The collapse of fin scaffolding: characterizing actinodin loss-of-function mutations in zebrafish

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Introduction

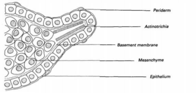

Figure 1A: Zebrafish pectoral fin bud

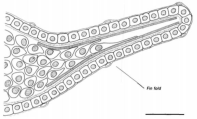

Figure 1B: Zebrafish pectoral fin fold

Zebrafish pectoral fins develop from fin buds (A) elongating into fin folds (B). The elongating fin folds are supported by ridged actinotrichia fibrils that span the length of the developing fold. These fibrils also guide the migration of mesenchymal cells as they migrate along the fold. A subset of these cells will differentiate into osteoblasts that secrete the bone matrix to create the skeletal structure of the fin rays. In the adult fin, actinotrichia fibrils are still present, located at the distal tip of the fin rays. Actinotrichia fibrils are composed of collagen and actinodin proteins which are encoded by the actinodin (*and*) family of genes containing four paralogues (*and1-4*).

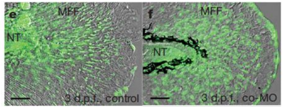

Figure 2: Median fin fold (mff) defects of double knockdown embryos at 3 days post fertilization (dpf)

Previous observations on embryos with a morpholino-mediated knockdown of *and1* and *and2* found that these morphant embryos presented with an absence of actinotrichia along with fin fold defects including a reduction in overall size (F). Morpholinos are unable to downregulate *and1/2* throughout fin development. Therefore a more permanent disruption of the gene expression using CRISPR/Cas9 can be used to observe the effects of the loss of actinotrichia at later developmental stages and adulthood.

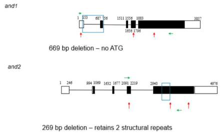

Figure 3: *and1* 669bp and *and2* 269bp deletions in the *actinodin* mutants.

CRISPR/Cas9 can be used generate partial deletions in the genes of interest to create a loss of function mutations. The 669 base pair (bp) deletion in the *and1* gene and the 269bp deletion in the *and2* gene (blue rectangles) remove the start codon and 8/10 of the actinodin's structural repeats respectively.

Hypothesis:

The disruption of actinotrichia formation during zebrafish development causes fin fold and mesenchymal cell migration defects that create fin ray defects in the adult zebrafish.

Objectives:

1. Analysing the actinodin 1 and actinodin 2 mutations in zebrafish
2. Identifying the molecular mechanisms involved in the mutant phenotype

Methodology

Generation of the *and1/2* double homozygous mutants

CRISPR/Cas9 to generate a partial deletion in an *and1* allele of the founder zebrafish

CRISPR/Cas9 to generate a partial deletion in an *and2* allele of the founder zebrafish

Crossed *and1* founder to produce homozygous offspring

Crossed *and2* founder to produce homozygous offspring

Crossed *and1* single homozygote with *and2* single homozygote to produce a double heterozygote mutants

Crossed double heterozygotes to produce double homozygotes

Gene and protein expression analysis

Fixed control and mutant embryos at 2 dpf

Performed whole mount *in situ* hybridization to visualize *and* gene expression

Performed whole mount immunohistochemistry to analyse expression of proteins forming actinotrichia: collagen type 2 and actinodin

Fin fold analysis

Took brightfield images of 5 dpf larvae

Fin skeleton analysis

Raised wildtype and double homozygous fish

Mutants were fixed and their scales were removed

Bones were stained using alizarin red staining

Results

Actinodin gene expression in WT vs *and1*$^{-/-}$*and2*$^{-/-}$ zebrafish

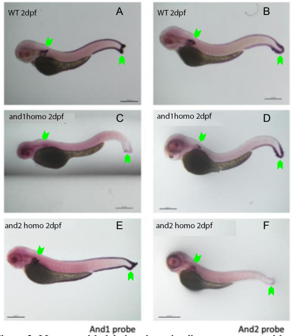

Figure 3: Mutants with deletions in *actinodin* genes present with decreased *actinodin* gene expression. Both the *and1* and *and2* single homozygotes present with a reduced expression of their respective genes compared to the wild type (see arrows in A,C,E and in B,D,F). This indicates that the deletions in the *and* genes caused by the CRISPR/Cas9 system, successfully disrupt the expression of the *and* genes during zebrafish development.

Fin fold analysis of WT vs *and1*$^{-/-}$*and2*$^{-/-}$ zebrafish

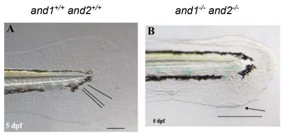

Figure 4: Double homozygous mutants present with deformed fin folds. The wildtype (A) larvae present visible actinotrichia (black lines). The double homozygous mutants (B,C) lack visible actinotrichia. Double homozygote deformities include folding at the distal end (black arrow), clustering of the mesenchymal cells (green arrow), and shortening of the fin fold. Scale bar, 10μm (A) and 20 μm (B,C).

Actinotrichia fibrils in WT vs *and1*$^{-/-}$*and2*$^{-/-}$ zebrafish

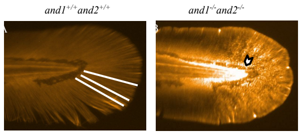

Figure 5: Double homozygous mutants present without actinotrichia fibrils. Immunohistochemistry using a type 2 collagen antibody on wildtype (A) and *and1/2* double homozygous mutants (B). The wild type embryos (A) display linear actinotrichia fibrils in a tight array along the fin fold (lines). The double homozygotes (B) present with an absence of the linear actinotrichia fibrils and increased col2 expression along the proximal base of the fin fold (arrow).

Caudal fin skeleton analysis of WT vs *and1*$^{-/-}$*and2*$^{-/-}$ zebrafish

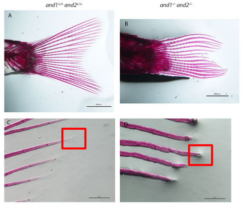

Figure 6.1: Double homozygous mutants present with fin ray defects. Alizarin red staining of bone revealed mutants (B,D) with thicker and less numerous fin rays, as well as a caudal fin that is less outstretched as compared to the wild type fish (A,C). The actinotrichia are visible at the tips of the wild type fin rays (see box in C) but not in the mutants (see box in D). Scale bar, 1000 μm (A,B) and 20 μm (C,D).

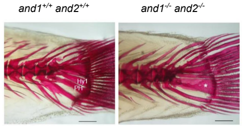

Figure 6.2: Double homozygous mutants present with endoskeletal defects. Alizarin red staining of bone revealed mutants with an apparent fusion (*) between the parhypural (PH) and hypural 1 (Hy1). Scale bar, 0.5 mm.

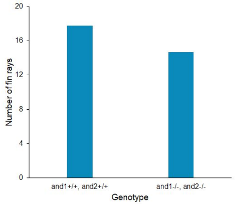

Figure 6.3: Double homozygous mutants present with a decreased number of fin rays. The wildtype fish (n=12) present with an average 17.75 fin rays while the double homozygotes (n=3) present with an average of 14.67 fin rays. The mutation resulting in the loss of an average 3.08 fin rays.

Conclusions

- The loss of function of actinodin proteins resulted in the disruption of actinotrichia fibrils;
- Actinotrichia fibrils are important for the development of the fin structure;
- The fin folds and migration of mesenchymal cells are disrupted during development;
- The lack of guidance for the osteoblast precursors results in their more proximal deposition and a disrupted fin phenotype in both the fin rays and endoskeletal structures of adult fish.

References

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