European Science Foundation

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF LESC EXPLORATORY WORKSHOP

Mechanisms of desiccation tolerance

Pembroke College, Cambridge, United Kingdom
21 - 22 September 2006

Convened by:
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1. Executive summary

The Workshop intended to bring together researchers studying resistance to desiccation at the biochemical and whole organism levels.
In view of identifying adaptations and mechanisms unique to desiccation tolerance the goals of the workshop were

- to collect a body of information on different organisms and at different levels of organisation (molecules, cells, organisms),
- to highlight similarities and differences between organisms,
- to define the *minimal desiccome* as the adaptations common and necessary to all anhydrobiotic organisms,
- to highlight different strategies for surviving that can represent the *comparative desiccome* as the differences between groups of organisms,
- to explore the possibility for active collaboration between laboratories and to look for new research directions.

Water is essential for life, but some organisms can survive desiccation for indefinite periods. This remarkable ability, called anhydrobiosis ("life without water"), is found across all biological kingdoms, including bacteria, fungi, animals and plants. Members of some taxonomic groups can survive desiccation at all stages of their life cycles: for instance, bdelloid rotifers, tardigrades and some nematodes, among invertebrate animals; certain angiosperms ("resurrection plants") and bryophytes (mosses and ferns), among the plants; and there are many examples among micro-organisms, including lichens, yeasts, algae and bacteria. Other taxa exhibit anhydrobiosis at specific life stages, such as the seeds of most higher plants and the embryonic cysts of the brine shrimp *Artemia* sp.
Current activity in this field aims to understand the mechanisms of desiccation tolerance at the molecular, biochemical, biophysical, cellular and whole organism levels. In nontolerant systems, desiccation causes irreversible damage to membranes and macromolecules, but such damage is either prevented or repaired in desiccation tolerant life forms.

The dry state is typified by biostability – extremes of temperature, radiation and pressure do not affect viability, and ageing is slowed – and there is considerable interest in the industrial application of desiccation tolerance. For example, drought-adapted crops, improved yeast strains, biostable pharmaceuticals, ambient temperature cell and tissue banks, and enhanced tissue engineering products are envisaged. There is a clear need for such technology, for example, in agriculture, where climate change will have an enormous impact: a new study by the Hadley Centre predicts that 50 per cent of the Earth's land surface will be affected by moderate drought by 2100; areas suffering severe and extreme drought will also see dramatic increases. Furthermore, the recent report from the European Plant Science Organisation concludes that research into drought stress "merits a higher investment of the scientific community".

Modern genetic and protein analysis techniques have begun to dissect out the molecular components of desiccation tolerance. For example, in the resurrection plant *Craterostigma plantagineum*, ~300 cDNAs representing genes regulated by desiccation have been cloned; and in anhydrobiotic nematodes and bdelloid rotifers, several hundred dehydration-responsive genes have been identified. Similar projects are underway in other plant and invertebrate species, and in the yeast *Saccharomyces cerevisiae*.

2. Scientific content of the event

The workshop wanted to assemble as many different models as possible for the tolerance to desiccation as a very widespread capacity that enables plants, animals and microorganisms to survive in unstable conditions. Most contributions focused on empirical results, in view of finding a common response of all organisms to a common problem.

The workshop was organised in four sessions.
(1) lessons from anhydrobionts: Plants, Animals and Microorganisms; similarities and dissimilarities of mechanisms and processes for drying.
(2) Desiccation at the whole organisms levels: advantages and disadvantages.
(3) A synthesis of the findings.
(4) Forward planning for future activities, projects for collaboration and grant applications.

The first session took the entire first day, the remaining three sessions were held on the second day.
The introduction to the workshop by Alan Tunnacliffe was followed by a presentation of dr. Arja Kallio about the strategy of the European Science Foundation and its role in organizing Exploratory Workshops that are mostly addressed to establishing new linkages, assemblages and collaborative interactions between scientists from European Countries.

(1) Lessons from anhydrobionts: Plants, Animals and Microorganisms.

