The Honors College at the University of Missouri – Kansas City

Smp1 and Meiosis Activation within Saccharomyces Cerevisiae

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INTRODUCTION

Within living organisms, the pattern in development occurs is of utmost importance. Patterning is crucial to organized and correct development, from mammalian organisms all the way down to fungi and microorganisms. Within yeast specifically, the development of a regular colony adheres to the general pattern of sporulating and non-sporulating cells. The colony initially starts with a thin layer of sporulating cells at the center of the colony, that eventually enlarges until it occupies the top half of the colony (Piccirillo et al., 2015). The bottom half of the colony consists of non-sporulating cells that are differentiated; these are termed feeder cells (Piccirillo et al., 2015). While development of the colony's layers is intriguing to say the least, of equal significance is the regulation of the colony's development, which keeps the process from running longer than necessary or taking place incorrectly.

REVIEW OF LITERATURE

When discussing meiosis activation within saccharomyces cerevisiae, extra-cellular pH is one environmental trait with wide-ranging effects for adaptation and differentiation. (1, 2, 3, 4). The pH outside the cell is primarily responsible for triggering the cell to undergo two fates: either invasive haploid growth or sporulation and, under high pH conditions, meiosis within saccharomyces cerevisiae depends on a regulatory pathway whose main actors are rim101 and ime1 (7, 3, 5, 6, 8, 9). Through this section, I will provide background knowledge on my thesis by answering four essential questions: how rim101 and ime1 work together, how rim101 and smp1 work together, what role do each of the three transcription factors play individually, and finally how do all three of the transcription factors cooperate to promote meiosis. Answering these questions will provide a firm basis from which we can then approach the research

hypothesis of whether or not rim101 activates ime1 through suppression of smp1. (Piccirillo, et al. 2019).

Within the context of meiosis activation of saccharomyces cerevisiae, each of the three aforementioned transcription factors plays an important role and it is crucial to understanding the thesis that we identify what role do each of the three transcription factors play individually. So far, it is understood that Rim101 has nutrient signaling capabilities, when activated, meaning rim101 functions in providing key nutrient signals to the yeast nucleus essential for meiosis initiation. Without ime1, however, there is no activation of meiosis. Ime1 is the master transcriptional activator of meiosis in that multiple nutritional and environmental signals converge upon Ime1 in order for action of meiosis to occur. Finally, although it is not known what specific function smp1 performs in the context of meiosis, it is known that smp1 is a transcriptional repressor and a target of rim101 activation that acts on ime1. Studies show that elevated smp1 expression in rim101Δ (rim101 deleted) mutants inhibits invasive growth and promotes smooth colony morphology (Lamb & Mitchell, 2003). When discussing whether rim101 activates ime1 through suppression of smp1, it is first imperative that we define each of the transcription factors and the roles each one plays in the rim101 – ime1 pathway and within meiosis initiation of saccharomyces cerevisiae.

It is key to a deeper knowledge of the rim101 – ime1 pathway that we first understand how it is that rim101 and ime1 work together, by first looking at what order these two transcription factors activate in then looking at what type of feedback loop the rim101-ime1 pathway works through. Experiments run on the sequential order of activation have shown that rim101 is activated after ime1 and works to turn on ime1. To further investigate what the type of relationship between rim101 and ime1 is, our lab compared the timing of rim101 activation to

the timing of ime1 activity. Response alleles requiring both a basic pH along with rim101 signaling to activate were utilized during this experiment and all strains had the ime1 gene deleted in order to prevent any unwanted side-effects. Results from this test told us that rim101 was activated slightly later in colony development than ime1 (Lamb et al. 2001; Frey et al. 2011). As to what type of feedback loop the rim101-ime1 pathway operates through, our lab hypothesized that a nonautonomous, or outside cell-signaling, positive feedback loop was the answer. We tested this idea through an assay comparing pure to wild type colonies lacking the presence of ime1. It was found that the level of Rim expression in colonies containing ime1 and lacking ime1 was approximately the same in chimeric and pure colonies meaning that ime1 activates rim101 through signals outside the cell instead of within the same cell.

