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A Transcriptome-based Perspective of Meiosis in Dinoflagellates

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Abstract

There is increasing interest in the possibility of sexual recombination in dinoflagellates, especially those symbiotic with coral, since recombination may be able to augment genetic diversity and reduce levels of coral bleaching. Several previous studies have addressed this in *Symbiodinium* by querying sequence databanks with a list of 51 genes termed a meiosis detection toolkit. Here, we have constructed an expanded list of 307 genes involved in meiosis in budding yeast. We find the genes involved in the major regulatory steps in yeast meiosis are also found in dinoflagellates, as are many of the genes involved in recombination. In contrast, few genes involved in forming the synaptonemal complex or forming spores are conserved. We further note that the meiosis-related genes absent in dinoflagellates are also as a general rule absent from other protists in the closely related apicomplexa and the ciliates. We conclude the symbiotic dinoflagellates are as able to undergo meiosis as are other protists.

Key words: Dinoflagellate; meiosis; conserved genes; *Symbiodinium kawagutii*; *Fugacium kawagutii*.

Introduction

The cell and molecular basis of meiosis is becoming well understood in a variety of model systems (Hong et al. 2019; Mercier et al. 2015; Owens et al. 2018; Yamashita et al. 2017). Meiosis is complex, as after replication of a diploid genome to make sister chromatids of each DNA molecule, the cell divides the genetic material twice. The first is a reductive cell division, in which pairs of homologous chromosomes are separated into two different haploid daughter cells. Each of these two daughters then separates the sister chromatids, using a mechanism essentially identical to a mitotic cell division, to produce four gametes. The independent segregation of homologous chromosomes, coupled with crossing-over events that occur during prophase of the first meiotic division, generates a population of gametes with an high genetic diversity. A hypothesis to explain the pervasiveness of meiosis among organisms is that the resulting increase in genetic diversity allows populations to better resist parasitic infections – this hypothesis is supported by both observations of natural populations and laboratory experiments (Lively and Morran 2014).

Despite the many studies on model systems, non-model organisms and dinoflagellates in particular are much less studied. It is clear that at least some dinoflagellate species are able to undergo meiosis. In *Lingulodinium*, fusion between two hap-

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loid gametes of different sexual types leads to formation of a diploid cell called a hypnozygote, which can form either resting cysts (under nutrient deficiency) or sexual cysts (formed in nutrient replete conditions) (Bravo and Fiigueroa 2014). *Lingulodinium* was also used for mating experiments between clonal lines to show the presence of only two sexual types (Fiigueroa and Bravo 2005). In *Alexandrium*, 4C cells were identified cells using imaging flow cytometry (Fiigueroa et al. 2015). In older studies with *Peridinium*, hypnozygotes with four nuclei following gamete fusion were reported (Pfiester 1976), and two successive divisions of a planozygote, suggestive of meiosis were reported in several species (von Stosch 1973). However, it is far from clear that all species are capable of sex, including the coral symbiont *Symbiodinium*. *Symbiodinium* has come under increased scrutiny because meiotic recombination may augment the functional diversity of symbionts and produce some strains more resistant to coral bleaching (Suggett et al. 2017).

