session 2013

Selection internationale

cell biology and genetics

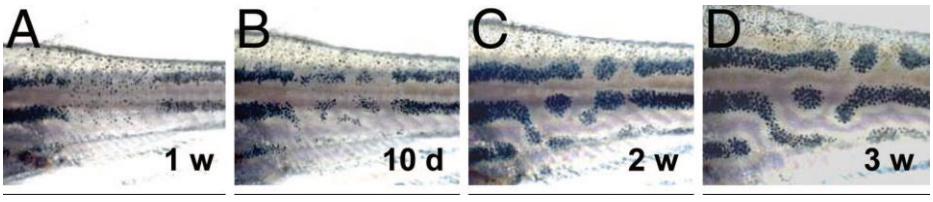
durée: 1h30

The zebrafish (*Danio rerio*), a small fish with distinctive stripes on the body trunk and fins, was selected as one of the model animal for molecular genetic research (Fig 1). The stripes of zebrafish are composed of a mosaic-like arrangement of 3 types of pigment cells: melanophores, xanthophores, and iridophores. Evidence from recent molecular and genetic studies on the altered patterns of mutant fish has suggested that the interaction between the melanophores (black) and xanthophores (yellow) are critical to the patterning process.

Figure 1. *Danio rerio*

The figure 2 presents laser ablation experiment of both melanophores and xanthophores on a square region on the left size of the body trunk above the anal fin base. In laser ablation method, each pigment cell was broken down by 4-5 laser pulses, leading to cell death. This shows that when all pigment cells in a wide area were killed, new pigment cells developed randomly in the vacant field regardless of their original position, followed by the segregation of pigment cells by cell type, through migration and cell death of pigment cells. These results suggest that the stripe pattern is generated in the skin by interactions between pigment cells, rather than by a prepattern mechanism.

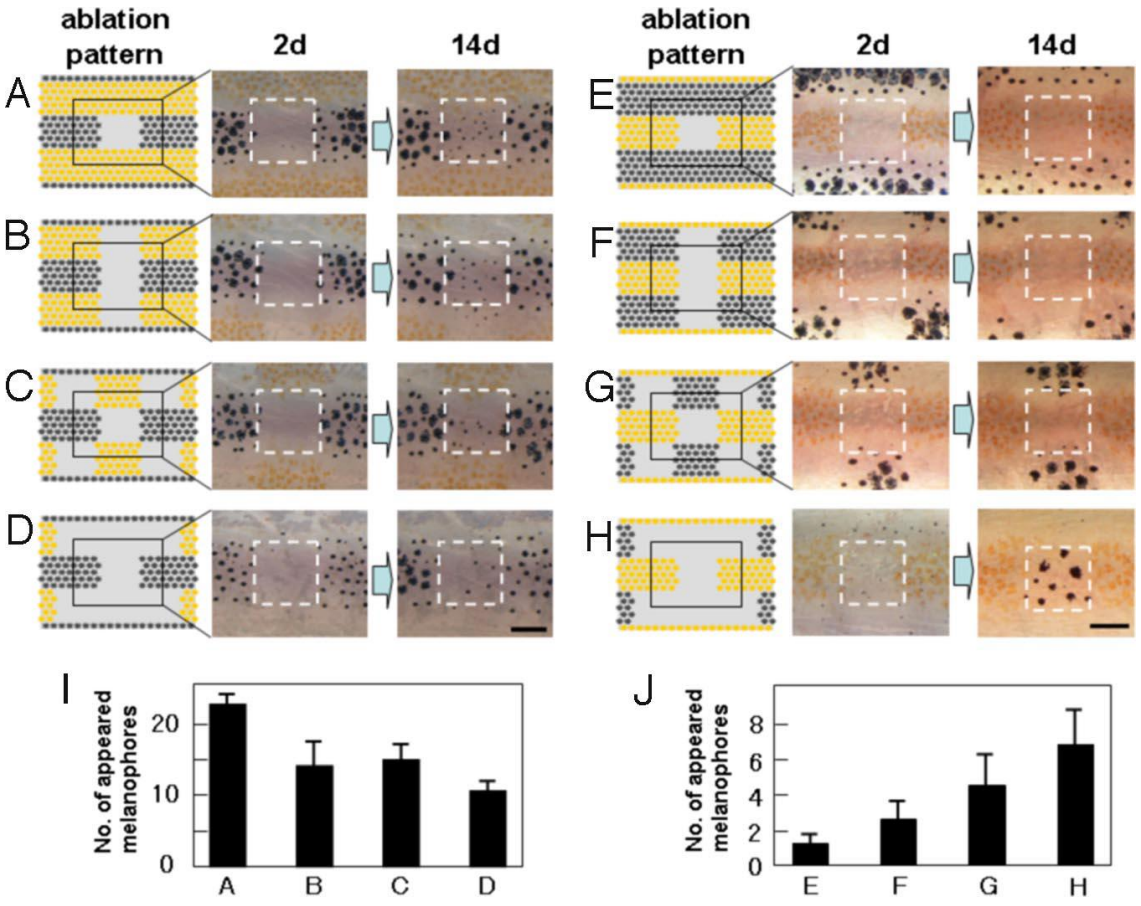
Figure 2.