As mentioned earlier, the rim101 transcription factor that functions in regulating ime1, and while that is important, it is equally important we understand how it is that rim101 and smp1 work together by looking at how rim10 acts on smp1 and how smp1 participates in biological activities dependent on rim101 expression. Rim101 controls the expression of a variety of genes, but in regard to smp1, it was assumed rim101 down-regulates, or suppresses, smp1. This assumption was verified through a gene expression analysis which tested for smp1 expression in yeast DNA strains containing and lacking rim101. Results told us that levels of smp1 were higher in strands lacking rim101 as opposed to strands containing rim101, verifying the assumption that rim101 does indeed down-regulate smp1. Even though the explicit function of smp1 is not known, to determine whether some defects in yeast strains lacking rim101 are the result of elevated Smp1 levels, (lamb & Mitchell, 2003) examined whether a mutation causing a deletion of the smp1 gene could suppress any mutant phenotypes having the rim101 gene deleted. Through an epistasis test checking for phenotypic results caused by genetic edits, it was

observed that the smp1 deleted mutation fully suppressed the rim101 deleted mutant defect in invasive growth and partially suppressed the defect in sporulation. It was also observed that the smp1D mutation restored a rough colony morphology to the otherwise smooth rim101D mutant. These observations tell us that smp1 is a key factor in inhibiting invasive and unnecessary growth and also promotes standard colony growth.

Now that we have addressed the roles each transcription factor plays individually, as well as how rim101 and ime1 work together, and how rim101 and smp1 work together, this now sets the stage to examine how all three of the transcription factors cooperate to promote meiosis. To study the relationship among rim101, smp1, and ime1 (Zhao et al. 2018) first monitored the expression levels of rim101 and smp1 in the deletion strains. In DNA strains with rim101 deleted, the protein levels of SMP1 were higher during sporulation than the strains with rim101 in them, however, in smp1 deleted strains, the rim101 protein levels were not significantly changed. Next, (Zhao et al. 2018) created double mutants to then test sporulation efficiencies and ime1 expression. After a 1-day incubation period, the sporulation efficiency of the mutant with both the rim101 and smp1 genes deleted was markedly higher than the rim101 deleted strain, implying that smp1 is an inhibitor of meiosis and must be repressed by rim101 for meiosis to commence. Furthermore, when double mutants were made in order study ime1 expression along with sporulation efficiency, the double mutant with the smp1 gene deleted showed not only a higher sporulation efficiency, but also a greater amount of ime1 expression than the mutant without the smp1 deletion, suggesting that sporulation efficiency and ime1 expression may be correlated. Next, additional experiments were performed to investigate the direct target gene of rim101 and smp1 during meiosis initiation. Although (Zhao et al. 2018) did not detect a direct interaction between rim101 and ime1 or smp1, they discovered that smp1 is highly enriched

upstream of the ime1 promoter transcription site. Results also showed that smp1 could directly bind to the ime1 promoter during mitosis and that the binding was weakened during meiosis. This suggests that smp1 represses ime1 transcription during mitosis by binding to the ime1 promoter and induces ime1 transcription during meiosis by detaching from the ime1 promoter. In rim101 deleted cells, the interaction between Smp1 and the ime1 promoter was much stronger than that in wild type cells, suggesting that Rim101 governs ime1 expression by regulating SMP1 expression during meiosis.

Findings from this and previous papers, are what have laid the groundwork for the Honigberg lab to engage in investigating what conditions un-sporulated "feeder cells" differentiate and which transcription pathways are responsible for colony differentiation. The following research and results will be in attempt to answer the research question of whether rim101 actives ime1 through suppression of smp1 as part of the rim101/alkaline response pathway. The methods to be used to approach this project will be creating a mutant smp1 deleted strain, followed by PCR, with chimera colony formation to soon follow.

METHODS

Streaking and strains

Following selection of the 5117 strain, we took a small sample of the frozen SH5117 strain and struck it out onto 10 milli-liters of YPDA media and incubated for approximately 2 days. We then organized the 5117 colonies from the incubated YPDA media onto a fresh YPDA media plate via patch plating. We then let our 5117-patch plate incubate for 2 days. All strains used in the study (supplementary table 1) were constructed using a W288C background and are prototrophic. Deletion mutants were constructed by designing PCR fragments to replace >90%

of the open reading frame (ORF) with the trp gene. All newly constructed genomic alleles were verified by diagnostic PCR with primers flanking the targeted region (Gray and Honigberg 2001; Gray et al. 2005).

