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

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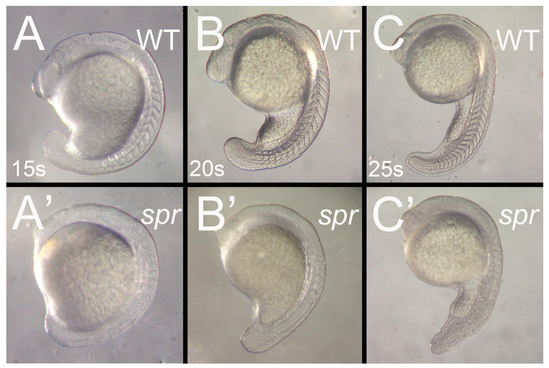
ABSTRACT

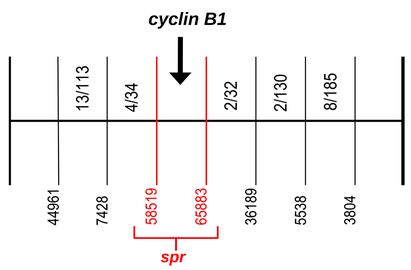
Progression through cell division is controlled by genes that regulate the cell cycle. As of now, the way embryos control cell division to regulate organ size remains unknown. Furthermore, mutations that cause cell cycle defects in these genes are often considered to be involved in cancer pathways. Here, we show that the zebrafish *specter* (*spr*) mutant is a cell division mutant that causes 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 to M transition of the cell cycle. Sequencing *spr* mutant cDNA showed that there is a nonsense mutation (C139T) in exon 2 of cyclin B1 gene. We hypothesize 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 grossly reduced in the mutant embryo at the 10-somite stage. Sytox 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. *In situ* hybridization of *deltaA*, a marker for neural precursors, revealed that there are fewer neural precursors in the *spr* mutant at the 20-somite stage. *In situ* hybridization of *pax2a*, a marker for midbrain-hindbrain boundaries, otic placode, optic stalk, pronephros and spinal interneurons, showed that there are also fewer neural precursors in the mutant at the 15-somite stage. Caspase-3 antibody staining revealed that a wild-scale apoptosis in the *spr* mutant occurs as early as 10 somite in the brain and tailbud, areas that first lose an optical transparency, and correlates with further development. Thus, 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. We are now using mRNA injections to see if we can rescue the mutant, part of the proof that the specter phenotype is caused by a mutation in *cyclin B1* gene.

THE *specter* MUTATION



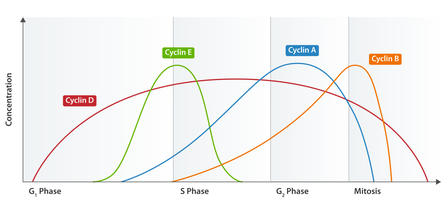
- Mendelian recessive mutation
- Mutant embryo arrests with the body shape of a WT embryo at 20 hours post fertilization
- Later, cells in the central nervous system die
- Cells that divide frequently (e.g., blood and neurons) appear to be larger in *spr*, which is a characteristic of all of the cell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

Mapping data indicates that the gene *cyclin B1* is a promising candidate for the *spr* mutation site



Cyclin B1:

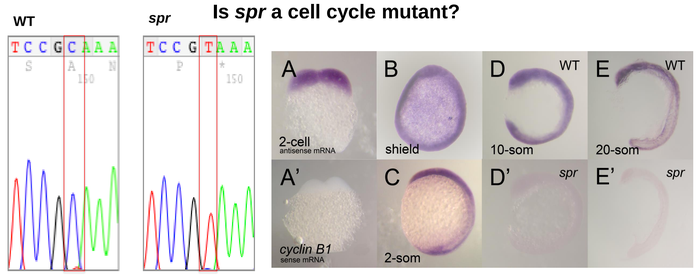
- Is essential for the control of the cell cycle at the G2 to M transition.
- One of the cellular controls of the cell cycle necessary to enter mitosis



Cyclins form complexes with cyclin-dependent kinases (CDKs) and are crucial regulators of the cell cycle.

