O-05

Germ Cell Transplantation in Fish: Can Salmon Make Trout?

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Primordial germ cell (PGC) is the progenitor cell of the germ cell lineage and eventually give rise to gametes that are responsible for creatingindividual organisms via a fertilization process. This means that PGC is a unique cell that can be converted into individual fish. This advantage of PGCs would make it possible to develop various applications in the field of fish bioengineering. First, PGCs may make it easier to preserve the genetic resources of fish. Cryopreservation of fish eggs or embryos has not been successfully achieved so far. Therefore, the only possible method to preserve genetic resources of fishes is to raise fish as live individuals. If PGCs isolated from various fishes could be cryopre-served, these cells could be converted into live fishes via germ-line chi-mera production. This is particularly useful for preserving genetic materials of endangered species. Even if the species of interest were to become extinct, itcould be recovered by thetransplantation of cryopreserved PGCs into the embryos of a closely related species. Another application of this technology is in what could be termed "surrogate broodstock technology". Seed production of fish that have a large body size and a long generation time, such as blue-fin tuna, requires high costs associated with extensive rearing space and intensive labor. However if PGCs of such target species could be transplanted into closely related species, whose body size is small and whose generation time is short (e.g., mackerel in the case of tuna), the PGCs could proliferate and produce mature eggs and sperm derived from the donor fish in the surrogate parent fish. In this study, as the first

step to complete the above-mentioned applications, we established a technique forinterspecies transplantation (xenotransplantation) of PGCs in salmonids.

Donor PGCs were prepared from transgenic rainbow trout carrying a green fluorescent protein (GFP) gene driven by a vasa gene promoter. Germline cells, including PGCs, of this transgenic trout were labeled by indelible green fluorescence. Approximately 20 PGCs were transplanted into the peritoneal cavity of a newly hatched yamame salmon embryo using a microinjector. Fluorescence observations of recipient gonads revealed that donor PGCs were incorporated, proliferated, and differentiated into the spermatogonia or oogonia in the xenogenic gonads. Further, the presence of donor-derived spermatozoa in the milt of mature salmon was confirmed by PCR analysis with GFP-specific primers. The PCR-positive milts were inseminated with trout eggs. Among the F1 offspring, embryos showing donor (trout)-derived phenotype were successfully produced. DNA finger-printing confirmed that the genotypes of such embryos were identical to that of the rainbow trout. To date, the donor-derived offspring have grown up normally.

Therefore, we concluded that surrogated salmon broodstock could produce live trout embryos in the offspring. Combined with *in vitro* culture, genetic modification, and cryopreservation of PGCs, the PGC transplan-tation technique will herald a new era in fish breeding technology.