Loss of function mutation for *tp53* does not rescue small eye phenotype in *Danio rerio*

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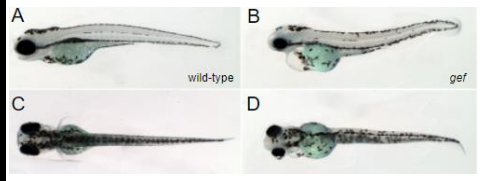
Abstract

In Zebrafish, the *chaf1b* gene is in part responsible for the development of the retina. When the *chaf1b* mutation, *t24412*, is homozygous, cell death is promoted through a Tp53-dependent pathway resulting in a small-eye phenotype. In our hands, knockdown of Tp53 via morpholinos failed to rescue the small-eye phenotype found in *chaf1b*^{nt2} homozygous mutants. Because morphants may not fully inhibit target gene function we crossed 2 fish heterozygous for *nt2* (*gef*) and a cell-death induction deficient allele of *tp53* (*zdf1*) and analyzed double homozygous mutants. Restriction fragment length polymorphism analysis was used to verify *nt2* and *zdf1* alleles. We found that loss of *tp53* function did not rescue the *chaf1b*$^{nt2/nt2}$ small-eye phenotype.

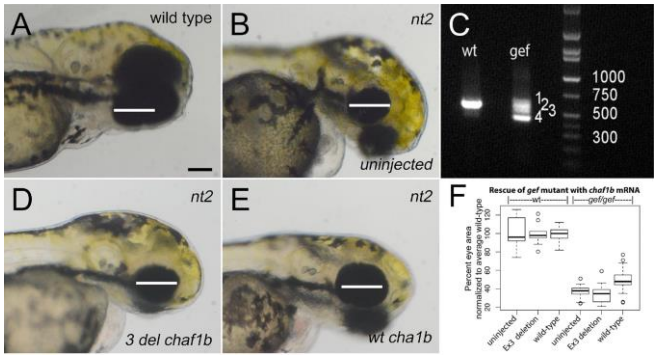
Introduction

The *chaf1b* gene codes for a subunit of the histone loading protein complex CAF-1 and is vital to the eye development of Zebrafish. The *good effort (gef* a.k.a. *nt2*) allele presents as a small-eye phenotype and results from a 3 bp deletion in intron 3 that overlaps the splice donor site, which leads to exon 3 splicing defects and non-functional protein (Bailey and Hyde). Chaf1b morphants phenocopy the *gef* small-eye phenotype and supports the idea that loss of *chaf1b* function is the cause of the *gef* phenotype. We tested whether addition of wild-type *chaf1b* mRNA could rescue *gef* mutant embryos. In vitro transcribed *chaf1b* mRNA, subcloned from *gef* and *wt* embryos, was injected into 1 to 2 cell embryos homozygous for *gef*. Mutants injected with normal *chaf1b* mRNA, but not those injected with mutant *chaf1b* mRNA, had bigger eyes than the mutants which did not receive injections. Loss of *chaf1b* function has been hypothesized to lead to failed histone loading on newly replicated DNA, leaving the DNA exposed so that it becomes damaged and leads to cell arrest in S-phase and apoptosis through a *tp53*-dependent pathway (Fischer *et al.*). However, knock down of *tp53* via morpholino injection resulted in a failure to rescue the *gef* phenotype. In order to more rigorously test this hypothesis, we tested whether a genetic loss-of-*tp53*-function allele, *zdf1*, which fails to activate apoptosis, could rescue the *gef* phenotype.

