Identification of Possible Phosphorylation Sites in Okp1 in Saccharomyces cerevisiae

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The regulation of the kinetochore proteins is one of the most critical events for chromosomal segregation during cellular reproduction. Deregulation of kinetochore proteins is one of the main causes of cancer and many genetic diseases, including sex-linked syndromes. One of the main proteins for outer kinetochore assembly is Okp1, which acts as a platform for the attachment of microtubules to centromeres; it is responsible for chromosomal alignment in metaphase, and chromosomal movement in anaphase. Okp1 also acts as a structural checkpoint, which monitors proper kinetochore attachments and regulators for an effective transition between the stages of mitosis. Scientific literature suggests that many sites in Okp1—mainly serine (S) and threonine (T) amino acids—are highly modified, and are most likely important for regulation, however, there is no known evidence on subsequent effectivity during cellular reproduction. The main objective of this study was centrally to focus on the mechanistic role of protein regulation (i.e., phosphorylation) in ensuring the accuracy of chromosome segregation. Achieving this objective requires two main steps: identifying possible phosphorylation sites and then performing site-directed mutagenesis targeting these sites. Site-directed mutagenesis is a technique that alters DNA sequence, resulting in an alteration of protein sequence, which will allow us to test for chromosome segregation defects due to impairment of function in a phosphorylation site. We initially hypothesized that the evolutionary conservation of phosphorylatable amino acids will reveal areas where phosphorylation is critical for the function of kinetochore proteins. This study was initiated by using Molecular Evolutionary Analysis-across computing bioinformatics software (MEGAX) for detecting conservation of possible phosphorylation sites—targeting serine (S) and threonine (T) amino acids—along Okp1 protein sequence. Multiple sequences alignment technique was pursued to align the polypeptide sequence of Okp1 in Saccharomyces cerevisiae with Okp1 in multiple closely related yeast species. The alignment allowed us to detect highly conserved areas—with almost 80% sequence similarity—that are mainly located around the center of the protein sequence. It was commonly thought that these highly conserved areas in the Okp1 protein sequence might be possible targets for phosphorylation, thus, possible contributors for the regulation of Okp1. The collected data will allow us to better design site-directed mutagenesis approaches to determine the mechanistic role of regulating these highly conserved areas in the process of chromosomal segregation.