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Topics in Yeast Sporulation

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Abstract of the Thesis

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Yeast sporulation is the process by which yeast cells under limiting nutrient conditions are able to alter their nuclear division cycle from mitotic division to meiotic division, resulting in development of up to 4 spores per diploid cell. Spores are a hardy cell type that can survive until nutrient conditions improve. Under particularly severe conditions, sporulating cells only produce 2 spores, in an arrangement known as a non-sister dyad (NSD). NSDs contain the two spores with the youngest spindle pole bodies (SPBs), which are produced in meiosis II. The SPBs are the central nucleating point of prospore membranes. A scaffolding component of the SPB, Nud1, has been shown to be phosphorylated at several sites, and be integral to the proper selection of NSD's during sporulation. I sought to test the hypothesis that Nud1 accumulates phosphorylations over time and this allows the cell to recognize the younger SPB's by their relative lack of phosphorylation. In an additional experiment we investigated the unknown sporulation function of four related GATA transcription factors induced late in sporulation.

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List of Abbreviations

Hyh	Hygromycin
MEN	Mitotic exit network
MOP	Meiotic outer plaque
Nat	Nourseothricin
NSD	Non sister dyad
PSM	Prospore membrane
SPB	Spindle pole body
TS	Temperature sensitive
WT	Wild Type

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Chapter 1

INTRODUCTION

1.1 Sporulation

When exposed to limited nutrient conditions, diploid cells of the yeast *Saccharomyces cerevisiae* have the ability to significantly alter their metabolic pathways and switch from normal mitotic "budding" division to a meiotic process, known as sporulation, which results in the formation of up to four spores (Freese, 1982). These spores are more durable than vegetative yeast cells, and can remain dormant until the spores encounter more favorable nutrient conditions for resuming vegetative growth (Neiman, 2011). Induction of sporulation has been shown to be dependent on many factors, notably nitrogen starvation in the presence of a poor carbon source (Freese, 1982). These requirements can be utilized experimentally to induce sporulation by culturing cells in appropriate sporulation media, typically using either acetate or glycerol as the carbon source.

The induction of sporulation is regulated by the transcription factor Ime1, which is sufficient to induce the entire process of sporulation in wild type cells (Kassir et al, 1988). Ime1 induces the expression of numerous genes, known as "early" sporulation genes, which regulate the initial stages of meiosis as well as activate the next wave of genes, the "middle" genes (Kassir et al, 1998). These "middle" genes are activated by the transcription factor Ndt80 and initiate meiotic divisions and the beginning of spore assembly (Hepworth et al, 1998). After the middle genes, the "mid-late" genes are induced, which are less well understood, but are known to be involved in the later

stages of sporulation, such as spore wall assembly. Finally, the “late” genes are induced, whose functions in sporulation have not been clearly defined (Neiman, 2011).

In terms of the mechanics of sporulation, the first key step is the *de novo* formation of a prospore membrane (PSM) around each of the haploid nuclei produced by meiosis (Neiman, 1998). The formation of these new membranes is nucleated by the spindle pole body (SPB) (Knop & Strasser, 2000). The SPB is the *S. cerevisiae* equivalent of the centriole, and normally functions as the microtubule organizing center of the cell (Jasperson, 2004). The SPB is situated in the nuclear membrane and has distinct nuclear and cytoplasmic faces, which are known as “plaques” (Muller et al. 2005). SPBs are complex structures which contain a large number of essential proteins, as illustrated in Figure 1. The core of the SPB is formed by a lattice of Spc42 trimers connected to the coiled-coil proteins Spc110 and Spc29 at the Spc42 N-terminus (Jasperson & Winey, 2004). These latter two coiled-coil proteins interact with each other through calmodulin (Cmd1) (Spang et al, 1996). Spc110 is also connected to the inner plaque, where it associates with Spc98, which is directly involved in microtubule dynamics, through its ability to bind γ-tubulin (Kilmartin et al, 1996). The C-terminus of Spc42 faces the outer plaque and binds to Cnm67, which in turn binds to Nud1 (Adams & Kilmartin, 1997). Nud1 is also bound to Spc72, which interacts with the cytoplasmic γ-tubulin binding complex (Jasperson & Winey, 2004).

During the process of meiosis, the mother cell SPB undergoes two duplication events, at the beginning of meiosis I and just before meiosis II, in order to produce the four SPBs required for meiosis (Moens & Rappaport, 1971). During the second duplication event, the protein components of the SPB begin to be altered, resulting in a

much larger cytoplasmic plaque, which is referred to as the meiosis II outer plaque (MOP) (Knop & Strasser, 2000). The MOP replaces Spc72, which connects the core SPB to the γ-tubulin binding complex, with a complex of proteins, shown in Figure 2, that are utilized in the process of the *de-novo* formation of the prospore membrane (Knop & Strasser, 2000). This signifies the shift in SPB function during sporulation from microtubule formation to membrane formation. The key MOP proteins are Mpc54, Mpc70, and Spo74; which are joined by Ady4, which is a secondary component needed for stability (Knop & Strasser, 2000)(Nickas et al, 2003). Mpc54 and Mpc70 interact directly with Nud1, through their C-termini, in the place of the displaced Spc72 (Knop & Strasser, 2000). Spo74 is also thought to bind to both Mpc54 and Mpc70 at their respective N-termini (Nickas, 2003). The core components on the assembled SPB promote PSM formation through promoting vesicle docking and fusion, serving as a nucleation point for *de novo* membrane formation (Mathieson et al, 2010) (Reidell et al, 2005). All three proteins have been shown to be required for proper MOP formation and ensuing PSM formation (Nickas et al, 2003). Without a proper MOP, yeast cells are unable to create the PSM, and spores do not form (Mathieson et al, 2010). It is interesting to note however, that Nud1 is not necessary for PSM formation (Gordon et al, 2006).

1.2 Dyad Formation

Under most sporulation conditions, cells will form four haploid spores, one inheriting each of the four nuclei produced by meiosis. However, under particularly conditions such as heat shock combined with limited nutrients, or the removal of all

nutrients after the induction of sporulations, *S. cerevisiae* cells will sometimes only form two spores instead of the usual four (Davidow et al, 1980)(Srivastrava et al, 1983). In wild type cells, these two spores are almost always “non-sister dyads” (NSDs), which refers to the fact that the nuclei within the two spores contain homologous chromosomes, rather than sister chromatids (Davidow et al, 1980). This response may help promote genetic diversity under difficult conditions where all four possible spores cannot be formed (Taxis, 2005). This fitness advantage is further increased due to linkage of the mating type gene to the centromere of Chromosome III. This ensures that the two spores in an NSD are usually of opposite mating type, enabling the haploid spores to mate with each other after germination (Jensen et al, 1983). Cells primarily judge how many spores to create by sensing the levels of available carbon and in instances of carbon depletion they will form NSD’s (Davidow et al. 1980). Further work indicated that the cells sense a metabolic intermediate of the glyoxylate pathway as opposed to directly detecting carbon levels (Nickas, 2004). One piece of evidence for this model is that cells sporulated in glycerol as a carbon source produce NSDs at high frequency (Nickas et al, 2004). This suggests that because glycerol is not metabolized through the glyoxylate pathway, the cell behaves as though carbon is limiting, even when the amount of glycerol present is considerable (Nickas et al, 2004). However, the precise identity of the metabolite in question, and the precise pathway by which it results in SPB alteration is still largely unknown. The same study also showed that NSD formation is regulated by the SPB, as the SPBs of the two non-spore forming nuclei were deficient for Mpc70p and Spo74p, and thus physically incapable of forming PSMs (Nickas et al, 2004).