Media Growth Conditions

All colonies in this study were grown as spot colonies inoculated with 0.5 µl of a 2 × 108 cells/ml suspension in water on Sp2% medium (2% potassium acetate, 0.5% yeast extract, 2% agar, pH 7.0) (Piccirillo et al. 2015). A total of 2 patch plates containing ten SH5117 colonies were grown in a row-arrangement with all colonies equally spaced and ~0.5 cm from one another. For my three transformations specifically, all inoculations contained an equal volume of cells from the 5117 strain.

Genotyping colonies

Following the two-day incubation period for the patch plate created after streaking the colonies, we then replica plate the patch plate onto Ade, Arg, Can, His, Leu, Lys, Trp, Ura, and YPG media and let incubate for 2-3 days. The 5117 strain is expected to contain: Ade, Arg, Leu, Lys, Ura, and YPG and be missing: Can, His, Trp, and Ura.

Designing a PCR fragment

To design the appropriate primer for my strain (SH5117), we utilized a gene editing program by which we were able to get 40 bases homologous to the gene and 20 bases homologous to our marker (trp). From Integrated DNA Technologies, we were then able to order the 5’ and 3’ smp1 diagnostic primers (specifications listed in supplementary table 2) as well as the 5’ and 3’ smp1 universal primers (specifications listed in supplementary table 2). Upon arrival of our primers, we then prepped them for PCR by adding H2O equal to the volume of the

primer, but in microliters, then diluting the primer/H2O mix 100x. Following the dilution, we iced then inserted our primers into the mini-cycler and ran a custom program specific to my PCR primers. We then formulated the cocktail to insert the primers into, using 240 microliters of 50% PEG, 36 microliters of lithium acetate, ssDNA, 24 microliters of H2O, and 10 microliters of my fragment. We preformed electrophoresis on the fragments soon after, then loaded eight tubes filled with 60 microliters of the cocktail-primer mixture for PCR. We chose the trp marker because the parent strain of the mutant is trp deleted, therefore the trp marker will be the simplest to insert. That way if properly inserted, we will be able to successfully grow the transformed fragment on trp- media (media lacking the trp gene).

Transformation

Adapted from the high efficiency transformation protocol by (Gietz, R.D. and R.A Woods 2002), we first inoculated 5mL SC liquid with cells then let the mixture sit overnight at 30°C. In the morning, we inoculated fresh SC liquid with the overnight culture for a final concentration of 5e6 cells/mL. We then grew the cells to a concentration of 2e7 cells/mL. Because we needed 1e8 cells for each transformation, we adjusted the volume accordingly. We then harvested and washed the cells with 1mL ddH2O before resuspending the cells in 1mL ddH2O. We then took 1e8 cells, spun them down, then resuspended them in 360 microliters of the cocktail. The cocktail was created using 240 microliters of 50% PEG, 36 microliters of lithium acetate, ssDNA, 24 microliters of H2O, and 10 microliters of my fragment. Heat shock then plate appropriate dilutions of the cell suspension onto SC selection medium. Dilute 10 microliters of the suspension into 1.0 mL of water and plate 10 and 100 microliters samples onto two plates each. The 10 microliter samples should be pipetted directly into 100 microliters puddles of sterile water on the SC selection medium. We then plated and incubated the mixture

for 3-4 days. We then calculated the transformation efficiency using the following equation: Transformation Efficiency = 500 × 1000 (plating factor) × 10 (plasmid factor) ×1 (cells/transformation × 108).

Diagnostic PCR/Electrophoresis

For electrophoresis, we performed a 10x dilution of 5X TBE in a 1000mL cylinder, added 1g agarose and 7 microliters of stain, then microwaved the mixture to then create the solution which once cooled will become our gel. Once the gel cooled, we inserted it into the electrophoresis tray. We then loaded 2 microliters of marker into the gel then proceeded to combine 5 microliters of my fragment with 2 microliters of dye then loaded each fragment into the gel until I had filled about twenty slots. We then let the gel run for about one hour then checked the results.

