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Atypical ploidy cycles, Spo11, and the evolution of meiosis

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Abstract

The Spo11 protein induces DNA double strand breaks before the first division of meiosis, enabling the formation of the chiasmata that physically link homologous chromosomes as they align. Spo11 is an ancient and well conserved protein, related in sequence and structure to a DNA topoisomerase subunit found in Archaea as well as a subset of eukaryotes. However the origins of its meiotic function are unclear. This review examines some apparent exceptions to the rule that Spo11 activity is specific to, and required for meiosis. Spo11 appears to function in the context of unusual forms of ploidy reduction in some protists and fungi. One lineage of amoebae, the dictyostelids, is thought to undergo meiosis during its sexual cycle despite having lost Spo11 entirely. Further experimental characterisation of these and other non-canonical ploidy cycling mechanisms may cast light of the evolution of meiosis.

Introduction

The main features of meiosis are remarkably well conserved across diverse eukaryotes. Underlying the complex structures and events that take place is a network of proteins that is more variable from lineage to lineage. Nevertheless, the pattern of conservation of the key genes across the eukaryotic tree indicates that the most recent common ancestor of eukaryotes must have possessed all of the components necessary for meiosis [1]. Along with the distribution of other genes involved specifically in the fusion of gametes and nuclei (Hap2/GCS-1 and GEX1 respectively), this implies that an orderly, recognisable sexual cycle including meiosis must be ancestral [2]. Conversely, although some of the important meiotic genes are related to DNA repair proteins present in all cellular lifeforms, no meiosis-specific genes have orthologues outside of the Eukarya. This suggests that the benefits of sex and recombination were important early in evolution specifically in the context of (proto-) eukaryotic biology, and that they enabled the diversification of extant eukaryotes. A number of favourable outcomes promoted by sexual recombination have been proposed to account for the prevalence of sex [3], and it is likely safe to assume that multiple benefits accrue in reality. More difficult to explain is how such a complicated sequence of events as meiosis evolved step-by-step to the immediate advantage of the cells in which they occurred (as opposed to longer term group-level advantages). Nevertheless plausible schemes have been proposed to account for this development, at least at the cytological level [4, 5]. The detailed early evolutionary history of individual genes involved in meiosis make up a separate set of questions that are no less interesting. This review will consider one of these proteins, Spo11, in an evolutionary context, focusing on atypical ploidy cycles and suggest how its history might illuminate broader questions.

During prophase I of meiosis, covalent links between homologous chromosomes, chiasmata, are generally formed. Chiasmata originate from DNA double strand breaks (DSBs), which are processed in an unusual form of repair that results in crossing-over between chromosomes. The mechanistic details of this process will be described in detail elsewhere in this issue. Central to the process is the unconventional endonuclease Spo11, which is used to trigger crossing over by catalysing the formation of DSBs prior to synapsis [6, 7]. This process is remarkable in employing a programmed form of DNA damage, although not uniquely so, since strand breaks are also induced during mating type switching in yeasts [8] and in V(D)J rearrangement of immunological genes in vertebrate immune cells [9]. Spo11 is closely related to type II topoisomerases

[7], which also make DSBs that are quickly religated, allowing decatenation and alteration of supercoiling [10]. All eukaryotes rely on topoisomerase II (Topo II) to decatenate DNA during its replication, and also use it to alter chromosome topology during transcription. A diverse subset of eukaryotes also possess Topo VI, a similar topoisomerase that is also found in Archaea [11-13]. Unlike Topo II, which is dimeric, Topo VI is tetrameric and composed of two A and two B subunits. The B subunits bind ATP, and are related to a segment of Topo II; A subunits are smaller, and contains a Toprim domain, found in all topoisomerases [14], as well as a winged helix domain that contains the catalytic tyrosine residue that acts as the nucleophile that attacks DNA phosphodiester bonds [15]. Spo11 is homologous to this catalytic A subunit of Topo VI [16]. A recent identification of a B-like subunit associated with Spo11 is described elsewhere in this issue (B. de Massy, personal communiction).