Another intriguing aspect of NSD formation is that the two haploid nuclei that are chosen to mature into spores are the ones that possess the younger, “daughter” SPBs formed at entry into meiosis II (Taxis et al. 2005) (Nickas et al, 2004). SPBs are duplicated conservatively; which means that during SPB duplication the older, “mother” SPB remains relatively unchanged, and is used as a template to assemble a new daughter SPB (Jasperson et al, 2004). The cell’s ability to selectively choose the younger SPBs indicates that the cell can not only sense when it should only form two spores, but it can also sense which two SPBs to use and which to abandon. This idea of age sensing is further supported by data on cells that produce either 1 or 3 mature spores (monads and triads). These data show that 96% of cells with 1 spore chose a “young” SPB over the two “older” SPBs. Most strikingly, in triads, 99% of cells ignored the oldest SPB from the original mother cell (Taxis et al. 2005). So, even when the cell isn’t forming dyads, it is still biased towards using the younger SPBs for PSM assembly. How the cell is able to determine the relative ages of the SPBs in order to discriminate between them is unknown; but some evidence suggests a role for Nud1 (Gordon et al, 2012).

Nud1 is a scaffolding protein of both the mitotic and meiotic SPB, located in the outer plaque. *NUD1* encodes an 851 amino acid long protein that contains multiple leucine rich repeats on the C-terminal half of the protein (SGD). Nud1 is phosphorylated and 51 different phosphorylation sites have been identified on the protein (Keck et al. 2011). The main kinase that phosphorylates Nud1 is thought to be Cdc15, which phosphorylates Nud1 at multiple sites during mitosis as a part of the mitotic exit network (MEN) (Rock et al, 2013).

The MEN is an important signaling cascade that begins during anaphase of mitosis and helps the cell properly exit from mitotic processes (Grunenberg et al, 2000). The key components of the MEN are assembled on the SPB through interactions with Nud1, which acts as a scaffold. Tem1 and Cdc5 recruit Cdc15 to the SPB, where it phosphorylates and activates Dbf2 and Mob1, which bind together to form an active kinase and then activate the phosphatase Cdc14 to trigger exit from mitosis (Shou et al, 1999). However, recent studies have shown that Nud1 does not simply act as a scaffold for the MEN, but is actively involved in the signaling process. Nud1 is first phosphorylated by Cdc15 at (at least) 3 specific sites, S53, S63, and T78. These phosphorylated residues are recognized by Mob1 and Dbf2, which allows them to bind Nud1 and the SPB in proximity to each other and to Cdc15, which then phosphorylates them and allows them to complex with each other (Mah et al, 2001). Thus Nud1 shows that it can have a dynamic function based on its phosphorylation state, while remaining stationary as a SPB scaffold (Rock et al, 2013). Another study showed that Nud1 probably also plays a role in regulating the age dependent inheritance of SPBs in mitosis (Hotz, et al. 2012). In mitosis SPBs are segregated according to age, so that the daughter cell always receives the older SPB (Yoder et al, 2003). This is mediated by the asymmetry of the SPB half bridge protein, Kar9. The younger SPB loses Kar9 during metaphase due to an increase in sumoylation and phosphorylation of the Kar9 on the SPB. This is dependent on a properly functioning Nud1, which triggers modification of Kar9 through the MEN machinery. (Hotz, et al. 2012). Unfortunately, the exact mechanism by which Nud1 selectively targets the new SPBs Kar9 is not known.

Nud1's dynamic roles in the SPB and mitosis raise questions about what additional functions Nud1 may have in meiosis. Some evidence suggests that Nud1 may be part of the system by which cells are able to differentiate the age of SPB's during sporulation (Gordon, et al. 2012). Using a temperature sensitive (ts) mutant of Nud1, Nud1-2, Gordon et al. (2012) showed that under NSD-inducing conditions, at the restrictive temperature for *nud1-2* (34^{o}C), spores were chosen almost completely statistically randomly (33% NSD), as opposed to wild type rates of 96% NSD formation. This suggests that Nud1 is necessary for recognition of daughter NSDs during sporulation.

One possibility for how Nud1 could allow the cell to distinguish younger and older SPBs would be that Nud1 accumulates phosphorylations at a steady rate over the course of meiosis, which can then be sensed by the cell in order to determine age; i.e. the SPB with the more phosphorylated Nud1 is older. This idea is consistent with multiple observations, such as: the abundant phosphorylation sites on Nud1 without any identifiable function (Keck et al. 2011), the ability of phosphorylated Nud1 to recruit and bind specific proteins that recognize phosphorylation sites (Mah et al, 2001), and the function of Nud1 in mediating age dependent segregation of SPB's in mitosis (Taxis et al, 2005). By experimentally challenging this hypothesis it could be possible gain more insight into Nud1's functions as well as the intriguing process by which the cell can determine the relative age of its parts.

1.3 Potential Role of GATA Transcription Factors in Spore Wall Assembly

Late in sporulation, after PSM formation is complete and nuclei are fully enclosed, the last major step of sporulation can begin; the assembly of the spore wall (Neiman, 2011). The spore wall is structurally different from the cell wall of vegetative cells and is also synthesized *de novo*, like the PSM (Neiman, 2011). The main components of the vegetative cell wall are layers of: β-1,3 glucan chains, mannan, and chitin; which are cross linked to each other by varying means (Lesage & Bussey, 2006). The spore wall also contains these components, except the positions of the β-1,3 glucan and mannan layers are switched (Kreger-Van Rij, 1978). The main difference between the spore wall and the vegetative cell wall is the addition of two additional layers outside of the three vegetative layers; these layers are composed of chitosan and dityrosine (Briza et al, 1988 & 1990). The spore wall layers are deposited in order, starting with the innermost mannan layer, and followed by the glucan layer, the chitosan layer, and finally the dityrosine layer (Tachikawa et al, 2001).

There are multiple waves of genes that are induced during sporulation that contribute to its progression. While the transcription factors regulating early and middle genes are well characterized, little is known about how the late genes are activated at the end of sporulation. These classifications of genes are based on time course analyses of protein and mRNA in sporulating yeast, in order to determine the levels of each gene's transcript over the course of sporulation (Chu, et al. 1998) (Brar, et al. 2011). These large scale screens identified numerous genes that had no described meiotic function but are tightly regulated and expressed only at specific times during sporulation. Four of these genes, *GAT3*, *GAT4*, *SRD1*, and *ECM23* were determined

by the screens to encode transcripts induced by *NDT80* as middle genes, but translated late in sporulation, around the end of meiosis II (Chu, et al. 1998) (Brar, et al. 2011).

