Regulation of meiosis in *S. cerevisiae* via PKA

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Abstract

The study of mitosis and meiosis is an essential part of learning biology. The budding yeast *Saccharomyces cerevisiae* is an outstanding system to study these processes. Diploid yeast cells divide when nutrients are abundant and enter meiosis and form spores in response to starvation signals. Following meiotic induction, cells duplicate the genome in meiotic S-phase and then enter prophase when the meiosis-specific processes of homolog pairing and genetic recombination take place. Subsequently, cells exit from meiotic prophase and undergo the meiotic divisions (MI and MII) to generate four haploid products that are then encapsulated in spore walls. If nutrients are added prior to the completion of prophase, cells will exit the meiotic program and return to mitotic growth. However, if cells have exited prophase, they will complete meiotic development and form spores irrespective of nutrients. Thus, exit from prophase marks the commitment point for meiotic development after which cells no longer require the inducing signal (starvation). Glucose is a potent inhibitor of meiosis that activates protein kinase A. In this study we have developed a genetic system for studying the role of PKA in regulating prophase exit and meiotic commitment. This system utilizes mutant forms of PKA that can be inhibited by the cell-permeable ATP analog, 1NM-PP1. We have developed strategies to induce meiosis and trap cells in meiotic prophase. Subsequently, cells were treated with glucose with and without 1NM-PP1. Our results suggest that glucose inhibits prophase exit through PKA and provide a system for studying how PKA controls meiosis using molecular, genetic, and cellular assays. These studies provide a well-suited experimental system for exposing talented undergraduates to hypothesis-driven scientific investigation.

Introduction

Diploid yeast cells divide mitotically when nutrients are abundant. They enter meiosis and form spores in response to starvation. Meiosis is tightly controlled by early, middle and late sporulation specific genes (Figure 1). Early genes are involved in meiotic S phase and prophase where homologs form synaptomenal complexes resulting in genetic recombination. Middle gene expression leads to meiotic divisions and spore wall formation. Late genes are expressed in spores. If glucose is added prior to completion of prophase, cells will exit the meiotic program and return to mitotic growth. If glucose is added after exit from prophase, the cells will continue the meiotic program and form spores. Therefore, exit from prophase marks the meiotic commitment point. The pathway that controls induction and progression of sporulation involves a complex signaling network that includes the Ras/cAMP pathway. In the presence of glucose, activated Ras increases the intracellular concentrations of cAMP. Elevated levels of cAMP activate protein kinase A, PKA, which phosphorylates targets that repress meiosis. Therefore in the presence of glucose, PKA is active and signals mitosis. In the absence of glucose, PKA is inactive resulting in sporulation.

In this study, we have developed an assay to study the role of PKA in the meiotic commitment point (Figure 3). Yeast strain, EWY155, containing key mutations in the ATP binding domain of PKA was used (Figure 2). All three PKA catalytic subunits, Tpk1, Tpk2 and Tpk3 have active sites that are wider than normal allowing the binding of the normal ATP as well as a "bulky" PKA inhibitor, INM-PP1 which renders PKA inactive. The role of PKA at this decision point was examined by growing cells in 0.5% glucose in the presence and absence of inhibitor (Figure 4).

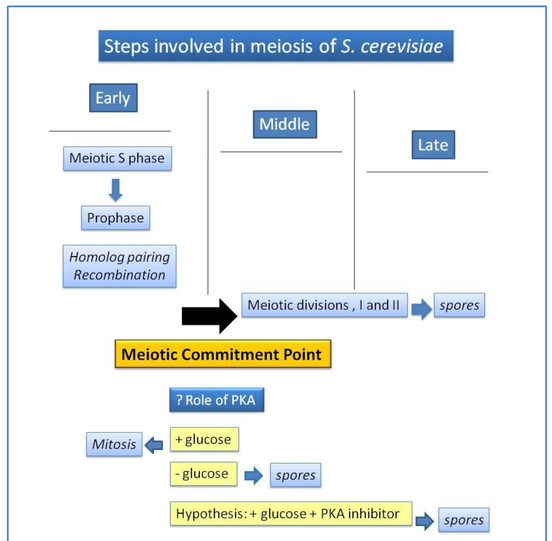

Figure 1 - Steps involved in meiosis of *S. cerevisiae*. In yeast, meiosis is controlled by early, middle and late sporulation specific genes. Early genes are involved in meiotic S phase and prophase where homologs base pair and undergo genetic recombination. Exit from prophase marks the meiotic commitment point as middle genes are expressed resulting in meiotic divisions and spore wall formation. The role of PKA at this commitment point is at question. In the presence of glucose, PKA is active and signals mitosis. In the absence of glucose, PKA is inactive and sporulation ensues. If PKA plays a role in exit from prophase then we hypothesize that in the presence of glucose and a PKA inhibitor that sporulation will occur.

