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## Effect of Dbf2 on Phosphorilation

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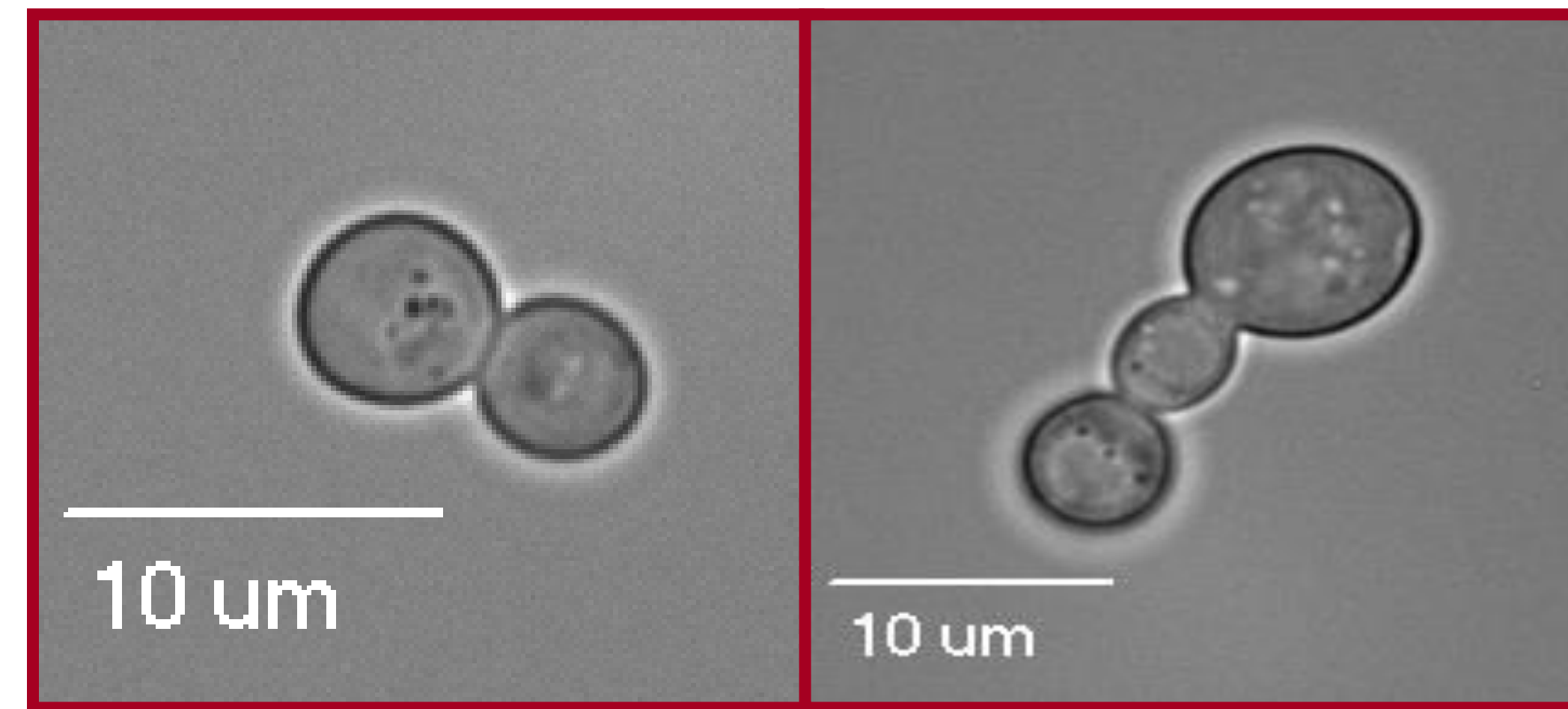
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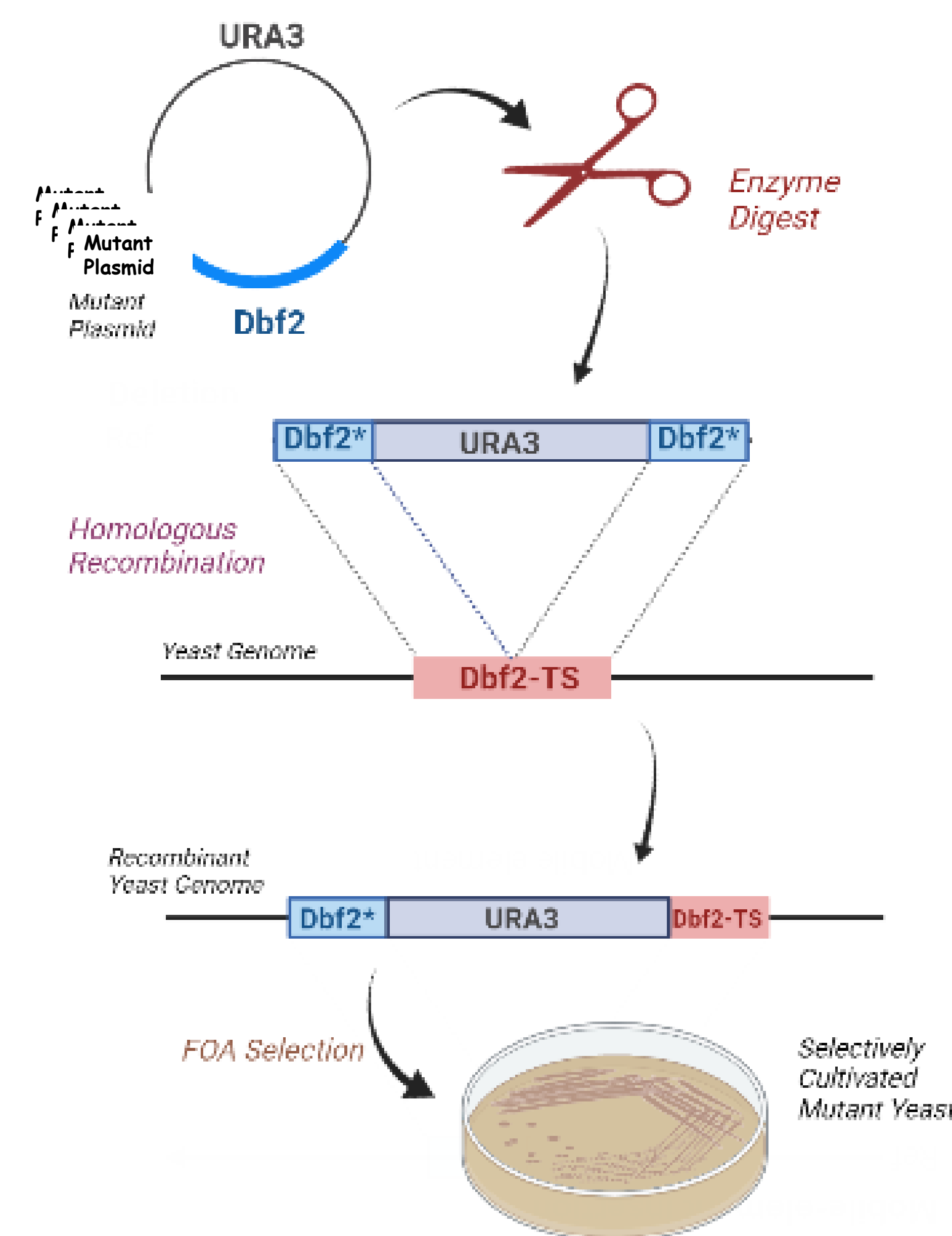
# Effect of Dbf2 on Phosphorylation in Yeast Cells:

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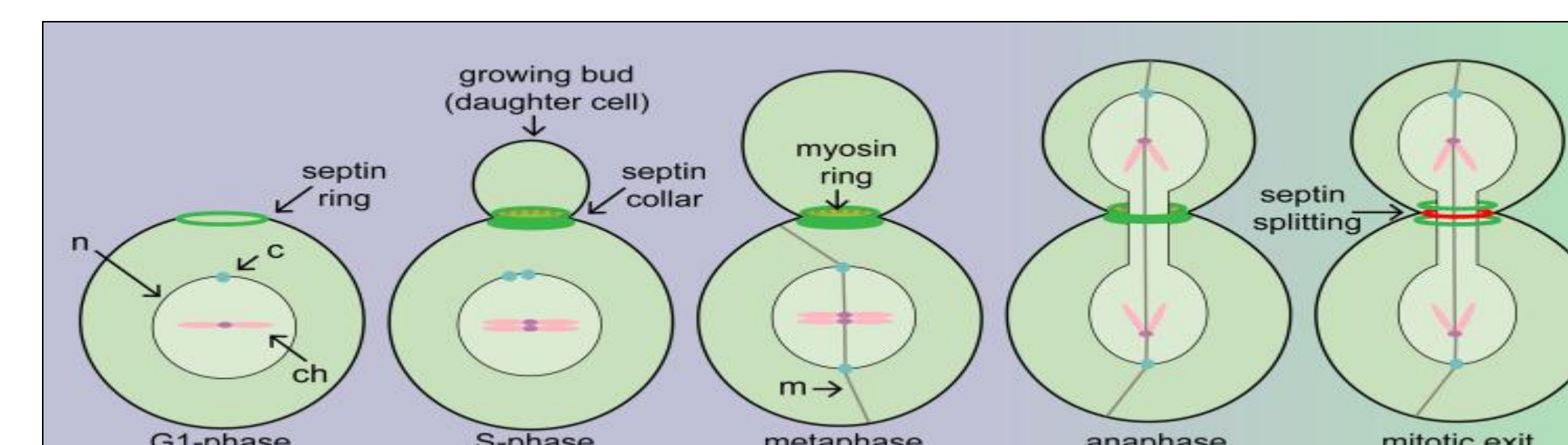
**Figure 2:** A Normal division on the left vs a "Chain" mutation on the right



