NIBB Center of the Interuniversity Bio-Backup Project (IBBP Center)

Head
KAWAGUCHI, Masayoshi

Associate Professor
NARUSE, Kiyoshi

Specially Appointed Assistant Professor:
KIMURA, Tetsuaki
TANAKA, Daisuke
Technical Assistant:
AKIMOTO-KATO, Ai
MATSUBAYASHI, Naomi
FUKUMOTO, Tatsuya
Secretary:
HAMATANI, Ayako

In order to realize a life sciences community resilient to natural disasters and calamities, the National Institutes for Natural Sciences (NINS) and Hokkaido University, Tohoku University, University of Tokyo, Nagoya University, Kyoto University, Osaka University, and Kyushu University concluded an agreement on June 1st 2012 to launch a system to 'back up' the biological resources essential to the work being done at universities and research institutions nationwide, called the 'Interuniversity Bio-Backup Project (IBBP)'.

The IBBP Center was established as a centralized backup and storage facility at NIBB, while IBBP member universities set up satellite hubs and work closely with the IBBP center to put in place reciprocal systems for backing up important biological resources that have been developed by researchers residing in the area each university satellite hub is responsible for.

Figure 1. IBBP Center

Figure 2. Cryogenic storage system. Liquid nitrogen tanks are monitored 24 hours a day and are refilled automatically.

The IBBP Center includes earthquake proof structures capable of withstanding even very large scale quakes (equipped with emergency backup power generators), cryopreservation facilities equipped with automatic liquid nitrogen feeding systems, deep freezers, and refrigerated storage (mainly for seed stocks), as well as all manner of automated laboratory equipment, cell culture tools, and the latest equipment necessary to back up the genetic resources in a collaborative manner. The specific methods of preservation are freezing of the sperm and eggs of animals, cultured plant and animal cells, proteins and gene libraries. Plant seeds are frozen or refrigerated.

University satellite hubs receive preservation requests of biological resources from researchers and report to the Managing Project Committee of IBBP (constituted of faculty of NIBB and satellite institutes), where the relevance of the request is reviewed. When the request is sustained, biological resources to be preserved will be sent to the IBBP Center by the requesting researcher, where they will be frozen (or refrigerated) and their particular information will be registered into a database. In the event of a disaster leading to the loss of a researcher's own biological resources, preserved samples will be promptly supplied back to the researcher so they can quickly resume their work.

Through the development of this backup system biological resources that had only been stored at individual research institutes can now be preserved at the state of the art facilities of the IBBP Center, and Japan's research infrastructure has been significantly strengthened.

I. Current status of back up for the biological resources

In 2015, IBBP Center stored 4,238 384-well and 69 69-well plates consisting of 1,634,016 clones as cDNA/BAC clones, 8946 tubes for animal cells, plant and animal samples, proteins, genes and microorganisms, 80 133mm-straw tubes for sperm and 606 seed samples. In total 1,634,702 samples are stored.

Figure 3. Cryo tube with 2D barcode. Each sample is printed with a unique barcode and is managed using a database.

II. Collaborative Research Project for the development of new long-term storage technologies and cryo-biological study

As the IBBP Center can only accept biological resources which can be cryopreserved in liquid nitrogen, researchers cannot backup those biological resources for which cryo-preservation methods are not well established, to increase the usability of IBBP, we started a collaborative research project for the development of new long-term storage technologies and cryo-biological study in 2013. This collaborative research includes two subjects 1) Establishment of new storage technology for biological resources for which long-term storage is unavailable. 2) Basic cryobiological research enabling us to improve low temperature storage for biological resources. In 2015 we had ten applications and accepted nine proposals. We are also working to establish a research center for cryo-biological study thorough this Collaborative Research Project. Accordingly, we organized the Cryopreservation Conference 2015 on October 28-29, 2015 at the Okazaki Conference Center, Okazaki, Japan. We had 100 participants from several fields covering physics, chemistry, biology, and technology.

