Transcriptional studies on stress-induced meiosis in the anaerobic gut fungi *Pecoramyces ruminantium* strain Hef-5

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Abstract

This study focused on the presence of a cryptic sexual life cycle of *Pecoramyces ruminantium* strain Hef-5. This strain of anaerobic gut fungi (AGF) can be found in the rumen of herbivorous animals and is known to enhance plant biomass metabolism via fermentation and saccharation. In all past research, this fungus has been described as strictly asexual; however, this research has been conducted based on the behavior of the organism in its natural habitat. Many other fungal organisms have been described to exhibit cryptic sexual life cycles which may only appear while the organism is under some stress. The presence of a cryptic sexual cycle can be sensed by the expression of core meiotic genes (CMGs). The three CMGs studied are Hop2, required for homologous pairing, Mnd1, required for recombination and nuclear division, and Dmc1, required for dsDNA break repair and homologous pairing. To impart stress on the organism, samples were flushed with oxygen for increasing increments of five minutes up to one hour, two samples for each time frame. The expression of Hop2 came first at the 10-minute mark and continues to be expressed with few discrepancies through the 60 minute time frame. Dmc1 expression begins at the 20-minute mark and stays, also with few discrepancies, through until the 55-minute mark. Mnd1 is the final CMG to be expressed after 25 minutes of oxygen exposure and continues to be expressed through the 60-minute trial. These results indicate that the AGF strain Hef-5 could have a cryptic sexual cycle under these conditions. The negative control showed no expression for any of the three CMGs, supporting the hypothesis. The continuous expression of these CMGs aligns well with the time frame for meiosis I is *S. cerevisiae*. Future research to expand this includes a full transcriptomic study of Hef-5 as well as the study of additional CMGs involved in other phases of meiosis.

Introduction

The *Pecoramyces ruminantium* strain Hef-5 is of genus Orpinomyces and phylum Neocallimastigomycota. This strain of anaerobic gut fungi (AGF) can be found in the rumen of herbivorous animals and is closely related to strain C1A, known to enhance plant biomass metabolism via fermentation and saccharation (Youssef et al. 2013). This fungus is anaerobic, and the described life cycle is strictly asexual; however, all prior studies of the AGF life cycle are based on the behavior in the anaerobic gut. It has been proposed that resistant structures unseen in the asexual life cycle of AGF exist, but have eluded discovery. The presence of these structures sparked investigation of a sexual life cycle in these strains through the study of expressed genes under certain conditions. Core Meiotic Genes (CMGs) are highly conserved genes which encode for a set of proteins that only function during meiosis, or are required for proper completion of meiotic recombination (Halary et al. 2011). The presence of CMGs in the strain Hef-5 would imply the presence of a cryptic sexual life cycle. The model organism for these CMGs is *Saccharomyces cerevisiae*, commonly known as yeast, a eukaryotic organism that undergoes both sexual and asexual reproduction. The three CMGs studied are Hop2, required for homologous pairing, Mnd1, required for recombination and nuclear division, and Dmc1, required for dsDNA break repair and homologous pairing (further detail on these CMGs found in the appendix) (Leu et al. 1998, Chan et al. 2014, Gerton et al. 2002, andRoeder et al. 1997).