The key question was: which of the adaptations found in the anhydrobionts in response to desiccation are necessary for survival? Desiccation-tolerant organisms must have special defence or repair mechanisms which are specific for desiccation stress. Research programmes are ongoing in many of the laboratories of the Workshop participants. One outcome of these studies should be the definition of the set of genes required for anhydrobiosis in particular species, and thereby to identify molecular mechanisms of desiccation tolerance. We might call this gene set the *desiccome,* and hope to define the *minimal desiccome* as the number of adaptations which are found in, and required by, all anhydrobiotic organisms. Thus, the minimal desiccome might include hydrophilic proteins like the LEA proteins.

Definition of a minimal desiccome would also highlight differences between organisms and might lead to the recognition of multiple variations on a basic survival strategy. Indeed, some organisms might have evolved unusual or unique strategies of desiccation tolerance. The Workshop might therefore lead to the concept of a *comparative desiccome*: phylogenetic variation in the mechanisms of desiccation tolerance.

For maximum progress during the Workshop, participants were organised into three teams according to their main field of research, i.e. Plants, Animals or Micro-organisms. Each participant was asked to prepare a poster for display during the Workshop summarising aspects of his work relevant to meeting aims. Each team had a coordinator who collected all the information from the participants prior to the Workshop and could thus organise a summary presentation for each team.

The *Plant Team* comprised Dorothea Bartels, Julia Buitink, Andy Cuming, Dirk Hincha, Folkert Hoekstra, Ilse Kranner and Patrick Van Dijk and was presented by Dorothea Bartels. After an introduction of the systematics of plants, showing that plant resistance to desiccation is independent from phylogeny, Bartels distinguished between tolerance to moderate water deficit, defined as tolerance to 80-60% of Relative Water Content, RWC, and tolerance to desiccation, i.e. capacity to survive a water potential inferior to 50% RWC. While tolerance to water deficit is rather common among plants, capacity of desiccation is typical of most seeds, of resurrection plants and is presumed to be an old trait in bryophytes. Common to all of them is presence of massive LEA (Late Embryogenesis Abundant proteins), of sugars and antioxidant defence, ABA switch and down-regulation of metabolism.

The common injuries a plant is exposed to when desiccating are 1) membrane and protein damages, 2) mechanical stress, and 3) oxidative stress. 1) is circumvented by synthesis of LEA and of sucrose, and by the 'vitrification' of cytoplasm (a change of physical status of the colloidal solution). 2) produces cell wall folding, vacuole fragmentation and filling with non-aqueous solution. 3) induces the increase of antioxidants that are synthesised ad hoc. Worthy of note is the observation that LEA of plants do not differ significantly from those of animals (e.g., *Aphelenchus avenae*, a nematode).

A peculiar aspect raised by Ilse Krammer is the 'longevity' of seeds, that is the longer the storage of dry seeds, the lower the viability. This cannot be ascribed to metabolism undergoing in the dry forms, as experimental results show that no metabolic activity is present in the dry seed, but oxidative damages are hypothesised. Remarkably, the same linkage between duration of dryness and lower viability is present in animals also.

The *Animal Team* included Ann Burnell, Ingemar Jönsson, Hans Ramløv, Claudia Ricci, Ralph Schill and Alan Tunnacliffe, and was summarized by Alan Tunnacliffe. As reported for the plants, also for the animals anhydrobiosis affects the molecular, cellular and organism organisation and animals' major adaptations consist in non-reducing disaccharides and hydrophilic proteins / peptides that are synthesised in response to desiccation. Trehalose is the common sugar present in the animals, but a representative group of desiccation-tolerant animals, the bdelloid rotifers, seems to lack this sugar as well as the genes for its synthesis. Among the other invertebrate groups, nematodes and tardigrades are known to possess large or small amounts of trehalose, while one tardigrade species has recently been found to lack trehalose.

These findings seriously challenge the current opinion of a central role for trehalose. Among the other organisms capable of desiccation, also oily seeds and prokaryots lack disaccharides. This body of results suggests that, similarly to plants, other adaptations may play an important role in desiccation tolerance of animals and the possible molecules conferring desiccation tolerance to animals cannot be restricted to a single one, but should include groups of molecules. As in plants, also among the animals hydrophilic proteins are expected to be implicated, and first experimental evidences point to LEA proteins, in addition to the widespread heat shock proteins (hsp). LEA have first been found in cotton seeds, and have been found in nematodes, in tardigrades and in bdelloid rotifers. To date not much is known about LEA function, but as are unfolded in the hydrated state and coiled in the dry state they are suggested to prevent aggregation of other proteins during the desiccation process.