All four of the genes belong to the GATA family of transcription factors, and share sequence homology with each other. They can be further separated into two pairs of homologs with extremely high sequence conservation; Gat3 and Gat4, which have 70% sequence conservation at the protein level; and Srd1 and Ecm23, which have 79% sequence conservation at the protein level. The conservation between the other combinations is between 46% and 52%. All four of these proteins appear to have similar DNA binding specificity as have all been shown to bind to the sequence AGATCT (Badis, et al. 2008). All four of these proteins are specific to the class *Saccharomycetes*, and Gat3 and Gat4 are specific just to the genus *Saccharomyces.* There is not much known about the functions of any of the proteins. Ecm23 is implicated in filamentous hyphae growth (Canizares, 2002), Srd1 has been shown to be involved with RNA processing of the Rrp1 gene (Fabian, et al. 1990), and little is known about the function of Gat4. Gat3 is involved with selecting the best nitrogen source for the cell to use, and has also been implicated in RNA processing through interactions with Pdr1, Msn4 and Fhl1 (Banerjee and Zhang, 2003). This at least suggests some tentative possible connection between Gat3 and sporulation, as Gat3 could be involved in preparing the cell to resume vegetative growth and nitrogen incorporation after the spores are released; but that is purely speculative.

The first direct experimental evidence that GATA family may play a role in sporulation came from a spore wall defect screen that found that a deletion mutant of *gat4* caused slight spore wall defects (Lin, et al. 2013). A *gat3*Δ *gat4*Δ double mutant

produced spores with reduced levels of dityrosine as well as increased ether sensitivity (Lin, et al. 2013). The fact that the double knockout further enhanced the *gat4*Δ phenotype indicates that Gat3 and Gat4 are both involved with proper spore wall formation, and are probably redundant with each other. The GATA family's known function as transcription factors, combined with nuclear localization of at least Gat4 during sporulation (Lam et al, 2014), suggest that Gat3 and Gat4 serve to promote spore wall formation through affecting the expression of sporulation genes; however, the exact mechanism by which this happens is still uncertain. Due to the similarly conserved natures of Ecm23 and Srd1, it stands to reason that these may also contribute to spore wall formation in a similar manner to Gat3 and Gat4. If so, and these proteins do make up two redundant pairs, investigating the quadruple knockout may lead to a much more pronounced sporulation phenotype, which could assist in ascertaining the mechanism by which these genes act.

Chapter 2

MATERIALS AND METHODS

2.1 Nud1 Project

2.1.1 PCR

Primers PS1 and PS3 were used to amplify the Nud1-2 gene off of the pRS314 Nud1-2 plasmid (Gruneberg, 2000), which contains an *kanMX6* marker, using a PCR mix containing: 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl each primer at 10µM, 1µl plasmid (at 347ng/µL) and .5µl Phusion polymerase. The reaction was run with an annealing temperature of 57.5ºC and an extension time of 2:15 for 35 cycles. The resulting product was tested by gel electrophoresis against the known size of the target fragment. The expected fragment was 2150bp, but unfortunately no amplification products of this size were observed.

Primers PS2 and ANO410 were used to amplify the YFP gene and a 200bp overhang of the end of the Nud1 gene from the *NUD1::YFP* strain MND44 (Tachikawa, 2001). This was performed using 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl each primer at 10µM, 1µl purified MND44 genomic DNA (347ng/µL) the reaction used an annealing temperature of 59ºC and a time of 65 seconds for 35 cycles. Gel electrophoresis showed that the product had the expected size (1030bp).

If both of the PCR's were successful, the two fragments would have been combined using Gibson assembly in order to create a plasmid consisting of the Nud1-2 gene with a YFP reporter and a kanMX6 selectable marker with a pUC19 backbone.

2.2 GATA Project

2.2.1 PCR

All the primers used and sequences are listed in Table 2. Primers PS15 and PS16 were used to amplify the antibiotic resistance gene *HphMX4*, from the plasmid pAG32 (Goldstein et al, 1999) with 50bp overhangs that are complementary to the -200bp upstream of ECM23 and also 50bp from the stop codon. This was done with a PCR mix containing: 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl of 10 µM primer, 1µl of 424ng/µl plasmid, and 0.5µl Phusion polymerase. The reaction was run with an annealing temperature of 57.5ºC and an extension time of 90 seconds for 35 cycles. Gel electrophoresis showed that the product had the expected size (1250bp).

Primers PS13 and PS14 were used to amplify the antibiotic resistance gene *natMX4* from the plasmid pAG35 (Goldstein et al, 1999) with 50bp overhangs that are complementary to the -200bp upstream of *SRD1* and also 50bp from the stop codon. This was done with a PCR mix containing: 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl 10 µM primer, 1µl 208ng/µl plasmid, and .5µl Phusion polymerase. The reaction was run with an annealing temperature of 57.5ºC and an extension time of 75 seconds for 35 cycles. Gel electrophoresis showed that the product had the expected size (950bp).

Primers PS11 and PS12 were used to amplify the *gat4ΔkanMX6* gene from genomic DNA of the yeast strain, AN522 (Lin et al. 2013) while also containing a 50bp overhang with the plasmid pUC19, using a PCR mix containing: 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl each µM primer, 1µl 279ng/µl purified AN522 genomic DNA, and .5µl Phusion polymerase. The reaction was run with an annealing temperature of

58.6ºC and an extension time of 60 seconds for 35 cycles. Gel electrophoresis showed that the product had the expected size (975bp).

Primers PS9 and PS10 were used to amplify the *gat3ΔHIS3MX6* gene from genomic DNA prepared from the yeast strain AN522 (Lin et al. 2013) while also containing a 50bp overhang with the plasmid pUC19, using a PCR mix containing: 10 µl HF buffer, 4 µl 2.5 µM dNTPS, 2.5µl 10µl µM primer, 1µl 226ng/µl purified AN522 genomic DNA, and .5µl Phusion polymerase. The reaction was run with an annealing temperature of 58.6ºC and an extension time of 60 seconds for 35 cycles. Gel electrophoresis showed that the product had the expected size (975bp).

2.2.2 Gibson Assembly

Plasmid pUC19 was digested with the restriction enzyme SbfI and the *gat3ΔHIS3MX6* and *gat4ΔkanMX*4 PCR products were separately incorporated into the vector using the NEB Gibson Assembly kit. Two *gat4* transformants and three *gat3* transformants were obtained from the inserts. All of the clones for each insert were miniprepped using the Roche High Pure Plasmid Isolation Kit and the candidates were digested with PstI and analyzed by gel electrophoresis, for proper incorporation of the two fragments, which has an expected size of 4.1kb. Unfortunately, this was unsuccessful, as only very faint bands around 2.5kb and under 500kb were found.

