATP is provided by cells surrounding the oocytes. The male sperm cells, in contrast, are being produced continuously, with meiosis as a repair mechanism. The spermatocytes are motile in many eukaryotes, and their active mitochondria already suffer from oxidative damage as a consequence of motility; thus, only the nucleus is transmitted to the zygote, while the mitochondria of male gametes are usually not inherited [42]. This division-of labor principle guarantees undamaged mitochondria for the offspring. Maternal inheritance of plastids in plants may follow similar principles.
3. Oxidative damage is repairable by recombinational repair. If the original function of meiosis is to repair DNA damages, then oxidative damage must be repairable by homologous recombination. Most damages affect just single strands of DNA, and repair can be conducted by the complementary strand which stores redundant sequence information for re-synthesis. Double-strand breaks and DNA cross-links cannot be easily repaired this

way, and these damages require information from a second, undamaged homologous DNA molecule by recombinational repair [20].

If oxidative damage is a trigger for recombinational repair at meiosis, then meiosis should be inducible by oxidative stress. The idea is supported by experimental work of the Bernstein group on the yeast *Schizosaccharomyces pombe* [20]. Strains of this free living, haploid, facultatively sexual-asexual organism have been treated with H₂O₂ in an adequate nitrogen medium, and the ratios of spores to colony forming units have been measured. The ratio was 4-18fold higher than for cells that were not exposed to H₂O₂. Most strikingly, this increase of sporulation was also observed in a nitrogen-rich medium, which means that starvation is not a primary cause for sporulation. DNA damage induces meiosis even under favorable nutritious conditions, which means that (1) recombination is here not conducted to create new gene combinations in the offspring that may adapt better to the changed environmental situation; (2) for excessive DNA damage, mitotic repair is insufficient. These experiments demonstrate that oxidative stress can induce meiosis in this yeast. Fission yeast can serve as a model for a simple, unicellular, haplontic protist that forms a diploid zygote only under stress conditions.

In the facultative sexual / asexual green alga *Volvox carteri*, sex is a response to increased levels of stress [43-44]. In this species, heat stress causes the production of a 30kDA glycoprotein inducer (SI). This inducer stimulates the gonidia to produce egg- or sperm bearing sexual spheroids. The fusion of gametes results in the formation of a desiccation-resistant, overwintering zygospore, which germinates and undergoes meiosis when favourable conditions return in the next spring. In this organism, sexual development in the gonidia is triggered by an approximately two-fold increase of reactive oxygen species after heat stress, and it could be demonstrated that ROS actually activate two sex genes, the SI gene and the clone B gene. These genes must have been ROS activated, because catalases decreased their transcript level [43-44]. The formation of the zygospore is likely a response to increased oxidative stress, and meiosis is the mechanism of recombinational DNA repair before the next haploid generation is formed. Nedelcu et al. [43, 44] suggest that sex might be one alternative as a stress response besides cell-cycle arrest and apoptosis.

Further support for an evolution of meiosis as a response to DNA lesions is available from research on the protist *Tetrahymena* by the group of Josef Loidl [45]. *Tetrahymena* has a micronucleus capable of meiosis, and a "vegetative" macronucleus. Elongation of the micronucleus and formation of a so-called "crescent" is the beginning of meiosis and normally induced by the enzyme spo11. However, micronucleus elongation can be also induced by DNA damaging agents such as UV irradiation and chemicals, even in spo11 deficient-mutants. These findings suggest that DNA damage is actually the trigger for spo11 activity. Interestingly, meiosis can be induced even by lesions other than DNA breaks, which is probably mediated by a phosphokinase signal transduction pathway [45]. These findings support the hypothesis that meiosis evolved in early eukaryotes as a response to several kinds of DNA damage.

The damaging effects of ROS on DNA, and their sex-inducing effects, strongly support a hypothesis that the need for a highly efficient DNA repair mechanism was the selective force for the evolution of homologous chromosome pairing and formation of chiasmata and crossovers at meiosis.