**Figure 3:** An Enzyme Restriction Digest procedure can be used to introduce a mutant allele into yeast cells. The mutant Dbf2 allele is retrieved from a bacterial genome that also has the URA3 gene to grow without external Uracil. The mutant plasmid is inserted into a sample of yeast cells which are then incubated on -Uracil Agar plates. Using FOA selection against the URA3 allele, we can then force homologous recombination to occur as the yeast grows. Only yeast colonies containing the desired mutant allele will grow. **Created with BioRender.com**

## IMPACT

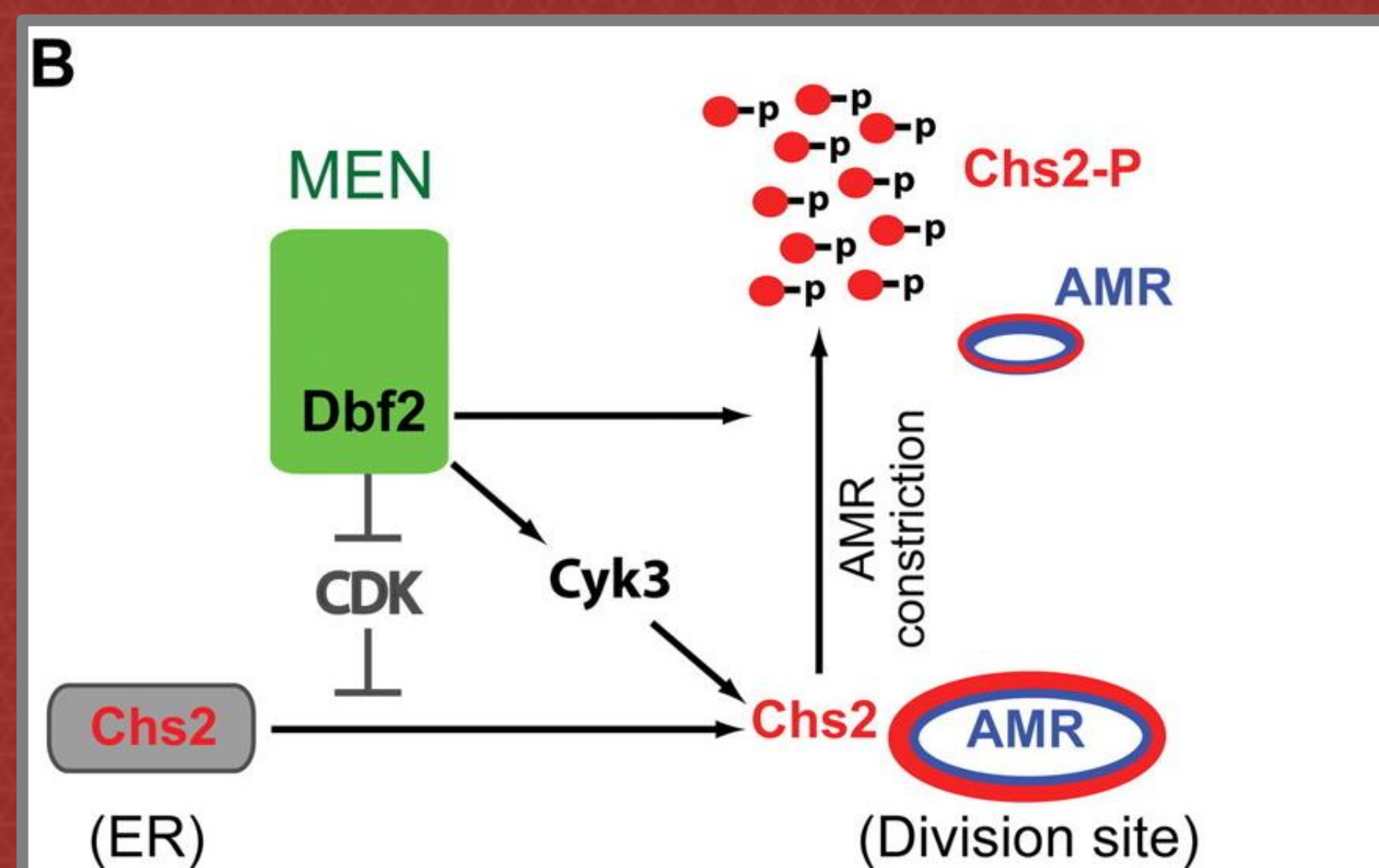
In cancer research, the study of proteins associated with the Mitotic Exit Network can serve to demonstrate how different genetic alterations can effect a cell's ability to successfully divide. However, mammalian cell lines are much more difficult to safely cultivate and analyze over a short period of time. In their stead, yeast cells are an excellent model organism. Additionally, many fundamental cellular functions seen in animal cells have a comparable homologue that can be observed in yeast cells. In this case, the DBF2 protein in yeast is an excellent experimental stand-in for the human cell equivalent, LATS2. LATS2 is a tumor suppressor protein that is integral in most mammals in the regulation of cell overgrowth. In humans specifically, its hypermethylation has been linked to astrocytoma, acute lymphoblastic leukemia, and breast cancer. Both DBF2 and LATS2 are serine/threonine-protein kinases that are phosphorylated during mitosis and are crucial to somatic cells' ability to successfully undergo cytokinesis. By understanding how to inhibit the proliferation of yeast through genetic alteration, we may very well gain a better understanding of new ways to treat some cancers in humans.