Since the sequence and structure of the Spo11 protein is constrained strongly by its catalytic function, genes encoding orthologues are easily recognisable. As genomes of eukaryotic organisms have been sequenced during the past twenty years, Spo11 has been an important part of the 'meiotic inventory' that has been used to indicate whether (often poorly studied) clades are likely to be capable of sex [1, 17]. Phylogenomic studies of this kind have been important in crystallising the idea of the antiquity and ubiquity of meiosis across the Eukarya. Spo11 homologues have been identified in all of the major eukaryotic supergroups, along with all of the other key meiotic genes [12]. Its homology with Topo VI A subunits suggests that Spo11 first evolved in ancient stem eukaryotes by duplication and divergence from one of these genes and becoming specialised in its current meiotic function [13]. The close similarity between Spo11 and Topo VI A subunits of both eukaryotes and Archaea suggests further that the ultimate origin of this meiosis-specific gene was an ancestral archaeal topoisomerase, consistent with recent placement of the root of the eukaryotic tree nested within the tree of the Archaea [18, 19].

The role of Spo11 in generating the DSBs that enable chiasmata was first demonstrated in a fungus, yeast [6, 7], and has also been analysed in fine detail in metazoans and plants [20-23]. Again, this widely conserved molecular function strongly implies that the protein has retained the same role since the most recent common ancestor of eukaryotes. Despite the clear mechanistic understanding of Spo11 function, and confident assessment of its broad evolutionary relationships, there is no consensus on how its function might first have

evolved, and the presence of Spo11 orthologues in organisms not known to carry out meiosis remains a puzzle. It seems also that it has a separate role prior to DSB formation, during the initial pairing of homologous chromosomes, not requiring its catalytic activity [24, 25]. Consideration of potential 'non-canonical' functions of Spo11 might provide hints about its evolution.

1. Spo11 without (clearly documented) meiosis

A number of relatively well-studied eukaryotes, including several parasitic protists, are not thought to undergo meiosis but nevertheless possess seemingly functional Spo11 orthologues encoded in their genomes. While it is likely that some of these species possess sexual cycles that remain cryptic because of unknown or difficult-to-study stages of their lifecycles, it is important to consider the possible implications of functions of Spo11 that are independent of meiosis. All eukaryotes, as outlined above, are believed to descend from sexual ancestors, so putative non-meiotic roles of Spo11 must be assumed to be derived rather than ancestral, in the absence of good evidence otherwise; nevertheless such roles might give clues about how this protein first evolved.

One example is the excavate parasite *Giardia*, which has a well-studied lifecycle in which trophozoites that contain two separate diploid nuclei form infectious quadrinucleate cysts. While this species possesses meiosis-specific genes including Spo11 [1], whose expression is upregulated during the nuclear divisions occurring during encystation, no direct evidence of meiosis has been found [26, 27]. Genetic exchange appears to occur between paired nuclei connected by bridges formed after partial fusion of their nuclear membranes [27, 28]. Tethering of telomeres to the nuclear envelope during this partial fusion is thought to reduce the risk of aneuploidies developing, but permits homologous recombination between nuclei that retain separate identities [27]. Further molecular genetic analysis of these exchanges should provides clues about functions for Spo11 during this process.

The parasitic amoebae *Entamoeba histolytica* and *E. invadens* also produce quadrinucleate cysts, and also infect the alimentary canal of vertebrates, but are very distantly related to *Giardia*, being members of the Amoebozoa rather than excavates [29, 30]. The whole infective lifecycle of *E. histolytica* has been examined in detail with no convincing reports of meiosis [31]. The expression of Spo11, along with other meiotic

genes, is induced during nutritional stress and encystment [32, 33], and homologous recombination also occurs under these conditions [33]. It seems likely that in *Entamoeba* recombination is stimulated between homologous chromosomes in an asexual process involving genome endoreplication instead of cell fusion. Many other amoebae have similar lifecycles morphologically to *Entamoeba*, although uninucleate cysts are most typical [34, 35]. The opportunistic pathogen *Acanthamoeba castellani* is another species with no reported sexual cycle, and that has as-yet unstudied Spo11 genes present in its genome [36]. *Acanthamoeba* is highly polyploid, with more than 20 copies of each chromosome. Intriguingly, the copy number of nuclear as well as mitochondrial DNA is thought to decrease when these amoebae encyst, without cell division [37]. How exactly this might happen in mechanistic terms remains unclear.