Figure 4. Group photo of Cryopreservation conference 2015

Publication List on Cooperation

〔Original paper (E-publication ahead of print)〕

- Tanaka, D., Ishizaki, K., Kohchi, T., and Yamato, K. T. Cryopreservation of gemmae from the liverwort *Marchantia polymorpha* L. Plant Cell Physiol.2015 Nov. 11.

Research activity by D. Tanaka

Assistant Professor:	*TANAKA, Daisuke*
Technical Assistant:	*AKIMOTO-KATO, Ai*
	FUKUMOTO, Tatsuya

Cryopreservation

Cryopreservation protocols contain components which are usually developed empirically using each biological resource's specific strategy to enhance survival (Benson 2008). The theory of cryopreservation encompasses several interconnected disciplines ranging from the physiological to cryophysical. Water status and cryopreservation, in combination with physiological factors, are the most influential determinants of survival (Figure 1).

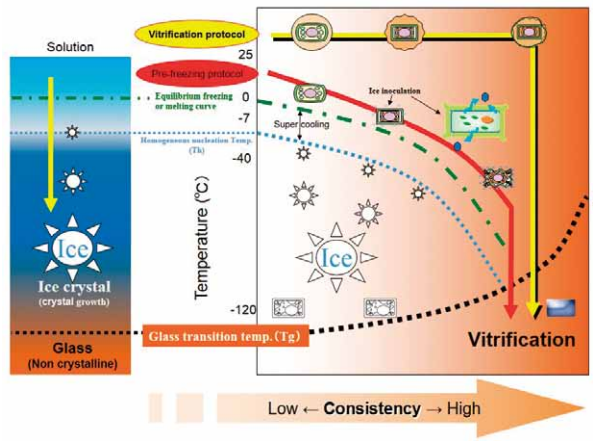

Figure 1. Phase diagram of vitrification of a solution.
Vitrification, a physical process, can be defined as the phase transition of an aqueous solution from a liquid into an amorphous glassy solid, or glass, at the glass transition temperature (Tg), while avoiding ice crystallization.

So far cryopreservation has been considered in terms of the liquid and solid (ice) phases of water. It is also possible to cryopreserve tissues by the process of "vitrification", the solidification of liquids without crystallization (Figure 2). This comprises a "glassy state" as the system is amorphous, lacks organized structure but possesses the mechanical and physical properties of a solid (Taylor *et al*. 2004).

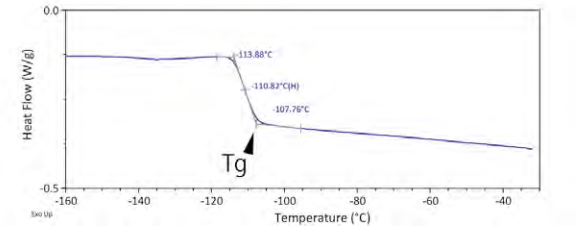

Figure 2. Differential Scanning Calorimetry (DSC) thermogram of Vitrified shoot apices of a Chrysanthemum plant treated with vitrification solution.
Glass transition temperature (Tg):-107

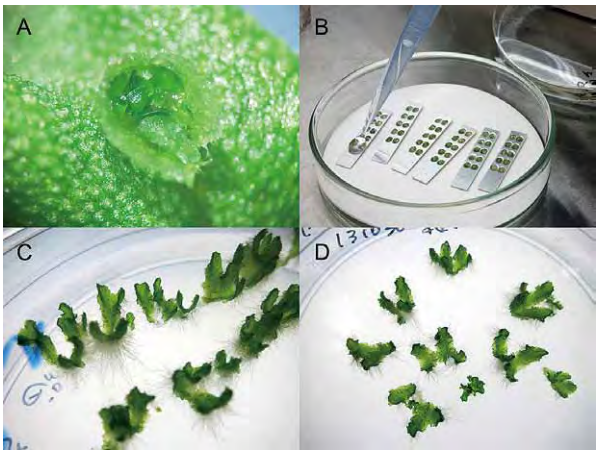

Figure 3. Cryopreservation protocol using aluminum cryo-plate.
A: Preparation of *in vitro* grown gemmae of liverwort. B: Placing pre-cultured gemmae in a cryo-plate's wells. C: Regrowth of cryopreserved livewort line 'Takaragaike-1' 30 days after rewarming. D: Control without cooling in liquid nitrogen).