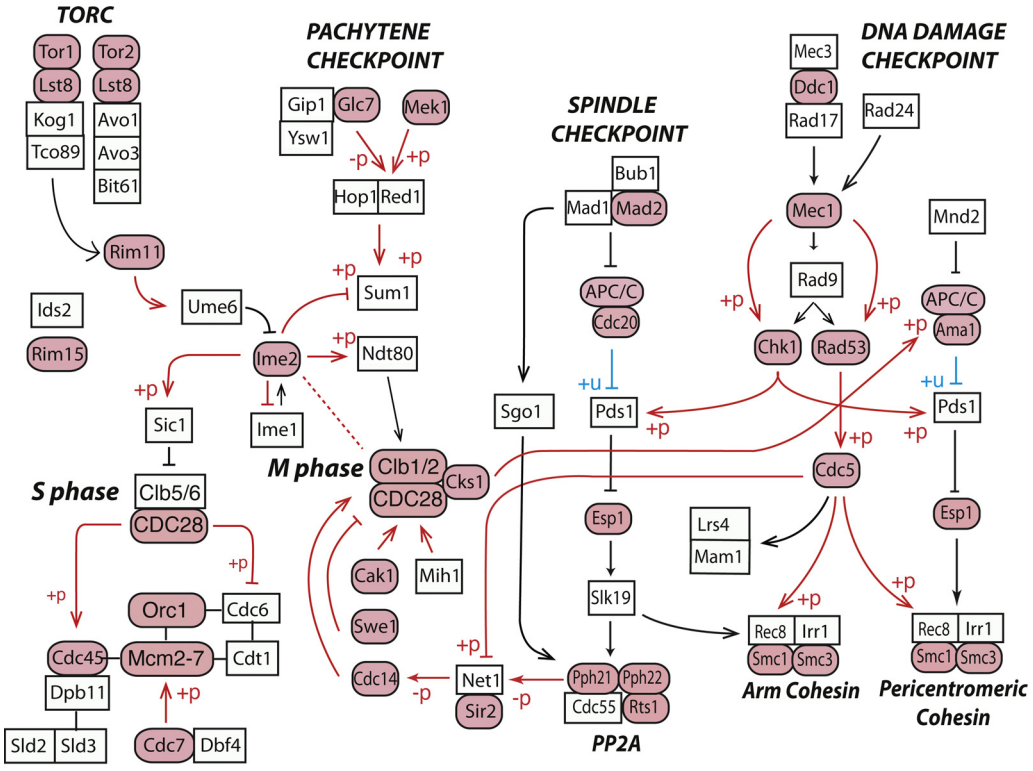
Even though there is little definitive evidence of sex from cytological or genetic evidence in coral symbionts (LaJeunesse 2001; Wilkinson et al. 2015), heat stress of *Symbiodinium* was observed to result in an up-regulation of MutS and Spo11, two essential meiotic genes (Levin et al. 2016). Several studies have queried sequence databases for the presence of genes known to be involved in meiosis. One query database containing 51 meiotic genes (Schurko and Logsdon 2008) showed that 31 of these were found in *Symbiodinium* (e-values $< 10^{-4}$) (Chi et al. 2014). A more recent re-examination of *Symbiodinium* for presence of these same 51 meiotic genes reported that between 46 to 48 genes were present depending on the species (Liu et al. 2018). However, the discrepancy between the two studies was not addressed, and in neither case was the conservation of these genes among different dinoflagellates explored.

In this study an extensive list of 307 genes involved in meiosis in budding yeast was used in BLAST searches to evaluate their possible presence in three dinoflagellate transcriptomes. One of the dinoflagellates chosen, *Lingulodinium polyedra*, is known to undergo meiosis (Fiigueroa and Bravo 2005). We further evaluated the presence of these genes in phylogenetically related protists, including apicomplexans, ciliates and diatoms, reasoning that absence of a gene among all these protists would reflect merely that the gene is not needed rather than being an impediment to the meiotic process in dinoflagellates. Similarity in gene content on the other hand would suggest the dinoflagellates are as likely to undergo meiosis as are the apicomplexans (Fritz et al. 2012; Guttery et al. 2012; O'Hara and Chen 2011), the ciliates (Loidl and Lorenz 2016) and the diatoms (Chepurnov et al. 2004). A comparison of the meiotic gene repertoire shows that the dinoflagellates *Lingulodinium* and *Symbiodinium* are practically identical. Furthermore, both show considerable similarity to other protists where meiosis has also been documented. These analysis thus support an ability of *Symbiodinium* to undergo genetic recombination.

Results and Discussion

An overview of meiosis, in a view similar to that in the *Saccharomyces cerevisiae* KEGG pathway, indicates that many of the yeast regulatory elements are also found in the dinoflagellate *S. kawagutii* (Fig 1) (*Lingulodinium polyedra* is shown in Supplementary Material Fig. S1). In this analysis, yeast genes that give significant hit to a *S. kawagutii* transcriptome are colored red. There are a few notable absences in this regulatory schema. First, the regulatory protein securin (Pms1p) is not found. Securin normally acts to prevent the transition from metaphase to anaphase by inhibiting the protease separin (Esp1p). However, it must be noted that securin is not found in any of the other protists tested, and furthermore, is also absent from fission yeast (Supplementary Material Table S1). Second, some elements of the cohesin complex holding homologous chromosomes together (Rec8p and Irr1p) are absent. This may suggest that dinoflagellate chromosomes also require alternative partners to Smc1p/Smc3p in the cohesin complex, although again, these two sequences are also absent in the majority of the other protists tested (Supplementary Material Table S1). Lastly, elements of the pachytene checkpoint, in particular the synapsis proteins Hop1p and Red1p, as well as the Sum1p transcription factor activated by them, are not present. This last is not surprizing, since there is no conservation of other budding yeast cell cycle-related transcription factor genes (including Ndt80 and Ime1) in other species.

