Interspecies Tissue Transplants in Planarians

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Background

Planarian flatworms have many stem cells called neoblasts which makes them adept at regenerating tissue after injury. After a planarian is injured, the stem cell proliferation surges immensely. This causes stem cells throughout their body to travel to the site of injury. An active topic in regeneration biology involves the identification of signals that stimulate and mobilize stem cells to the site of injury. The aim of this project is to test whether stem cells from two different planarian species—*Schmidtea mediterranea (S. med.)* and *Dugesia japonica (D. jap.)*—can engraft, respond to injury cues, and integrate into tissues after transplantation of cells between species.

Colorimetric in situ hybridization is a process that can be used to visualize these stem cells. In colorimetric in situ hybridization, RNA probes for stem cells bind to their target sequences in the planarian. The animals are then stained which allows visualization of these stem cells.

Visualization of stem cells in the planarian can help to determine their migration patterns. We want to determine, after injury, which neoblasts migrate to the site of injury and if tissues from one or both species are regenerated.

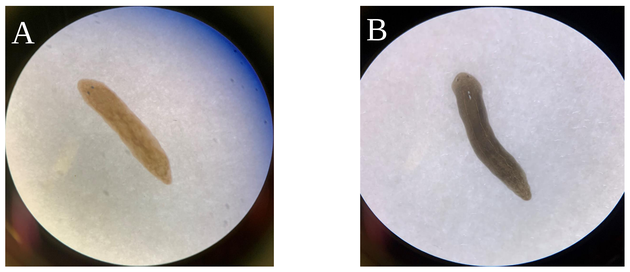

Figure 1: Two species of Planarians: These are the two species of planarians that were used. (A) is *D. jap*. (b) is *S. med.*

Methods

Each 60-mm-diameter petri dish was prepared by placing a piece of 60-mm-diameter Whatman grade 3 filter paper on the bottom which was moistened with Holtfreter's rinse. Two rectangular pieces of black filter paper were placed in the dish, moistened with Holtfreter's, and it was placed on ice. One planarian of each species was placed in chloritone. After 3.5 minutes, the planarians were transferred to Holtfreter's Rinse. After 30 seconds, each planarian was placed on a piece of the black filter paper in the petri dish. A circular hole was cut in the D. jap. using a 0.8-mm-diameter capillary tube and that tissue was discarded. The same size hole was cut in the S. med. and that tissue plug was placed in the hole in the D. jap using forceps. The planarian was then covered using OSB cigarette paper dipped in casein-saturated Holtfreter's. Four pieces of rectangular Whatman Grade 3 filter paper were dipped into casein-saturated Holtfreter's and placed on top of the cigarette paper. Four pieces of kimwipe were dipped into casein saturated Holtfreter's solution and placed on top of the filter paper. The dish was placed into a 4℃ freezer overnight. The next day the planarian was unwrapped, and the dish was flooded with planarian salts. Then, it was determined whether the transplant had been successful.

Methods cont.

If the transplantation was successful or arguably successful, the black filter paper was removed from the petri dish and the planarian was left to heal at room temperature.

Colorimetric in situ hybridization was performed on uninjured D. jap and S. med planarians. Three different procedures were investigated and attempted. The first was only using *S.med.* with the protocol for NAC fixation. The second was only for *D. Jap* with the protocol for HCl fixation. The third was for both *S. med.* and *D. jap.* with the protocol for nitric acid/formic acid fixation.[1] The riboprobes that were used were PIWI for *D. jap* and H2B DIG for *S. med.*

Figure 2: Materials for Tissue Transplantation

Results

By observation, we think that we were able to generate a transplantation success rate of about 75%. While only a few were ideal transplants, most seemed to have some sort of transplanted tissue. Only one neoblast is required to be transplanted to be visible after any sort of staining. The colorimetric in situ hybridizations have not been successful thus far. The first trial was not finished due to time constraints. There was no development in the second trial. The third trial is currently in progress.

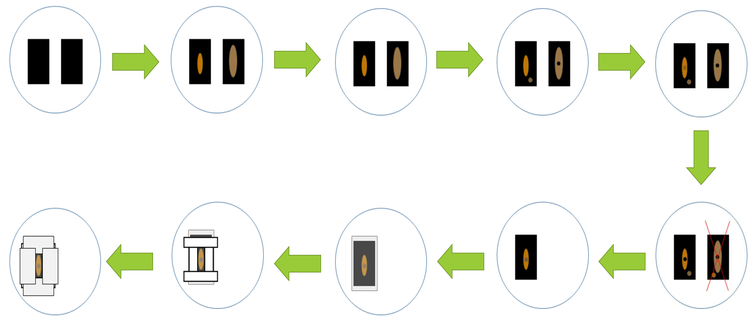

Figure 3: Schematic of the transplantation protocol. The brown oval represents *S. med.*, and the orange oval represents *D. jap.*

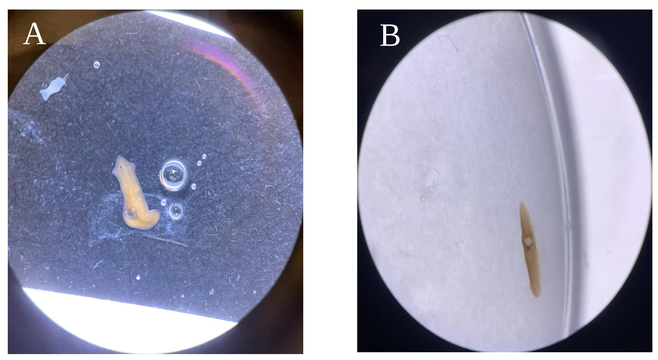

Figure 4: Identification of transplanted tissue, post-transplantation (A) An ideal transplant (B) A partial transplant as evidenced by the ring of dark tissue around the white center. For regeneration to be effective, only a small amount of tissue is required.

Future Direction

We want to continue to perform colorimetric in situ hybridizations until we get a protocol that gives optimal staining of neoblasts in both *S. mediterranea* and *D. japonica*. This may require additional research. We want to continue the process of optimization of the colorimetric in situ hybridization. After confirming that we have the optimized protocol, we will begin the staining process with the planarians containing transplanted tissue. The purpose of these colorimetric in situ hybridizations will be to qualitatively determine if the transplanting process is efficient and successful. We already believe that the transplantation process is successful as a result of visual observation, but this would just be a second confirmation. We will then begin amputation of the planarians. After transplantation, we will amputate the heads of the planarians just above the eye spots. We will then use colorimetric in situ hybridization to look at the migration of stem cells in various levels of regeneration to determine which stem cells—those from the host or those from the donor— get integrated into the regenerated tissue. We will also use fluorescence in situ hybridization to better visualize the neoblasts. Finally, we will complete the transplantation process in the opposite direction with *D. japonica* serving as the donor *a*nd *S. mediterranea* serving as the host.

Acknowledgements

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References

1. Guerrero-Hernández, C. et al. (2021). A Powerful and versatile new fixation protocol for immunohistology and in situ hybridization that preserves delicate tissues in planaria. Preprint copy under review. DOI: 10.1101/2021.11.01.466817.