

with significant hydrogen bonding. Thus the observed apical P–O bond lengths appear reasonable. Stabilization of penta-coordinated phosphorus intermediate through hydrogen bonding has also been demonstrated recently in the X-ray structure of the complex (**8**) of human α -thrombin with the inhibitor (α -aminoalkyl) phosphonate¹⁰.

It is important to note that the first direct observation of metaphosphate **2** in a condensed aqueous phase in the active site of fructose-1,6-bisphosphatase is also reported early this year¹¹. Fructose-1,6-bisphosphatase (FBPase) is a key regulatory enzyme that catalyses the hydrolysis of fructose 1,6-bisphosphate to fructose-6-phosphate and orthophosphate (PO_4^{3-}). An X-ray structural study by Honzatko and coworkers reveals that while crystals of FBPase grown at neutral pH have an orthophosphate at the active site, those grown at pH 9.6 (or higher concentrations of K^+) have metaphosphate and water (or OH^-) in equilibrium with each other.

It remains to be seen how the structural evidence thus obtained for both the transition state species (intermediates (?)) proposed in Scheme 1 in *pure biochemical processes* can be gainfully exploited in future.

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Transplanting the fish

T. J. Pandian

Cryopreservation of fish eggs and embryos has been unsuccessful¹. Establishing a transgenic strain in a species of conservation and/or commercial importance demands time, labour and money, as the site of integration of a foreign gene in the chromosome of the host remains uncontrollable and this results in unpredictable expression². To overcome these difficulties, Yoshizaki has achieved – what I proposed to call – transplanting the fish.

Primordial germ cell (PGC) is the progenitor of the germ cell lineage and is committed to differentiate into either spermatogonia or oogonia after the completion of gonadal differentiation³; as such the PGCs have the potential to develop into complete individuals. Hence they were extracted from the rainbow trout embryos, marked with green fluorescent protein (GFP) gene and transferred into host fry of the same species or closely related species. Because the *vasa* transcripts are restricted to germ cell lineage, a transgenic strain carrying *GFP* gene driven by the *vasa* regulatory regions was generated⁴. Consequently, the expression of *GFP* gene was limited to the PGCs alone^{5,6}.

The genital ridge, isolated from the transgenic embryos, was dissociated by trypsin and flow cytometrically sorted into GFP-positive and GFP-negative cells. On transplantation of these exogenous GFP-positive cells into the peritoneal cavity of the recipient hatchlings, the exogenous GFP-positive cells were incorporated into the genital ridge of about 20% host fry, very much like the endogenous PGCs. Subsequently, 4% of the exogenous cells proliferated, underwent meiosis and differentiated into eggs and sperm, in synchrony with the endogenous PGCs. Thus the donor-driven gametes produced the normal progenies through fertilization.

This new technique provides the scope for transgenesis, and *ex situ* conservation of PGCs, from which progeny of the concerned species can be derived. The introduction of foreign gene into the PGCs and selection of transformants, using selected markers, facilitate the *in vitro* selection of transformants. The method is advantageous over the conventional transgenic technique, as cells carrying foreign gene can be selected in a petri dish, instead of rearing hundreds of fishes and making a

large number of DNA analyses to identify the suitable/desired candidate transgenic. The selected transformant can then be developed into an individual fish, using chimera technique⁷.

Yoshizaki proposes to rapidly mass produce tuna sperm and eggs by transplanting its PGCs into *sterile* mackerels, which are cheaper to maintain, and to conserve fish species by cryopreserving their PGCs. Not only Yoshizaki, but also another group led by Strussmann is on the job. During the early nineties, he introduced sperm cells into *sterile* pejerrey *Odontesthes argentinensis* and found them to be alive until the 10th week after introduction. In view of the global warming and thermal pollution from nuclear plants, he was attracted to devoting more of his time on sex determination, differentiation and reversal; he is one, who has made several, impressive contributions on thermal impact on sex reversal in fishes. However, Robert J. Gold, a student of Strussmann is now engaged in deriving sperm from sterile testis of a recipient, into which bits and pieces of testis of the donor species have been

Table 1. Comparison of techniques used by Yoshizaki and Pandian

General technique	Transcloning (Yoshizaki)	Androgenesis (Pandian)
Donor	<i>Onchorhynchus mykiss</i>	<i>Puntius conchonius</i>
Recipient	<i>O. mosou</i>	<i>P. tetrazona</i>
Germplasm source	Primordial germ cells (PGCs)	Spermatozoa
Transfer technique	By microinjection of PGCs	Natural fertilization/activation
Requirements	Isolation of PGCs Marking with vasa and GFP Tracing the marked PGCs in recipient	Collection of cadaveric sperm from post-mortem specimens preserved at -20°C Incubation of milt in 2.5% PEG for 10 min to promote dispermy Genome inactivation of the recipient eggs by UV irradiation
Advantages	Restoration of nuclear and mitochondrial genomes	Restoration of nuclear genome alone

earlier introduced. On my suggestion, he has chosen the Siamese fighting fish *Beta splendens*, an airbreathing fish amenable to microsurgery. The idea is to use the sterile gonad of the recipient species as a surrogation chamber for exogenous gonad to function normally and to produce its sperm. Much of our understanding of the role played by one or other sex steroid was based on surgical removal of testis or ovary of fish. Surgical removal of gonad in *B. splendens* resulted in regeneration of the entire gonad (see ref. 8); briefly, the gametes, from which progenies could be derived through fertilization, can more easily be obtained by transplantation of the essential fraction, i.e. 'stem cells' of the gonad of the donor, rather than transferring the PGCs, as has been made by Yoshizaki.

The techniques of Yoshizaki and Strussmann pose great challenges to taxonomists and immunologists; How do the fry and adult of a recipient species tolerate the presence and/or proliferation of PGCs and pieces of testis of donor species, respectively, especially in the absence of immuno-suppressants like cyclosporin? It is assumed that the

basal laminar layer of testis prevents the entry of phagocytes. Incidentally, there are also claims for *ex situ* culturing of human sperm in the rat gonad, using the technique which Strussman conceived 10 years ago and is now engaged in. Controversial claims have also been made in a report by Sofikitis *et al.*⁹

When so many advances are made in Japan, what is the status in India? Well, a beginning has been made at Madurai Kamaraj University. Choosing the rosy barb *Puntius conchonius* as a model fish, we have induced dispermic interspecific androgenesis, a simple technique to restore genome of a fish species, for instance, rosy barb using its sperm drawn from postmortem preserved specimen and genome-inactivated eggs of tiger barb *P. tetrazona*¹⁰. A simple and widely practicable method of obtaining live sperm from specimens, that were preserved at -20°C for 240 days has been reported earlier¹. Table 1 presents a comparison between the highly skilled sophisticated method of Yoshizaki, and the simple, widely practicable method of ours.

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COMMENTARY

Rainwater harvesting, a time-honoured practice: Need for revival*

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'Rainwater harvesting' and 'groundwater recharge' are now two catchwords which are commonly used by most people, without realising their full significance. Harvesting of rainwater through impounding

it where it falls by means of ponds, check dams and in large-sized tanks is a well-understood technique practised for at least the last 1000 years. The landscape, especially of South India, is virtually studded with such structures. Unfortunately, village communities deprived of ownership rights have lost all interest in their upkeep, with

the result that the tanks have remained neglected, become filled with silt and incapable of harvesting water to any significant extent.

It is only in recent years that there has been much talk of rainwater harvesting. Thanks to the crusading efforts of Anil Agarwal and his team at the Centre for

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