4. The mechanisms of recombination enzymes should be more adapted to repair than to simple random genetic exchange. This was one of the key arguments of Bernstein et al. [18] for the hypothesis that meiosis evolved as a repair mechanism of DNA. In the majority of cases, recombination does not result in an exchange of flanking regions of the DNA strands, which are much larger than the recombined regions and would efficiently create new gene combinations. Meiosis is consequently not optimized for creating genetic diversity, but for recombinational repair. Recent findings on the evolution of meiosis proteins strongly support this idea.

4. The evolution and function of meiosis proteins revisited

Many enzymatic activities related to DNA repair existed in prokaryotes before the evolution of eukaryotes with their onset of meiosis, namely, the cut- and paste activities of topoisomerases, recombinational break repair activities, including RecA-type recombinases, mismatch repair activities, and the clustering of telomeres and their dragging across the nuclear envelope [46]. Arguments in favour of a predisposition to meiosis in prokaryotes are that the core genes involved in meiosis have homologs in prokaryotes [46-48]. For instance, Rad51, which is found in almost all major groups of eukaryotes, is homologous to RecA in bacteria and RadA in Archaea [47-48]. RecA type recombinases are present in all living cells, and act in eukaryotes during mitotic recombinational repair [46]. Recombinational repair is already crucial for bacteria for viability despite the availability of other repair mechanisms. However, recombinational repair in circular DNA bears the danger of inversions or split of the ring-like genome into parts, while linear chromosomes can be more easily repaired [49]. Research in the last decade has elucidated the functions of eukaryotic proteins involved in meiosis [38-39,46-48,50]: Many of them are not specific for meiosis, but also act at recombinational repair at mitosis, such the mismatch repair proteins (Mlh1-3, Msh 2, 6). Some proteins represent a “core” meiosis-specific subset: Hop1, Hop2 and Mnd1 are responsible for homologous pairing; Spo11 is associated with double-strand breaks at the beginning of meiotic recombination; REC8 is a meiosis-specific paralog of mitotic RAD21, that is involved in sister chromatid cohesion; RAD52 mobilizes single-strand DNA for homology search. Rad51 is a key protein for heteroduplex formation. It is highly conserved among eukaryotes and acts during mitotic repair, and interacts with the meiosis-specific Dmc1 protein for strand exchange during meiosis. Rad51 is homologous to the bacterial RecA recombinase, an important repair enzyme. During meiosis, Rad51 is assisted by its meiosis-specific paralog Dmc1 for the formation of Holliday-junctions. Dmc1 is required for the formation of inter-homolog joint molecule recombination. Rad51 and Dmc1 probably work together promoting meiotic recombination events. The function of the Rad51 paralogs is not yet clear, but they likely play a role in homologous recombination. Heteroduplex DNA and crossing over is processed by mismatch-related repair proteins (Msh4, Msh5). Brca2 plays an early role in homologous recombination and very likely controls the formation of the Rad51/single-strand DNA nucleofilament. The MRX complex is a tripartite complex, with Mre1 and Rad50 being strongly conserved among eukaryotes. Both proteins

are probably involved in double-strand end processing. The MRX complex probably has multiple roles during meiosis.

The key proteins of meiosis have obviously originated out of repair proteins, and still act during meiosis. They provide for successful pairing, Holliday junction formation, homologous repair and resolution of Holliday junctions. *Arabidopsis*-mutants with defects in these key recombination proteins show chromosomal instability during meiosis, which is frequently associated with sterility [38]. The question is – is modern meiosis also a consequence of oxidative stress, or is the protein activity at meiosis a cause of DSBs?