RESULTS

In order to test the hypothesis of whether SMP is a repressor, we needed to design a transformed SMP strain using haploid yeast strains that were trpΔ with a smp1Δ background, genetic transformation utilizing homologous end recombination was used to knockout smp1 and replace it with a Trp marker. A transformation refers to the uptake of DNA by a cell, causing a change in its phenotype (ksu). Since the efficiency of a fragment inserting into the genome is innately low, we needed to select a marker that will prove the easiest to insert. After creating the transformed smp strain, we had to verify insertion of the trp fragment, and this is done via electrophoresis as seen in figure 1.

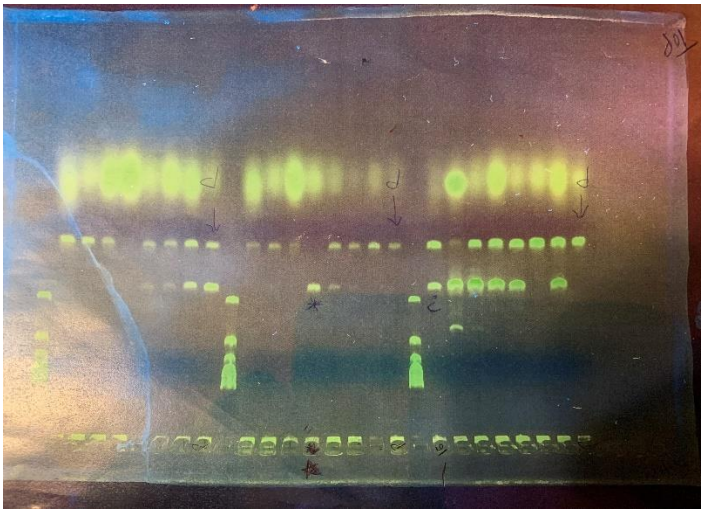

Figure 1: This electrophoresis gel is composed 30 wells total. 21 of these wells are filed with an aliquot of the transformed DNA strand, 3 of the wells contain parent strains of the transformed DNA strand, and another 3 of these wells are reserved for our DNA ladder, also known as the kilobase marker. The remaining 3 wells of the gel were left blank.

Because of our gene map indicated by figure 2, we were able to identify if the trp fragment had inserted using our kilobase marker.

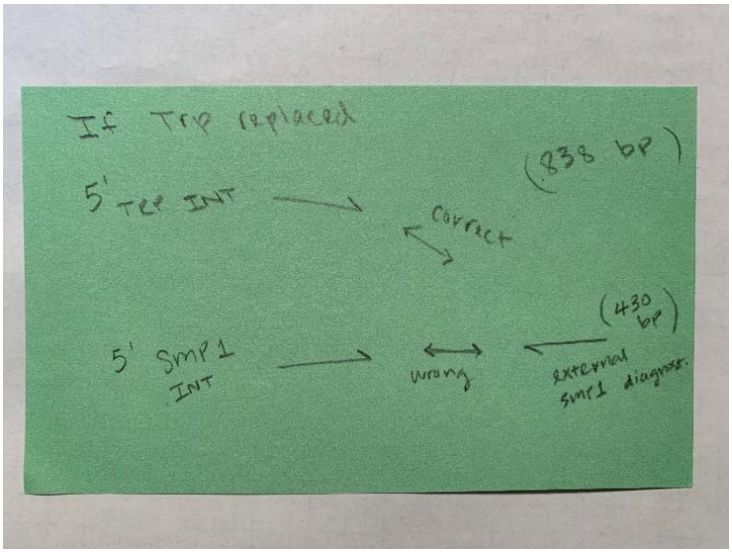

Figure 2: The gene map shows us that, upon insertion of the trp fragment, the transformed DNA should measure out to be 838 base pairs. If the trp fragment does not insert however, the DNA stand will measure out to be 434 base pairs, the same length as the parent strain.

We ran into an issue with the electrophoresis strains, in which we saw two different lengths of bands on all but one of the strands, number 11. After consultation, a retest via electrophoresis of one mixed colony as well as colony number 11 was in order and we chose mixed colony number 15. The results of the second round of electrophoresis are shown below in figure 3.