Candida albicans, another human pathogen, provides an intriguing example of a documented meiosis-independent role for Spo11. *Candida* species were originally thought to be entirely asexual fungi, but recently have been found to be capable of mating and meiosis in some cases [38-41]. *C. albicans* is capable of mating, but apparently as part of a parasexual cycle that does not include meiosis [42]. Parasexuality is defined by an increase in ploidy involving cell-cell fusion but a reduction back to the starting ploidy level by random chromosome loss rather than a balanced reductional division [43]. *C. albicans* normally grows as a diploid, but can mate in a controlled way to form tetraploids, which return to the diploid state by this parasexual route [42]. This non-meiotic ploidy cycle enables some diversification of genotypes [44], and *C. albicans* can also reduce to the haploid level after random, concerted chromosome loss, which likely promotes the purging of deterimental alleles [45]. Like *Entamoeba, C. albicans* was found to express Spo11 outside of meiosis. Remarkably, recombination between homologous chromosomes in this parasexual system was detected in wildtype but not in Spo11 null strains [46].

These putative non-meiotic roles for Spo11 across different eukaryotic lineages hint at co-option of this protein to promote recombination in the absence of conventional sex. Exploration in other lineages will be of interest to determine whether these phenomena might be more widespread than currently appreciated, as will more mechanistic analysis in the systems mentioned above. It is also worth emphasizing the correlation with tetraploidy (or higher levels of ploidy). Topo VI is required for endoreplication in plants [47-49], and since Spo11 has a wider phylogenetic distribution than Topo VI it is possible that it takes over a more

conventional topoisomerase-like role in some organisms that need to maintain many chromosome copies; experimental investigation of this possibility will be an important advance.

2. Meiosis in the absence of Spo11

The presence of Spo11 in genomes across the tree of eukaryotes, and its conserved role in meiosis, as discussed above, implies that it evolved very early in eukaryotic history and has had a crucial and very specific role in promoting meiotic recombination ever since. Its apparent non-meiotic function in some organisms suggests scenarios in which Spo11 might have evolved before the advent of meiosis. Conversely, there is also evidence concerning meiosis in the absence of cross-overs or of Spo11 that raises the possibility that meiosis could have arisen before Spo11. The best-studied examples are the achiasmatic meiosis of some metazoa, notably in male *Drosophila* flies [50]. Chiasmata are normally thought of as being necessary for orderly segregation of homologous chromosomes in meiosis I, but deviations from this rule show that alternative mechanisms can provide sufficient accuracy. Furthermore, in Spo11 null mutants, chiasmata can be formed after the induction of nicks or DSBs by Spo11-independent means [51-53], suggesting how meiotic recombination might have been triggered in a pre-Spo11 evolutionary context [54].

As genomes of diverse eukaryotes are sequenced and the inventory of meiotic genes checked, lineages are typically found to have lost one or more meiosis-associated genes (either due to genuine loss of the gene or through sequence divergence making it unrecognisable). This is unsurprising as a general point since gene loss is an unavoidable consequence of genome attrition as mutations accumulate and compensatory changes become fixed [55]. And specifically, many meiotic genes have paralogues that can presumably be co-opted without difficulty [56, 57]. Spo11 will doubtless be found to have been lost in a number of lineages, perhaps most frequently in newly asexual lineages. In the social amoeba, the genera *Dictyostelium*, *Polysphondylium*, and *Acytostelium*, Spo11 appears to have already been absent in their most recent common ancestor [17] (and unpublished results), which is thought to have lived several hundred million years ago [58]. This is noteworthy because these organisms are believed to be sexual [59]. The absence of Spo11 sequences was noted in the earliest *Dictyostelium* genomes and the possibility raised that this could have been the result of incomplete assemblies [60]. However now seven *Dictyostelium* genomes and one each of *Polysphondylium* and *Acytostelium* are available in the public databases in near-complete states, and no vestige of Spo11 is

discernible in any [61-68]. Topo II enzymes are however clearly present as would be expected in each case. These species span the root of the dictyostelid tree, implying an ancient loss of Spo11 before the initial radiation of these amoebae [69].

The evidence for meiosis in these organisms is good, if not incontrovertible [70]. The dictyostelid sexual cycle involves the fusion of haploid cells that can be hetero- or homothallic, giving rise to a diploid zygote that grows by feeding cannibalistically on surrounding amoebae. As it grows, a wall is laid down around the zygote, forming a dormant (or at least immotile) structure, the macrocyst. After several weeks' maturation these cysts break open to release many haploid progeny. Ultrastructural studies have provided evidence of synaptonemal complexes within the zygote nucleus [71, 72]. Analysis of progeny carrying genetic markers suggests that recombination occurs at relatively high frequency and in patterns consistent with meiosis [73]-77] (and unpublished results). Often only one progeny class is produced per germinated cyst, apparently randomly selected since typically all possible combinations occur from multiple cysts in any given cross [74] [75]. A population genetic study of wild *D. discoideum* isolates also suggested that recombination is frequent in natural populations [78]. Along with the presence of several meiosis-specific genes in dictyostelid genomes [17], these data support a consensus that some form of meiosis takes place in macrocysts.