Below the schema of the regulatory elements are a series of yeast genes grouped in boxes (Fig. 1, bottom). Again, those genes with hits to the S. *kawagutii* transcriptome are colored in red. Interestingly, among these groupings, the genes involved in recombination show a high proportion of conserved sequences (Fig. 1). To assess how well gene presence in *S. kawagutii* reflected what is observed in other protists, the subset of genes whose prod-



APC/C

AMA1	APC13
APC1	CDC16
APC2	CDC20
APC4	CDC23
APC5	CDC26
APC9	CDC27
APC10	MND2
APC11	

Synaptonemal complex

ECM11	MEI4	TOP2
GMC1	MND1	ZIP1
GMC2	REC8	ZIP2
HED1	RED1	ZIP3
HOP1	SPO16	ZIP4
HOP2	SPO22	

Recombination

CSM2	MND1	PSY3	RFA3
DMC1	MMS4	RAD51	RMR1
DNL4	MSH2	RAD52	SAE2
EXO1	MSH4	RAD54	SHU1
FOB1	MSH5	RAD55	SHU2
HFM1	MSH6	RAD57	SKI8
LIF1	MUS81	RDH54	SPO11
MEI5	NEJ1	REC107	SRS2
MLH1	PPH3	REC114	XRS2
MLH2	PMS1	RFA1	
MLH3	PSY2	RFA2	

Spore formation

ADY1	MPC70	SPO19	SPO77
ADY2	MUM2	SPO20	SPR3
ADY3	MUM3	SPO24	SPS1
ADY4	SMA1	SPO71	SSP1
ATG9	SMA2	SPO73	SSP2
HHO1	SPO1	SPO74	YVH1
MPC54	SPO14	SPO75	

Chromosome separation

CSM1	CSM3	CTF19	IRC15	MCK1
CSM2	CSM4	IPL1	KAR3	PBP2

Figure 1. Overview of *Symbiodinium* (*Fugacium*) *kawagutii* genes mapped onto the yeast KEGG meiosis pathway. Genes found in the *S. kawagutii* transcriptome by BLAST searches with meiosis related genes from budding yeast are colored in red, while genes not found are in black. The schema at the top shows the principal regulatory steps, while the boxes at the bottom group together genes associated with different processes. Some lines are marked with +/− p to indicate changes in phosphorylation status, or with +u to indicate addition of ubiquitin.

