Is specter a mutation in the cell cycle gene cyclin B1?

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ABSTRACT

Progression through cell division is controlled by genes that regulate the cell cycle. As of now, the way embryos control cell division is regulated in one common way: known as the G2/M transition. Furthermore, mutations that alter cell cycle division in these genes are often considered to be involved in cancer pathways. Here, we show that the specter specter spr mutation is a cell division mutant that creates mitotic abnormalities and later becomes developmentally arrested at about 20 hours of development. We mapped the spr mutation to an interval on linkage group 5, which includes the cyclin B1 gene. Cyclin B1 is necessary for the G2/M transition of the cell cycle. Sequencing spr mutant cDNA showed that there is a nonsense mutation (C570T) in exon 2 of cyclin B1 gene. We hypothesized that the spr mutation is caused by a non-functional cyclin B1 protein: cell cycle progression and developmental abnormalities are seen as soon as maternal cyclin B1 mRNA is depleted. In situ hybridization of cyclin B1 revealed that the expression is greatly reduced in the mutant embryos at the 10-somite stage. Systox Green staining of DNA showed nuclear fragmentation in the mutants at the 15-somite stage. Phospho histone H3 antibody staining showed that fewer cells enter mitosis in the mutants compared to the wild type embryos, and that neural stem cells do not migrate properly to the midline to divide. An in situ hybridization of deltaA, a marker for neural precursor cells, revealed that there were fewer neural precursors in the spr embryos at the 20-somite stage. Number of caspase-3 positive cells reveals a wave of apoptosis in the spr mutant at an early stage of the hindbrain border (bracket) to quantify our results. The number apoptotic cells significantly increases with time. The CONCLUSION for spr is that it has fewer neural precursors compared to WT. The FUTURE DIRECTIONS for spr are to continue to work on my Master’s project in the United States. This study was funded by a National Science Foundation grant to Don Kane.

THE specter MUTATION

- Mendelian recessive mutation
- Mutant embryo arrests with the body shape of a WT embryo at 20 hours post fertilization
- Later, cells in the central nervous system die
- Cells that divide frequently (e.g., blood and neurons) appear to be larger in spr, which is a characteristic of all the cell cycle gene mutants in our lab

Morphological changes described in spr mutant can be explained by the mutation in the cell cycle gene cyclin B1 seems like a really good candidate

Yes, antibody staining of caspase-3 shows cells are dying by apoptosis. We counted the number of caspase-3 positive cells between the forebrain and the midbrain-hindbrain border (bracket) to quantify our results. The number apoptotic cells significantly increases with time.

If spr has bigger neurons, does it also have fewer neural precursors?

No, antibody staining of phospho histone H3 shows fewer mitotic cells in spr.

Cyclins B1:
- Is essential for the control of the cell cycle at the G2 to M transition.
- One of the cellular controls of the cell cycle necessary to enter mitosis

CONCLUSION

Morphological changes described in spr mutant can be explained by the mutation in the cell cycle gene and cyclin B1 seems like a really good candidate

FUTURE DIRECTIONS
- Confirm that the spr phenotype results from a disruption in cyclin B1 by rescuing the mutant with injections of WT cyclin B1 mRNA. By knocking down cyclin B1 mRNA by antisense oligonucleotide and CRISPR-Cas system.
- Determine at what stage in the cell cycle and what kind of cells are affected by a loss of cyclin B1.

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