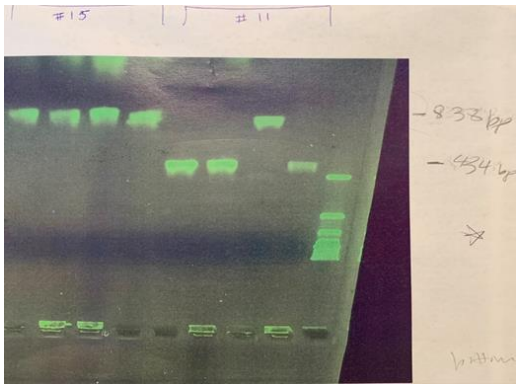

Figure 3: As seen here on the 11-well electrophoresis gel colonies 11 and 15 each had 4 wells apiece. Mixed colony number 15 had strains all equal in size to the 838 bp marker outlined to the right of figure 3, meaning that the trp fragment did insert into the smp plasmid and is therefore of interest to us.

Additionally, colony number 11 had three different wells with sizes approximately near our 434 bp marker along with one well at our 838 bp target point. Based off the retest, we identified both colonies to be successfully transformed with the trp fragment we inserted, and further diagnostic PCR confirmed our assumptions. We now have our strains stocked and stored in our yeast databases for further testing, as seen in (supplementary table 1).

DISCUSSION

Rationale to methods

When determining a yeast strain to streak out, our laboratory kept three criteria in mind that guided our selection: is the yeast strain rim 101 deleted, is the strain ime1 deleted and, lastly, is the strain trp deleted? We wanted a strain deleted for rim101 because rim 101 represses smp1 which would inhibit our ability to identify whether our trp fragment implanted from our transformation. There was a need for the strain to be ime1 deleted as well because at this point, we were not looking to see activity of ime1 but rather the confirmation of our trp fragment. Finally, we were looking for a strand that had the trp gene deleted because we are going to insert a trp marker as part of our transformation procedure then grow the transformed yeast strain on trp deleted media. Taking these considerations into mind, we selected strain 5117 because met all the required criteria. We patch colonies in order to organize the single colonies grown from the 5117-yeast strain into a square shaped arrangement which will serve to be reference plate for all future tests performed on the 5117 strain. In our lab, single colonies are of utmost importance and although we could test single colonies directly from the streak plate, our process of patching gives us approximately 4 by 4 rows of square patches which we then sample from when running any further experiments. Genotyping is the process of determining differences in the genetic make-up (genotype) of an individual yeast strain by examining the strain's DNA sequence using biological assays and comparing it to another strain's sequence or a reference sequence. It was necessary to perform a genotyping of the 5117-yeast strain to verify that the strand possesses the qualities we expect it to have.

Next Steps

Future research on this hypothesis will require the creation of several more smp1 deleted strains in a rim101 background, along with the creation of chimera colony assays using the LacZ operon as part of the signal strains due to its ability to turn blue if the ime1 gene, which we insert into the LacZ operon, is activated. If smp1 is a crucial component in the regulation of ime1, we would expect future chimera colony assays to turn blue in the presence and absence of smp1. In its presence, smp1 is repressed by rim101 and prevented from repressing ime1 activity, hence activation. In its absence, smp1 is removed, therefore it cannot inhibit ime1 activity, hence activation. This proposed assay would, of course, need to be coupled with the results of previous assays performed by Abbigail McCune, a former researcher under the Honigberg lab, in order to draw meaningful conclusions on whether smp1 is the factor through which rim101 acts on to activate ime1.

If both past and future assays conclude that Rim101 does repress smp1 to allow the expression of Ime1, then future assays w to determine will be created to see whether the repression of smp1 is done within or outside of the same cell. Theoretically, we would want a signal strain lacking a wildtype Rim101 copy but possessing wildtype smp1 alongside Ime1-LacZ in order to simulate normal environmental signaling conditions. The two signal strains would also need to possess wildtype Nrg1 copies, but one would need to have Rim101, and the other would need rim101Δ in order for us to see whether or not a deletion of rim101 causes the yeast cell ime1 to get its activation signal from another cell with rim101 already present. Within the Rim101 signal strain chimeric colony, blue coloration, as a result of ime1 activation within the LacZ operon, would indicate the wildtype copies signal moving between neighboring cells and repressing Nrg1 not only within their individual cell. White coloration, as a result of no ime1

activation within the LacZ operon, would indicate that the Rim101 of the signal strain failed to reach the Nrg1 of the neighboring reporter strain equating to cell-autonomous signaling.

