Genetics of body shape: Connexin43 is the key to two zebrafish mutants with shorter backbones and fins

The variation in body shape within and among animal species has long been an intriguing question. D'Arcy Thompson, a pioneer of mathematical biology in the early 20th century, put forth the idea that body shape variation in animal species could be explained by the extension, contraction, and bending of the body frame of a standard species. Today, thanks to great progress in molecular biology, we can directly address this question to ask about the underlying molecular mechanisms driving these processes.

The zebrafish (*Danio rerio*) is a model organism for developmental and genetic studies, and several mutants related to bone formation have been identified and analyzed. In this study, we aimed to challenge D'Arcy Thompson's proposition and gain a better understanding of the molecular mechanism guiding body shape variation. To do this, we focused on the zebrafish body shape mutant named *steopsel* (*stp*) (Fig. 1).

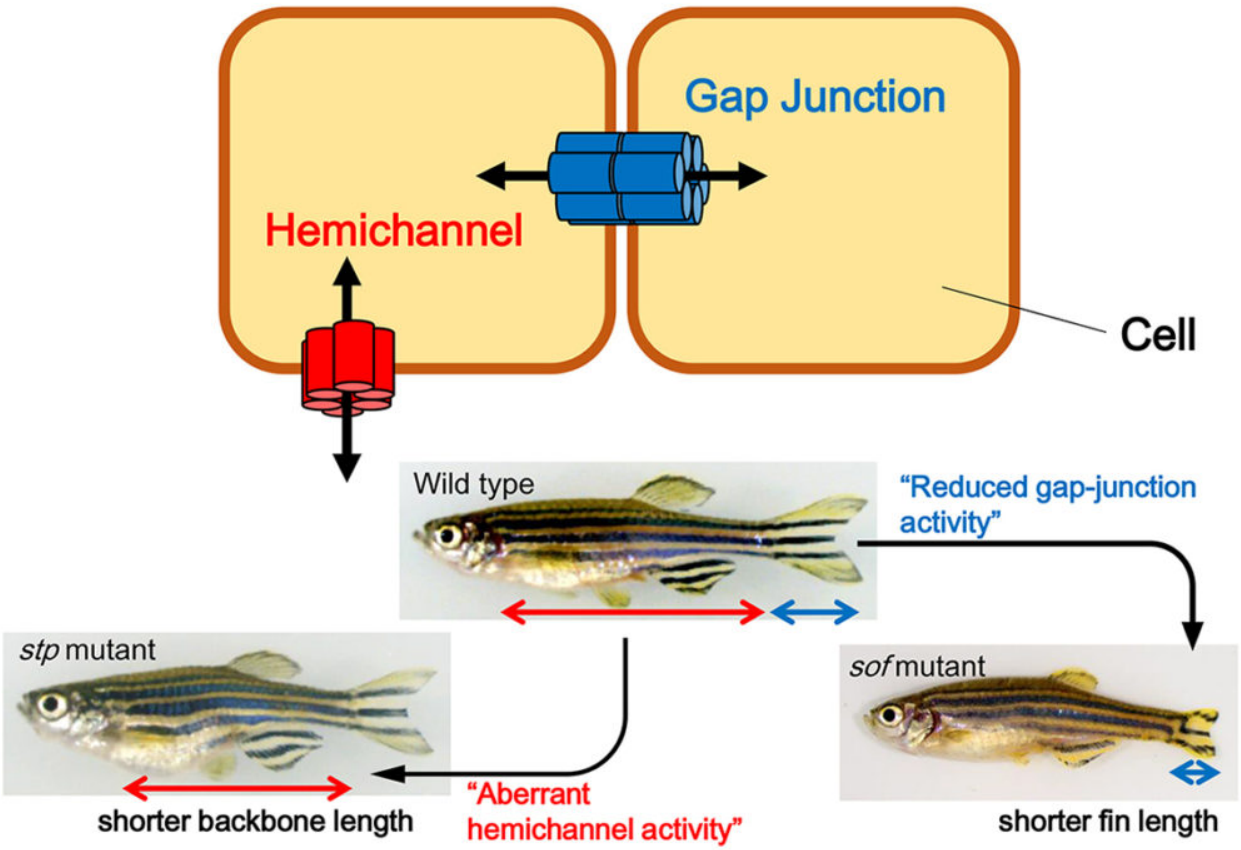

Fig. 1. Dual functions of Connexin43 protein cause two different bone phenotypes in zebrafish.

The *stp* mutant was originally isolated by a large-scale screen of zebrafish mutants induced by ENU, a chemical reagent that causes mutations in DNA. The *stp* mutant grows normally until ?35 days after fertilization; however, the body length of the mutant fish ultimately becomes shorter than that of the wild type (i.e., non-mutant) fish at later growth stages. Micro-computed tomography scanning analysis revealed that the overall shape of each vertebra of the mutant is almost identical to that of the wild type, whereas the vertebra length (in the head-to-tail direction) was shorter. To reveal the molecular mechanism causing this shorter vertebra phenotype, we performed genetic mapping analysis and found a mutation in the connexin43 gene (*cx43*).

Connexin protein is a component of hemichannel and gap junctions, important protein complexes that regulate traffic for a cell. Six connexin proteins form a hemichannel, which functions as a channel molecule and allows small molecules and ions to move in and out of a cell. Docking of two hemichannels on adjacent cells forms a gap junction to allow for the movement of small molecules between neighboring cells.

cx43 is one of the most widely studied connexin genes because it is linked to several human diseases. In zebrafish, *cx43* was previously identified as a corresponding gene for a short fin segment length mutant called short-of-fin (*sof*). A defect in gap junction function due to a mutation in *cx43* causes the short fin phenotype.

To identify the cause of the substantial difference in phenotype between the *sof* and *stp* alleles of *cx43*, we compared the functions of the gap junction and hemichannel between wild type, *stp*, and *sof* fish. We performed electrophysiological experiments and found that the growth-dependent malformation of the vertebrae in *stp* fish is likely caused by aberrant hemichannel activity of Connexin43 rather than by its reduced gap junction function due to the *stp* mutation (Fig. 1).

Masakatsu Watanabe [1] *and* ***Shigeru Kondo*** [1,2]
[1]*Graduate School of Frontier Biosciences, Osaka University, Japan*
[2]*CREST, Japan Science and Technology Agency*

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