Here, we try to understand the interaction between melanophores and xantophores leading to pattern formation and the genetics underlying this mechanism.

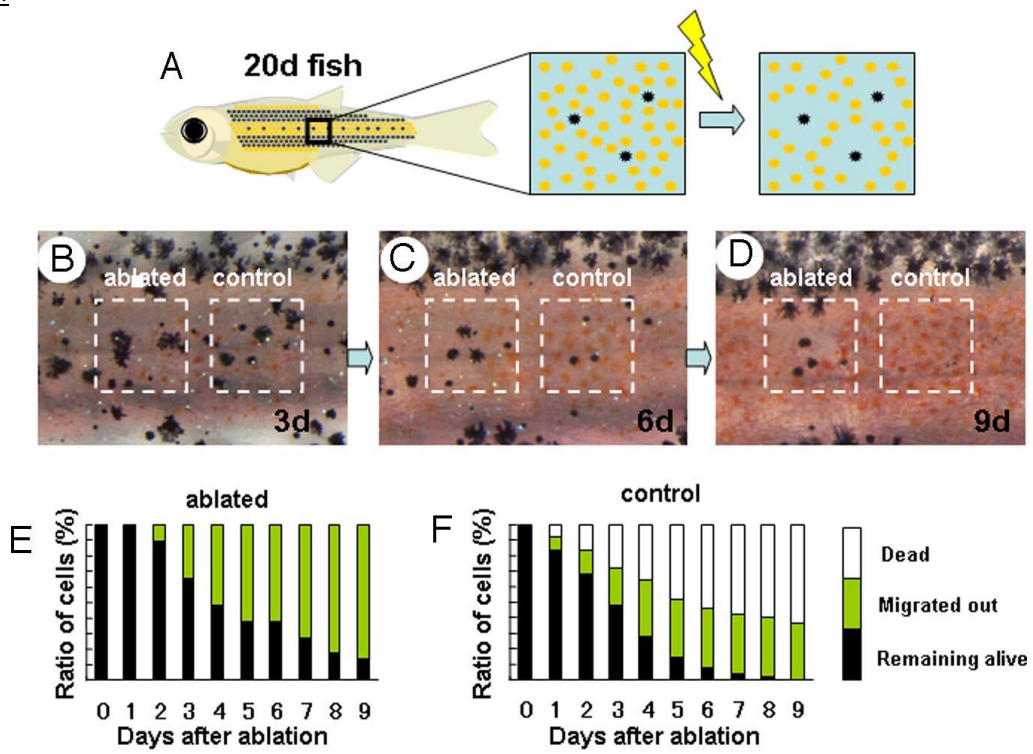
Question 1 (2 points) - To study the long-range interaction (activation versus inhibition) between melanophores and xanthophores that influences the development of new pigment cells, we perform specific laser ablation experiments. The figure 3 presents de novo pigment cell development induced by laser ablation. (*A*–*H*) To measure the influence of distant region on cell regeneration in the center region, pigment cells in the neighboring stripes were ablated by laser with the pattern shown (*Left*). The ablated pattern (day 2) and the regenerated pattern (day14) are shown (*Center* and *Right*). (*I*) The number of melanophores that appeared in the center square area for the ablation patterns A–D. (*J*) The number of melanophores that appeared in the center square area for the ablation patterns E–H. what conclusion can you make on the long-range interactions (activation versus inhibition) between melanophores and xanthophores.