In extant organisms, DSBs are thought to be generated “on purpose” by the meiosis-specific Spo11 orthologs [41]. This seems to be a paradox – why should intact DNA strands be broken only to be repaired afterwards again? Double strand breaks belong to the most serious lesions of DNA, and different solutions exist to resolve Holliday junctions (HJ): only some of them lead to cross-over, ie. an exchange of genes on the two homologous chromatids, which potentially creates new beneficial gene combinations. HJ dissolution, but also some forms of double strand break repair do NOT result in an exchange of flanking regions (which would have efficiently created new gene combinations) [38]. HJ are thus not efficient processes for creating new gene combinations, which questions whether the function of spo11 is to make “programmed” breaks. In many extant organisms, spo11 is present together with DSBs and caps the cleaved DNA strand ends. The function of spo11 must be understood from a perspective of evolutionary history and its chemistry. Spo11 has evolved from archaeal topoisomerase VI via several gene duplications [48]. All eukaryotic lineages were preceded by the origin of Spo11 from topoisomerase VI homologs, which means that spo11 originated before eukaryotes diversified.

The evolution of Spo11 suggests that this protein most probably originally did *not* have a function of “creating” double strand breaks [48]. First, a gene duplication generated spo11-3, which is not meiosis-specific. Spo11-3 is present in protists, the choanoflagellate *Monosiga*, and in plants; in other eukaryotic lineages, this ortholog was probably secondarily lost. The function of this basic spo11-3 homolog is still not well understood, but it is *not* involved in meiotic recombination. Later on, gene duplications separated spo11-3 from the meiosis-specific homolog spo11-2, which is again found mainly in protists and plants, and is phylogenetically sister to spo11-1 which is found in protists, plants, animals and fungi. Only the meiosis-specific orthologs act during meiosis in animals, plants and fungi, and they appear together with double strand breaks. Mutants deficient for spo11 show a failure of meiosis. However, induced breaks are regarded as part of a later evolution of meiosis [51]. The evolution of spo11 suggests that in early eukaryotes, the model of programmed double strand breaks generated by modern spo11 homologs is not applicable. In early protists, DSBs caused by oxidative damage might have been the direct trigger for spo11-like topoisomerase activity to cap the lesions which would prevent further lesions by ROS. Topoisomerases form tyrosyl phosphodiester links to the DNA backbone [52]. If the DNA backbone or the bases were already attacked and damaged by ROS before, then free oxygen radicals would hang around on loose ends either on the sugar-phosphate backbone or on the bases. Damaged DNA thus becomes a highly reactive radical by itself. In this situation, the electrons that are required to scavenge

the radicals can be taken from the spo11 tyrosine, while a transesterase reaction links to the DNA backbone, thereby cleaving and sealing the end [53]. This action initiates the further steps of recombinational repair whereby spo11 remains initially attached to the cleaved ends and is later removed by endonucleases. Thus, spo11 may have a capping and marking function of oxidative damages, which initiates recombinational repair. According to this hypothesis, DSBs would not be *caused* by topoisomerase activity, but represent a consequence of topoisomerases reacting to DNA base oxidation, which marks the chromosome region requiring urgent repair. This is simply a chemical reaction following an oxidative damage, not a break on purpose. The enzyme machinery of meiosis then just needs to repair the DSB which confers an immediate selective advantage.

A repair function for DNA damages other than breaks can be inferred from the observations on *Tetrahymena* [45]. Thus, the main function of spo11 would be to seal ends and mark the damaged site before starting with repair. Other proteins, such as the MRN complex, then conduct single strand invasion, resulting in Holliday junctions and recombination during zygotene and early pachytene. These processes require all mitotic homologous repair components together with the meiosis-specific enzymes. Mismatch repair enzymes are normally important for eliminating replication errors to keep mutation rates down; MMR related enzymes act during meiosis for the resolution of Holliday junctions, or act in the rejection of crossing-over between divergent (non-homologous) chromosomes [46].