Results

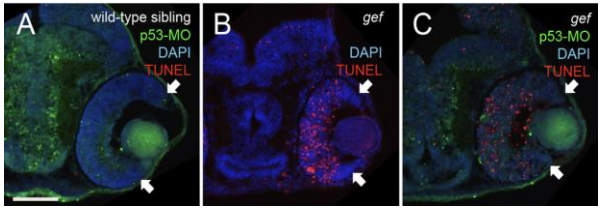


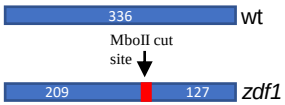
The *gef* (*nt2*)mutation results in a small-eye phenotype
Figure (A). Lateral view of *wt* zebrafish. (B) Lateral view of *gef* mutant. (C) Dorsal view of *wt* zebrafish. (D) Dorsal view of *gef* mutant. Note the large unaffected lens protruding from the retina.



Injections of wild-type *chaf1b* mRNA partially rescue small-eye phenotype
(A) Right, lateral view of wild-type embryo. Black bar represents 100 microns. White bar is average A-P diameter of *gef* mutants (B) Uninjected *gef* mutant. (C) *chaf1b* exon 3 deletion mRNA *gef* mutant. (D) *wt chaf1b* mRNA injected *gef* mutant. (E) Gel of the RNA sequences, 2 being leftover maternal *wt* RNA, 4 being the truncated *gef* RNA, 1 and 3 are other RNA splice sites. (F) size difference of zebrafish eyes after injection relative to wild-type.

Morpholino knockdown of *tp53* does not rescue small-eye phenotype
Figure (A) Image of *wt* phenotype with *tp53* morpholino knockdown and TUNEL to label cells undergoing apoptosis. (B) *gef* mutant without *tp53* morpholino injection and TUNEL to label cells undergoing apoptosis. (C) *gef* mutant with *tp53* morpholino injection and TUNEL to label cells undergoing apoptosis. Note that the dividing cells of the margin (arrows) do not apoptose in any condition.

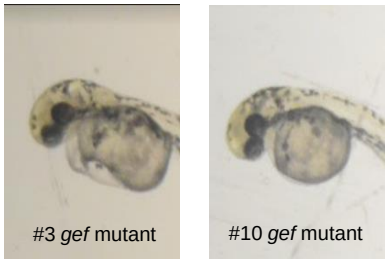




Base change of defective *tp53* allele, *zdf1*, creates a second cut site for MboII
The *zdf1* allele is caused by a single T to A point mutation. This creates a second sequence of 5'GAAGA3' that MboII can cut the DNA at. This results in 2 bands being present when amplicons of the *zdf1* is cut by MboII into 209 bp and 127 bp bands. The wild-type amplicon fails to cut and is seen on a gel as a 336 bp band.

Zygosity of *tp53* was verified via restriction fragmentation length polymorphism analysis (RFLP)
Loss of Tp53 function only occurs when homozygous *zdf1* alleles are present in an embryo.

Lane 1 contains the FisherSci 1kb plus DNA lane marker.
Lane 2 is a heterozygous control.
Lane 3 is a homozygous control.
Lane 4 is uncut DNA from the homozygous control.
Lanes 5-8 are test DNA.
Embryo 3 (lane 5) is heterozygous.
Embryo 10 (lane 6) is homozygous mutant.
Embryos 18 and 19 (lanes 7 and 8) are homozygous wild-type embryos.



Loss of Tp53 function does not rescue the *gef* (*chaf1b*$^{nt2/nt2}$) small-eye phenotype
All embryos with small eyes were analyzed at 3 dpf for tp53 zygosity using RFLP. The left panel represents embryo #3 in the gel shown before, whereas the the right panel represents the #10 *zdf1* homozygote shown in the same gel. The size of zebrafish retina at 3 dpf is comparable to that of zebrafish which have homozygous *chaf1b*^{nt2}. Therefore, knockout via loss of function mutation failed to rescue the small-eye phenotype.

Conclusions

Knockout of *tp53* via a loss of a function mutation in homozygous *zdf1/zdf1* embryos is not sufficient to rescue *gef* mutant fish (small-eye phenotype).

Rapidly dividing cells, those predicted to die by apoptosis in *gef* mutants, persist longer than those which have left the cell cycle.

References

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