Figure 3



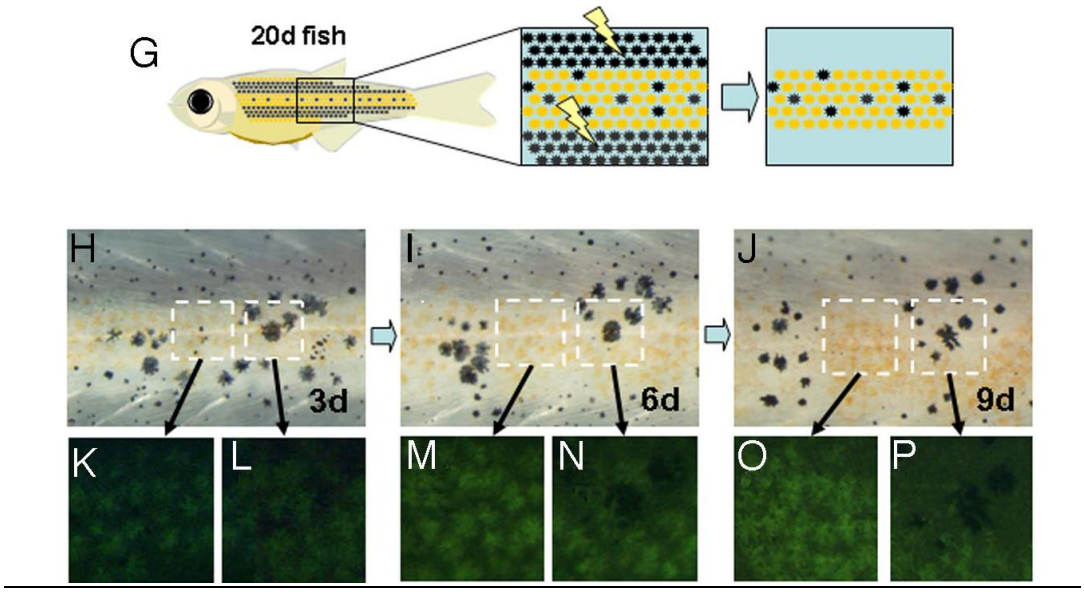
Question 2 (2 points) **-** to study the short range effect of xanthophores on melanophores, we performed experiments on young zebrafish [20 days after fertilization (dpf)]. Before the adult stripes are completed, the melanophores are sparsely distributed in the region of future black stripe but also in the future yellow stripe. Xanthophores adjacently surrounding each melanophore were ablated, and the survival of these cells was tracked. results are shown in figure 4. what can you deduce from it?

Figure 4



Question 3 (2 points)- To study the short range effect of melanophores on xanthophores, we also used 20-dpf zebrafish because these specimen have a mixed distribution of pigment cells. Melanophores in the black stripe region were continuously ablated to enhance the survival of melanophores in the middle region. the figure 5 depicts the survival of the pigment cells at days 3, 6, and 9 after ablation of black stripes. What can you deduce from it?

Figure 5



Question 4 (2 points)**-** draw a general scheme of the interactions between melanophores and xanthophores.

Question 5 (2 points)**-** This activator - inhibitor process leading to pattern formation has been first proposed by Alan Turing in 1952. He proposed that two morphogens, *u* and *v*, can interact and freely diffuse at different rates. Under certain conditions, patterns of morphogens can emerge leading to stripes, spots or labyrinths pattern. A typical form of Turing equation system leading to pattern formation in one dimension is:

$$\frac{\partial u}{\partial t} = A - Bu + \frac{u^2}{v} + D_u \frac{\partial^2 u}{\partial x^2}$$

$$\frac{\partial v}{\partial t} = u^2 - v + D_v \frac{\partial^2 v}{\partial x^2}$$

where D_u and D_v are the diffusion coefficients of u and v respectively. Can you explain the different terms of the equation and the interactions between the morphogens u and v in this system.