It is still unclear why spo11-induced cleavage is targeted to certain sites, so called “hot spots”, while other regions are recombinationally rather stable [52]. In *Saccharomyces cerevisiae*, 150–300 DSBs are formed during prophase I prior to formation of the synaptonemal complex [54]; these DSBs are inducers of homologous recombination, and the lack of DSBs in mutants deficient for Spo11 blocks recombination. But, in fact, most of DSBs do NOT result in cross-overs. In plants, it is meanwhile established that around 90% of Holliday junctions are resolved as non-crossovers. That is, only 10% of spo11 induced DSBs would serve for efficient recombination. From this small percentage again only a part of new gene combinations in the flanking regions might be beneficial. Under the assumption that meiosis is good for creating new gene combinations, DSBs would resemble a lottery with perhaps 2-3% potential winners among the recombined products of meiosis. The recombined gametes further have to merge with gametes of another individual – without any guarantee that the new gene combination in the zygote would be favored by selection. That is, in the light of new findings on meiosis - recombination at meiosis becomes like a lottery where only few of the offspring would have the winners ticket, the great majority would have no benefit or even a disadvantage form an unfavorable new gene combination. From mathematical modelling, the lottery model for maintenance of sex [55] in general has been shown to be wrong as it would require a strong, truncating selection to give a benefit to a very small proportion of the offspring, that of the fittest, while the great majority of the offspring would have to be purged by selection. Since the assumption of truncating selection is an unrealistic assumption and the proportion of winners of meiotic recombination is far too small, the model was rejected as a general explanation for maintenance of sex.

A further paradox arises from a functional perspective: how should *spo11* “know” whether the recombination at these sites would be beneficial? The “foresight” of evolution is already unrealistic for behavior of individuals, it is even more unrealistic for the activity of enzymes which simply follow the chemistry inherent in their molecular structures. So how could such an elaborate enzyme machinery evolve that would make a serious DNA lesion for the likelihood to win in a lottery? This lottery, is, as we know, a very expensive one, as a number of proteins have to be synthesized to conduct recombinational repair of an induced DSB. In fact, in the light of new findings on meiosis it seems that an additional cost has to be calculated, that of producing a big set of proteins for the whole meiosis machinery. From the perspective of a selective advantage, such a costly lottery ticket is unrealistic. It would be much more convincing to assume a direct and immediate benefit of DNA repair to meiotic products that can be immediately favored by selection. If DNA lesions are the trigger for the initiation of recombination then it is easier to explain that “hotspots” for recombination exist at sites where previous oxidative damage is severe; otherwise the observed topological distributions of recombination sites do not have any convincing functional explanation [50]. And, it is reasonable that in multicellular organisms, the costly and accurate homologous recombinational repair during meiosis I is focused on the germline cells, while other, less accurate and potentially mutagenic non-recombinational repair is good enough for somatic cells.

Meiosis is thus crucial for eukaryotic life. Even in asexual lineages, meiosis is hardly ever eliminated completely: in angiosperms, apomixis is usually facultative, i.e. sexual processes occur in parallel to apomictic reproduction, with low frequencies of meiotic offspring [56]. However, meiosis by itself has not been silenced, it takes place but is just by-passed by a somatic cell or altered to avoid the reduction of ploidy. Occasional sex, ie. normal meiosis and production of a reduced gametophyte, happens regularly at low frequencies. In animals, automixis is a widespread form of asexuality, but automixis maintains meiosis with meiotic products fused again [6]. Most strikingly, many forms of automixis result in homozygous offspring, with all its detrimental effects. To some extent automixis is more similar to self-fertilization of flowering plants (autogamy) which is usually regarded as a sexual process. In such cases, meiosis cannot be maintained because of recombination – it requires another function which is most likely DNA repair. That is, the key component of sex, the repair at meiosis I, is still present. Animals with cyclical parthenogenesis like *Daphnias* alternate between sexual and asexual generations, the former being produced under environmental stress situations [57]. All these forms of asexual or parasexual reproduction demonstrate that meiosis I has a crucial function other than creating new gene combinations.

