Journal of Biology
J. Biol.
BioMed Central

Minireview

How do terrestrial Antarctic organisms survive in their harsh environment?

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Published: 29 April 2009

Journal of Biology 2009, **8**:39 (doi:10.1186/jbiol142)

The electronic version of this article is the complete one and can be found online at http://jbiol.com/content/8/4/39

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Abstract

Anhydrobiosis, or extreme desiccation tolerance, is one of the strategies that allows terrestrial Antarctic organisms to survive in a harsh environment. A new study in *BMC Genomics* analyses gene expression in an Antarctic nematode during desiccation, and sheds new light on this phenomenon.

Antarctic terrestrial organisms live permanently on the continent (unlike penguins and seals that only breed there) and survive in one of the harshest environments on Earth. Sites that support life are largely limited to regions that are ice free, for at least part of the year, and which receive meltwater in spring and summer. Living at the limits of life, these organisms may be particularly sensitive indicators of climate change and are good models for studying how life survives in extreme environments. Antarctic species show high levels of endemicity and recent molecular studies suggest that many terrestrial Antarctic organisms have ancient origins, dating from before the break up of Gondwana [1]. Although controversial, there is increasing interest in bioprospecting amongst Antarctic organisms for molecules with practical uses.

Life without water

Although the most obvious stress faced by organisms in Antarctica is cold and the risk of freezing, there are a variety of other stressors that are significant [2]. The most important factor determining their distribution is the presence of liquid water, to which organisms must have at least occasional access in order to grow and reproduce. When liquid water is absent organisms survive in a dormant state known as anhydrobiosis - life without water - in which their metabolism comes reversibly to a standstill. Anhydrobiosis is a feature of many organisms in habitats where they are exposed to desiccation. Among animals, it is found in rotifers, tardigrades, nematodes and some arthropod larvae. Many species of nematodes are capable of anhydrobiosis and nematodes have proved to be good models for the study of this phenomenon. Anhydrobiotic nematodes are important components of the Antarctic terrestrial fauna [3].

The disaccharide trehalose has long been thought to be important for anhydrobiosis; especially by acting as a replacement for water, preserving the function of membranes and proteins. More recently, other mechanisms have been recognized. In particular, a group of proteins called late embryogenesis abundant (LEA) proteins, first identified from plant seeds, are associated with anhydrobiosis in a number of animals. They may play a role in preventing protein aggregation during desiccation [4]. However, focusing on specific adaptations, such as trehalose

Figure 1
The Antarctic nematode *Plectus murrayi*. Photo: DA Wharton.

Figure 2
Lake Canopus in the Wright Valley, Dry Valleys area of East Antarctica is one of the locations where *Plectus murrayi* is found, in the mat of cyanobacteria at the edge of the lake. Photo: DA Wharton.

and LEA proteins, may result in important mechanisms being overlooked. The construction and screening of cDNA libraries and cDNA arrays have proved successful in identifying freezing-responsive gene expression in a freezing-tolerant frog, *Rana sylvatica* [5]. Similar approaches have been used to study the responses of plants to a variety of stressors, including desiccation, and have been applied to desiccation survival and anhydrobiosis in nematodes [6]. An Arctic springtail (Collembola), *Onychiurus arcticus*, over-winters by desiccating at low temperatures (cryoprotective dehydration). An expressed sequence tag (EST) analysis has indicated that a number of biochemical pathways are associated with desiccation and recovery [7].

A recent paper in *BMC Genomics* describes the first EST and differential expression analysis of the response of a terrestrial Antarctic animal to environmental stress [8]. In this work, Adhikari *et al*. describe changes in gene expression in response to desiccation in the free-living nematode *Plectus murrayi* (Figure 1). This nematode is found in the Dry Valleys and coastal sites in the McMurdo Sound region (Figure 2), and from several other areas of continental East Antarctica, where it is the most widely distributed and abundant free-living terrestrial nematode. Despite this abundance, little is known about its survival strategies.

Desiccation-induced gene expression in *P. murrayi*

In the study by Adhikari *et al*. [8] a total of 2,486 ESTs were generated comprising 1,387 unique transcripts. The *Caenorhabditis elegans* genome comprises about 20,000 genes and the *P. murrayi* genome is probably of a similar size. The transcripts reported in this paper are therefore likely to represent only a small proportion of those expressed overall.

The unique transcripts from *P. murrayi* were compared with known sequences. Of these, 38% were considered to have homologs in *C. elegans*, 7% showed matches with other nematode databases (typically *C. briggsae*), and a further 11% of transcripts were similar to sequences from other organisms. The remaining 44% did not match any known sequence.