2.2.3 Transformations

The *ecm23ΔhphMX4* and *srd1ΔnatMX4* PCR products were transformed separately into AN117-4B, AN117-16D, A14134, and A14135. AN117-4B and A14134

are *MAT**a***, while AN117-16D and A14135 are *MAT*□. The A14134, and A14135 strains contain an estradiol-inducible *NDT80* gene. Transformations were performed by first incubating the cells at 30ºC in YPD on a roller overnight, then diluting the cells 1:50 in 50mL of YPD and placing them on a 30ºC shaker for 3hrs. The cells were then spun down and resuspended in 150µl of 0.1M LiAc/ 1xTE and separated into a control and 2 experimental tubes for each. 700µl of 40%PEG/0.1M LiAc/1xTE was then added to each tube as well as 10µl of 5µg/µl boiled carrier DNA and 10µl of designated PCR product; 428 ng/µl for *ecm23* and 319 ng/µl for *srd1*. The tubes were then incubated at 30ºC for 35 minutes, and 42ºC for 20 minutes. The cells were then spun down and resuspended in 1ml YPD on the 30ºC shaker for 2 hours, then spun down again, resuspended in 150µl YPD, plated on their respective selective media, and incubated at 30ºC. After 3 days the plates were examined and any colonies on the experimental plates were patched onto a new selective medium plate and incubated at 30ºC overnight. The resulting candidates were tested by colony PCR. To prepare the template for the colony PCR, 0.1 µl of .02M NaOH was added to cells gathered with a pipette tip in order to lyse the cells while heating for 5 minutes at 100 ºC. The cells were then allowed to cool and then spun down and resuspended in TE. PCR was then performed using 10 µl Taq buffer, 4 µl 2.5 µM dNTPs, 2.5µl each 10µM primer, 3µl of cells, and 0.5µl Taq polymerase. The colony PCR was tested by gel electrophoresis, and the samples that matched the expected fragment size of 1250bp for *ecm23*Δ*hphMX4* or 950bp for *srd1*Δ*natMX4* were then plated out on their own plate and named. This resulted in successful replacement of *ecm23* with *hphMX4* in AN117-16D (PS01), as well as *srd1* with *natMX4* in AN117-4B (PS02) and in A14134 (PS03) (Table 2). The transformation of *ecm23ΔhphMX4* into A14135 was unsuccessful.

2.2.4 Genetic Crosses

The haploid mutants were then crossed with their corresponding mating partner, resulting in PS01 crossed to PS02, which was named PS005.

Chapter 3

RESULTS

3.1 Nud1

We hypothesized that accumulating phosphorylations on Nud1 contributes to the cell's ability to determine the relative age of meiotic SPB's. If Nud1 is phosphorylated during sporulation at a constant rate, the older SPBs (from the mother cell and meiosis I) will have more phosphate groups than the younger SPBs that are formed from new components during meiosis II. Under dyad conditions the cell is then able to, through an unknown mechanism, sense the relative levels of phosphorylation of Nud1 on each SPB and select the youngest SPBs to utilize to form spores. In the *nud1-2* temperature sensitive (ts) mutant, cells lose the ability to determine the relative age of spores while retaining their ability to reduce spore number, and form random dyads instead of NSDs. We theorize that this is due to the *nud1-2* mutant being unable to be properly phosphorylated, which causes all SPBs to be equally phosphorylated and appear the same age to the cell.

The goal of the project was to test this hypothesis by comparing the phosphorylation states of Nud1 and Nud1-2 through the use of isogenic WT and *nud1-2* temperature sensitive strains. The *ts* strain is used because *NUD1* is necessary for vegetative growth but not for sporulation; thus, a *nud1* knockout is not viable while a *nud1-ts* mutant allows for the strain to be cultured and sporulated while still being able to test the effect of a loss of *NUD1* on sporulation by sporulating the cells at the mutant's restrictive temperature. After the *nud1-2* strain alongside an isogenic *NUD1* strain were sporulated at restrictive temperature, the extracts of each strain would be analyzed by western blotting in order to determine the relative phosphorylation levels. Phosphorylation of a protein can retard its mobility in SDS-PAGE, resulting in an upwards shift in the observed band. This would allow for the comparison of the amount of phosphorylation between the Nud1 and Nud1-2 proteins. Anti-Nud1 antibodies are not available so it was further necessary to incorporate an epitope tag (YFP) at the 3' end of both *NUD1* and *nud1-2* so that the proteins could be detected on the western blot.

Unfortunately, construction of the isogenic *NUD1::YFP* and *nud1-2::YFP* strains was not completed due to a failure in the assembly of the *nud1-2::YFP* construct. Thus the hypothesis has not yet been tested.

3.2 GATA

The hypothesis regarding the four GATA family members was that they constituted two pairs of redundant transcription factors that are activated late in sporulation and are involved with spore wall assembly by altering the expression of their

target genes. In addition, it is thought that while Gat3/Gat4, and Ecm23/Srd1 represent two evolutionarily distinct redundant pairs, all four may be at least partially redundant with each other. Because the *gat3 gat4* double knockout only exhibited a weak spore wall phenotype, creating a quadruple knockout of all four genes may cause a much stronger phenotype that could help shine light on the mechanics of the process by which these genes assist in spore wall assembly.

The purpose of this experiment was to create a quadruple knockout and all four combinations of triple knockouts of the four GATA genes, and sporulate the strains to test for any spore wall defects. The knockout strains would be tested for potential spore wall defects through a variety of methods that test for deficiencies in cell wall structure: such as simple microscope observation, ether sensitivity testing, and calcofluor white staining.

The knockouts were made in two separate strain backgrounds, SK1 for enhanced sporulation, and an inducible *NDT80* strain which arrests at the entrance to meiosis in the absence of estradiol. The *NDT80* inducible strain was utilized to synchronize the sporulation of the entire cell cultures by allowing the cells to arrest in meiotic prophase before treating them with estradiol and triggering them to sporulate at approximately the same time. Synchronization allows for all the cells to progress through the process of sporulation together, and follow the same transcriptional schedule, allowing for more accurate measurements of the change in RNA transcripts over time of the entire population. This would allow us to compare the transcript levels of the knockout strains to isogenic WT sporulating cells in order to see which genes' expression levels are different after the time where the GATA genes are turned on

during sporulation. Any genes found by this screen would be potential targets of the GATA members, and could help elucidate the mechanism by which the GATA genes affect spore wall assembly based on their role.

Unfortunately, the quadruple knockout and the triple knockouts were not completed in time to provide data on its sporulation phenotype, or the differences in RNA levels during sporulation. However, the necessary double knockouts in the SK1 background and the *srd1* knockout in the synchronized background necessary for building the quadruple knockout are available for future use.

CHAPTER 4

DISCUSSION

The process of yeast sporulation involves multiple distinct events and several layers of regulation. Many mechanistic steps and the factors involved remain elusive. Two of these were studied here: the mechanism by which cells can recognize the age of SPB's, and the control of late gene expression.

We hypothesize that Nud1 allows the cell to determine the age of SPBs by measuring the amount of phosphates added to Nud1. This would mean that as soon as SPBs form, their Nud1 proteins begin to be phosphorylated at multiple sites. There

would then be another protein or complex of proteins that would be able to recognize the level of Nud1 phosphorylation and somehow compare the phosphorylation state of all four SPBs in meiosis II in order to determine the two youngest NSDs to form into spores. This working model is illustrated in Figure #3.