5. The purging of deleterious mutations in diploid-haploid cycles

The DNA repair hypothesis explains the origin of programmed DSBs, the need for a second chromosome for recombinational repair, and the evolution of Holliday junctions. But, it does not explain the second important part of meiosis, ie. segregation of chromosomes at anaphase I, the lack of a second synthesis phase, and the subsequent mitotic division at meiosis II which results in four haploid meiotic products. For conducting recombinational repair, permanent

diploidy would suffice [11]. This was one of the major points of criticism of the original repair hypothesis. The processes after prophase I cannot be related to DNA repair but must have another functional background. From a mechanical view, segregation could be a direct consequence of homolog pairing at prophase I, as synapsis and correct homolog pairing are required for correct segregation [51]. Wilkins and Holliday [51] discuss that the evolution of meiosis out of mitosis requires just a few alterations, basically the homolog pairing of chromosomes at prophase I, while the processes, i.e. skipping of centromere splitting and segregation of chromosomes instead of chromatids, and skipping the second S phase, are just consequences of homolog pairing. The authors discuss that these processes would have evolved to limit erroneous recombination. Although this hypothesis is convincing from a mechanical point of view it does not provide an evolutionary explanation of a selective advantage to maintain this as a regular process.

Here I will briefly summarize an idea proposed earlier by Hörandl [12] that reductional division is needed to eliminate mutations. Mutations, as changes in the DNA sequence, cannot be actively repaired; in fact they are mostly products of an erroneous non-homologous DNA repair mechanism [34]. Most mutations are either neutral or deleterious. Asexual lineages would accumulate deleterious mutations from generation to generation, while genotypes with low mutational load would be lost by drift in small populations; this process would in the long run lead to extinction of asexual lineages (Muller's ratchet; [15]). Recombination in a sexual population can reconstitute the genotypes with a lower load of mutations in the progeny, which was seen as a major advantage of sexuality. However, a lot of questions remain unanswered in this hypothesis. First, mutation accumulation happens far too slowly to give an immediate benefit to recombination; second, Muller's ratchet is only effective in small populations; third, the existence of ancient asexuals that had no sex for millions of years questions the generality of the model. Again, an evolutionary perspective helps to develop a causal model.

Early eukaryotes were probably haplontic, and so are algae, many fungi, and bryophytes. In haplonts and haplodiplonts, the diploid stage was just needed to conduct recombinational repair, but it was not the predominant stage for the normal cellular functions which were optimized for haploidy. Initially, segregation might have just evolved to return to the default haploidy, whereby skipping the second synthesis phase before the start of meiosis II could have evolved as a response to lack of nutrition or in a situation of starvation. Hunger was the trigger for meiotic cell divisions which is consistent with observations that e.g. yeasts start sporulation under starvation [58-59]. After mixis, two chromosome sets are available for repair during prophase I, but for continuing the cell cycle on the diploid level, resources were not sufficient, and the gene regulatory network was not yet adapted to work in the diploid condition. In this situation, a cell cycle mechanism without the need of a second synthesis phase, resulting in four haploid daughter cells, conferred a selective advantage over continuing a solely mitotic cell division on the diploid level. The shift between diploid and haploid stages was initially probably just a side effect of recombinational repair under oxidative stress and nutrient-poor conditions.

