Pigment Pattern Formation by Contact-Dependent Depolarization

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The zebrafish, a tropical freshwater fish, has distinctive pigmented stripes that span the length of the body. Molecular genetic studies with this model organism have identified and characterized many genes involved in the formation of skin patterns and suggest that the interactions between the two pigment cell types, melanophores and xanthophores, play a key role (*1*–*3*). However, this molecular interaction network is unclear. Because the formation of pigment patterns occurs only in the skin of adult fish, most experiments have been performed by observing the distribution or migration of pigment cells in intact fish. However, these observations do not allow for the elucidation of specific interactions. To overcome this difficulty, we used gene mutation analysis to examine pigment cell interactions in an in vitro system.

In wild-type fish, melanophores and xanthophores segregate precisely (Fig. 1A). However, these cells intermingle in some regions of fish with a mutation in the *jaguar* gene (Fig. 1A), suggesting that communication between the pigment cells is disturbed (*2*, *4*). The *jaguar* gene encodes an inwardly rectifying potassium channel 7.1 (Kir7.1) (*5*), and this gene is expressed and required in melanophores (fig. S1). Prior works have shown that loss of Kir channels often leads to the depolarization of cells (*6*). Therefore, mutation of *jaguar* may result in the depolarization of melanophores. To test this hypothesis, we studied cell membrane potential by using a voltage-sensitive dye, $DiBAC_4(3)$. Pigment cells were dissociated from the fins and incubated with $DiBAC_4(3)$. As expected, the intensity of fluorescence was much higher in the *jaguar* melanophores than in the wild-type melanophores, indicating that the membrane undergoes constant depolarization in the *jaguar* melanophores (fig. S2). Moreover, we observed wild-type melanophores undergo transient depolarization when in contact with the dendrites of a xanthophore (Fig. 1B and movie S1). On the other hand, collision of fibroblasts did not induce contact-dependent depolarization of melanophores (movie S1), suggesting that a specific membrane-associated signal exists within the dendrites of the xanthophores. Long-term observation also revealed that most melanophores rarely migrate when they were not in contact with xanthophores. However, when melanophores were depolarized by contact with a xanthophore, they frequently (6 out of 10) moved away from the xanthophore (Fig. 1C and movie S3). This "repulsive" behavior should be considered important, because melanophores from the *jaguar* mutant always (10 out of 10) maintained contact with the xanthophore (movie S4). The repulsive behavior between melanophores and xanthophores may be responsible for the segregation of the two cell types in the skin of zebrafish.

Contact-dependent depolarization as well as the repulsion behavior shown by the melanophores may form part of the interaction network for stripe patterning. Mathematical studies have suggested that animal skin patterns can be formed by a reaction-diffusion (RD) mechanism (*7*, *8*). Theoretically, diffusion can be replaced by the combination of other signaling mechanisms that have long and short functional distances (*9*). If the interaction observed in this study serves as a short-range interaction, it does not contradict the RD hypothesis. Signal transfer via dendrites is more likely to be the mechanism underlying zebrafish skin pattern formation rather than diffusion because it is a more stable and direction-oriented process compared to a concentration gradient.

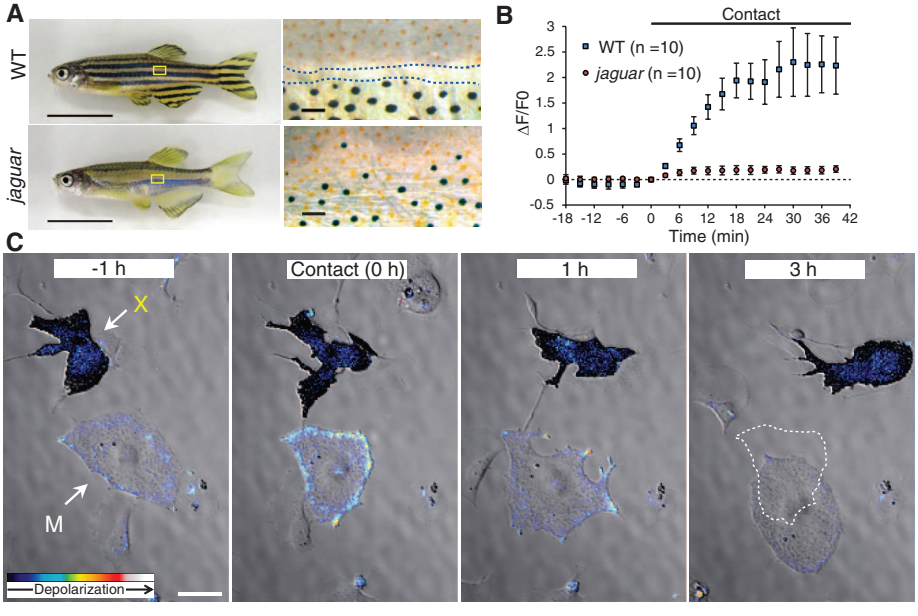

Fig. 1. (**A**) Pigment pattern of wild-type (WT) and the homozygous kir7.1 mutant (*jaguar*). Black cells and yellow cells are melanophores and xanthophores, respectively. Scale bars in overall views indicate 1 cm; enlarged views, 100 μm. Blue dotted lines indicate the edge of each group of pigment cells. (**B**) Time course of melanophore depolarization (ΔF/F0, change in fluorescence (F) level) by contact with xanthophores. Changes in membrane potential in WT are much larger than in *jaguar* mutant. See also movie S2 with regard to *jaguar* cells. Error bars represent SEM. (**C**) Snapshots showing repulsive behavior of WT melanophores (M) when contacted by xanthophore (X). White dotted outline indicates the position of melanophore at 0 hours. Scale bar, 25 μm.

References and Notes

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Acknowledgments: We thank K. Kawakami for providing the Tol2 vector and Y. Kurachi, K. Furutani, M. Okada, A. Ogura, and K. Ida for helpful discussions. This study was supported by grants-in-aid for Scientific Research from the Ministry of Education, Culture, Sports, Science, and Technology of Japan [Grant-in-Aid for Scientific Research on Innovative Areas, Scientific Research on (B)] and grants from the Uehara Memorial Foundation and Mitsubishi Foundation.

Supporting Online Material

www.sciencemag.org/cgi/content/full/335/6069/677/DC1
Materials and Methods
Figs. S1 and S2
References (*10*–*13*)
Movies S1 to S4

17 August 2011; accepted 9 January 2012
10.1126/science.1212821

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