Regulation of Protein Kinase A (PKA) activity to mediate chromosome segregation in Saccharomyces cerevisiae

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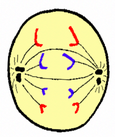
Abstract

Chromosome segregation results in the partitioning of sister chromosomes into two new cells with an equal number of chromosomes. This process occurs during mitosis and meiosis and errors can lead to aneuploidy, a karyotypic state in which cells carry the incorrect number of chromosomes. Most of the intrinsic factors that regulate the chromosome segregation process such as the multi-subunit kinetochore complex that connect chromosomes to the mitotic spindle are highly studied and virtually all of the protein subunits have been identified in yeast, flies, worms, and humans. However, much less is known about how extrinsic factors, which include nutrients, temperature, and pH might act on the intrinsic chromosome segregation machinery. Recently, the major glucose signaling pathway (Ras/Protein Kinase A) was shown by our lab to phosphorylate the outer kinetochore subunit, Dam1, and contributes to chromosome segregation fidelity in the budding yeast, *S. cerevisiae*. While this is the first description of a molecular pathway connecting glucose to chromosome segregation many questions remain. In particular, what is the mechanism regulating PKA-mediated phosphorylation of the kinetochore. Is the known Ras/PKA pathway that responds to glucose also directing PKA toward the kinetochore or are there additional signaling components that are specific to PKA's role in kinetochore phosphorylation. As a first step toward determining the mechanism of PKA regulation we measured the expression and activity of PKA at different cell cycle stages. Our initial results show that PKA activity increases through the cell cycle although protein levels remain constant. Associated with the increase in PKA activity we also observe increasing levels of Dam1 phosphorylated at the PKA site. Our next step is to determine if the known Ras/PKA pathway components are required for the cell cycle regulation of PKA activity and Dam1 phosphorylation. These studies will bring new insights into how extrinsic factors such as glucose can directly modulate kinetochore function and chromosome segregation fidelity.

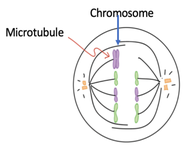
Background

Extrinsic Factors → Chromosome Segregation

How do microtubules attach to chromosomes?

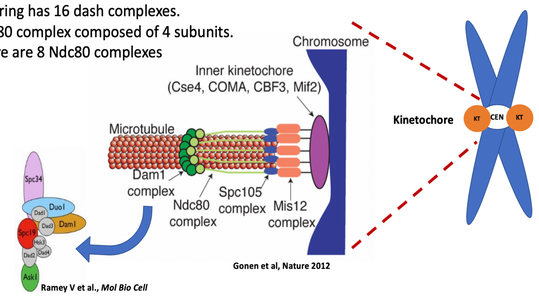


How does a cell use glucose to fix attachment errors?

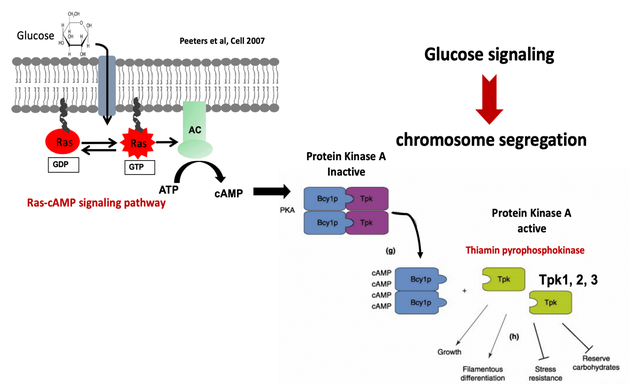


MT-Chromosome Attachment

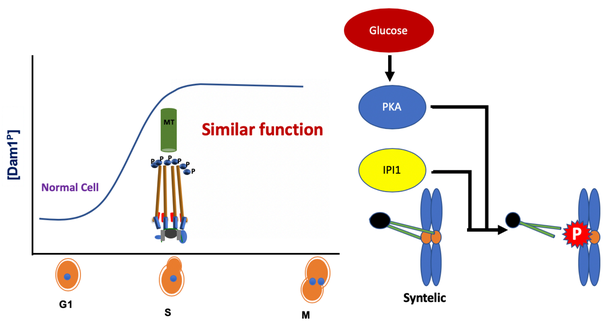
- ✓DASH complex (Dam1 complex)composed of 10 subunits.
- ✓The ring has 16 dash complexes.
- ✓Ndc80 complex composed of 4 subunits.
- ✓There are 8 Ndc80 complexes