The absence of Spo11 in these amoebae thus raises several questions. Are chiasmata formed during meiosis I, and if so how are they induced? One can speculate that dormant cells in the soil might be exposed to many kinds of stress that could induce spontaneous DNA damage, for instance desiccation or radiation, that makes the active induction of DSBs redundant. The ecology of macrocyst production is not well understood, and it is possible that the environment that they subsist in will suggest an explanation. Equally, other features of these species' genomes provide some clues. *Dictyostelium* is highly radiation resistant, and has low mutation rates, suggesting very effective DNA repair mechanisms, as might be expected for a soil-dwelling organism [79, 80]. Surprisingly, as well as lacking Spo11, all dictyostelid genomes sequenced so far lack orthologues of the ATM gene, which encodes a protein kinase critical in most organisms in the DSB repair response, as well as its interaction partner nibrin (unpublished results). Orthologues of the related kinases ATR and DNA-PKcs are both present [61, 81]. This suggests an unusual configuration in the initiation and control of DSB repair processes, perhaps relying more heavily on DNA-PK or other damage-responsive kinases than is

usually the case. Since ATM is important in controlling meiotic DSB formation and resolution [82, 83], its loss along with Spo11 is unlikely to be coincidental. This raises another question: did loss of ATM make the retention of Spo11-mediated DSB formation too dangerous a process, triggering a reconfiguration of meiosis? Or if the most important role for ATM in the ancestral amoeba was during meiosis, did loss of Spo11 cause it to become dispensable? DNA-PK has been reported to have a role in mouse spermatocytes [84]; is this a conserved function in gametogenesis in species that have retained this kinase?

The macrocysts of the most widely used social amoeba species, *D. discoideum*, have characteristics that make the study of meiosis difficult: cysts are large and difficult to image, and germination frequencies are usually very low [75]. Examination of different species with different characteristics will very likely prove worthwhile. Further genomic exploration of Amoebozoan clades that are related to the dictyostelids and that face similar selective pressures may also bring interesting examples to light.

3. The emergence of Spo11 and the evolution of meiosis

To reiterate: the only well-attested conserved function of Spo11 is in the generation of DSBs during meiosis. All of the other phenomena described above occur in derived rather than ancestral circumstances, and so none can with any confidence be held to reflect any ancestral aspect of Spo11 function. Nevertheless if we wish to understand how this conserved function came to exist, we must consider scenarios that do not fit the pattern of 'standard meiosis' and that could resemble primitive or intermediate stages of the evolution of this complex process (see [85]).

The Spo11 polypeptide is clearly evolutionarily related to the topoisomerase VI A subunits found in all Archaea and a subset of eukaryotes, as discussed above. Topo VI is the only type II topoisomerase found in many archaeal genomes (though some Archaea also possess a gyrase homologue), while all eukaryotes possess Topo II proteins [13]. The phylogenetic distribution of Topo VI suggests that it was present in the most recent common ancestor of eukaryotes (Figure 1). The range of functions of Topo VI in eukaryotes is not clear, but it has a role distinct in plants in endoreduplication that is distinct from Topo II function. Endoreduplication is a widespread phenomenon in which DNA replication is uncoupled from mitosis, allowing ploidy increases. In plants, this facilitates increased cell size, and frequently occurs as a response to

stress [86]. Intriguingly, endoreduplication is stimulated by DSBs in *Arabidopsis* [87]. The ubiquity of Topo II in eukaryotes compared to the patchy distribution of Topo VI might suggest that the latter enzyme had a more specialised role early in evolution, and it seems not unlikely that its main function was in facilitating increases in ploidy.

