CRISPR-Cas9 mutagenesis on suspect Dsn1 phosphorylation sites and its impact on functionality

Abstract:

The kinetochore is a large protein complex that is attached to the centromere of the chromosome and interacts with microtubules during cell division. It is required to separate duplicate DNA molecules. Some kinetochore proteins are phosphorylated and it is known that phosphorylation of proteins can impact their function. The Dsn1 protein, a subunit of the kinetochore; was found to be phosphorylated through mass spectrometry analysis. Two sites were of interest; serine 567 and 569. These sites contact the Ndc80 complex, a protein group that aids in chromosome segregation and the microtubule itself. We aimed to determine whether these sites were necessary for proper kinetochore function through CRISPR gene editing technology to mutate serine 567 and 569 of the *DSN1* gene so that the Dsn1 protein can no longer accept phosphate groups at those positions. We used a vector that codes for a Cas9 enzyme which allows for CRISPR gene editing as well as a gene encoding a green fluorescence protein (GFP), and an aph(3')-IA gene. We identified a sequence in the *DSN1* gene that allowed us to design a guide RNA sequence for the CRISPR system. We ordered that DNA and attempted to insert it in place of the GFP gene. We transformed this DNA into *E.coli* and selected non-glowing colonies which should contain the vector with the GFP gene replaced by sgRNA. Plasmid DNA was purified and sent out for sequencing. Sequencing results were inconclusive. We are awaiting DNA sequence confirmation of insertion of sgRNA.