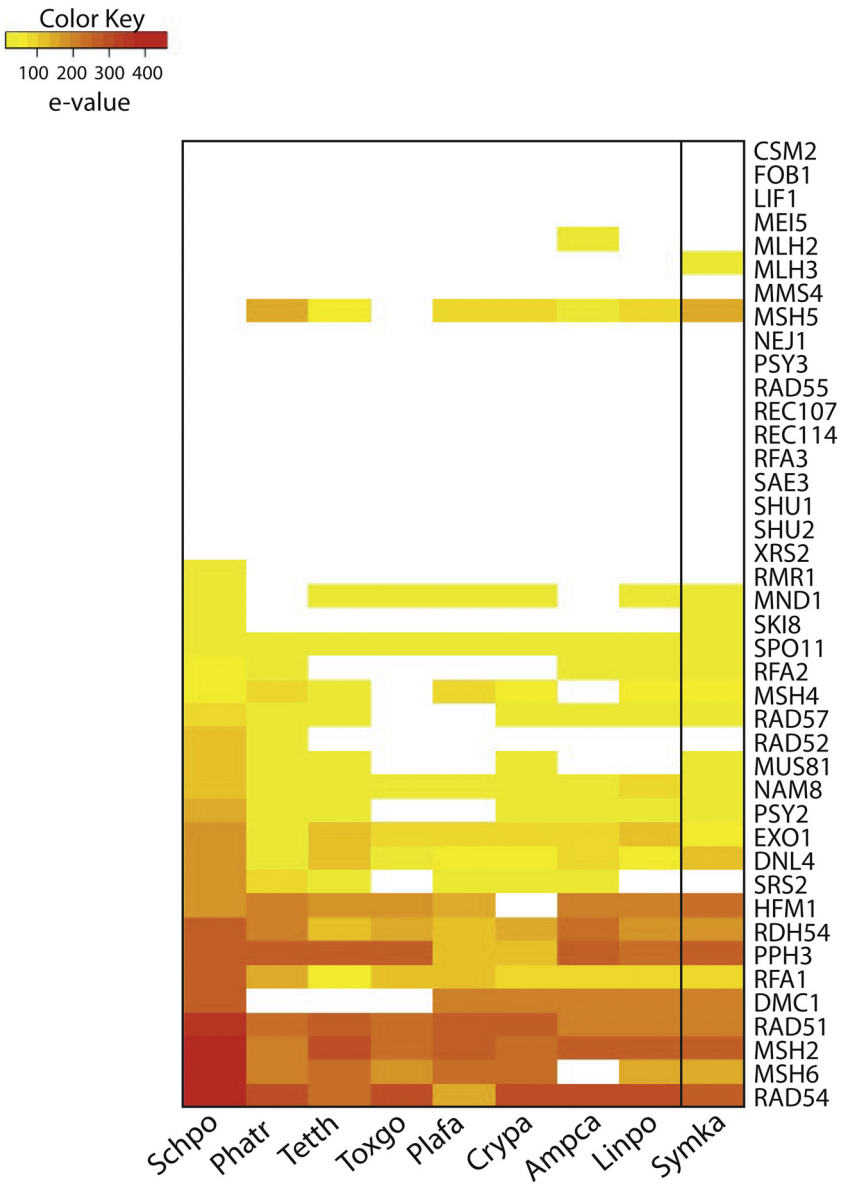


Figure 2. Heat map illustrating best BLAST e-values after screening a variety of organisms with yeast genes involved in recombination. From left to right, the organisms are fission yeast *Schizosaccharomyces pombe*, the diatom *Phaeodactylum tricornutum*, the ciliate *Tetrahymena thermophila*, the apicomplexans *Toxoplasma gondii, Plasmodium falciparum* and *Cryptosporidium parvum*, and the dinoflagellates *Amphidinium carterae*, *Lingulodinium polyedra* and *Symbiodinium* (*Fugacium) kawagutii*. White squares have no BLAST hit (>e^{-05}); the color scheme runs from yellow (poor BLAST hit) to red (good BLAST hit) using values calculated from the e-value with a −ln function. E-values of zero were arbitrarily assigned a value of e^{-200} for ease of graphing. Genes are ordered by decreasing e-values in *S. pombe*.

ucts are involved in recombination was also used to query apicomplexan, ciliate and diatom databases. The results of this analysis (Fig. 2) are presented as a heat map, where the colors pass from yellow to red with a lowering of the e-value (e-values of 0 were arbitrarily set at e^{-200} to allow them to be presented graphically). Absent genes lack color. The different genes are ordered according to an increase in similarity with the budding yeast query to the fission yeast database (leftmost column). We observe here that the degree of similarity to budding yeast genes in BLAST searches is very similar between the different protists. Other than the apicomplexans, most of the protists surveyed

Table 1. Number of meiotic genes with similarity to budding yeast.

Species	Number of similar genes
Schizosaccharomyces pombe	65.7%
Phaeodactylum tricornutum	50.5%
Tetrahymena thermophila	53.7%
Toxoplasma gondii	43.3%
Plasmodium falciparum	40.4%
Cryptosporidium parvum	44.3%
Amphidinium carterae	46.9%
Lingulodinium polyedra	49.5%
Symbiodinium kawagutii	49.5%

have roughly 50% of the budding yeast meiotic gene repertoire, while fission yeast has genes similar to 66% of the budding yeast gene repertoire (Table 1). In particular, the similarity between *Symbiodinium* gene inventories and what is observed with the other protists clearly supports the notion that the recombination machinery is sufficient to support meiosis in this dinoflagellate.

It was interesting to note that the group of genes implicated in formation of the yeast synaptonemal complex suggests that almost all are missing (Fig. 1, Supplementary Material Fig. S2). Among these, the only highly conserved gene is the topoisomerase (Top1), as the Mnd1 gene has only a weak similarity to the yeast query. However, this is a characteristic shared by all other protists tested (Supplementary Material Fig. S1), and is thus unlikely to reflect any adaptations to the particularities of dinoflagellate chromosomes (Wong 2019). The genes encoding structural maintenance of chromosomes (Smc) proteins, ATPases that act as either cohesins (Smc1 and Smc3) or condensins (Smc2 and Smc4), were not listed as part of the synaptonemal complex, but are present and highly conserved over all the organisms surveyed (Supplementary Material Fig. S2).

