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Study of the planarian phototaxis during brain regeneration

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Planarians show negative phototaxis and have extensive regenerative ability, including the ability to regenerate the brain. Recently the process of regeneration of the planarian brain has been divided into three steps based on the expression of neural markers. In this study, we have analyzed the process of recovery of the light response during head regeneration. Although morphological observations indicated that regeneration of the eyes and optic nerves appeared to be completed by the fourth day, the recovery of the evasion behavior against light was not recovered within 4 days after amputation. Functional recovery of the evasion behavior could be detected starting 5 days after amputation and then gradually recovered. We previously identified genes which are specifically expressed in the brain after the recovery of morphological structures. This characteristic suggested that these genes may be involved in functional recovery of the brain. To investigate the function of these genes, we performed gene knockout analysis using the RNA interference method. The results clearly indicated that these genes are involved in the functional recovery of the visual system.

Key words : planaria, phototaxis, brain regeneration, RNA interference

INTRODUCTION

The planarian has an inverted U-shaped brain with nine branches extending from each side and a pair of eyes composed of pigment and visual cells on the dorsal side [1]. The pigment cells form an eye cup and the visual cells are located outside of the eye cup. The visual cells project optic nerve fibers on the dorsal-inside region of the brain and some of the fibers form an optic chiasma[1]. It is known that planarians have strong regenerative ability. strong regenerative ability. When an animal is cut posterior to the auricle, the head is regenerated, including a brain, a pair of eyes and optic nerves, within a few days after amputation. Recently the process of regeneration of the planarian brain has been divided into three steps based on the expression of neural markers, which have been classified as early, mid-regeneration and late expressing [2]. In this study, we demonstrated that there is a time gap between the morphological recovery and functional recovery of the light response during head regeneration, and we analyzed the function of late-expressing genes by the RNA interference method.

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MATERIALS AND METHODS

Animals. A clonal strain (GI) of the planarian *Dugesia japonica*, derived from the Iruma river (Gifu, Japan), was used in this study.

Phototaxis assay. A planarian was put into a 60 x 30 x 10 mm container which was painted black except on one clear side and exposed to light from a horizontal position on the clear side of the container. Planarian behavior was recorded using a video camera (Sony) for 90 seconds. Using a computer and behavior analysis software (Panlab), we analyzed the time spent in a target quadrant that was located in the dark end of the container opposite the clear side (Figure 1A).

RNA interference. The RNAi method was basically performed as previously described [3]. After synthesis of double-stranded RNA (dsRNA) using the1020_HH or eye53 plasmid clone as a template, respectively, dsRNAs were injected once a day for 7 days using a Nanoject injector (Drummond Scientific). Four hours after the third injection, planarians were amputated at the posterior portion of the auricles and trunk pieces were used for the phototaxis assay.

RESULTS AND DISCCUSION

Recovery of phototaxis behavior during head regeneration. In order to understand planarian phototaxis, we established a phototaxis assay system and analyzed the process of recovery of phototactic behavior during head regeneration. In previous studies eye regeneration was detected within 1.5 days after amputation, and connection of the right and left visual neurons was shown to recover within 2 days. Projection of visual neurons to the brain is established around 3-4 days after amputation. Morphological regeneration was almost complete within 4 days [2 and our unpublished data]. However, when we traced the movement of planarians in response to light irradiation during the process of head regeneration, functional recovery was clearly delayed compared to morphological regeneration. Recovery of the ability to move to the dark side of the assay chamber could be detected in 2-day regenerates and was almost complete within 4 days (data not shown). However, the negative phototactic response was still weak in 4-day regenerates. Interestingly, strong fuctional recovery of negative phototaxis was observed starting at 5 days (Figure 1B). We thus found a large time gap between morphological and functional recovery in the process of brain regeneration.

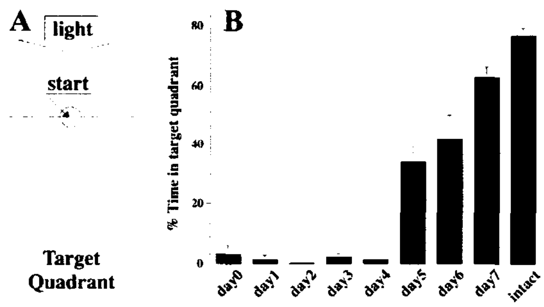

Figure 1 Position of the target quadrant (A). Mean percentage of time spent in the target quadrant by normal planarians during head regeneration (B).

Recovery of phototactic response in gene knockout planarians. To investigate the molecular mechanisms involved in functional recovery of the brain or visual system, we produced several gene knockout planarians and analyzed their phototactic behavior. As the first target, we produced knockout planarians of *Djsyt*, a planarian synaptotagmin homolog [4]. *Djsyt(-)* planarians could not distinguish the direction of the light and moved in a random way, although morphologically normal brain and visual connections were formed. This suggests that synapse formation is essential for recognition of the direction of the light. Secondly, we focused on the late-expressing genes. Based on previous studies [2, 5], brain regeneration of planarians has been divided into three stages, early-, mid- and late-stages, distinguished by the expression of brain-marker genes. Two of these marker genes, 1020_HH and eye53, are classified as late-expressing genes, since their expression is detected from 5 days. Notably, both genes are expressed in the central-posterior region of the brain where the optic chiasma is formed, and eye53 is also expressed in visual cells. The structure of the products of these two genes is similar, although we can not find sequence similarity between them and any other known protein. To investigate the relationship between the up-regulation of the phototactic response and the expression of these genes on day 5, we produced gene knockout planarians of these genes by RNAi. Interestingly, the functional recovery of the phototactic response normally seen in 5-

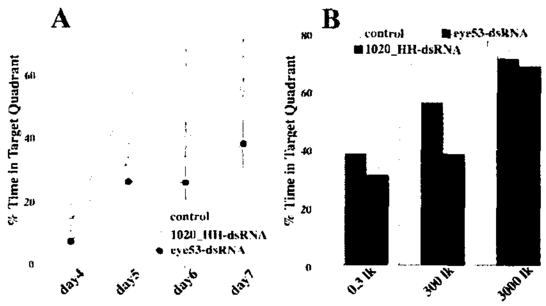

Figure 2 Comparison of mean percentage of time spent in the target quadrant by control planarians, 1020_HH-dsRNA- and eye53-dsRNA-injected planarians.

day regenerates was suppressed in the knockout planarians of both of these genes (Figure 2A). This effect was more strongly observed in eye53-dsRNA-injected planarians. However, these planarians could recognize the direction of the light, suggesting that these two genes may be involved in functional recovery of the visual system in 5-day regenerates. The effect of gene knockout of these genes became more prominent when we used a lower level of light (Figure 2B).

In conclusion, in the planarian visual system, synapse formation is essential for recognition of the direction of light, and the 1020_HH and eye53 genes may have important roles in the response to light signals.

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