There is some evidence that lends support for this hypothesis; notably, Nud1 is necessary for proper NSD selection while not being required for sporulation itself. This suggests that the reason Nud1 stays in the meiotic SPB, as opposed to other proteins such as Spc72 that are removed in favor of meiosis specific factors, may be primarily to regulate the age-dependent sorting and NSD formation. Nud1 is also known to be phosphorylated, and phosphorylating specific sites on Nud1 has been shown to play a key role in regulating the signaling function of the SPB (Rock, 2013). In addition, multiple proteins such as Dbf2 and Mob1, which are known to only recognize and bind to specific phosphorylation sites of Nud1 (Rock, 2013), may function as adaptor proteins that can integrate the phosphorylation state of Nud1 over time. The large number of phosphorylation sites also lends credence to the possibility of a Nud1 phosphorylation gradient over time, as each protein molecule could conceivably be phosphorylated dozens of times over hours without the need for regulatory phosphatases. Alternatively, it may be that only a specific few phosphorylation sites are actually important in terms of Nud1 activity, and it is more of a phosphorylation switch than a gradient by which Nud1 selects daughter SPB's. For instance, Nud1 may be phosphorylated in-between meiosis I and II at a specific site, which would result in only the parent SPB's possessing Nud1 with the key phosphorylation, signaling them as old.

If the experiment was successful, it would have shed some light on this process by determining the difference in phosphorylation state between the wild type and *nud1-2* alleles. If the two different strains showed an identical distribution of phosphorylated protein in the western blot, it would imply that the hypothesis was incorrect as it would indicate that the Nud1-2 protein is not obvioiusly different in its phosphorylation than Nud1. However, if the *nud1-2* strain showed less phosphorylation than the WT strain it could mean that the mutated Nud1-2 protein cannot be properly phosphorylated, which could be the cause of the inability of *nud1-2* cells to distinguish SPB age.

If Nud1 is indeed a sensor of SPB age, we would follow up with further experiments further investigating the mechanism of action. For instance, mutagenesis and mass spectrometry will be performed to determine which phosphorylation sites are important for the NSD response. This could help determine if it's specific phosphorylation sites that are involved in the pathway or a more global accumulation of modifications. This question could also be tackled by mimicking phosphorylated sites on Nud1 through the substitution of aspartate at phosphorylation sites, and determining if any specific combination of sites causes a change in NSD percentage. In addition, if it was proven that Nud1 phosphorylation is the signal the cell uses to determine SPB age, the next logical question would be to understand how the cell reads and reacts to that signal. Therefore, it could be useful to determine if there are any meiotic proteins that can associate with the hyper-phosphorylated Nud1 found in NSD's. Thirdly, it would be interesting to see if there are any meiotic specific kinases that are involved with phosphorylating Nud1 during sporulation other than Cdc15.

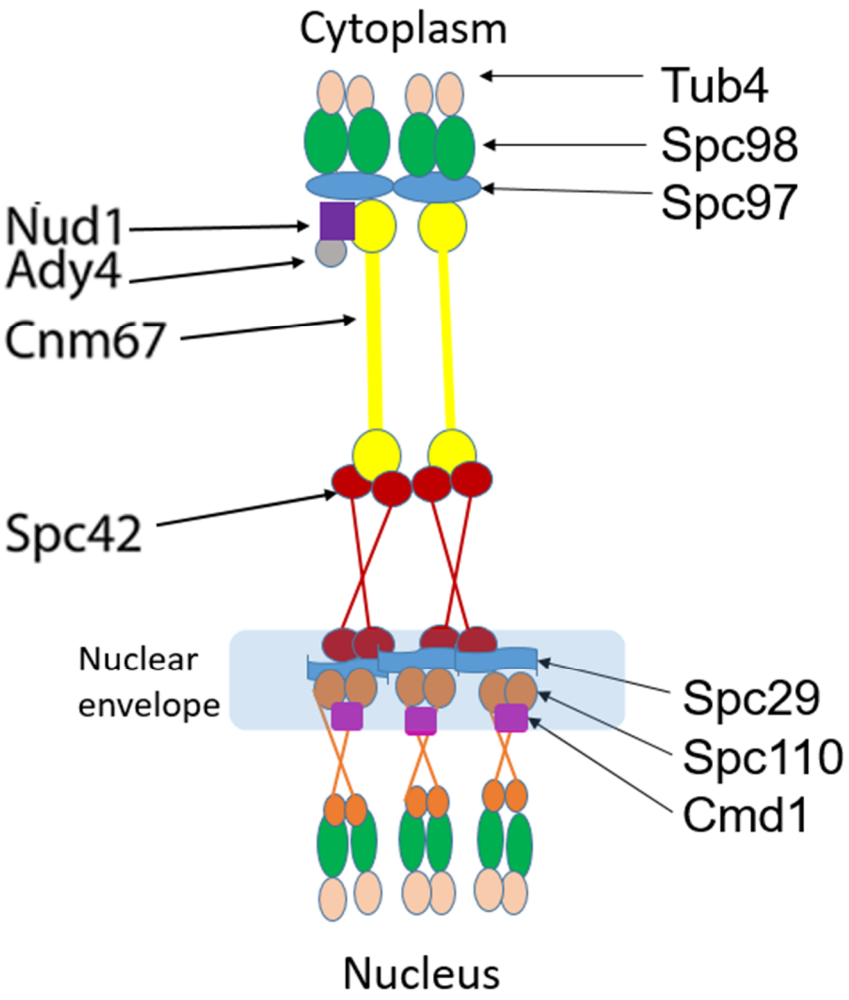
The GATA transcription factors may be an even more mysterious, as not much is known about their functions in vegetative cells, and even less is known about their role in meiosis. In sporulating cells all that is known is that Gat3 and Gat4 play a redundant role in spore wall assembly, probably through acting as transcription factors (Lin et al, 2013)(Lam et al, 2014). The high conservation between the proteins, and in particular between the two pairs of extremely conserved proteins, Gat3/Gat4 and Srd1/Ecm23, may indicate that these are redundant gene pairs which probably arose as a result of one of the many gene duplication events over the evolutionary history of yeast (Kellis et al, 2004). The relatively mild spore wall phenotype of the *gat3 gat4* double knockout might be due to overlapping function with *SRD1* and *ECM23*. That is why the quadruple knockout mutant may be required to fully reveal the function of these genes. However, there is also a chance that the quadruple knockout mutant may be completely lethal if all four genes do happen to be redundant for some vital process. The redundancy may allow cells to survive with only one or two of the four genes; but, if the process that the four genes controls plays a role in vegetative processes too, cells may not be able to function without at least a small vestige of functionality in the single/double/triple knockouts. That is why creating the triple knockouts would also be useful, in case the quadruple knockout was lethal due to a complete lack of redundancy, but maybe 1 of the 4 would be enough to let the cell grow, and exhibit the strongest possible phenotype for diagnostic purposes.

The use of an *NDT80* inducible strain would also allow for further experiments such as RNA-seq analysis of gene expression over a time course of sporulation. This would be possible because of *NDT80's* role as a master regulator of meiotic genes

(Hepworth, 1998). When sporulation is induced in the *NDT80*-inducible strains, all the cells will eventually arrest right before entering Meiosis, as *NDT80* is required to activate the transcription of essential meiotic genes. Addition of estradiol induces *NDT80* expression and causes the population to enter the meiotic divisions very synchronously (Carlile, et al. 2008). When RNA-seq experiments are performed on the synchronized samples, it allows for a reduction in noise provides temporal information of the changes in gene expression over the course of sporulation. This method could provide information on the specific mRNA levels that are altered when the different GATA transcription factor genes are knocked out. Knowing the downstream targets of the Gat transcription factors may be even more informative than their knockout phenotype in determining their role in sporulation, as hopefully, they would have a downstream effect on other genes involved in sporulation.