To the *Microrganism team* contributed Ilse Kranner, Francesc Posas, Alexander Rapoport, Alan Tunnacliffe and Patrick Van Dijck who presented the summary of the data about microrganism desiccation resistance. Also for microrganisms the molecules involved in the stress response are trehalose, inositol and sucrose, but a very important role is played also by the aquaporins and by the activation of antioxidant mechanisms such as the synthesis of molecules like hsp, glutathione, ascorbate and tocopherol to prevent damages. Concentration of trehalose was found to be related to stress resistance as the deficiency of fermentation capacity resulted in the loss of stress resistance. However, accumulation of sugars might be related to osmotic stress and not strictly to the desiccation process. And mutants of the yeast *Saccharomyces cerevisiae* disabled for trehalose biosynthesis still could tolerate desiccation.

On the basis of the observation that metabolic activation of yeast determines lack of stress resistance, it is advanced the idea that metabolic activity and stress resistance can be alternative, mutually exclusive processes. It is also advanced the hypothesis that osmoprotectant molecules may confer protection also if they are located externally to the organism. This permits the organisms to avoid the biosynthesis but still be protected.

(2) Desiccation at the whole organisms levels: advantages and disadvantages.

This session was a round-table discussion, introduced by a short presentation by Claudia Ricci.
The focus was addressed to the difference between drought sensitive and drought resistance, remarking that the amount of water retained by the organism is the major difference. However, this fact can have very dramatic consequences on the internal organisation: one thing is to have devices to keep internal water and thus keep internal structures and/or cytostructures in place, and another thing is to lose internal water and adapt external shape and internal organisation to absence of water. Apparently multicellular organisms, plants and animals, can tolerate lower water content than bacteria do. This difference could be ascribed to the cascade of responses that occur for 1) shape modification, 2) tissues and 3) cells in a multicellular organism while a single cell can respond only at the cell level.

All animal organisms capable of desiccation are minute in size, have a fixed small number of cells (eutely) and most of their tissues are syncythial. But this does not fit plants, that can be relatively large in size, do not have the ‘eutely’ trait and do have relevant borders between individual cells (no syncythium). Another aspect that is worthy to be considered is the fact that desiccation means dormancy, that is absence of metabolic activity, also because in absence of water no molecule transfer is possible. However, oxidative processes can continue, in addition to structural damages to molecules such as DNA or chromosome integrity. This implies that at reactivation after re-hydration repair mechanisms should be activated as well. In line with this is the observation that dry animals seem to better tolerate stresses like radiation, brief exposure to high temperatures, high pressure or even immersion in organic solvents, as documented for dry tardigrades.

In summary, a cascade of events leads to protect external and internal body structures (organs, tissues, cells) against damages. Among these we can list morphological adaptation, like folding up, possibly useful for controlling rate of water loss, and chemical adaptation, that are chemicals produced at the start of desiccation, stored, and metabolized at the end. These chemicals may work as osmoprotectant, but also to protect structures like membranes against fusion or disintegration. Several molecules are good candidates: trehalose, sucrose, LEA, polyols, prolin, octulose, HSP. However none of them seems to be unique for desiccation resistance.

3. Assessment of results: A synthesis of the findings
Future directions

Peter Alpert was asked to present this synthesis, and he pointed at the most relevant aspects raised during the discussions and evidenced the many discrepancies among the different organisms that were presented. His synthesis follows.

A new conclusion from the workshop was that none of the mechanisms of desiccation tolerance are unique to desiccation tolerance. For example, the disaccharides and LEA proteins associated with desiccation tolerance also serve other functions and occur in many desiccation-sensitive organisms.

While it may seem disappointing that the remarkable ability to tolerate desiccation does not involve unique processes, this conclusion has encouraging implications for the engineering of tolerance. Making sensitive organisms tolerate desiccation is likely to mean changing the regulation of existing processes rather than introducing wholly new processes.

A caveat here is that inherent conflicts between tolerance and size, morphology, and productivity may limit ability to engineer tolerance in many large organisms. For example, rigidity seems to be antithetic to tolerance in animals though not in plants. Tolerance also seems linked to low productivity, although this link may have been broken in work on yeast.