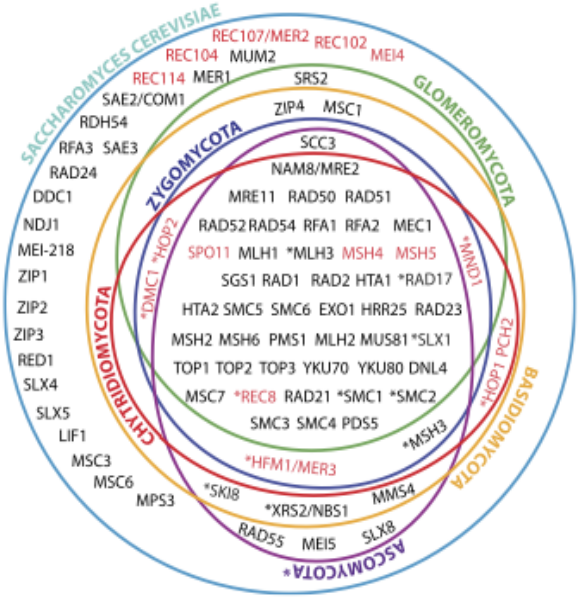

Figure 1: Taken from Halary 2011, a catalog of fungal core meiotic genes in a Venn diagram distinguishing the presence or absence of CMGs which are directly or indirectly involved in the meiotic process of *Saccharomyces cerevisiae*. Taken from Halary et. al. 2011.

Many organisms which have previously been classified as strictly asexual have recently been reported to have cryptic sexual life cycles which are not recognizable or not usually present, unless under stress (Ni et al. 2011). Such stressful conditions can include starvation from essential nutrients, unfavorable oxygen levels, temperatures outside of the thermal neutral zone, etc. The shift from an asexual reproductive cycle to sexual reproduction allows organisms to accelerate adaptation to their environment through shuffling of homologous genes, as well as facilitating beneficial mutations (Zeyl and Bell 1997). A study conducted by Goddard et. al. illustrated the advantages of sexual reproduction over asexual reproduction in a stressful environment in *S. cerevisiae,* done by comparing populations limited to mitosis or meiosis. Results of this study showed an overall increase in fitness for populations limited to meiosis and a static or decrease in fitness for mitosis populations (Goddard et. al. 2005). This investigation focuses on measuring the presence of three CMGs (hop2, mnd1, dmc1) in strain Hef-5 while the

organism is exposed to oxygen. These were selected due to their presence not only in *S. cerevisiae*, but also in *Batrachochytrium dendrobatidis* (phylum Chytridiomycota), a close phylogenetic relative to the strain in question (Hibbet et al. 2007).

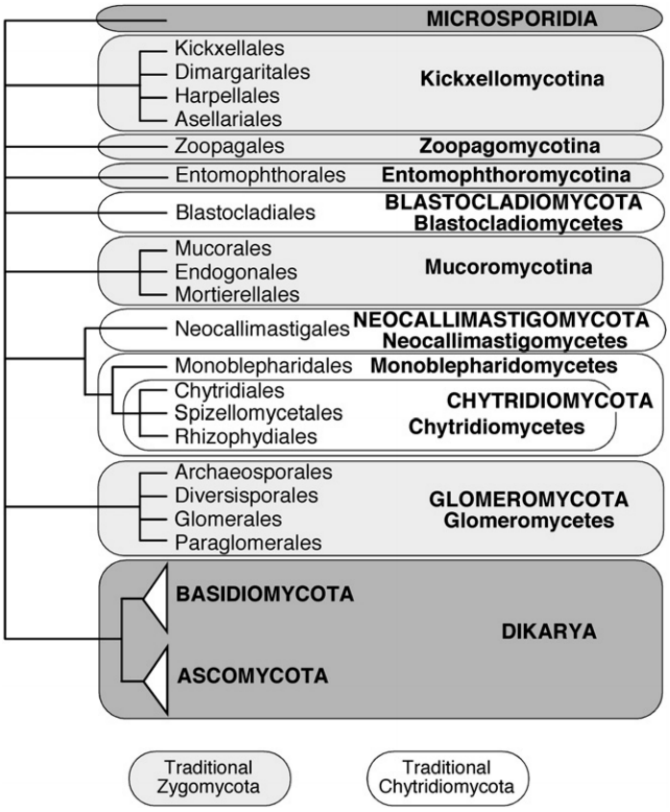

Figure 2: A phylogeny and classification of fungal phyla. Length of branches is not proportional to the genetic distances between phyla. Taken from Hibbet et. al. 2007.

