

SLTB Newsletter



Hello SLTB members, I hope this newsletter finds you well and just before you head off for your well earned Christmas holiday.

In this edition, we hear details of the Amphibian Biobanking Workshop held at the University of Portsmouth earlier this year. One of our PhD student members has written a small article on the role of proteins in cryopreservation, and we bring you details of the first of two SLTB events this coming year.

Merry Christmas and Happy New year to you all!

Jon Green, General Secretary

TOWARDS A BIOBANKING STRATEGY FOR AMPHIBIAN CONSERVATION

Organised by Rhiannon Lloyd, Matt Guille, Kevin Zippel, Oliver Ryder and Dominik Lermen

September 6-8th 2010

Approximately one-third of the 6,260 amphibian species assessed to date are described as critically endangered, endangered or vulnerable, a situation that

is causing a great deal of international concern. One of the primary reasons for this precipitous decline in amphibian populations is the global spread of the chytrid fungus, *Batrachochytrium dendrobatidis*, which is believed to have emerged from Africa (as suggested by examination of archive samples from the 1930s) due to global trade in clawed frogs *Xenopus laevis* for use in laboratories. The Amphibian Ark (AArk), which aims to coordinate a global response to the amphibian extinction crisis, envisages that zoos around the world will maintain at least one threatened species in biosecure captivity for possible reintroduction to its natural habitat in the future; assuming that the habitat can be cleared of the fungus. As this strategy is only likely to protect 10% of the threatened species, owing to space constraints and the significant annual recurrent costs, there is a clear conservation role for biosecure genetic resource banks containing cryopreserved germplasm and tissue culture cells. Coupled with reproductive biotechnologies these could play a significant role in ensuring the long-term conservation of amphibians. This approach could reinforce species-specific ex-situ breeding programmes by maximizing the available gene pool and eliminating the reliance on maintaining numerous individuals within the living populations. However, until

now, there has been relatively little interest in this approach from groups in charge of amphibian conservation.

Biobanking is one of the key interventions highlighted in the “Amphibian Conservation Action Plan (2007)”, formulated in order to help abate the Amphibian Extinction Crisis. To take this strategy forward the Amphibian Ark Biobanking Advisory Committee (ABAC) was formed in 2008, at a meeting in Trier, Germany. This group, consisting of individuals actively engaged, or with a strong interest, in amphibian biobanking has since expanded and now includes over 30 members worldwide. The objective of the Amphibian Biobanking workshop, held partly at the Zoological Society of London and partly at the European Xenopus Resource Centre, Portsmouth, in September 2010 was to bring together experts in different aspects of amphibian biology, conservation and cryopreservation so that they could develop a coherent approach to this topic, meet each other and share their expertise. The workshop was mainly organized by Dr Rhiannon Lloyd, a Research Fellow at the Institute of Zoology (the research arm of the Zoological Society of London) and involved delegates from as far away as Canada, Panama, Australia, Japan and the USA.

The meeting itself was a huge success. The first two days was organized as a series of talks which covered “just about everything you need to know to set up a biobank”. While it is obvious that there is a considerable requirement for state of the art cryobiology, it is also important to know about genetic sampling from small populations (how many samples are needed to represent a species?); what are the disease risks associated with storing and using cryopreserved tissues and germplasm for breeding (would we simply be preserving the disease as well and then redistributing it?); how should amphibian species be prioritized for attention, given

the global scale of the task? Practical issues were also discussed such as how to culture amphibian cells and how to collect sperm and eggs from amphibians and then produce tadpoles for either captive breeding and/or reintroduction.

The final day of the workshop consisted of various practical demonstrations set up in Portsmouth for a small group of delegates. Apart from having practical demonstrations about technical aspects of cell culture for amphibians, there was an element of discovery and experimentation built in to the programme. As it is not yet possible to cryopreserved amphibian embryos, there was an attempt to replicate a novel technique developed in fish, whereby live primordial germ cells are removed from vitrified (‘donor’), but essentially dead, embryos and transplanted into different (‘host’) embryos where they subsequently develop into gametes. If this approach were successful it would be possible to exploit the germplasm within the embryos that is currently regarded as defunct.

Two major outcomes of the workshop were discussed and are currently in progress. One is the preparation of an amphibian biobanking guideline document, which would include straightforward amphibian biobanking protocols, for the wider conservation community and the other is the development of an internationally authoritative white paper stressing the need for research and capacity building in this area. It is envisaged that this would be distributed to national and international funding agencies and conservation organizations.