The workshop also concluded that we are now confident that we have identified the basic problems that must be overcome to achieve desiccation tolerance: disintegration and fusion of membranes, denaturation and aggregation of macromolecules, oxidation, and probably DNA damage. We are also confident that we have identified the general solutions to these problems: compatible solutes, protective proteins, anti-oxidation systems, downregulation of metabolism, and immobilization of cytoplasm without crystallization. It is likely that no one of these mechanisms is either sufficient or essential for tolerance.

If the applied goals of research on desiccation tolerance are to engineer tolerance and to develop biostable materials, then we need to accomplish three major tasks. First, we must develop quantitative, standard in vitro and in vivo assays for key mechanisms of tolerance. One way to do this will be to hold pairs of conferences and technical courses on each of the key mechanisms. The conference will compare potential assays and select the best one; the course will train researchers to conduct the selected assays on their study organisms. Second, we must conduct genomic, transcriptomic, epigenetic, and proteonomic on a small but diverse set of model tolerant organisms. Third, we must use the results of these “polyomic” studies to construct a database for comparison of sequences between organisms.

Two main functions of a research network on desiccation tolerance will therefore be the first and last of these tasks, which cannot be accomplished by any one research group, but only by a network of groups, through a series of conferences and courses and construction of a website that will maintain a pooled database and repository for results.

Forward planning for future activities

The project for organizing this workshop derived from the interactions established at a symposium organised by Peter Alpert in the frame of the Annual Meeting of the Society for Integrative and Comparative Biology, at the beginning of 2005 in San Diego, USA entitled *Drying without Dying: The Comparative Mechanisms and Evolution of Desiccation Tolerance in Animals, Microbes, and Plants.*

Many leading research groups investigating desiccation tolerance are based in Europe but there has not previously been any coordinated research network. Thus a core Research Consortium was assembled and intends to apply for further funding at national, European and international levels. We have already submitted, and has accepted, an application to the new EuroBioFund where the consortium will be presented in a plenary session. In addition we have also applied to ESF for a Research Networking Programme (LESC) submitting a project entitled ‘Biology and Biotechnology of desiccation tolerance in eukaryotic cells’. This project, if approved, could allow, in addition to the planned research activities of each group, to have scientific and technical conferences and workshops, to exchange visits of senior and junior researchers to use facilities to collaborate and to produce joint publications.

4. Final programme

Wednesday 20 September 2006
Accommodation available from 14.00 at Pembroke College
18.00 *Welcome drinks and poster display in Nihon Room, Foundress Court, Pembroke College*

Thursday 21 September 2006
09.00 - 13.00 **Session I** *(including refreshment break)*

Presentation of the European Science Foundation (ESF)
Dr Arja Kallio (Head of Unit of Life, Earth and Environmental Sciences of ESF)

Team presentations (1h 30m each)
1. **Plants team**
2. **Animals team**
3. **Micro-organisms team**

Lunch
14.00 – 17.30 *(including refreshment break)*

Round table discussion and synthesis of findings
1. What is similar between organisms? The minimal desiccome
2. What is dissimilar between organisms? The comparative desiccome

Dinner in College

Friday 22 September 2006

09.00 – 10.30 **Session II** *(including refreshment break)*

Implications for the whole organism (**Claudia Ricci**): **round table discussion**

10.30 – 13.00 **Session III**

Summary of findings (**Peter Alpert**)

Lunch
14.00 – 17.30 **Session IV** *(including refreshment break)*

Forward planning for future activities
1. Collaborative projects
2. Funding for collaborations: plans for grant applications

Close of workshop

Due to travel time, some participants remained till Saturday 23. Accommodation was made available till Saturday at Pembroke College

Departure

5. List of Participants

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6. Statistical information on participants

The invited participants came from 12 countries: Germany (3), United Kingdom (3), Sweden (1), Denmark (1), Italy (1), Belgium (1), The Netherlands (1), Ireland (1), France (1) Spain (1), Latvia (1), USA (1).
The meeting was also attended by dr. Arja Kallio, the Head of Unit of Life, Earth and Environmental Sciences of ESF, and by dr. Chiara Boschetti, Inst. Biotechnology, Cambridge, in addition to the organizers.
In total the attendants were 18, of which 11 males and 7 females.