

MEETING REPORT

Sleeping Beauties, dormancy and resistance in harsh environments: molecular, proteomic and metabolomic aspects – Berlin, Germany, 18–20 May 2008

Dormancy, diapause, rest and quiescence are all terms that are in use to indicate a state of (temporary) lowered metabolic activity ('hypometabolic state') in living organisms. Dormancy occurs in many organisms from all kingdoms, ranging from bears to mice and frogs, from fish to buds and seeds, and from crustaceans to yeast and bacteria. Oddly enough, interaction among the research groups studying the phenomenon in the various systems has been rare. The aim of the Sleeping Beauties Workshop was to bring these groups together, to discover similarities among the systems under study and to initiate discussions and collaborations. The Sleeping Beauties Workshop attracted some 70 participants from all over the world at the Max Planck Institute in Dahlem-Berlin. The pleasant surroundings and inspiring history of this location were very conducive to a lively meeting.

An excellent keynote lecture was delivered by Kenneth Storey (Carleton University, Ottawa, Canada), in his characteristic and animated idiosyncratic style, in which he addressed mechanisms of metabolic suppression during hibernation of mammals. During hibernation only 1–2% of the genes are turned on, protein synthesis is reduced to c. 1% and energy production to 5%. The hibernation-related pathways appear to be controlled by phosphorylation/dephosphorylation, e.g. via eukaryotic initiation factor-2 α (eIF2 α) and eukaryotic elongation factor-2 (eEF-2), as well as epigenetic inhibition. The few genes that are 'switched on' during hibernation are mitochondrial genes, genes encoding antioxidant and stress proteins, and transcription factors.

In his keynote address, Felix Franks (BioUpdate Foundation, London, UK) discussed the importance of the physical properties of water in water stress responses of living organisms. For example, did you know that chilling increases the pH of water from 7 to 8 and that this may uncouple metabolic pathways in living cells? Also, freezing damage of cells is not usually the result of growth of ice crystals but of an

increase in solute concentration, as in drying. From these two keynote lectures, it already became clear that dormancy and stress responses are intimately intertwined, a feature that seems obvious, but which is not commonly addressed in seed research.

The 30 or so shorter talks were devoted to such themes as Dormant forms, Quiescence and hibernation, Survival in harsh and extreme environments, LEA proteins and dormant forms, Ecological aspects of dormancy and germination, and Exit from dormancy. These themes were addressed in a limited number of model systems. The model systems reported on for dormancy research from other kingdoms included the tardigrades (or waterbears), a fascinating phylum of approximately 750 species. Tardigrades are cryptobiotic multicellular organisms (size 0.3–0.5 mm) with precise muscle control, which move like higher animals. In the dry state, they survive acid and solvent attack, very high and very low temperatures, high pressure and radioactive radiation. They have been found under layers of ice 5 m thick, in oceans 6000 m below the surface and on 6000 m high mountains (www.tardigrades.com). Like the tardigrades, the rotifers are multicellular animals. They have specialized organ systems and a complete digestive tract that includes both a mouth and anus. Most species of rotifers are about 0.2–0.5 mm long. Of the three classes of the Rotifera phylum, the Bdelloidea can survive drying for extended periods (<http://www.ucmp.berkeley.edu/phyla/rotifera/rotifera.html>).

Other speakers discussed the killifish, which are mostly 2.5–5 cm long, are oviparous and may produce diapause embryos that are resistant to drying and anoxia. In this way, they survive their sometimes harsh habitats, such as shallow lakes that may remain inundated for several weeks and subsequently dry up for months. The killifish are considered an excellent model to study effects of hypoxia and anoxia in vertebrates.

Suspended animation in insects (arctic springtail, an arctic species tolerant of freezing desiccation), *Artemia franciscana* (brine shrimp) cysts, cyanobacteria and yeast was also discussed. The plant contingent of the meeting was well represented by talks devoted to seed dormancy (Julia Buitink, Marc Cohn, William Finch-Savage, Henk Hilhorst, Dirk Hinch, Olivier Leprince, Gerhard Leubner, David Macherel and Peter Toorop) and bud dormancy (James Anderson).

Despite the substantial phylogenetic distances among these organisms, as the meeting progressed striking similarities became apparent among the dormant and/or dry states. Since many of these organisms are now being studied with the aid of modern molecular ‘-omics’ technology, we are able to compare complete transcriptomes or extensive expressed sequence tag (EST) libraries across species. Also high-resolution protein structure and binding properties of proteins, as well as metabolic aspects, were among the presented results. So, what were the similarities?

As mentioned, dormancy and stress responses are closely associated. Many of the above organisms become dormant before they are exposed to stress conditions. The timing of this may depend on developmental phase (e.g. sexual versus asexual), seasonal rhythms, environmental cues and food supply, among others. The common denominators appear to be (epigenetically) controlled reduction of metabolic activity, arrest of the cell cycle and the initiation of protective mechanisms. Most of the presentations addressed aspects of these key events. Several examples were given of metabolic control mediated by AMP-activated protein kinase (AMPK), a highly conserved eukaryotic protein serine/threonine kinase. It controls energy homeostasis and is activated

by metabolic stresses in mammals. In *Medicago* seeds, the AMPK analogue MtSNF4b is a subunit of the SnRK1 (sucrose-non-fermenting-related kinase) complex and is activated by energy deprivation. It is associated with both dormancy and protection of seeds against adverse conditions. Many of the protective mechanisms among various organisms include late-embryogenesis abundant (LEA) (-like) proteins and heat-shock proteins (HSP). Although the functions of LEAs may appear undisputed from a superficial scan of the literature (i.e. stabilizers of proteins and membranes, antioxidants, ion sinks and hydration buffers), there is very little conclusive evidence for these functions *in vivo*. However, in a fascinating presentation, Alan Tunnacliffe (Cambridge University, UK) showed that a group 3 LEA protein, which is expressed in mammalian cells, ameliorates formation of inclusion bodies by aggregation-prone proteins.

A number of lectures discussed the induction and release from dormancy on the level of the transcriptome. Both in bud and seed dormancy, co-expression analysis of dormant and non-dormant transcriptomes is being attempted to identify ‘super-nodes’ within regulatory networks in order to pinpoint the key genes in the regulation of dormancy. Although these *in silico* experiments should be approached with caution, they may yield valuable information that can be verified by ‘wet’ experiments. In his presentation, *Seed Science Research* Editor, Marc Cohn, gave valuable information as to how this could be done in a less ambiguous way. It is clear that systems biology is here to stay.

One of the sessions included a number of talks on the ecology of some of the model organisms. Again, some stunning similarities among organisms became apparent. For example, the dynamics of dormant egg banks of the water flea in underwater



LEA summit (left to right): David Macherel, Dirk Hinch, Olivier Leprince and Alan Tunnacliffe. Photo by M.A. Cohn.



Esther Lubzens. Photo by M.A. Cohn.

sediment layers seem almost congruent with that of soil seed banks.

From the above examples, it should be clear that dormancy, and its regulation and mechanisms, is a common feature of many extremophiles across all the kingdoms of life. Bringing together researchers of the different organisms and disciplines in the Sleeping Beauties Workshop was a splendid initiative of Professor Esther Lubzens (National Institute of Oceanography, Haifa, Israel). The Sleeping Beauties Project website is <http://www.gmm.gu.se/SB/>, where most of the PowerPoint slides from the oral presentations, as well as photographs from the meeting, will be posted in due course. I am certain that this meeting was an inspiration to all of us and

will hopefully stimulate 'across borders' collaborations. The next Sleeping Beauties is planned in Israel in 2010.

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