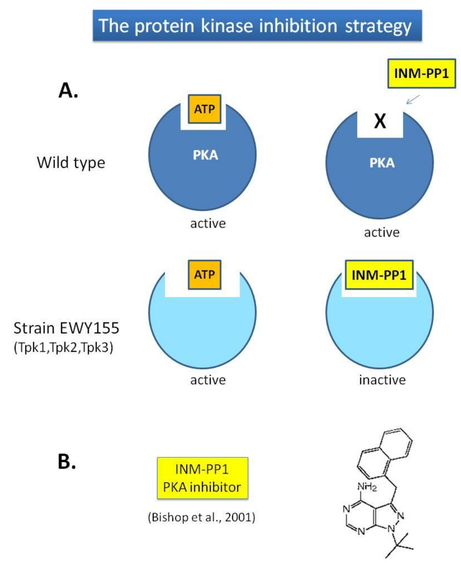

Figure 2 - Schematic diagram of the protein kinase inhibition strategy. **A.** Wild-type PKA binds to ATP but does not bind to INM-PP1 inhibitor. The *S. cerevisiae* strain EWY155 is a PKA inhibitor sensitive strain. All three PKA catalytic subunits, Tpk1, Tpk2 and Tpk3 contain a wider active site allowing for ATP to bind as well as INM-PP1 inhibitor. Binding of the inhibitor renders PKA inactive. **B.** Chemical structure of INM-PP1 inhibitor.

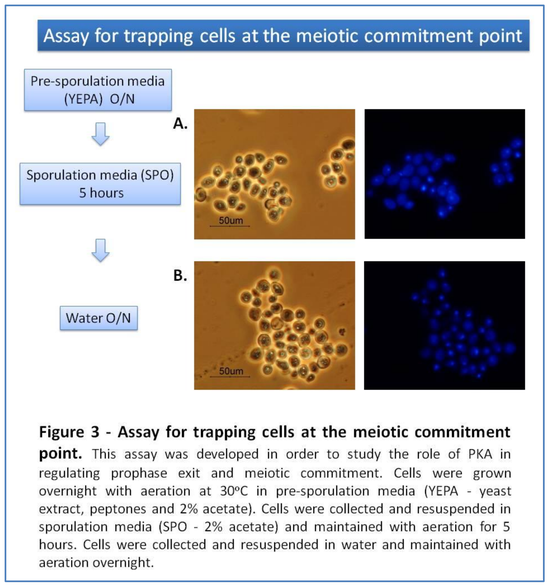

Figure 3 - Assay for trapping cells at the meiotic commitment point. This assay was developed in order to study the role of PKA in regulating prophase exit and meiotic commitment. Cells were grown overnight with aeration at 30ºC in pre-sporulation media (YEPA - yeast extract, peptones and 2% acetate). Cells were collected and resuspended in sporulation media (SPO - 2% acetate) and maintained with aeration for 5 hours. Cells were collected and resuspended in water and maintained with aeration overnight.

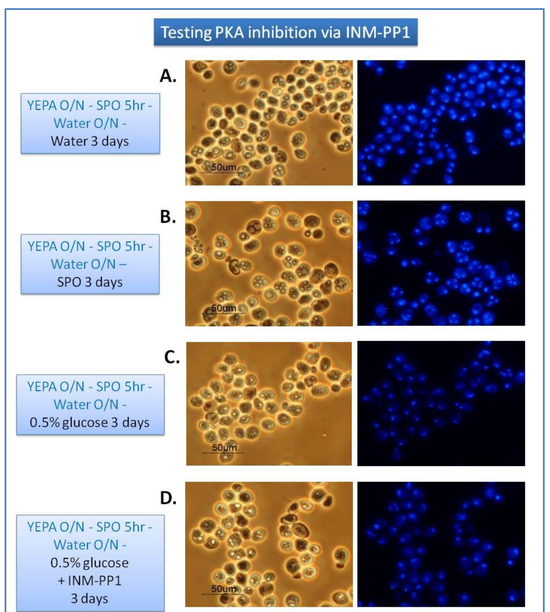

Figure 4 - Testing PKA inhibition via INM-PP1. Cells were grown overnight with aeration at 30ºC in pre-sporulation media (YEPA), collected and resuspended in sporulation media (SPO) and maintained with aeration for 5 hours. Cells were collected and resuspended in water and maintained with aeration overnight. Cells were collected and resuspended in (A) water, (B) SPO, (C) 0.5% glucose and (D) 0.5% glucose + 0.1 mM INM-PP1 and maintenied with aeration for 3 days. Cells were DAPI stained and photographed using phase contrast and fluorescent microscopy.

Conclusions

- The assay used for trapping cells in the meiotic commitment point demonstrated that incubation of cells in sporulation media for 5 hours followed by overnight incubation in water inhibits meiotic divisions from occurring (Figure 3B).
- Continued incubation of cells in water for a total of 4 days resulted in meiosis eventually taking place producing dyads (Figure 4A).
- The assay did not affect the ability to produce spores when cells were first arrested and subsequently placed in sporulation media for three days. As seen in Figure 4B, the majority of cells produced 4 spores per cell as expected.
- After being arrested in prophase I, cells placed in 0.5% glucose for three days reverted back to mitosis (Figure 4C). No spores are seen indicating that in the presence of glucose, PKA is activated thereby repressing meiosis.
- Interestingly, when 0.5% glucose plus INM-PP1 inhibitor was added to arrested cells again no spores were seen (Figure 4D). This is contrary to what we had expected. Our hypothesis was that the PKA inhibitor would render PKA inactive so that even in the presence of glucose the cells would enter meiosis and form spores. It is possible that other kinases may complement the function of the three PKA proteins, Tpk1, Tpk2 and Tpk3. Sch9 is a homolog of mammalian protein kinase B and there is evidence that Sch9 and PKA may be involved in the same cellular processes. (Yorimitsu et al., 2007)
- We currently have obtained a cell strain that is INM-PP1 inhibitable for all three PKA kinases and Sch9 kinase. Repeating the assay with this strain will help us examine the role of these kinases in regulating the meiotic commitment point.

References

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