Vitrification-based protocols are known to be effective for long-term, stable preservation of plant germplasm; this protocol can reduce the cost and manpower for maintaining a large number of germplasm lines and keep many valuable genetic lines for a long term under genetically stable conditions. However, it is still not widely employed as a reliable long-term preservation protocol due to the lack of basic knowledge on cellular and water behavior in tissues when immersed in liquid nitrogen.

In the present study, electron microscopy combined with freeze-substitution was employed to examine the ultrastructure of cells of the gemmae of the liverwort, *Marchantia polymorpha* L., strain Takaragaike-1, which were cooled to the temperature of liquid nitrogen after exposure to various steps of the Cryo-plate protocol (Figure 3).

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Research activity by T. Kimura

Assistant Professor: *KIMURA, Tetsuaki*

Leucophores are similar to xanthophores

Mechanisms generating diverse cell types from multipotent progenitors are crucial for normal development. Neural crest cells (NCCs) are multipotent stem cells that give rise to numerous cell-types, including pigment cells. Medaka has four types of NCC-derived pigment cells (xanthophores, leucophores, melanophores and iridophores), making medaka pigment cell development an excellent model for studying the mechanisms controlling specification of distinct cell types from a multipotent precursor cell. However the genetic basis of chromatophore diversity remains poorly understood.

We reported that *leucophore free-2* (*lf-2*) which affects leucophore and xanthophore differentiation, encodes *pax7a*. Since *lf-2*, a loss-of-function mutant for *pax7a*, causes defects in the formation of xanthophore and leucophore precursor cells, *pax7a* is critical for the development of the chromatophores. This genetic evidence implies that leucophores are similar to xanthophores, although it was previously thought that leucophores were related to iridophores, as these chromatophores have purine-dependent light reflection.

Many leucophores-3 (*ml-3*) mutant embryos exhibit a unique phenotype characterized by excessive formation of leucophores and an absence of xanthophores. We show that *ml-3* encodes *sox5*, which is expressed in premigratory NCCs and differentiating xanthophores. Cell transplantation studies reveal a cell-autonomous role of *sox5* in the xanthophore lineage. *pax7a* is expressed in NCCs and required for both xanthophore and leucophore lineages; we demonstrate that Sox5 functions downstream of Pax7a.

We propose a model in which multipotent NCCs first give rise to *pax7a*-positive bi-potent precursor cells for xanthophores and leucophores; some of these precursor cells then express *sox5*, and as a result of Sox5 action develop into xanthophores (Figure 1). Our findings provide clues for revealing diverse evolutionary mechanisms of pigment cell formation in animals.

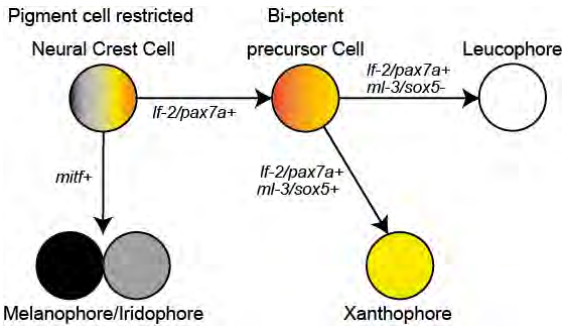

Figure 1. Model for leucophore and xanthophore development from neural crest cell.