UNIVERSITY of WASHINGTON TACOMA

IDENTIFICATION OF POSSIBLE PHOSPHORYLATION SITES IN Okp1 IN *SACCHAROMYCES CEREVISIAE*

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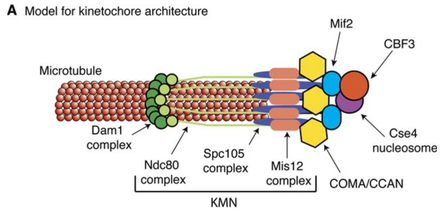
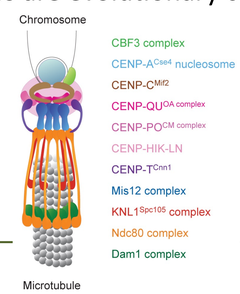
Protein phosphorylation is a mechanism of regulation that is extremely important a variety of cellular process (protein synthesis, cell division, cell growth, development, etc). Cells often use protein phosphorylation to change the activity or shape of a protein. In this project, we are going to look at the potential conservation of phosphorylation sites of the Okp1 protein, involved in chromosome segregation in *Saccharomyces Cerevisiae*. *Saccharomyces cerevisiae* is fungus known as yeast that is responsible for alcohol production and bread formation.

Introduction

Okp1 s one of the proteins that helps make up the *Saccharomyces Cerevisiae* kinetochore. It is an outer Kinetochore Protein and part of the complex called COMA (Ctf19p, Okp1, Mcm21p, and Ame1p).



OKP1 gene is an ortholog to the centromeric protein CENP-Q in humans. This protein is involved in kinetochore assembly in humans. There is biochemical evidence that Okp1 protein in yeast is phosphorylated, and we would like to understand more about which phosphorylation cites are evolutionary conserved.



Objective

Our objective is to identify possible phosphorylation sites in Okp1 protein in *Saccharomyces Cerevisiae* by looking for amino acid sequence conservation surrounding Serine and Threonine.

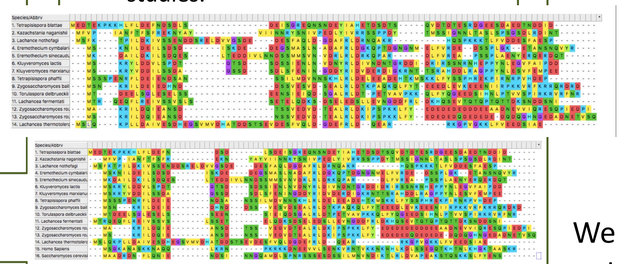
Scientific Methodology

1. We did a BLAST search to identify possible homologs in Okp1 from other yeast species. We identified some potential homologs with relatively low identity indicating that Okp1 has changed thru evolution.

Percent Identity	Name/Description
30.42%	*Tetrapisispora blattae*
30.63%	*Kazachstania naganishii*
32.58%	*Lachance nothofagi*
33.08%	*Eremothecium cymbalarie*
34.27%	*Eremothecium sinecaudum*
34.74%	*Kluyveromyces lactis*
35.23%	*Kluyveromyces marxianus*

36.02%	*Tetrapisispora phaffii*
37.17%	*Zygosaccharomyces bailii*
37.79%	*Torulaspora delbrueckii*
38.05%	*Lachancea fermentati*
38.67%	*Zygosaccharomyces rouxii*
39.18%	*Zygosaccharomyces rouxii*
39.92%	*Lachancea thermotolerans*

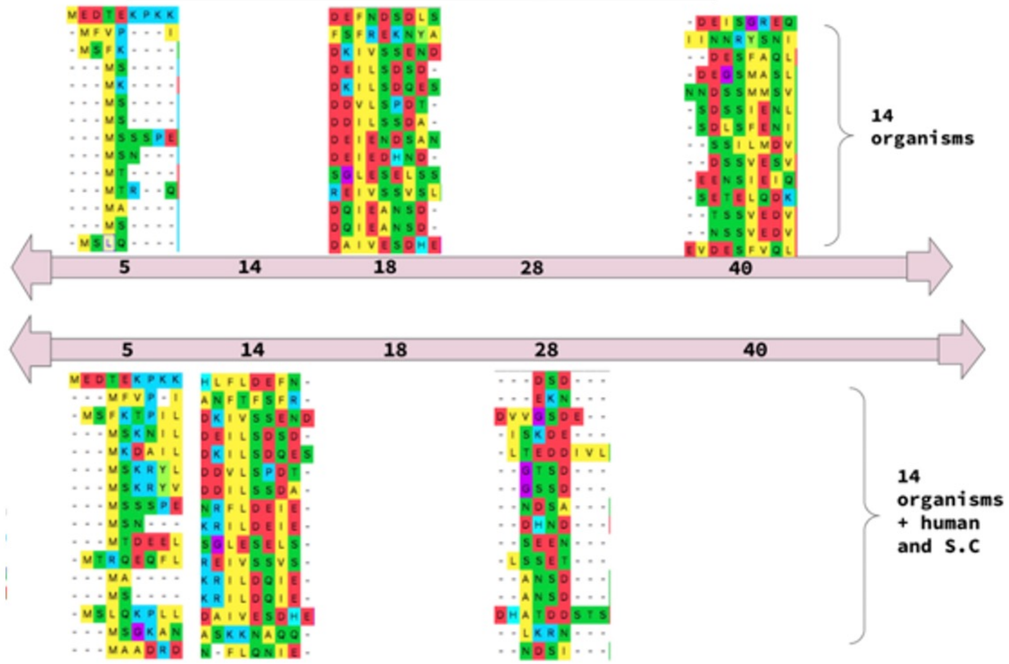
2. We took the amino acids from those possible homologs and used Sequence Alignment with a program called MEGA X to try to help us identify areas of sequence conservation.
3. We identify sites where amino acid sequences were conserved around Serine and Threonine which represent possible phosphorylation sites that we can identify for future studies.



Results

Conservation sites with Serine/Thereonine

Boxes with letter T represents Threonine, Boxes with letter S represent Serine. We can observe, none of the site numbers completely have all Serine or Threonine.



We identified 3 conservation sites with conservation surrounding Serine and Threonine residues out of the 16 organisms that we analyzed which were yeast species and human. We identify 3 potential phosphorylation sites that seem to be somewhat conserved among yeast species or potential phosphorylation sites, however, the sequence identity between Okp1 and homologs on other species was not very extensive. In order to confirm this phosphorylation site, we would then have to follow up this experiments with some biochemical identification of phosphorylation in those positions.

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