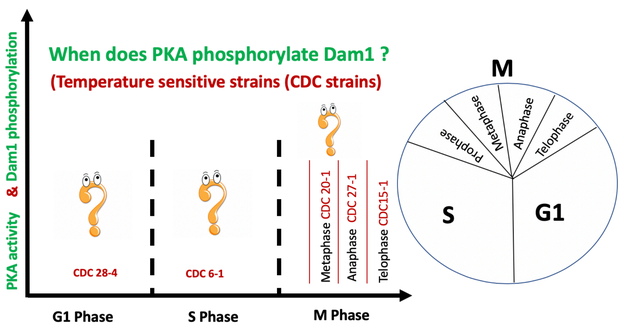
Glucose signaling



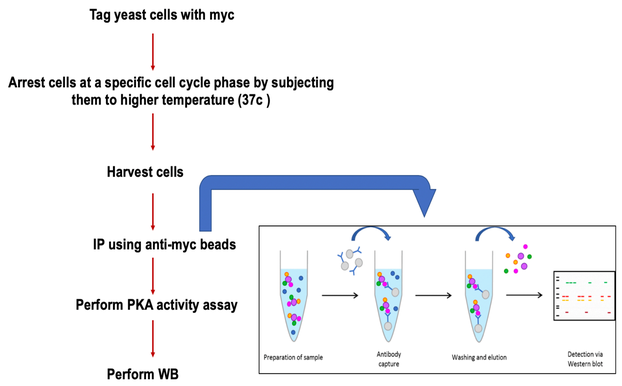
Hypothesis



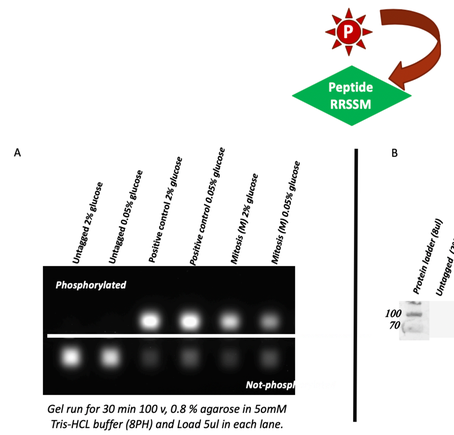
Questions



Approaches & Methods



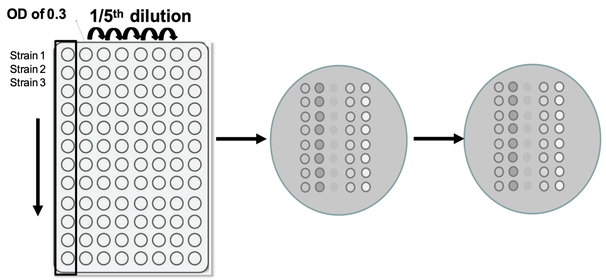
Glucose amount and PKA activity



Gel run for 30 min 100 v, 0.8 % agarose in 5omM Tris-HCL buffer (8PH) and Load 5ul in each lane.

Fig1: PKA activity assay in (A) was performed to look at the actual activity of the isolated PKA toward phosphorylation of the peptide substrate (Pep Tag). Once the Pep Tag is phosphorylated it will migrate up, and it is not phosphorylated it will migrate down. The expression level of PKA protein after immunoprecipitation prosses in (B).

Growth Assay



Cohesin mutation show more sensitivity to high copy TPK1

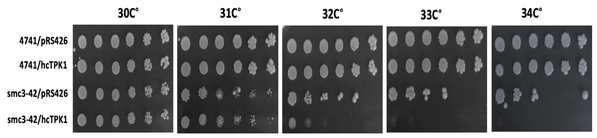
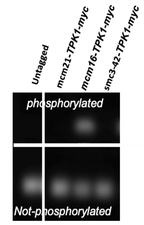


Fig2: Growth assay (spot test) was performed to look at how the combination of high copy TPK1 and sensitive mutation either on cohesin (smc3-42) affects the cell growth at a temperature higher than 30C°. smc3-42/high copy TPK1 cells were more sensitive than smc3-42/-pRS426 and that indicates high copy TPK1 increases the sensitivity of the mutated strain.

Cohesin mutation & inner kinetochore mutation reduce PKA activity



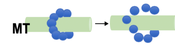
Gel run for 30 min 100 v
0.8 % agarose in 5omM Tris-HCL buffer (8PH)
Load 5ul in each lane.

Fig3: PKA activity assay was performed to look at the actual activity of the isolated TPK1 from either inner kinetochore mutated strain(mcm21) or cohesin mutated strain(smc3-42) toward phosphorylation of the Pep Tag peptide. Once the Pep Tag is phosphorylated it will migrate up toward the + charge end, and once it is not phosphorylated it will migrate down toward – charge end. A high reduction in the TPK1 activity was seen in both mcm21 & smc3-42 mutated strains.

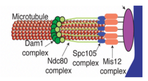
Mcm21 & smc3-42 mutations seem to reduce the activity of TPK1 by increasing its interaction with its regulatory domain BCY1 (need further study)

Future work

- ✓ **What is the phosphorylation of dam1 doing to the kinetochore?**
- ✓ **Is it affecting oligomerization of the Dam1 ring ?**



- ✓ **Does it have a function in Dam1 interactions with other kinetochore proteins (e.g. Ndc80)?**



Acknowledgements

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