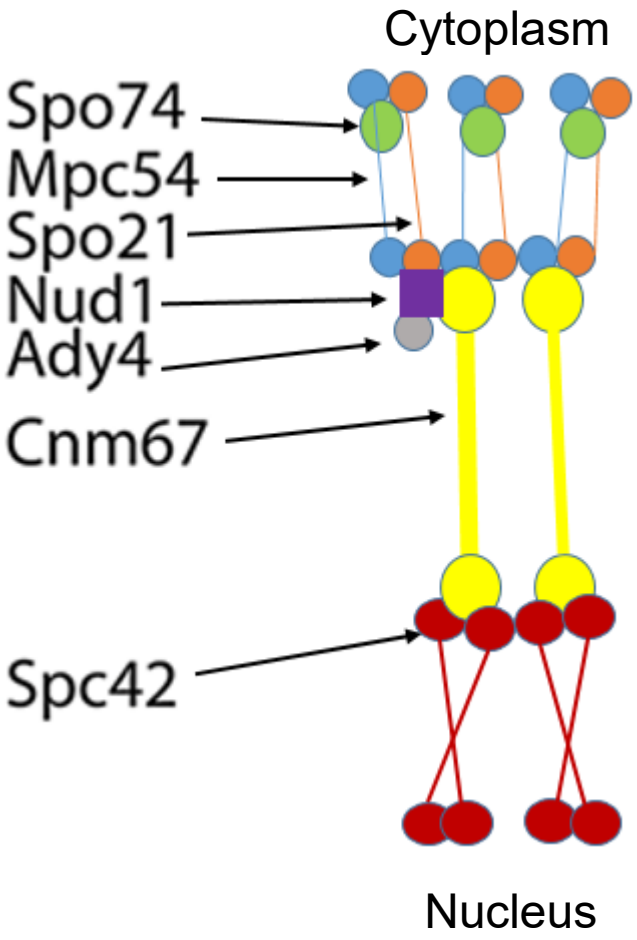
Figures

Figure 1: Mitotic Spindle Pole Body of S. Cerevisiae



Figure#1 was adapted from (Neiman, 2011) and (Jasperson, 2004) and shows the structure of the protein components of the mitotic spindle pole body in relation to the nuclear envelope. This is how the spindle body is structured during vegetative growth and mitosis.

Figure#2: Spindle Pole Body Meiotic Outer Plaque of *S. cerevisiae*



Figure#2 was adapted from (Neiman, 2011) and shows the protein components of the meiotic outer plaque of *S. cerevisiae*. This shows the cytoplasmic outer plaque spindle pole body as it appears during the process of sporulation.

Figure 3: Nud1 Phosphorylation Hypothesis for Non Sister Dyad Formation

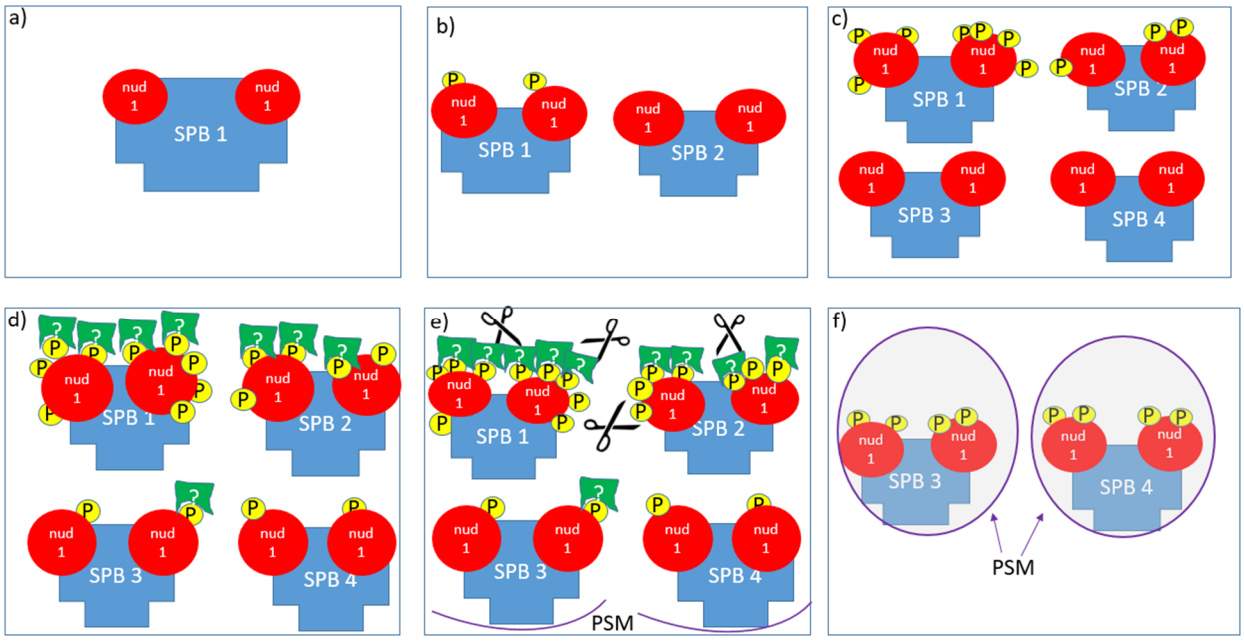


Figure 3: shows the proposed hypothesis for how sporulating cells choose which SPB's to use to form NSD spores under limiting conditions. The figure shows the SPB's of a single sporulating cell in NSD forming conditions over the course of meiosis and the proposed mechanism of Nud1 phosphorylation leading to the production of NSD's. The proposed model represents the gradual phosphorylation of Nud1 over time, with allows the cell to select the least phosphorylated SPB's to package into NSD spores. Each blue box represents a SPB of a single cell; each red circle on the SPB's represents nud1 molecules present in the SPB, and each yellow circle represents a phosphate group that has been added to a Nud1 molecule. Panel a) represents the cell prior to the onset of meiosis, where it has only a single SPB (SPB 1). Panel b) shows the cell during meiosis I after SBP1 has been replicated to produce SPB2. The Nud1 molecules on SPB1 begin to accumulate phosphorylations as meiosis is ongoing. Panel c) shows the cell during meiosis II after SPB1 and 2 have been replicated again to form 4 SPB's. The panel shows that SPB1 has continued to accumulate more phosphorylations while SPB2 also begins to be phosphorylated. panel d) shows the cell at a later time point where all 4 SPB's have accumulated a bit more phophorylations. The green boxes with the "?" represent the unknown mechanism by which the cell is able to sense the phosphorylation levels of Nud1. In this figure the green boxes are shown recognizing and attaching to phosphorylated Nud1. Panel e) represents how, as the cell continues to progress through sporulation, NSD conditions trigger the cell to only attempt to produce 2 spores, resulting in the cell targeting SPB 1 and 2 for destruction (as indicated by the scissors). The 2 younger SPB's with the least phosphorylations, 3 and 4, continue the process of sporulation and begin to nucleate PSM formation. The cell chooses to destroy SPB1 and 2 because they have the strongest phosphorylation signal, represented by the green boxes. The fact that SPB3 also has a green box reflects that all 4 SPB's accumulate phosphorylation's that cell can sense, but that only the 2 oldest SPB's that have been accumulating phosphorylations the longest are chosen for destruction. Panel f) shows the cell at the end of meiosis with SPB 3 and 4 fully encased by the PSM and ready to continue sporulation.