The strong connections between these three genes, and their lack of function outside of meiosis signifies that the presence of such genes in the transcript of an organism could lead to an expressed sexual cycle of the organism. These three proteins all have functions that are essential to recombination, which occurs during prophase 1 of meiosis. A study conducted by Goyon and Lichten observed that stable hDNA appear late in meiotic prophase, shortly before the first cytokinesis (1992). This hDNA appears quickly after double-strand breaks and are formed before the mature crossover molecules are formed. The time between these two structures is a mere 18

minutes. Moreover, the time that it takes for double-strand breaks until the appearance of these mature crossover products is one hour and 12 minutes. With the idea that double-strand breaks initiate meiotic recombination, there is roughly 1 hour that these three genes should be in function (Goyon et al. 1992).

The expression of CMGs Hop2, Mnd1, and Dmc1 is expected to occur while samples of Hef-5 are subjected to prolonged exposure of oxygen for periods up to an hour. Negative controls will be used to compare the expression of the CMGs without oxygen exposure to the samples exposed. The time period in which the CMGs will remain expressed has been thus far unstudied, as well as the concentration of expression.

Methods

To test the idea that a cryptic sexual cycle in the AGF operates in the presence of stress, enrichments were set to follow the transcription of the CMGs. Presence of oxygen in AGF strain Hef-5 was done by inoculating the organism into serum bottles containing 45 mL of bovine rumen fluid media. Each bottle is inoculated with 1 mL of antibiotic solution and 4 mL of fungal sample. The samples are then incubated at 39 degrees Celsius until they reach maximum growth (48-96 hours). Each sample except for the negative control was exposed to oxygen with the use of a filter, to avoid exposure to any microbes in the atmosphere. The color indicated the presence of oxygen in the media, yellow is anoxic, pink oxygenic. The media was kept pink for various specified times (increments of 5 minutes up to one hour). During this time the samples were kept in the 39 degrees Celsius incubator to avoid temperature as an added stress. After all trials are exposed for the allotted time, each is dried and crushed using liquid nitrogen in order to extract RNA. Once the RNA is collected from all samples, cDNA is synthesized to run an RT-qPCR

and melt curve to measure the transcription level of Mnd1, Hop2, and Dmc1 relative to the housekeeping gene GAPDH.

Results

The CMGs Hop2, Mnd1, and Dmc1 were found to have varying time of onset and periods of expression. Hop2 was found to be expressed at the 5-minute mark, followed by Dmc1 at 20 minutes, and Mnd at 25 minutes. With some discrepancy, all three CMGs are expressed from the 25-minute mark up to the full 60-minute trial (Table 1). No expression shown with no exposure to oxygen.

	Expression Present												
CMG	0 min	5 min	10 min	15 min	20 min	25 min	30 min	35 min	40 min	45 min	50 min	55 min	60 min
Hop2	-	-	✓	-	✓	✓	-	✓	-	✓	✓	✓	✓
Mnd1	-	-	-	-	-	✓	✓	✓	✓	✓	✓	✓	✓
Dmc1	-	-	-	-	✓	✓	-	✓	✓	-	✓	✓	-

Table 1: Displays the presence or absence of expression as shown in the RT-qPCR melt curve for all Hef-5 samples when exposed to oxygen for specified increments of time.

Discussion

The results collected suggest that the earliest period that meiosis I could be occurring in AGF strain Hef-5 is around the 20-minute mark after oxygen exposure. This aligns with the time frame seen in the study by Goyon and Lichten, as the gene expression increases within an hour of the onset of the stressor (1992). The concentration of each of the CMGs did not seem to

increase over the time allotted, however, this could be due to their ability to use the same RNA strand to code for multiple proteins over a period of time, or the constant breakdown and construction of new mRNA to keep the concentration the same. The continuous expression of these CMGs aligns well with the time frame for meiosis I in *S. cerevisiae*. The negative control showed no expression for any of the three CMGs, supporting the hypothesis. These results indicate that the AGF strain Hef-5 could have a cryptic sexual cycle under these conditions.