These considerations are relevant to the emergence of Spo11 not only because of its homology, but also because an asexual ploidy cycle is often held to have likely existed before the evolution of meiosis [4, 88, 89]. Empirically, this seems a very probable scenario, given that polyploidy is common in bacteria and Archaea as well as eukaryotes [90, 91]. A preexisting asexual ploidy cycle implies periodic increases in ploidy by endoreplication, or by nuclear division unaccompanied by cytokinesis, followed by some mechanism to reduce ploidy. The introduction of regular cell fusion and the regularisation of reduction makes a plausible broad step-by-step scenario in which sex and meiosis might have evolved [4]. Crucially, ploidy cycling alone should be capable of reducing the mutation load of an asexual cell, suggesting how an immediate selectable advantage could have accrued even before the introduction of organised recombination based around syngamy and crossing-over [89]. If such an asexual ploidy cycle involving endoreduplication existed in the lineage that led to the first meiotic cells, it seems not unreasonable to suggest that a role for Topo VI similar to that in *Arabidopsis* was the reason for those genes' presence along with Topo II. One can then hypothesize that duplication and divergence of the A subunit from this ancestral function was the initial origin of Spo11. Reports of partial fusion of archaeal cells accompanied by genetic exchange supports these ideas [92, 93]; it will be important to confirm the ploidy of these cells, and examine whether any ploidy cycling occurs.

How and why programmed DSB formation first evolved remains a separate question. One theory for the emergence of crossing-over and recombination is that it was a defence against selfish elements that bias the outcome of divisions so as to be over-represented among the progeny, known as meiotic drive loci [94]. These elements are common across eukaryotes as diverse as plants, yeasts, and metazoa [95, 96]. Typically they involve two components, one detrimental and one providing resistance. Crossing-over (and uncertainty about whether and where crossing-over occurs) can therefore act to break these loci apart, negating their advantage [94, 97]. Given this widespread phenomenon, and the ubiquity of conflict more generally in the

genetics of sex, perhaps it would not be surprising if a potent DNA-damaging enzyme like Spo11 first emerged as a weapon in a genetic arms race of this kind.

Conclusions

Spo11 evolved early in evolution before the initial radiation of eukaryotes into the extant 'supergroups', along with the other core meiotic genes. It must have arisen from a Topo VI A subunit, which originally may have had a role in maintaining a polyploid genome in an organism that periodically cycled its ploidy level. In certain extant eukaryotes, Spo11 appears to have roles not directly connected to its canonical function in meiosis I. Further mechanistic investigations into these examples will be important, especially in cases where the evidence consists only of gene expression data. But it seems likely that these phenomena will prove to be widespread in eukaryotes that have non-meiotic ploidy cycles. Social amoebae, on the other hand, appear to undergo a form of meiosis not involving Spo11. Again, further molecular characterisation of this process is required to clarify whether crossing-over is promoted by some other means, whether meiosis is achiasmatic, or if some novel reductional process is used.

Deeper understanding of conserved features of Spo11 and the proteins that interact with and regulate it will be valuable in progressing towards a fuller model of its evolution as well as its function. And more unusual uses of this conserved protein should not be ignored, since the possibility remains that ancestral aspects of its biology might be retained in some protist lineages. Recent confirmation of a meiotic sexual cycle with transient reduction to haploid gametes in the parasitic excavate *Trypanosoma* is an important addition to our understanding of the full evolutionary context of these processes [98, 99]. One of the first theories of the deep history of meiosis was prompted by studies on excavate protists [100]. The examination of a range of protists with experimentally accessible lifecycles, using modern tools, is to be encouraged.

Acknowledgments

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Figure Captions

Figure 1. The phylogenetic distribution of topoisomerase VI, Spo11, ATM, and sex within eukaryotes.

The tree illustrates approximate positions of selected taxa mentioned in the text within the tree of eukaryotes, showing those which retain Topo VI genes. For additional information, some organisms not mentioned in the text that retain this enzyme are also shown. Proteins clearly orthologous to plant Topo VI enzymes and their archaeal homologues are included, although the possibility that these proteins have alternative activities remains possible. The fact that clades as distant as plants and the holozoan *Capsaspora* possess this gene makes it almost certain that they inherited it ultimately from the last common ancestor of eukaryotes. The presence of close homologues in Archaea reaffirms this conclusion. Spo11 is more widely conserved, as is ATM, although both are absent from dictyostelid genomes. While *Entamoeba* has been well studied and so far found to be asexual, the life-cycles of *Thecamonas, Sphaeroforma,* and *Capsaspora* are still being explored, and the presence of a sexual phase is not unlikely. *Emiliania* has haploid and diploid phases is is presumed to undergo meiosis, but direct evidence is lacking [101]. *Giardia* has a parasexual-like cycle not involving out-crossing, as described above, but has also been inferred from population genetic data to undergo sex [102].