**Figure 4:** A Diagram showing the main stages of mitotic cell division. Chs2, a chitin synthase protein phosphorylated by Dbf2, is essential in the formation of the primary septum in *Saccharomyces cerevisiae*.

## ABSTRACT

Cytokinesis refers to the separation of two daughter cells after mitotic division. An important signaling pathway that enables this process is called the Mitotic Exit Pathway, or MEN. Of particular interest in this pathway is the Dbf2 protein kinase. Dbf2 essentially acts as a middleman within the signal transduction cascade of and activates proteins that physically separate the cells. (Figure 1.3) The kinase activity of Dbf2 is regulated by other cellular kinases, including the cell cycle regulator kinase. Using mutant alleles of the dbf2 gene, we can manipulate this protein's ability to be phosphorylated or dephosphorylated, thereby altering the downstream effects of the entire MEN. After introducing the mutant alleles of Dbf2 into yeast cells, the effects of the mutations can be quantitatively observed under a microscope, since defects in cytokinesis result in "chains" of unseparated cells. (Figure 1.1)



**Figure 1:** Phosphorylation is essentially the "switching on" of a protein kinase by the active binding of a phosphate ion. A Phosphorylation Cascade refers to when a phosphate ion is quickly passed down a series of kinases, each kinase passing off the phosphate to its neighbor and so on. Pathways like these are biologically efficient as they do not require signal molecules to travel long distance through the cytosol. Shown above is a representation of such a signal cascade in the MEN of *Saccharomyces cerevisiae*. Once phosphorylated by Cdc15, Dbf2 phosphorylates Chs2 to regulate cytokinesis.

## APPROACH

In order to analyze the downstream effects of the DBF2 kinase, we first need to successfully grow mutant yeast strains. We accomplish this through a series of genetic procedures in which we selectively cultivate the mutant Dbf2 allele over the Temperature Sensitive Dbf2 allele. (Figure 1.2) Subsequently, we can control the DBF2 kinase's ability to be phosphorylated and dephosphorylated by inserting point mutations in one of the serine phosphorylation sites. We measure the number of "chains" across each of the mutant strains to measure which alterations of Dbf2 inhibit cytokinesis most effectively.

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