Tables

Table1: Primers Used

ANO410	CGACGGCCAGTGAATTGTAATACGACTCACTATAGGGCGAATTGGAGCT CAGATCTATATTACCCTGTTATCC
PS1	ACGCCAAGCTCGGAATTAACCCTCACTAAAGGGAACAAAAGCTGGGTAC CTGCTGGTAGCTTATATCTCTG
PS2	CTCTGGTTAGATGACACTCCT
PS3	CTCTTGGTTAGATGACACTCCT
PS9	GCCAAGCTTGCATGCCTGCAATAATGAAAGTATCACTTACAGCTC
PS10	TCCTCTAGAGTCGACCTGCATAGCTCGAAGAATCATGATCA
PS11	GCCAAGCTTGCATGCCTGCAAGCAGGTCAGGTTTTGTACC
PS12	TCCTCTAGAGTCGACCTGCATAAGACACAAGTTTGTTCTCG
PS13	CTTTGTTAAGAGGTTGCTAGGTAAATAACCAGACATTTACTATTGCTAGT CACATACGATTTAGGTGACAC
PS14	ATATCCTAGTGGAAAAATATCATCTATGCGGTGAAAGAATTAAATAGATA AATACGACTCACTATAGGGAG
PS15	ATAAAAATACTGCGTTGTAGTGCTGAGTGATAGAACTTTTAAATCATATTC ACATACGATTTAGGTGACAC
PS16	AAAAATAGAGGAAGAATCCGCTGAATATACCATCTTTACAAACTACTTAA AATACGACTCACTATAGGGAG

Table#1 shows the name and sequence of each PCR primer that was used.

Table 2: Strains Used

AN117-4B	*MATα ura3 his3ΔSK leu2 trp1::hisG arg4-NspI lys2 hoΔ::Lys2 rme1Δ::LEU2*
AN117-16D	*MATa ura3 leu2 trp1-hisG his3ΔSK lys2 hoΔ:: LYS2*
A14134	*MATa ura3 trp1 his3 leu2 lys2 hoΔ::LYS2 GAL4-ER::URA3 P_{Gal}-NDT80 (TRP1)*
A14135	*MATα ura3 trp1 his3 leu2 lys2 hoΔ::LYS2 GAL4-ER::URA3 P_{Gal}-NDT80 (TRP1)*
AN522	*MATa ura3 leu2 trp1 arg4 rme1Δ::LEU2 gat3Δ::kanMX6 gat4Δ::HIS3 lys2 HO* *MATα ura3 leu2 trp1 arg4 rme1Δ::LEU2 gat3Δ::kanMX6 gat4Δ::HIS3 lys2 HO*
MND44	*MATa ARG4 his3 ho::LYS2 leu2 lys2 NUD1::YFP-HI3MX6 RME1 trp1 ura3* *MATα arg4 his3 ho::LYS2 leu2 lys2 NUD1::YFPHI3MX6 rme1::LEU2 trp1 ura3*
PS001	*MATa ura3 leu2 trp1-hisG his3ΔSK lys2 hoΔ:: LYS2 ecm23ΔhphMX4*
PS002	*MATα ura3 his3ΔSK leu2 trp1::hisG arg4 NspI lys2 hoΔ::Lys2 rme1Δ::LEU2 srd1ΔnatMX4*
PS003	*MATa ura3 trp1 his3 leu2 lys2 hoΔ::LYS2 GAL4-ER::URA3 P_{Gal}-NDT80 (TRP1) srd1ΔnatMX4*
PS004	*MATα ura3 his3 trp1::hisG arg4-NspI lys2 ho::Lys2 rme1::LEU2 leu2 ecm23ΔhphMX4* *MATa ura3 leu2 trp1-hisG his3ΔSK lys2 ho:: LYS2 srd1ΔNatMX4*

Table#2 shows the name and genotype of each yeast strain used.

REFERENCES

Adams IR, Kilmartin JV. 1999. Localization of core spindle pole body (SPB) components during SPB duplication in Saccharomyces cerevisiae. J. Cell Biol. 145:809–23

Badis, G., Chan, E. T., Bakel, H. V., Pena-Castillo, L., Tillo, D., Tsui, K., . . . Hughes, T. R. (2008). A Library of Yeast Transcription Factor Motifs Reveals a Widespread Function for Rsc3 in Targeting Nucleosome Exclusion at Promoters. Molecular Cell, 32(6), 878-887. doi:10.1016/j.molcel.2008.11.020

Banerjee N, Zhang MQ. Identifying cooperativity among transcription factors controlling the cell cycle in yeast. Nucleic Acids Res 2003; 31:7024–7031.

Brar, G. A., Yassour, M., Friedman, N., Regev, A., Ingolia, N. T., & Weissman, J. S. (2011). High-Resolution View of the Yeast Meiotic Program Revealed by Ribosome Profiling. Science, 335(6068), 552-557. doi:10.1126/science.1215110

Briza P1, Ellinger A, Winkler G, Breitenbach M. (1988) Chemical composition of the yeast ascospore wall. The second outer layer consists of chitosan. J Biol Chem. 1988 Aug 15;263(23):11569-74.

Briza P1, Ellinger A, Winkler G, Breitenbach M. (1990) Characterization of a DL-dityrosine-containing macromolecule from yeast ascospore walls. J Biol Chem. 1990 Sep 5;265(25):15118-23.

Cañizares, J.V., Pallotti, C., Saínz-Pardo, I. et al. Arch Microbiol (2002) The SRD2 gene is involved in Saccharomyces cerevisiae morphogenesis 177: 352. doi:10.1007/s00203-002-0400-

Carlile, T. M., & Amon, A. (2008). Meiosis I is established through division-specific translational control of a cyclin. Cell, 133(2), 280–291. http://doi.org/10.1016/j.cell.2008.02.032

Chu S, DeRisi J, Eisen M, Mulholland J, Botstein D, et al. (1998) The transcriptional program of sporulation in budding yeast. Science 282: 699–705. doi: 10.1126/science.282.5389.699

Davidow L. S., Goetsch L., Byers B., 1980 Preferential occurrence of nonsister spores in two-spored asci of Saccharomyces cerevisiae: evidence for

regulation of spore-wall formation by the spindle pole body. Genetics 94: 581–595.

Fabian GR, Hess SM, Hopper AK. 1990. srd1, a *Saccharomyces cerevisiae* suppressor of the temperature-sensitive pre-rRNA processing defect of rrp1-1. *Genetics* 124:497–504

Freese EB, Chu MI, Freese E (1982) Initiation of yeast sporulation of partial carbon, nitrogen, or phosphate deprivation. *J Bacteriol. 1982 Mar; 149(3):840-51.*

Goldstein AL, McCusker JH. Three new dominant drug resistance cassettes for gene disruption in Saccharomyces cerevisiae.. Yeast. 1999 Oct;15(14):1541-53

Gordon, O., Taxis, C., Keller, P. J., Benjak, A., Stelzer, E. H. K., Simchen, G., & Knop, M. (2006). Nud1p, the yeast homolog of Centriolin, regulates spindle pole body inheritance in meiosis. The EMBO Journal, 25(16), 3856–3868. http://doi.org/10.1038/sj.emboj.7601254

Gruneberg U, Campbell K, Simpson C, Grindlay J, Schiebel E. Nud1p links astral microtubule organization and the control of exit from mitosis. The EMBO Journal. 2000;19:6475–6488. doi: 10.1093/emboj/19.23.6475

Hepworth SR, Friesen H, Segall J (1998) NDT80 and the meiotic recombination checkpoint regulate expression of middle sporulation-specific genes in Saccharomyces cerevisiae. Mol Cell Biol. 1998 Oct; 18(10):5750-61.