This side effect became important after a shift from haplontic to diplontic or diplohaplontic life cycles which occurred in metazoans and in vascular plants (ferns, lycophytes, and seed plants). A predominant diploid life cycle allows for the buffering of deleterious or disadvantageous mutations in somatic cells as a second homologue without this mutation is left [60-62]. The masking hypothesis states that diploidy will be favored over haploidy under free recombination because of masking deleterious recessive mutations, but is less likely to be favored when recombination rates are low [63]. Even if somatic deleterious mutations are not inherited, they decrease the fitness of a population. Masking confers an advantage to diploidy compared to haploidy in both sexual and asexual organisms [64]. Thus, masking increases temporarily the fitness of diploid or polyploid populations [62]. But, diploidy increases the mutational load. It was already recognized by Haldane in 1937 [65] that the mean fitness of a population depends more on the genome-wide deleterious mutation rate than on the effects of mutations. The equilibrium mean fitness of a population is reduced by approximately c/U , where c is the ploidy level and U the mutation rate per haploid genome [66]. Under the conditions of equivalent mutation rates per base pair, the mutational load is thus higher in diploid than in haploid organisms. Under these conditions and in the absence of epistasis, haploids will have the lowest mutational load. The masking effect also reduces the efficiency of selection against such mutations as individuals carrying such masked mutations are not targeted by selection. Mutations can accumulate. But, in the haploid stage a deleterious mutation in a functional gene will be expressed and exposed to selection. If the mutational load strongly reduces the fitness of that individual, selection can purge the population from the mutations by eliminating the individual that carries the mutation. This way a haploid line can keep the mutational load low, especially if it has a rapid generation turnover, while a diploid line will accumulate mutations.

The diplontic or diplohaplontic organism would carry a lot of “masked” mutations after a prolonged diploid phase. After a return to the haploid phase, previously masked mutations are immediately exposed to selection which increases the efficiency of selection against mutants in the haploid gametes or gametophytes [12,49]. Segregation is quantitatively more important for the creation of new combinations of alleles than physical recombination [62]. Therefore, segregation at meiosis has a two-fold effect: creating genetic variation in the products of meiosis, and consequently among gametes or gametophytes, and expressing genes with deleterious mutations, which allows selection to act efficiently upon the fitness of haploid stages. This hypothesis would infer a strong selective advantage for a regular return to the haploid stage. In fact, selection on haploid gametes is always quite strong as few gametes, and only the fittest ones, reach syngamy and karyogamy; most of them are lost. In anisogamous organisms selection is usually very hard on male gametes, as spermatozooids usually must actively move and compete to win the race to the egg cell for fertilization. In flowering plants, competition is known even for the immobile male gametophyte: competition among pollen tubes occurs as they grow through the style and selects for the fastest ones [68]. It also makes sense that selection is usually weaker on the immobile female gametes, as their function is to keep cell organelles inactive to protect from oxidative stress [42]. Gametes or gametophytes of vascular plants are small, short-lived and thus can be produced in high numbers; it does not matter too much to lose the great majority of them in a hard truncating selection process which purges defective, non-competitive mutants as they have no chance to proceed to fertilization.

The mutations carried by these gametes or gametophytes are eliminated from the lineage, and the zygote can combine two “purged” chromosome sets. Thus, meiosis becomes a comprehensive DNA restoration process which eliminates not only direct defects of ROS but also indirect consequences, ie. mutations in germline cells.

The establishment of a reductional division requires mixis to re-establish the diploid stage. In all higher eukaryotes, such meiosis-mixis cycles become obligate from generation to generation as a regular DNA restoration process is essential for maintenance of a lineage [12].

6. Conclusions and directions for future research

Recent evidence strongly supports that meiosis not only originated for DNA restoration, but also that meiosis is maintained for this purpose in extant organisms. In addition to the benefits of DNA repair at prophase I the alteration of diploid-haploid cycles helps to purge mutations from the germline in diplontic or diplohaplontic organisms. Allelic recombination is just a by-product, a side effect of this process which does have other important evolutionary consequences, but is not the reason why sex is maintained [12].

The hypotheses proposed here are derived from observations of quite different research fields: redox chemistry, DNA function and repair, karyology and chromosome research, proteomics, eukaryotic bauplan (generalized body plan) and physiology, and evolutionary history. Further relevant information is needed in all these fields, but the challenge to combine results from different fields into a coherent theory is even greater. The main aim of this review is to stimulate broader interdisciplinary thinking, and to develop research projects to specifically test the different hypotheses of this theory.