The breakdown of functions was assessed by Gene Ontology (GO database) and by assignment to metabolic pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. These analyses showed a wide range of functions associated with the EST transcripts, representing most of the functions that might be expected of an eukaryotic organism. Analysis of abundant transcripts suggested that metabolic genes and those associated with the processing of environmental information were highly expressed. The authors note that ribosomal protein transcripts were abundant; findings consistent with protein expression. In particular, the most abundant transcript detected in the desiccation cDNA library was S28, a ribosomal small subunit component. This is perhaps not too surprising, as any increase in protein synthesis associated with environmental stress is likely to be associated with an increase in ribosome number, and therefore the synthesis of ribosomal proteins (and RNA). KEGG analysis also indicated that protein degradation was active. In particular, a cathepsin-L-like protease was identified that is implicated in protein turnover during

development and differentiation in *C. elegans*, especially in molting.

In addition to sequencing a library of transcripts present in desiccated nematodes, a library of differentially expressed transcripts was also made. Two rounds of subtractive hybridization using cDNAs from desiccated and hydrated *P. murrayi* produced 80 sequences specific to the desiccated sample (in subtractive hybridization, two samples are hybridized to remove cDNAs present in equal amounts in both samples). About a quarter (22) of these were considered to be associated with metabolism, 15 were involved in environmental information processing (including a homolog of type II antifreeze protein from fish (GenBank accession number FK670242)), 23 with genetic information processing, 17 were considered to encode novel proteins, and three matched hypothetical proteins of unknown function.

Fourteen of the genes identified by subtractive hybridization were further examined by real-time PCR. Many of these showed a significant increase in mRNA abundance after desiccation. Among this group were LEA, trehalose-6-phosphate synthase (TPS), aldehyde dehydrogenase and glycerol kinase. Both glycogen synthase and the clone identified as an antifreeze protein homolog showed a decrease in expression (along with an unidentified protein). The heat-shock proteins Hsp70 and Hsp90 did not alter expression during desiccation.

Water stress increases the formation of reactive oxygen species so the production of antioxidants may be a part of an anhydrobiotic response. Genes associated with antioxidant production and that are stimulated during desiccation in *P. murrayi* include superoxide dismutase, Ras-related protein and glutathione S-transferase. An aquaporin (proteins that regulate the flow of water across cell membranes) is one of the most abundant transcripts in the cDNA library, which is consistent with the need to control water flow as osmotic strength changes during desiccation in *P. murrrayi*.

The use of subtractive hybridization imposes an inherent limitation on the data. Only those genes that are differentially expressed in desiccation are likely to be detected. It is possible that some genes are constitutively expressed, rather than induced by stress, but are nonetheless important in desiccation (and perhaps freezing). For example, in notothenioid fish, which are highly represented in the Antarctic, Hsp70 is not induced by environmental challenge but instead is constitutively expressed at a high level [9]. The finding of Adhikari *et al*. [8] that Hsp70 and Hsp90 expression is not increased by desiccation suggests that this might be the case in *P. murrayi*.

Further thoughts and future directions

The distribution of genes expressed during desiccation raises a number of issues. Those genes that had homologs in other animals suggest the usual range of metabolic activities that might be expected of a nematode. Does this mean that desiccation tolerance reflects subtle modifications to the normal suite of metabolic processes? Alternatively, does the as-yet unidentified 44% of the total EST library encode components of new pathways that confer resistance to desiccation in *P. murrayi*? It is not unusual for organisms newly sequenced to reveal unique reading frames and transcripts, and it is not always clear whether these new transcripts are expressed and play a role. However, it is likely that at least some of these unique sequences play some interesting role in the metabolism of the organism that is their host.

About a quarter of the transcripts identified by subtractive hybridization belong in the category of new sequences, and these may provide a fruitful set of candidates to answer this question. Identifying the roles of these genes will prove challenging. We will need some integrated molecular, physiological and biochemical studies to see if some of the potential mechanisms identified by EST and similar approaches do in fact play an important role in anhydrobiosis.

The desiccation survival abilities of *P. murrayi* are ill defined. Adhikari *et al*. [8] exposed the nematodes to a relatively mild desiccation stress of 87% relative humidity at 23°C for 2 days. Are they capable of anhydrobiosis, which we may practically define as surviving exposure to 0% relative humidity? Are further mechanisms invoked when nematodes are exposed to more severe desiccation? Are different pathways involved during repair and recovery upon rehydration? What effect does lowered temperature have and is the response to freezing similar to that to desiccation?

As perhaps might be expected, enzymes involved in trehalose synthesis (such as TPS) and LEA proteins are upregulated during desiccation of *P. murrayi*. However, a note of caution may be that *C. elegans* has several *tps* and *lea* genes and yet is not particularly desiccation tolerant. It has only been shown to survive exposure to 97% relative humidity, which can hardly be considered anhydrobiotic. Nematode species vary widely in their ability to survive desiccation [10]. To identify the mechanisms that are important in anhydrobiosis, rather than looking at changes in gene expression in response to desiccation in a particular species, it may be more instructive to compare the responses to desiccation between species that are not capable of anhydrobiosis, those that will survive anhydrobiotically if they are dried slowly, and those that will survive immediate exposure to severe desiccation. Nevertheless, this study is an

important first step in understanding the survival mechanisms of terrestrial Antarctic organisms.

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