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LEA PROTEIN EXPRESSION DURING COLD-INDUCED DEHYDRATION IN THE ARCTIC COLLEMBOLA *MEGAPHORURA ARCTICA*.

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Abstract - The Arctic springtail *Megaphorura arctica* (Tullberg, 1876) employs a strategy known as cryoprotective dehydration to survive winter temperatures as low as -25 °C. During cryoprotective dehydration, water is lost from the animal to ice in its surroundings as a result of the difference in vapour pressure between the animal's supercooled body fluids and ice (Worland et al., 1998; Holmstrup and Somme, 1998). This mechanism ensures that as the habitat temperature falls, the concentration of solutes remains high enough to prevent freezing (Holmstrup et al., 2002). In *M. arctica*, accumulation of trehalose, a cryo/anhydro protectant, occurs in parallel with dehydration. Recent studies have identified a number of genes and cellular processes involved in cryoprotective dehydration in *M. arctica* (Clark et al., 2007; Clark et al., 2009; Purać et al., 2011). One of them includes late embryogenesis abundant (LEA) proteins. This study, together with that of Bahrndorff et al. (2008), suggests that LEA proteins may be involved in protective dehydration in this species.

Key words: Collembola, cryoprotective dehydration, LEA proteins, SCP, water

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INTRODUCTION

LEA proteins are a heterogeneous group of small molecular weight proteins (usually 10-30 kDa) that were first identified in cotton seeds, but subsequently have been found in many other organisms (Hundertmark and Hincha, 2008). They are produced in seeds in abundance during late embryo development, but their expression is also upregulated under different stress conditions. LEA proteins are highly hydrophilic and intrinsically disordered in solutions. They have been divided into different groups although classification and nomenclature have not been consistent in the literature (Tunnacliffe and Wise, 2007). A number of putative mechanisms have been proposed for LEA protein action (Wise and Tunnacliffe, 2004; Goyal et al., 2005), however their precise role has not been defined. In order to identify the presence of LEA protein in *M. arctica* we induced cryoprotective dehydration under laboratory conditions. We measured the water content, the supercooling point (SCP) and performed Western blot analysis.

Specimens of the springtail *Megaphorura arctica*, were collected under bird cliffs near Ny Ålesund, Svalbard (78°56'N, 11°53'E). Groups of about 100 animals were placed in sealed tubs on moist plaster of Paris. The animals were acclimatized to 5°C for one week, followed by one week at 2°C. The temperature was then reduced manually by 2°C per week to -14°C. Once the temperature was below zero a few ice chips were added to the tubes. For each experimental temperature Differential Scanning Calorimetry (DSC) was used to measure the SCP and water status as de-

scribed in Worland et al. (1998). Animals exposed to -2°C and control animals from 5°C were prepared for protein extraction. Proteins were separated by SDS-PAGE electrophoresis (Laemmli, 1970), then blotted and examined by immunoblot analysis (Towbin at al., 1979) with AriLEA1-3 antibody.

RESULTS AND DISCUSSION

DSC analyses revealed that the 10 week gradual cooling treatment resulted in a decrease in the SCP (from -5.95°C to -31.34°C) and total water content (from 3.43 to 0.39 g/g dry weight (DW)). There was also a large reduction in the osmotically active water content over the same period (from 2.73 at 5°C to 0.08 g/g DW at -14°C). The content of osmotically inactive (unfreezable) water decreased slightly as the samples went through the initial stages of dehydration (from 0.70 in control to 0.44 g/g DW at -2°C), but then remained fairly constant with further reductions in temperature falling to 0.31 g/g DW at -14°C. Western blot analysis using the LEA-specific antibody AriLEA1-3 identified one band in the sample taken from the -2°C, while no band was identified in the control group of animals from 5°C. The molecular weight of the LEA protein was ~10 kDa (Figure 1).

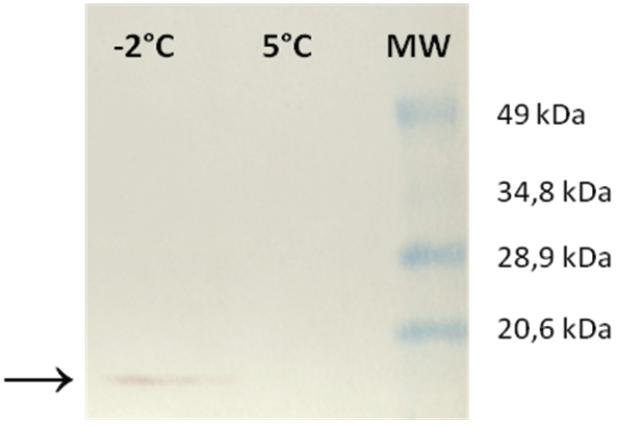

Fig. 1. Expression of putative LEA protein in *Megaphorura arctica*. Western blot analysis of proteins extracted from control (5°C) and animals exposed to low temperature (-2°C). Anti-AriLEA1-3 antibody was used. MW – molecular weight.

Previous studies on *M. arctica* have shown that acclimation to -5.5°C was accompanied by a decrease in total water content from 70% to 40% of fresh weight, mostly by the loss of osmotically active water, accompanied by a decrease of SCP from -7 to -17°C. Trehalose concentration increased 100-fold while glycogen reserves declined (Worland et al., 1998). In the present study we exposed the springtails to more extreme conditions by reducing the acclimation temperature to -14°C which caused a decrease in SCP from -6°C to -31°C and a loss of about 88.5% of the total water content, 97% of which was osmotically active.

The presence of Group 3 LEA protein in *M. arctica* was first found by bioinformatic analysis of Bahrndorff et al. (2008). Furthermore, cross-species Western blot analyses with ArLEA1A antibody showed drought-induced expression of putative LEA proteins in six species of Collembola and their species specific sizes (Bahrndorff et al., 2008). Also, their results showed that drought-exposed individuals of *M. arctica* expressed higher level of several bands compared to the control, of which the 60 and 10 kDa bands were expressed at considerably higher levels. The presence of several bands of LEA proteins in their study could be due to oligomerization, different post-translational processing of the same protein or from different sized LEA proteins. The LEA specific antibody AriLEA1-3 used in the present study showed the presence of a putative LEA protein of about 10 kDa induced by treatment at -2°C, but not in control animals at 5°C. These results are in accordance with previous LEA studies (Bahrndorff et al., 2008; Goyal et al., 2003), where these proteins were highly expressed during dehydration implicating their potential cellular protective role. The absence of other bands of LEA proteins in our experiments, especially 60 kDa, is very interesting and might be explained by different experimental conditions. Namely, in our experiment dehydration was induced by cold and in the Bahrndorff et al. (2008) experiment by drought. This could suggest the presence of different cellular stress responses to dehydration induced by drought and cold. Our results confirmed the presence of 10 kDa

LEA protein in cold-induced dehydration, but the true identity and role of such LEA proteins in cold and drought tolerance in the Arctic springtail *M. arctica* has to be explored.

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