Redox chemistry is a relatively young field, and it is still a challenge to reliably trace and measure reactions and results of oxidative damage on organic molecules. Most ROS are extremely unstable, with msec half times and thus difficult to measure in living tissues [69]. ROS may induce various different reactions not only with DNA and RNA molecules but also with other components of the cells. At low levels, ROS can be molecular messengers for many life-history traits [70], but at high levels they exert damaging effects. Most important, the role of oxidative stress and ROS on the onset and during meiosis, and the effects of ROS on meiosis protein-DNA interactions need further investigations.

DNA repair has become a major field of research and allowed for the recognition of the functional and evolutionary origin of meiosis out of DNA repair mechanisms. Nevertheless, the role of DNA repair during meiotic recombination in extant, modern eukaryotes is still not well understood. Most authors take it for granted that DSBs at meiosis are done “on purpose” but they disregard the chemical processes during the origin of DSBs. Recombination hotspots need to be investigated as to whether they represent damaged sites, as hypothesized here. Further studies on meiosis proteins and chromosome behavior under different levels of oxidative stress are needed to understand the postulated repair functions of meiosis.

Meiotic sex is ubiquitous in eukaryotes, but nevertheless many different physiological constraints and life forms exist in various eukaryotic groups. Most observations on sex as a response to oxidative stress have been made on organisms with a simple bauplan, such as algae, fission yeasts and protists. Detailed studies on the correlation of oxidative stress and sex in metazoans and land plants are still scarce. A major problem is the scarcity of suitable model systems with both sexual / asexual reproduction in higher eukaryotes to see effects of oxidative stress on mode of reproduction. In animals, abiotic stress affects the reproduction mode in animals with cyclic parthenogenesis, e.g. daphnias and aphids [57]. However, for mammals as the best established lab organisms, no natural parthenogenesis is known. The germline of metazoans separates very early in development, and is thus kept apart from the tissues with the highest oxidative stress (e.g., muscles, liver cells). It will be crucial to investigate stress responses and putative signalling or messenger pathways between germline and somatic cells, and different constraints on male and female gamete formation. In plants, environmental stress leads to increased frequencies of homologous recombination, both in meiosis and mitosis, and epigenetic instability [71]. Plants have the advantage that germline cells (megaspore and microspore mother cells) differentiate late in the development in adult individuals; thus, meiosis can potentially be directly correlated to environmental stress factors, which makes them suitable for experimental approaches. Nevertheless, few model systems allow for a comparison of sexual and asexual reproduction. Transcriptomic studies on sexual / asexual *Boechera* show a significant increase of oxidoreductase gene activity during the promeiotic to the meiotic stage. Genes that were overall significantly over-represented in meiotic stages in sexual plants compared to apomictic plants include those related to redox regulation [72-73]. It will be promising to study oxidative stress, gene expression, and mode of reproduction in facultatively apomictic flowering plants under different environmental stress conditions.

The hypothesis on purging mutations in haploid stages can only be tested on diplontic or diplohaplontic organisms, i.e. animals or seed plants. Ferns are also theoretically interesting, but high ploidy levels might complicate methodological setups. It might be promising to study model organisms forming unreduced gamete and gametophytes, and their offspring with respect to mutational load compared to the parental organisms. Unreduced gametes are expected to result in offspring with a higher mutational load than in model systems with reduced gametes / gametophytes as the purging effect should be weakened. Genomic approaches will be needed to detect changes among generations. Moreover, it will be important to study gene expression in gametes / gametophytes to understand genes that set the fitness parameters on which selection can act upon during the insemination / pollination and fertilization process. Finally, mathematical modeling is promising for understanding long term effects of gamete selection over many generations, as diplontic organisms mostly have a slow generation turnover.

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