Atlas of Science
another view on science
https://atlasofscience.org

Exploring gap junction networks required for zebrafish skin pattern formation

The study of pattern formation has advanced to a new phase involving channel molecules acting on bioelectric signals, with gap junctions being major relevant factors.

Gap junctions are composed of connexin proteins: six connexins form hemichannels/connexons on the surface of the plasma membrane, and gap junctions are formed between cells when two connexons on adjacent cells dock. However, understanding the role of gap junctions is challenging because of their complexity. Approximately 20 connexin genes exist in mammals and 40 exist in fish, and different types of connexins may form gap junctions. The resultant heteromeric or heterotypic gap junctions exhibit different characteristics from homomeric gap junctions, including the size of the gap junction pore, selectivity of small molecules passing through it, and even changes in the directionality of the current flow.

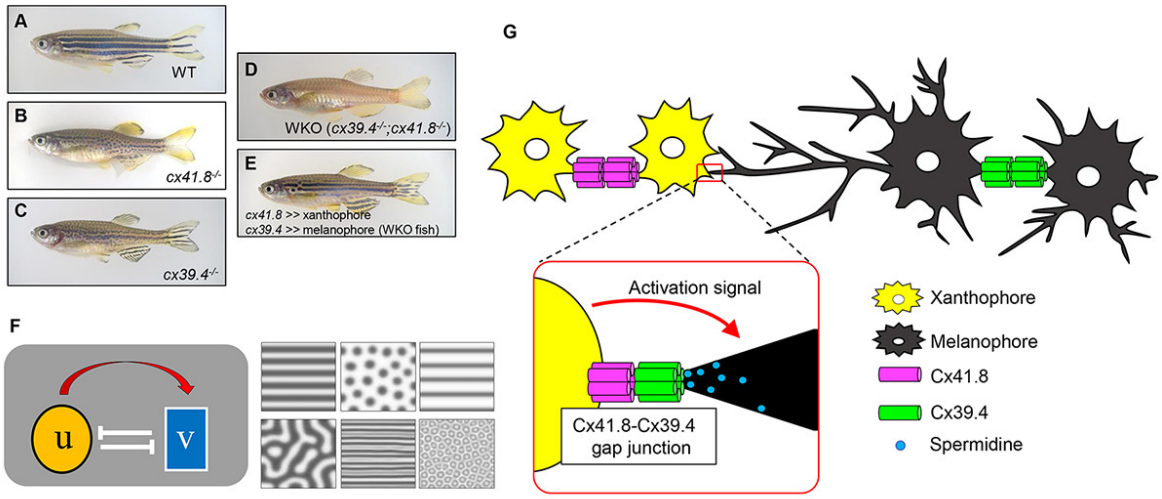

Fig. 1. Minimal gap junction network model for zebrafish skin pattern formation.

The zebrafish exhibited black and yellow stripes composed of melanophores and xanthophores, respectively (Fig. 1A). This striped pattern is considered a model case for pattern formation studies. The reaction-diffusion (R-D) model is a well-known theoretical model of pattern formation, in which the reaction and diffusion of two factors create a stable pattern in space (Fig. 1F). One well-known model with long-range activating and short-range inhibitory effects is applied to zebrafish pigment pattern formation.

Gap junctions link zebrafish skin patterns to the R-D model. Loss of *connexin41.8* (*cx41.8*, Fig. 1B) results in a spotted pattern, while loss of *connexin39.4* (*cx39.4*, Fig. 1C) results in a wavy stripe/labyrinth pattern. These patterns are well produced by the R-D model (Fig. 1F). When both genes are deleted, the pattern is lost (double knockout, WKO, Fig. 1D).

This study delves into the complexity of gap junctions. To understand the gap junction network required for the skin patterning, we controlled connexin expression in zebrafish pigment cells using transgenic techniques. *cx41.8* and *cx39.4* were expressed in various combinations in WKO fish. Thus, the striped pattern was reproduced by expressing *cx41.8* in xanthophores and *cx39.4* in melanophores, which is the minimal requirement for stripe patterning (Fig. 1E).

Furthermore, we performed electrophysiological experiments and found that the Cx39.4-gap junctions exhibited spermidine-dependent rectifying properties. The involvement of spermidine in pattern formation has been previously described: a) the Kir7.1 channel involved in pattern formation requires polyamines for its function; b) defects in spermidine synthesis alter the skin pattern; and c) Cx39.4 and Cx41.8 have polyamine-sensitive motifs at their N-termini. Taken together, these results suggest the existence of the gap junction network shown in Figure 1G, in which the gap junctions between xanthophores and melanophores mediate an activating signal from the former to the latter.

In conclusion, the minimal gap junction network required for striped pattern formation in zebrafish is the expression of *cx39.4* and *cx41.8* in melanophores and xanthophores, respectively. The gap junctions of Cx39.4 and Cx41.8 have potential rectification properties that are important for the transfer of activating signals from xanthophores to melanophores.

Yuu Usui [1,2], ***Masakatsu Watanabe*** [1]
[1]*Graduate School of Frontier Biosciences, Osaka University, Japan*
[2]*First Department of Physiology, Kawasaki Medical School, Japan*

Publication

The minimal gap-junction network among melanophores and xanthophores required for stripe pattern formation in zebrafish
Yuu Usui, Toshihiro Aramaki, Shigeru Kondo, Masakatsu Watanabe
Development. 2019 Nov 15