Bill Holt and Rhiannon Lloyd
Institute of Zoology

November 2010

SLTB WEBSITE

We have debated about how to make better use of the SLTB website in AGMs and in past newsletters, but I will talk briefly about it again. At present, the SLTB website is basically only used to download the forms for upcoming events or looking at previous newsletters in the archive section. We need to make our website more informative and interesting, with up-to-date information so that it becomes a point of call for low temperature biology. This cannot be achieved without your help. If you have any low temperature biology information, perhaps you have or will present at an event other than the usual SLTB or CYRO meetings, then tell me about it. Either let me know before the event and I can advertise it on our site, or send me a small paragraph after and I will put this on the website and in the next newsletter. Not only would this fulfil part of our obligations of being a charity, but people will be interested to hear about it. It is our intention to eventually revamp the webpage, however, as nice as it is to have a lovely new page, it will not bring more people to the webpage unless we have content to put on it. So next time you receive an email about an event or an open position, please click that forward button and let me know about it at:

j.e.green@uel.ac.uk

WE HAVE BEEN LISTENING (AND SOON YOU CAN TOO)

During the AGM held at CRYO2010 in Bristol in July, members said they would be interested in having a "Members Only" Section of the website where they could download occasional podcasts, perhaps of invited speakers from SLTB symposiums. We have spoken to the invited speakers of the upcoming "Regenerative Medicine and Cryobiology Symposium" and are happy

to report that there will be some podcasts of the event made available on the website.

THE ROLE OF PROTEINS IN CRYOPRESERVATION

Antifreeze proteins (AFPs) refer to a class of polypeptides that are produced by some species including vertebrates, plants, fungi and bacteria. These proteins enhance survival in subzero environments by binding to small ice crystals inhibiting growth and re-crystallisation, hence also referred as ice-binding proteins (IBPs) (Jia Z: *et al*, 2002). Consequently they have potential for application in preventing frost damage to plants and in cryo-preservation of food and organs.

As presented in the Annual Scientific SLTB meeting (September 2009), Braslavsky and his coworkers applied fluorescence microscopy in order to detect the binding planes of hyperactive and moderately active IBPs to ice surfaces. They observed that the hyperactive IBPs have affinity for the basal plane, unlike the moderately active IBPs. Moreover, they performed experiments with microfluidic devices where solution can be exchanged around small ice crystals in a controlled temperature environment. The experiments showed that ice crystals were highly stabilized by bound hyperactive IBPs even in reduced concentrations. Fluorescently tagged IBPs demonstrated that thermal hysteresis is determined by the surface density of prebound IBPs and that these do not equilibrate with IBPs in solution.

An example of survival enhanced by AFPs is the desert beetle *Microdera punctipennis* (Coleoptera: Tenebrionidae). Hou *et al* (2010) studied for a 13-month period, seasonal changes in supercooling point (SCP), body water content, haemolymph osmolality and antifreeze protein gene (Mpafp) expression in order to investigate its cold survival strategy. Haemolymph was incubated at 70°C to denature most protein components except AFPs,

centrifuged and the supernatant was measured for osmolality. Supercooling point was determined by thermal analysis, water content was calculated gravimetrically and real-time quantitative PCR was applied. The results obtained showed changes in SCPs from -8.0°C in summer to -18.7°C in winter. Additionally, during winter total water decreased from 65.36% to 55.88%. Mpafp mRNAs level increased by 13.1 fold from summer to early winter, and haemolymph osmolality increased accordingly from 550mOsm to 1486mOsm. Correlation of Mpafp mRNAs level and SCP indicates explained 65.28% of the variation in SCPs while the correlation between Mpafp mRNA level and total water reflected an indirect influence of antifreeze protein on water content via reducing SCP.

In parallel, Late Embryogenesis Abundant proteins (LEA proteins) are proteins in animals and plants that protect other proteins from desiccation induced aggregation or osmotic stresses associated with low temperature (Goyal K *et al*, 2005). Although the causes of LEA protein induction have not yet been determined, conformational changes in transcription factors or integral membrane proteins due to water loss have been suggested (Caramelo *et al*, 2009).

In order to come to a full understanding of the role of LEA proteins in desiccation and freezing tolerance, a genome-wide characterisation of all LEA proteins and their encoding genes in the model plant species *A. thaliana* is under development by Hinch *et al* (2010). Fifty-one LEA protein encoding genes have been identified, which could be classified into nine groups according to their amino acid sequence similarity. Expression studies at different developmental stages and organs, under different stress and hormone treatments showed expression of all genes. Real time PCR showed higher expression levels in seeds while in vegetative tissues many genes were induced by cold or drought. Several LEA genes were cloned

and expressed for recombinant protein production in order to gain structural and functional information. Further studies are under development from the team where recombinant proteins are used to study secondary structure and the effect of LEA proteins on the stability and structure of model lipid membranes under stress conditions is also studied.

