Report

Objectives of the proposal:

The present research project conducted during my stay at the INRA Institute was aimed to test the technical feasibility of the germ cell transplantation technique in zebrafish embryos and fry. This will allows us to evaluate the stemness of GFP positive germ cell populations collected from the vasa:eGFP transgenic zebrafish male gonads.

Transplantation of germ stem cells:

Purification of germ cells

Germ cells were purified and collected from the vasa:eGFP transgenic zebrafish lines using Percoll gradient after dissociation with collagenase. Purification experiments were conducted 2 times with 6 males and about 20 to 40 million cells were collected for each experiment.

Injection in recipient zebrafish embryos and fry

Recipient zebrafish were obtained by natural mating of the vasa:DsRed2 transgenic zebrafish line. This line expresses the DsRed reporter gene in the premeiotic spermatogonial cells and in the oocytes. Transplantation of purified germ cells from the vasa:eGFP transgenic zebrafish lines were conducted into 3 stages (blastula, 6-day old larva, and 21-day old fry) with 1 to 3 cell concentration ($36 \cdot 10^6$ cells/ml to $320 \cdot 10^6$ cells/ml) to find the best conditions for producing germline chimeras in zebrafish.

Unfortunately, all embryos transplanted with germ cells at the blastula stage developed abnormally and died at 24 hpf. On the other hand, 6 days old larvea and 21 days fry were successfully injected with the donor cell suspension and the mortality induced by the anesthesia and/or germ cell transplantation was estimated to 15%.

Perspectives/conclusion:

It is too early to determine whether the transplanted cells are capable to colonize the gonads of the recipient fish. The transplanted fish are being breed until they reach the sexual maturation (about 60 dpf at least). An external examination of the GFP fluorescence will be carried out to detect the colonization of the gonad.

Taiju SAITO, PhD, Researcher
Laboratory of Reproductive Physiology
Faculty of Fisheries and Protection of Waters
Research Institute of Fish Culture and Hydrobiology
University of South Bohemia in Ceske Budejovice
Zatisi 728/II, 389 25 Vodnany, Czech Republic

Jean-Jacques Lareyre, PhD, Researcher
Directeur de Recherches
INRA L.P.G.P. (Laboratoire de Physiologie et Genomique des Poissons)
Campus de Beaulieu, Batiment 16A, Allee Henri Fabre, 35042 Rennes Cedex
France