To assess if there were patterns in the identities of the genes conserved, genes were assigned to different stages of the recombination pathway and gene products that function together in complexes (as in yeastgenome.org) were grouped together, linked by hyphens (Supplementary Material Fig S3). However, this does not reveal any overarching rule dictating the presence or absence of the different components. In some cases, all complex components are present (such as Msh2-Msh6 or Msh4-Msh5), while in another, entire complexes are absent (such as the complex containing Rec107, or that containing Csm2). Alternatively, some complexes contain some but not all of the components (such as the complex containing Mre11 or that containing Mlh1). Out of nineteen complexes, 6 have retained all components and 6 lack all components.

Previous assessments of the ability of *Symbiodinium* to undergo meiosis have used BLAST analyses of *Symbiodinium* databases using a meiosis detection toolkit (Schurko and Logsdon 2008), a set of 51 meiosis-specific and meiosis-related genes. One study (Chi et al. 2014) recovered 31 of the 51 genes using a genome derived protein list of *S. minutum* (Shoguchi et al., 2013) while a second (Liu et al. 2018) recovered between 46 and 48 genes depending on the *Symbiodinium* species (47 for *S. minutum*). However, our BLAST searches with the same toolkit genes found only 31 of the 51 sequences in our *S. kawagutii* transcriptome assembly. While the reason for the discrepancy between the different analyses of *S. minutum* is unknown, we conclude the lower proportion of the meiotic gene toolkit genes appears more likely. It should be noted that the 31 of 51 genes found here in *S. kawagutii* are not all identical to the 31 of 51 genes found in *S. minutum*. Hop2, BRCA1, Ku80 and Sae2 were found in *S. minutum* but not in *S. kawagutii*, while Mer3, Dna2, Mph1 and Msh3 were not found in *S. minutum* but were found here. Overall, the agreement between the two species is 84%. We also note that for the most part, the genes missing in *S. kawagutii* are also missing when BLAST searches are performed with the NCBI transcriptome shotgun assembly (TSA) database limited to dinoflagellates.

In conclusion, we have found an inventory of meiotic genes in *Symbiodinium* that is similar to what is found in dinoflagellates known to undergo meiosis, such as *L. polyedra*, as well as to apicomplexan and ciliate species that undergo meiosis. These analyses thus support the contention that *Symbiodinium* can undergo meiotic recombination.

Methods

A list of 307 genes involved in meiosis in budding yeast *Saccharomyces cerevisiae* was compiled from genes listed in the KEGG meiosis pathway (sce04113), genes identified through a meiosis key word search in the *Saccharomyces* genomes database (www.yeastgenome.org), genes found to be required for meiosis in a genetic screen (Okuda et al. 2008; Rabitsch et al. 2001), and genes comprising a meiosis detection toolkit (Schurko and Logsdon 2008). The full list, along with the best e-value hits for all searches, can be found in Supplementary Material Table S1. When two or more yeast genes gave hits to the same transcriptome sequence, the transcriptome sequence was placed only once and corresponded to that with the best (smallest) e-value. The yeast gene list was

used in BLAST searches of Trinity assemblies of Illumina reads from *Lingulodinium polyedra* (CCMP1936) (Beauchemin et al. 2012; Roy et al. 2014), *Symbiodinium kawagutii* (CCMP2468) (Illumina reads available from NCBI with Accession number PRJNA517819), and *Amphidinium carterae* (Lauritano et al. 2017). *S. kawagutii* has been renamed *Fugacium kawagutii* (LaJeunesse et al. 2018) but for simplicity the name *Symbiodinium* is used here. Datasets also included genome derived protein datasets for the apicomplexans *Plasmodium falciparum* 3D7 (from PlasmoDB.org), *Toxoplasma gondii* ME49 (from ToxoDB.org) and *Cryptosporidium parvum* (from CryptoDB.org), the ciliate *Tetrahymena thermophila* (from ciliate.org), the diatom *Phaeodactylum tricornutum* (from ensemblegenomes release 42) and the fission yeast *Schizosaccharomyces pombe* (from PomBase.org) were also screened. Depending on the target database, either BLASTp or tBLASTn was used, both with a cut-off of $1e^{-05}$. The best BLAST hit e-value was recorded for each screen. Yeast genes without hits in the three dinoflagellate assemblies above were also tested using tBLASTn to query the TSA database at NCBI (limited to dinoflagellates).

Declarations of Interest

None.

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Appendix A. Supplementary Data

Supplementary material related to this article can be found, in the online version, at https://doi.org/10.1016/j.protis.2019.06.003.

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