Further continuations of this research include focusing on two specific time frames in which these CMGs are expressed, this will allow more samples to be used, providing further data and interpretation of the expression levels. Including more genera of AGF is also planned for the future. After further trials with Hop2, Mnd1, and Dmc1 are conducted, it would be advantageous to include additional CMGs found in Figure 1 such as a protein that initiates double-strand breaks (Spo11) as well as a protein that is involved in other phases of meiosis (Rec8). These two proteins, Spo11 and Rec8, have been found in all of the listed families mentioned in the Halary study (2011) (further detail on these CMGs found in the appendix). The presence of these genes in the transcriptome of Hef-5 is required for functional meiotic recombination, as their roles include double-strand break catalysis and maintaining chromosomal adhesion during metaphase (Allers 2001 and Buonomo 2000)

Appendix:

Hop2

In the *S. cerevisiae* genome, the Hop2 gene is found on the q arm of chromosome seven. The Hop2 gene is named for its function, **Ho**mologous **P**airing. It is essential to preventing synapsis from initiating between two non-homologous chromosomes (Leu et al. 1998). The gene contains two introns, one on the 3’ end which has canonical splice sites, and the other at the 5’

end with non-canonical splice site. The different splicing between these introns may play a role in meiotic recombination. Both sites have been observed to be efficiently spliced during wild type meiosis (Chan et al. 2014).

Mnd1

In the *S. cerevisiae* genome, the Mnd1 gene is found downstream of the Hop2 gene on the q arm of chromosome seven. The Mnd1 protein is required for recombination and meiotic nuclear division. Mnd1p is required for the stable formation of heteroduplex DNA (hDNA), the combination of two strands from two different DNA molecules. This hDNA is formed during genetic recombination and is essential to this process (Gerton et al. 2002).

Dmc1

In the *S. cerevisiae* genome, the Dmc1 gene is found on the q arm of chromosome five. It has been found that Dmc1 is a meiosis-specific protein that is required for double-strand break repair (Bishop et al. 1992) and homologous chromosome pairing (Roeder et al. 1997).

Hop2 Mnd1 complex

Hop2 has been documented to work with Mnd1 to create a complex which prevents non-homologous pairing, as well as the repair of meiotic double-strand breaks (Tsubouchi et al. 2002). These double strand breaks can be detrimental to the health of the organism if unmended. The heterodimer of Hop2p-Mnd1p stimulates the Dmc1 D-loop activity. This insinuates that the ability of Dmc1 to cause joint molecule formation in the organism is entirely dependent on the Hop2 and Mnd1 complex that is formed (Chan et al. 2014).

Spo11

Spo11 is a meiosis-specific protein which initiates recombination through catalyzing double-strand break formation in the DNA using a transesterification reaction. This process is

required for the pairing of homologous chromosomes as well as the formation of the synaptonemal complex. Without the presence of Spo11, an organism would be incapable of initiating double-strand breaks (Allers 2001). The gene coding for Spo11 is located on the *S. cerevisiae* chromosome eight, being 398 amino acids in length with a molecular weight of 45430.4 Da. Spo11 is included in a protein family with others found in nematodes, fission yeast, and archaebacteria is highly conserved (Keeney 1997).

Rec8

The Rec8 protein is a Recombination protein which is a component of the meiosis-specific sister chromatid cohesion complex. This complex maintains cohesion between chromosomal homologs during meiosis I until the Rec8p is cleaved during the metaphase to anaphase transition (Buonomo 2000). Other than essential roles of Rec8 include the promotion of allelic collisions and prevention of nonspecific interactions between chromosomes (Klein 1999). This protein-coding gene is located on chromosome XVI of *S. cerevisiae* and is 680 amino acids long with a molecular weight of 77178.7 Da.

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