Is the zebrafish *zombie* mutant caused by a mutation in *CDC20*?

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The zebrafish, *Danio* rerio is a model organism for embryonic development due to its rapid development, optical transparency, ability to lay around 200 eggs a day, and access to its genome [2]. In our laboratory, we analyze mutants isolated from the Tübingen screen for zebrafish mutants that arrest in the first 24 hours of development [14]. The *zombie* mutant was identified because its body shape arrests in development at about 14 hours of development. Closer inspection revealed that cells in the mutant arrest in the cell cycle once the chromosomes begin to condense during mitosis in either prophase or metaphase. This defect appears as early 11.6 hours of development [14]. A similar phenotype is seen in the Drosophila melanogaster cell cycle gene *fizzy*, whose gene product was identified to be a homolog of the *Saccharomyces* cerevisiae gene, cell division cycle 20 (*cdc20*) [4]. CDC20 is an activator protein of the anaphase promoting complex/cyclosome (APC/C), an ubiquitin E3 ligase that is responsible for cell cycle progression. Activation of APC/C targets securin degradation which ultimately results in sister chromatids belonging free to move to opposite poles for anaphase [12, 20]. Loss of function of *cdc20* in S. cerevisiae causes mitotic arrest before or during early anaphase [4]. In previous unpublished studies, *zombie* was mapped to Chromosome 2 (Kane, unpublished) and it was fine mapped to the vicinity of *cdc20* (Musaev, unpublished), suggesting mutant *cdc20* as a candidate gene. Rescuing the *zombie* mutant phenotype using wild-type zebrafish *cdc20*mRNA however, proved unsuccessful (Johnston, unpublished). The zombie mutant is a homozygous recessive; in more recent experiments we mutagenized the wild-type cdc20 chromosome in heterozygous *zombie* mutant embryos using the CRISPR/Cas 9 system (Johnston, unpublished). If zombie is cdc20, this should produce mosaic patches of cells that are homozygous mutant and arrested in either prophase or metaphase in heterozygous embryos, the cell cycle is normal and most cells are in interphase. This is precisely what we observed (Johnston, unpublished). We have now begun performing complementation testing between homozygous wild-type zebrafish whose germ-line has been mutagenized for the wild-type cdc20 chromosome using the CRISPR/Cas 9 system and heterozygous *zombie* mutant carriers to determine whether *cdc20* is the gene mutated in *zombi*e.