Braslavsky I, Celik Y, Pertaya N, Graham L, Mok YF, Liu J, Qin Y and Davies PL (2010) *CryoLetters* **31(2)**, 169-197

Caramelo JJ, Iusem ND (2009) *Progress in Biophysics and Molecular Biology* **99(1)**, 1-6.

Goyal K, Walton, LJ & Tunnacliffe A (2005) *Biochemical Journal* **388(1)**, 151-157.

Hincha DK, Hundertmark M, Thalhammer A and Popova AV (2010), *CryoLetters* **31(2)**, 178-179

Hou F, Ma J, Liu X, Wang Y, Liu XN and Zhang FC (2010) *CryoLetters* **31(5)**, 359-370.

Jia Z, Davies PL (2002) *Trends Biochem Sch* **27(2)**, 101-106

Article provided by Christina Vogiatzi, University of Copenhagen

 NOMINATIONS FOR NEW
 COMMITTEE MEMBERS

In September next year, three members of the SLTB committee (Hugh Pritchard, Chairman, Jon Green, General Secretary and Roland Fleck, general committee member) will retire. This may seem rather early to be asking for nominations, but we have decided to bring this to your attention now so you have plenty of time to think about it. The Nominees must be in good standing and have agreed to stand as a candidate for election to the Society's committee.

Nominations should be sent to SLTB General Secretary (Jon Green, j.e.green@uel.ac.uk) no later than Friday 24th June 2011.

If more than two nominations are received for the same post a postal ballot will be held.

UPCOMING EVENTS:

SLTB SYMPOSIUM - REGENERATIVE
MEDICINE AND CRYOBIOLOGY

The symposium that was due to take place earlier this month will now take place on 22nd-23rd March at the Linnean Society, London. The Tuesday will be a day of invited speakers followed by a dinner. On Wednesday morning there will be a free communications session. The program will be emailed shortly to members and posted on the webpage <http://www.sltb.info>

FINAL COST 871 MEETING

Final meeting of COST 871 will take place at AGROCAMPUS OUEST INHP, Angers, FRANCE, 8-11th February, 2011.

This meeting marks the final culmination of the COST871 CryoPlanet, which is concerned with the cryopreservation of crop species in Europe. COST has two workgroups

WG1 Fundamental aspects of cryopreservation/cryoprotection and genetic stability

WG2 Technology, application and validation of plant cryopreservation.

During the meeting there will be specific sessions dedicated to each of these Workgroups. The invited plenary speaker for WG1 will be Prof. Olivier Leprince who will be discussing Desiccation tolerance mechanisms, while the invited plenary speaker for WG2 will be Dr H.H. Kim. Other speakers will be members of COST 871. There will also be poster sessions related to both workgroups.

The meeting is being organised by Dr Agnès Grapin (grapinagnes.grapin@agrocampus-ouest.fr) and Dr Florent Engelmann

(florent.engelmann@ird.fr) from who will provide further information if you are interested in attending.

Further information about COST 871 is available at:

<http://www.biw.kuleuven.be/dtp/tro/cost871/home.htm>

CRYO-2011

The 48th Annual Meeting of the Society for Cryobiology
24-27th July, at the LaSells Stewart Center on the campus of Oregon State University in Corvallis, Oregon.

Abstract Submission Deadline: 2nd May
Early Registration Deadline: 1st June

See website for full details:
<http://www.cryo-2011.org/>

THE 2ND INTERNATIONAL CONGRESS ON CONTROVERSIES IN CRYOPRESERVATION OF STEM CELLS, REPRODUCTIVE CELLS, TISSUE & ORGANS (CRYO)
Palacio de Congresos, Valencia, Spain, April 7-9th, 2011.

Abstract Submission Deadline: 5th February
See website for full details:
<http://www.comtecmed.com/cryo/2011/>

Gift Aid Declaration

As many of you are aware, the Society is a registered, UK charity, that can reclaim the income tax paid on UK members' subscriptions. Members must declare, formally, that they agree to the Society treating their subscriptions as Gift Aid donations by completing a Gift Aid Declaration form (www.sltb.info/forms.html), which will greatly help the Society's balance sheet!

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