Jaspersen, S. L., & Winey, M. (2004). The Budding Yeast Spindle Pole Body: Structure, Duplication, and Function. Annual Review of Cell and DevelopmentalBiology, 20(1),1-28.doi:10.1146/annurev.cellbio.20.022003.114106

Jensen, R., Sprague, G. F., & Herskowitz, I. (1983). Regulation of yeast mating-type interconversion: feedback control of HO gene expression by the mating-type locus. Proceedings of the National Academy of Sciences of the United States of America, 80(10), 3035–3039.

Kassir Y, Granot D, Simchen G IME1, (1988) a positive regulator gene of meiosis in S. cerevisiae. Cell. 1988 Mar 25; 52(6):853-62

Keck, J. M., Jones, M. H., Wong, C. C., Binkley, J., Chen, D., Jaspersen, S. L., . . . Winey, M. (2011). A Cell Cycle Phosphoproteome of the Yeast Centrosome. Science, 332(6037), 1557-1561. doi:10.1126/science.1205193

Kellis, M., Birren, B. W., & Lander, E. S. (2004). Proof and evolutionary analysis of ancient genome duplication in the yeast Saccharomyces cerevisiae. Nature, 428(6983), 617-624. doi:10.1038/nature02424

Kilmartin JV, Goh P. 1996. Spc110p: assembly properties and role in the connection of nuclear microtubules to the yeast spindle pole body. EMBO J. 15:4592–602

Knop, M., & Strasser, K. (2000). Role of the spindle pole body of yeast in mediating assembly of the prospore membrane during meiosis. The EMBO Journal, 19(14), 3657–3667. http://doi.org/10.1093/emboj/19.14.3657

Kreger-Van Rij. Arch Microbiol. 1978 Apr 27;117(1):73-7. Electron microscopy of germinating ascospores of Saccharomyces cerevisiae.

Lesage, G., & Bussey, H. (2006). Cell Wall Assembly in Saccharomyces cerevisiae. Microbiology and Molecular Biology Reviews, 70(2), 317–343. http://doi.org/10.1128/MMBR.00038-05

Lin, C. P., Kim, C., Smith, S. O., & Neiman, A. M. (2013). A Highly Redundant Gene Network Controls Assembly of the Outer Spore Wall in S. cerevisiae. PLoS Genetics, 9(8). doi:10.1371/journal.pgen.1003700

M. Hotz, C. Leisner, D. Chen, C. Manatschal, T. Wegleiter, J. Ouellet, D. Lindstrom, D.E. Gottschling, J. Vogel, Y. Barral. Spindle pole bodies exploit the mitotic exit network in metaphase to drive their age-dependent segregation Cell, 148 (2012), pp. 958–972

Mah AS, Jang J, Deshaies RJ (2001) Protein kinase Cdc15 activates the Dbf2-Mob1 kinase complex. Proc Natl Acad Sci U S A. 2001 Jun 19; 98(13):7325-30.

Mathieson E. M., Suda Y., Nickas M., Snydsman B., Davis T. N., et al., 2010 Vesicle docking to the spindle pole body is necessary to recruit the exocyst during membrane formation in Saccharomyces cerevisiae. Mol. Biol. Cell 21: 3693–3707

Moens P. B., Rapport E., 1971 Spindles, spindle plaques, and meiosis in the yeast Saccharomyces cerevisiae (Hansen). J. Cell Biol. 50: 344–361

Muller E. G., Snydsman B. E., Novik I., Hailey D. W., Gestaut D. R., et al., 2005 The organization of the core proteins of the yeast spindle pole body. Mol. Biol. Cell 16: 3341–3352

Neiman, A. M. (1998). Prospore Membrane Formation Defines a Developmentally Regulated Branch of the Secretory Pathway in Yeast . The Journal of Cell Biology, 140(1), 29–37.

Nickas M. E., Schwartz C., Neiman A. M., (2003) Ady4p and Spo74p are components of the meiotic spindle pole body that promote growth of the prospore membrane in Saccharomyces cerevisiae. Eukaryot. Cell 2: 431–445.

Nickas, M. E. (2004). Regulation of Spindle Pole Function by an Intermediary Metabolite. Molecular Biology of the Cell, 15(6), 2606-2616. doi:10.1091/mbc.e04-02-0128

Pak J., Segall J., 2002 Regulation of the premiddle and middle phases of expression of the NDT80 gene during sporulation of Saccharomyces cerevisiae

Palmer R. E., Sullivan D. S., Huffaker T., Koshland D., 1992 Role of astral microtubules and actin in spindle orientation and migration in the budding yeast, Saccharomyces cerevisiae. J. Cell Biol. 119: 583–593

Riedel CG, Mazza M, Maier P, Körner R, Knop M Differential requirement for phospholipase D/Spo14 and its novel interactor Sma1 for regulation of exocytotic vesicle fusion in yeast meiosis. J Biol Chem. 2005 Nov 11; 280(45):37846-52.

Rock, J. M., Lim, D., Stach, L., Ogrodowicz, R. W., Keck, J. M., Jones, M. H., . . . Amon, A. (2013). Activation of the Yeast Hippo Pathway by Phosphorylation-Dependent Assembly of Signaling Complexes. Science, 340(6134), 871-875. doi:10.1126/science.1235822

Shou W1, Seol JH, Shevchenko A, Baskerville C, Moazed D, Chen ZW, Jang J, Shevchenko A, Charbonneau H, Deshaies RJ. (1999) Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex. Cell. 1999 Apr 16;97(2):233-44.

Spang A, Grein K, Schiebel E. 1996b. The spacer protein Spc110p targets calmodulin to the central plaque of the yeast spindle pole body. J. Cell Sci. 109:2229–37

Srivastava PK, Harashima S, Oshima Y.Two-spored asci produced by interrupted sporulation: a novel approach to linkage analysis in yeast. Mol Gen Genet. 1983;191(1):165-6.

Tachikawa H, Bloecher A, Tatchell K, Neiman AM (2001) A Gip1p-Glc7p phosphatase complex regulates septin organization and spore wall formation. J Cell Biol. 2001 Nov 26; 155(5):797-808

Taxis C., Keller P., Kavagiou Z., Jensen L. J., Colombelli J., et al., 2005 Spore number control and breeding in Saccharomyces cerevisiae: a key role for a self-organizing system. J. Cell Biol. 171: 627–640

Yoder, T. J., Pearson, C. G., Bloom, K., & Davis, T. N. (2003). The Saccharomyces cerevisiae Spindle Pole Body Is a Dynamic Structure. Molecular Biology of the Cell, 14(8), 3494–3505. http://doi.org/10.1091/mbc.E02-10-0655