A few shortcomings led to my not being able to complete chimeric colony assays over the year in which I worked on this project. First among them would be the fact that we use peer-to-peer teaching when learning a new procedure. The lab assistant teaching me the procedure had a very full schedule in the fall, as did I, which lead to many days and weeks going by without any of us being able to commit 4-5 hours to lab procedures, which in turn extend the time at which I would be able ascertain results. Another area that slowed me down was the incubation time between when I finished the procedure and when I was able to see results. The incubation time for our media is usually 2-3 days, and there were many times during the lifetime of this project that I made mistakes in either over-diluting a solution or adding too much of a compound to mixture. All of these mishaps throughout my work on this project definitely slowed my progression rate and, therefore, led to me accomplishing less than what I had initially anticipated.

Overall, there is much work still to be done with the Rim101-smp1 assays. If smp1 is associated with the Rim101-Ime1 signaling pathway, there will be further questions on how this signal travels. If smp1 is not found to be the missing piece in Rim101 mediated activation of Ime1, then there will be questions on just what this missing factor is. Either way, there is a rather promising future for this research project as it will be passed on to the next generation of undergraduate researchers within the Honigberg laboratory.

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Strain Specifications

(Supplementary Table 1)

SH 6348

MAT @ ade2 can1:ADE2:CAN1 his3-11, 15 leu2-3. 112 trp1-3'del ura3-1 ime1D rim101D:LEU smp1D::TRP

SH 5117 transformed with smp1D::TRP fragment. Isolate 1 of 2

SH 6349

MAT @ ade2 can1:ADE2:CAN1 his3-11, 15 leu2-3. 112 trp1-3'del ura3-1 ime1D rim101D:LEU smp1D::TRP

SH 5117 transformed with smp1D::TRP fragment. Isolate 2 of 2

SH 5117

MAT @ ade2 can1:ADE2:CAN1 his3-11, 15 leu2-3. 112 trp1-3'del ura3-1 ime1D rim101D:LEU

SH 4547 transformed with 5'URA univ(pS305 LEU) URA-3'univ fragment

Primer Specifications

(Supplementary Table 2)

Sequence – 5 SMP1 INT diag 100 nmole

DNA Oligo, 20 Bases

5’ – AAT ATT CCG AAG GCC ACA AA – 3’

Properties:

Melting temp (50nM NaCl): 52.2°C

GC Content: 40.0%

Molecular Wt: 6,103 kg/kmol

Amount of Oligo: 108.7 nmoles

Sequence – SMP1 diag - 3 100 nmole

DNA Oligo, 20 Bases

5’ – CTT AAA TGC GCC AAC ATA CA – 3’

Properties:

Melting temp (50nM NaCl): 53.3°C

GC Content: 45.0%

Molecular Wt: 6,039 kg/kmol

Amount of Oligo: 107 nmoles

Sequence – 5’ SMP1 univ 100 nmole

DNA Oligo, 60 Bases

5’ – AAT CGT ACA GTT ACT TTC ATA AAG CGA AAA GCA GGA CTA TTA ACT ATG CGG CAT CAG AGC – 3’

Properties:

Melting temp (50nM NaCl): 67.1°C

GC Content: 40.0%

Molecular Wt: 18,508.1 kg/kmol

Sequence – 5’ SMP1 univ 100 nmole

DNA Oligo, 60 Bases

5’ – TGA ACT AGC TAC CGA GGA AGG AAA AAC CAC AAT AGA TGA ACC TGA TGC GGT ATT TTC TCC – 3’

Properties:

Melting temp (50nM NaCl): 68.1°C

GC Content: 43.3%

Molecular Wt: 18,509.1 kg/kmol