Identification of Conserved Phosphorylation Sites Through Evolutionary Conservation of the Ndc80 Protein in the Kinetochore
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Phosphorylation of the *Saccharomyces cerevisiae* Ndc80 protein and its N-terminal tail is important for the kinetochore-microtubule (MT) attachments, stabilization, and proper chromosome segregation. There is evidence that many sites within the Ndc80 protein are phosphorylated and modified. However, we do not know which of these modifications and phosphorylation sites are critical for the function of the Ndc80 protein. Site-directed mutagenesis is a technique where DNA gets altered so that it encodes for an amino acid of a protein that is different from the original amino acid. The mutated amino acid is non-phosphorylatable; this allows us to observe whether or not there is a function for the phosphorylation of that particular amino acid. However, site-directed mutagenesis has a drawback in which creating too many mutations will mess up the structure of the protein to the point where it will not function anymore. Thus, it is essential to rank the previously identified phosphorylation sites of the Ndc80 protein in the order they are critical to the function of the protein to reduce the number of mutations. We aim to determine which of the previously identified phosphorylation sites in the Ndc80 protein are crucial for the functioning/regulation of the Ndc80 protein, and which phosphorylation sites should have higher priority for further research into the mechanistic role of those modifications. We will search for evolutionary conservation of the phosphorylated amino acids within the Ndc80 of *Saccharomyces cerevisiae* to direct our site-directed mutagenesis. Evolutionary conservation will imply the sites that are most likely to have critical-functional phosphorylation. First, we identified evolutionarily conserved sequences of the *Saccharomyces cerevisiae* Ndc80 protein sequence using a combination of P–BLAST and PSI-BLAST searches. Second, we performed multiple sequence alignments and pairwise alignments to compare the conservation across the Ndc80 protein of *Saccharomyces cerevisiae* and the targeted evolutionary sequences. Our analysis indicates that the phosphorylation sites located within the N-terminal domain (first 257 amino acids) of the Ndc80 protein are the most essential phosphorylation sites for the functioning of the protein. Our research showed the phosphorylation site located at position 205 (serine) as the most conserved site within Ndc80. Analysis of Serine/Threonine at positions 21/22, Serine/Threonine at positions 37/38, and Serines at positions 4, 6, and 100 revealed strong conservation as well. We have concluded that the phosphorylation site located at position 205 may be the most important phosphorylation site for the functioning/regulation of the Ndc80 protein, and it should have the highest priority for further research. Moreover, phosphorylation sites located at positions 21, 22, 37, 38, 4, 6, and 100 should have second priority for further research.