

pattern of gene expression observed in regenerates are similar to the development of a joint.

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Program/Abstract # 29

The Retinal Homeobox (Rx) gene is necessary for retinal regeneration in *Xenopus laevis* tadpoles

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The Retinal Homeobox (Rx) gene is essential for retinal development. Rx functions to promote maintenance of retinal progenitor cells (RPCs). Loss of Rx function early in eye development results in impairment in or lack of eye development. In the mature retina, Rx is expressed in photoreceptors and, in lower vertebrates, in RPCs in the ciliary marginal zone at the anterior periphery of the retina. The mature *Xenopus* retina can regenerate after resection. In the second week after resection, the wound fills with RPCs that are proliferative and express RPC markers, including Rx. To investigate the role of Rx in retinal regeneration, we knocked down Rx expression using a transgenic shRNA approach. We found that Rx expression was essentially normal at late tailbud/early tadpole stages (st 38) but was decreased by st 41. There was no obvious effect on eye development until st 50 when tadpoles exhibited impaired visual function and photoreceptor degeneration. Rx shRNA tadpoles also exhibited deficits in regeneration when 25% of the retina was removed at st 44. The wound did not heal normally and RPCs did not develop normally, if at all, in the wound. Regeneration was rescued by expression of exogenous Rx, suggesting that the impairments in regeneration observed in Rx shRNA transgenic tadpoles were specifically due to knockdown of Rx expression. These results demonstrate that Rx expression is necessary for retinal regeneration. Further, these results suggest that Rx is necessary for RPC development/recruitment during retinal regeneration.

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Program/Abstract # 30

The mechanism underlying the switching of reproductive strategies in planarian: Pluripotent stem cell transplantation using microsatellite markers to identify donor-derived cells

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Planarians can reproduce asexually and sexually. Asexual (AS) strains reproduce only by fission, innate sexual (*InS*) strains

reproduce only by cross-fertilization, and some strains seasonally switch reproductive strategies. However, the mechanism underlying the switching of reproductive strategies in metazoans remain unknown. Sexual reproduction can be experimentally induced in AS worms by administration of sexualizing substance(s), resulting in acquired sexual (*AcS*) worms. Hence, we compared the pluripotent stem cells (neoblasts) of *InS* and *AcS* by transplantation in order to determine the mechanism of underlying the switching of reproductive strategies. Recently, we indicated that the determination of their reproductive strategies depend on whether they have the ability to start generating sexualizing substance(s) without administration of sexualizing substance(s). In this study, a neoblast-rich fraction obtained from donors was transplanted into X-ray-irradiated recipients. We discovered microsatellite markers and successfully estimated the ratio of donor-derived cells in recipients. By tracing donor-derived cells using these markers we demonstrated that the ratio of donor-derived cells in recipients was gradually increased with time by 10 weeks of transplantation and then the donor-derived cells were stably maintained. Then neoblasts obtained from *InS* or *AcS* worms were transplanted into AS worms. Only the recipients of *InS*-derived neoblasts exhibited sexual reproduction. This suggests that the neoblast itself determined reproductive strategy.

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Program/Abstract # 31

Genetic cellular studies of regeneration in the planarian *Schmidtea mediterranea*

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Metazoans have evolved a series of renewal and repair mechanisms to respond to both injury and normal wear and tear in order to maintain the form and function of their body plans. As important as such mechanisms are to the survival of multicellular organisms and the obvious relevance to regenerative medicine, we know little about how these processes are effected and regulated at the cellular and molecular levels. One aspect of such mechanisms garnishing much attention is populations of adult somatic stem cells, which appear to play key roles in growth, renewal, and regenerative events. Here, I will discuss how the study of a simple metazoan, the planarian *Schmidtea mediterranea*, is beginning to shed light on the way adult somatic stem cells are regulated in animals to maintain tissue homeostasis and to replace missing body parts lost to injury.

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