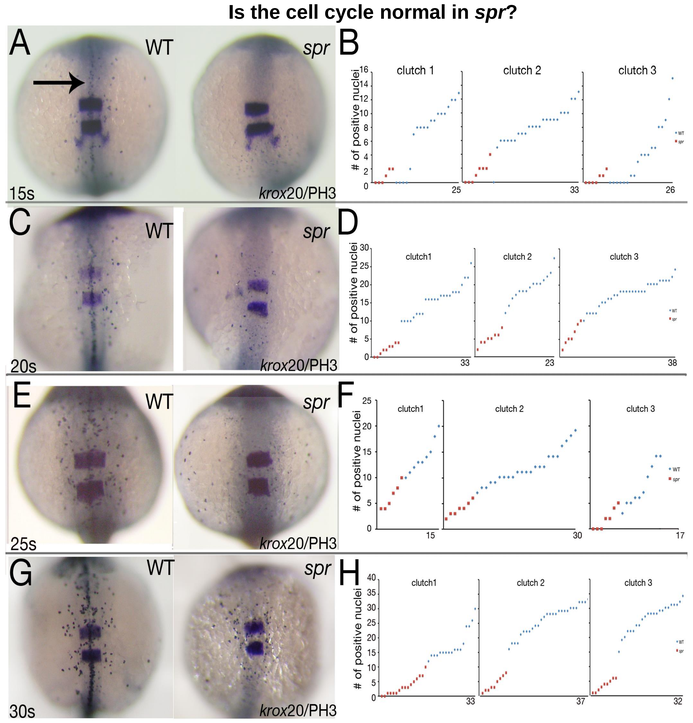
RESULTS

Is *spr* a cell cycle mutant?



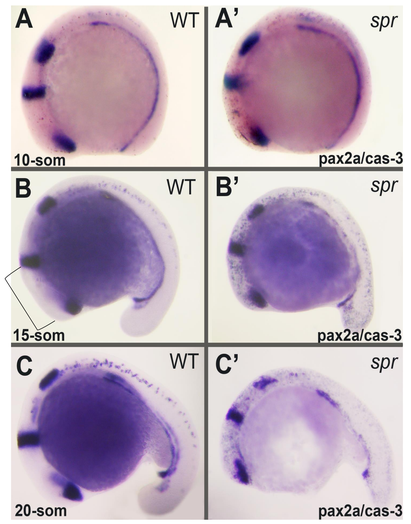
It seems so. Sequencing data reveals a nonsense mutation early in exon 2 of the *cyclin B1* gene on linkage group 5. It is likely that there is no expression of cyclin B1 in later somite stages.

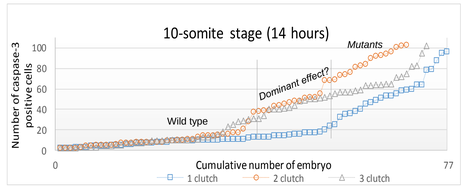
Is the cell cycle normal in *spr*?

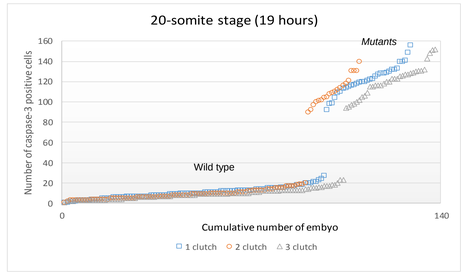


No, antibody staining of phospho histone H3 shows fewer mitotic cells in *spr.*

Are cells dying in the central nervous system in *spr* by apoptosis?

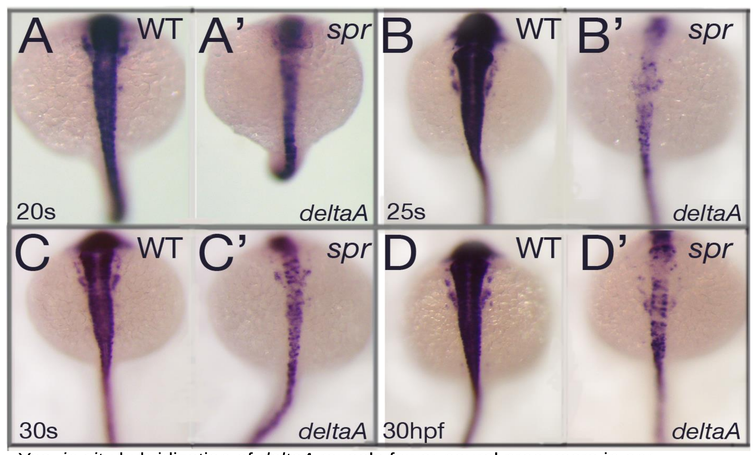






Yes, antibody staining of *caspase-3* shows cells are dying by apoptosis. We counted the number of *caspase-3* positive cells between the forebrain and the midbrain-hindbrain border (bracket) to quantify our results. The number apoptotic cells significantly increases with time.

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



Yes, *in situ* hybridization of *deltaA* reveals fewer neural precursors in *spr*.

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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