

## Extra Views

# A Zebrafish Model of Germ Cell Aneuploidy

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## KEY WORDS

zebrafish, aneuploidy, meiosis, nondisjunction, Mps1, mitotic checkpoint

## ABSTRACT

The high frequency of chromosomal nondisjunction in human germ cells impacts society in many ways. Yet, the etiology of chromosome disorders remains unclear. Using a zebrafish strain with a hypomorphic mutation in the kinase Mps1, a genetic association between reduced germ cell mitotic checkpoint activity and aneuploid progeny was recently established. This work highlights the exquisite sensitivity of vertebrate germ cells to disruptions in Mps1 function and mitotic checkpoint activity. In addition, it introduces the zebrafish as a promising tool with which to further investigate the origins of aneuploidy.

Aneuploidy originates primarily from errors in meiotic chromosome segregation, a phenomenon that is predominantly maternal and displays a striking increase in frequency with maternal age. In fact, among women over 40 years of age, ~35% of all clinically recognized pregnancies are trisomic or monosomic.<sup>1,2</sup> How germ cell aneuploidy originates is largely enigmatic; however, a candidate for influencing the etiology of aneuploidy has emerged in the past several years. The mitotic, or spindle, checkpoint is a sensing mechanism that detects spindle errors or misaligned chromosomes in dividing cells and initiates a metaphase arrest to permit error correction. Such a checkpoint ensures that genomes are distributed to daughter cells with high fidelity. The mechanisms of this checkpoint, during which kinetochore-chromosome attachment is coordinated with anaphase onset, are currently being investigated by several groups.<sup>3</sup>

It is not a surprising prediction that a process that restricts mitotic error would also operate in human germ cells undergoing meiosis, and that age-related reductions in mitotic (or meiotic) checkpoint activity might be responsible for prominent characteristics of aneuploidy. Indeed, genetic evidence for the importance of mitotic checkpoint regulation during meiosis was provided four years ago in experiments using *S. cerevisiae*.<sup>4,5</sup> However, the extent to which this checkpoint prevents meiotic error in vertebrate germ cells has been difficult to judge, as mice with null mutations in mitotic checkpoint genes have failed to survive past several days of development.<sup>6-8</sup>

We addressed this issue using a zebrafish mutant (originally called “nightcap”) that we had serendipitously discovered via a mutagenesis screen for weak, temperature-sensitive mutations disrupting regeneration of the adult caudal fin. While adult *nep* mutants are normal in virtually all aspects at the permissive temperature, they were extremely difficult to breed as homozygotes. Only about 1% of progeny from homozygous matings survived to adulthood, with most animals showing highly variable defects as early-stage embryos. A significant percentage of dysmorphology was also seen in progeny from outcrosses of homozygous mutant males or females to wildtype fish. By using defective fin regeneration as a trait for genetic mapping, we positionally cloned the responsible mutation, a missense change in the Mps1 kinase.<sup>9</sup> Mps1 has a universal role among eukaryotes in the mitotic checkpoint, and is essential for meiotic chromosome segregation in yeast.<sup>5,10-12</sup>

Upon gene identification, the most obvious explanation for the heterogeneous embryonic dysmorphology was aneuploidy. We went on to show that *mps1* message is highly concentrated in male and female germ tissues, mainly in immature meiotic cells, as opposed to minimal expression in all somatic tissues examined. When adult mutant males were kept briefly at the restrictive temperature for the mutation, they demonstrated a deficiency in spermatocyte mitotic checkpoint activity. Furthermore, at the permissive temperature, they displayed an unusually wide range of sperm size and DNA content by flow cytometry, suggestive of monosomies and trisomies. Both fluorescent in situ hybridization and PCR-based chromosome analysis confirmed aneuploidy in embryos derived from *mps1* mutant parents.<sup>13</sup>

Thus, by possessing a hypomorphic mitotic checkpoint mutant that permitted viability, a clear link was made between reduced germ cell checkpoint activity and aneuploidy. Several interesting questions remain, however. For instance, why is meiosis, and specifically meiosis I, so exquisitely sensitive to a reduction in Mps1 function? Because spermatocytes with an *mps1* mutations are error-prone, it appears that an extended arrest in meiosis I (as in oocytes) is not essential for vulnerability to error. Second, can the aneuploidy generated by this model be modulated? This might be attempted chemically or through transgenic augmentation of germ cell mitotic checkpoint activity. Third, do *mps1* missense mutations in mammals have similar effects? Here, one could use homologous recombination and embryonic stem cell technology to introduce an identical mutation into the murine germline. Similarly, it is tempting to speculate whether certain forms of human infertility in otherwise normal men and women are caused by missense mutations in mitotic checkpoint genes like *mps1*.

Perhaps most importantly, our study highlights a promising conduit, the zebrafish, for further investigation into the etiology of aneuploidy. Admittedly, detailed analysis of cell cycle machinery would be inadequately served by the zebrafish model system. However, it is a proven tool for vertebrate gene discovery. Forward genetic screens may be conducted with relatively little space, personnel, and cost. Mutant identification is assisted by large clutch size, ex utero development, and an embryonic transparency that facilitates detailed inspection of live progeny. All of these advantages, plus the fact that gametes are easily collected from anaesthetized males, forecast successful genetic screens for additional zebrafish aneuploidy mutants. Potentially fruitful strategies include a flow cytometric screen of sperm from mutagenized families, or detection of high frequency loss of a transgene that constitutively expresses green fluorescent protein in progeny. As the zebrafish community continues to expand, the fish should emerge as another powerful tool with which to address the origins and pervasiveness of human chromosome disorders.

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