Dehydration-regulated processing of late embryogenesis abundant protein in a desiccation-tolerant nematode

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Abstract Late embryogenesis abundant (LEA) proteins occur in desiccation-tolerant organisms, including the nematode *Aphelenchus avenae*, and are thought to protect other proteins from aggregation. Surprisingly, expression of the LEA protein AavLEA1 in *A. avenae* is partially discordant with that of its gene: protein is present in hydrated animals despite low cognate mRNA levels. Moreover, on desiccation, when its gene is upregulated, AavLEA1 is specifically cleaved to discrete, smaller polypeptides. A processing activity was found in protein extracts of dehydrated, but not hydrated, nematodes, and main cleavage sites were mapped to 11-mer repeated motifs in the AavLEA1 sequence. Processed polypeptides retain function as protein anti-aggregants and we hypothesise that the expression pattern and cleavage of LEA protein allow rapid, maximal availability of active molecules to the dehydrating animal.

Keywords: Anhydrobiosis; Desiccation stress; Desiccation tolerance; Late embryogenesis abundant protein; Molecular shield

1. Introduction

Anhydrobiotic organisms can survive almost complete desiccation by entering a state of metabolic arrest which is reversible on rehydration [1]. One response to desiccation common to many, perhaps all, anhydrobiotes is the production of highly hydrophilic proteins. The best characterised of these are the late embryogenesis abundant (LEA) proteins which are linked to the acquisition of desiccation tolerance in orthodox seeds, pollen and anhydrobiotic plants [2, and references therein]. Several groups of LEA proteins have been defined on the basis of expression pattern and sequence; for example, Group 3 LEA proteins are characterised by a repeating, loosely conserved, 11-mer amino acid motif. In addition, Group 3 LEA proteins are highly hydrophilic and natively unfolded in solution; unusually, they show increased folding on drying, but become unstructured again on rehydration [3,4].

Recently, LEA and LEA-like proteins or their genes have also been found in micro-organisms and invertebrate animal species [5], including the anhydrobiotic nematode *Aphelenchus avenae*, where expression of a Group 3 LEA protein gene is induced by dehydration [6]. The widespread occurrence of LEA proteins and genes suggests that plants, animals and micro-organisms might use these proteins in similar ways to combat water stress. LEA proteins have a stabilising function (reviewed in [7]) and recently several groups have shown that LEA, and other hydrophilic, proteins can preserve enzyme activity after desiccation and rehydration [8–10]. A possible mechanism for the protection observed is the prevention of water stress-induced aggregation of sensitive proteins [11]. Hydrophilic proteins might also function as membrane stabilisers [12] and it has been proposed that folding of LEA proteins on membranes occurs in a manner similar to that of α-synuclein, which has a role in vesicle management [13].

These studies improve our understanding of how LEA proteins behave in vitro, but there is a need for corresponding investigations of their in vivo biology, particularly in desiccation-tolerant animals, where there are no data on regulation of LEA proteins. Therefore, we have performed experiments to determine the distribution and behaviour of the LEA protein AavLEA1 in both hydrated and dehydrated nematodes. We report the surprising finding that, during nematode anhydrobiosis, AavLEA1 expression is partially out of phase with that of its mRNA, and that the protein is processed to smaller polypeptides which nevertheless retain anti-aggregation activity.

2. Material and methods

2.1. Immunostaining

Adult and juvenile nematodes, including embryonated eggs, were ruptured by freeze-cracking, stained with immunopurified anti-AavLEA1 and detected with either biotin-conjugated goat anti-rabbit serum (1:200), followed by a streptavidin/diaminobenzidine treatment, or a goat anti-rabbit FITC (fluorescein isothiocyanate; 1:200) [14]. In some preparations, cell nuclei were stained with DAPI (4′,6-diamidino-2-phenylindole). Negative controls were performed using primary antibody preabsorbed with AavLEA1 (5 μM). A positive control comprised incubation of freeze-cracked worms with FMRFamide-specific antibodies (Affinity) which stained neurons strongly. Microscopy was performed with a Leica DMRB optical microscope and a digital Nikon Coolpix camera or a laser scanning confocal microscope (Leica TCSNT).

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2.2. RNA analysis

A. avenae was exposed to 90% RH for various times (saturated solution of $BaCl_2$) at 25 °C prior to storage at −80 °C; controls were untreated. RNA isolation, Northern hybridisation and quantitative PCR were as described [15].

2.3. SDS–PAGE and Western blotting

About 15 μg of total protein was loaded per lane of 11% SDS slab gel and run in Bio-Rad mini-Protean 3 electrophoresis cells, with 25–50 ng of recombinant LEA protein as positive control [4]. Anti-14-3-3 (rabbit polyclonal) and lysates of EGF-stimulated A431 cells were used according to supplier's instructions (Upstate).

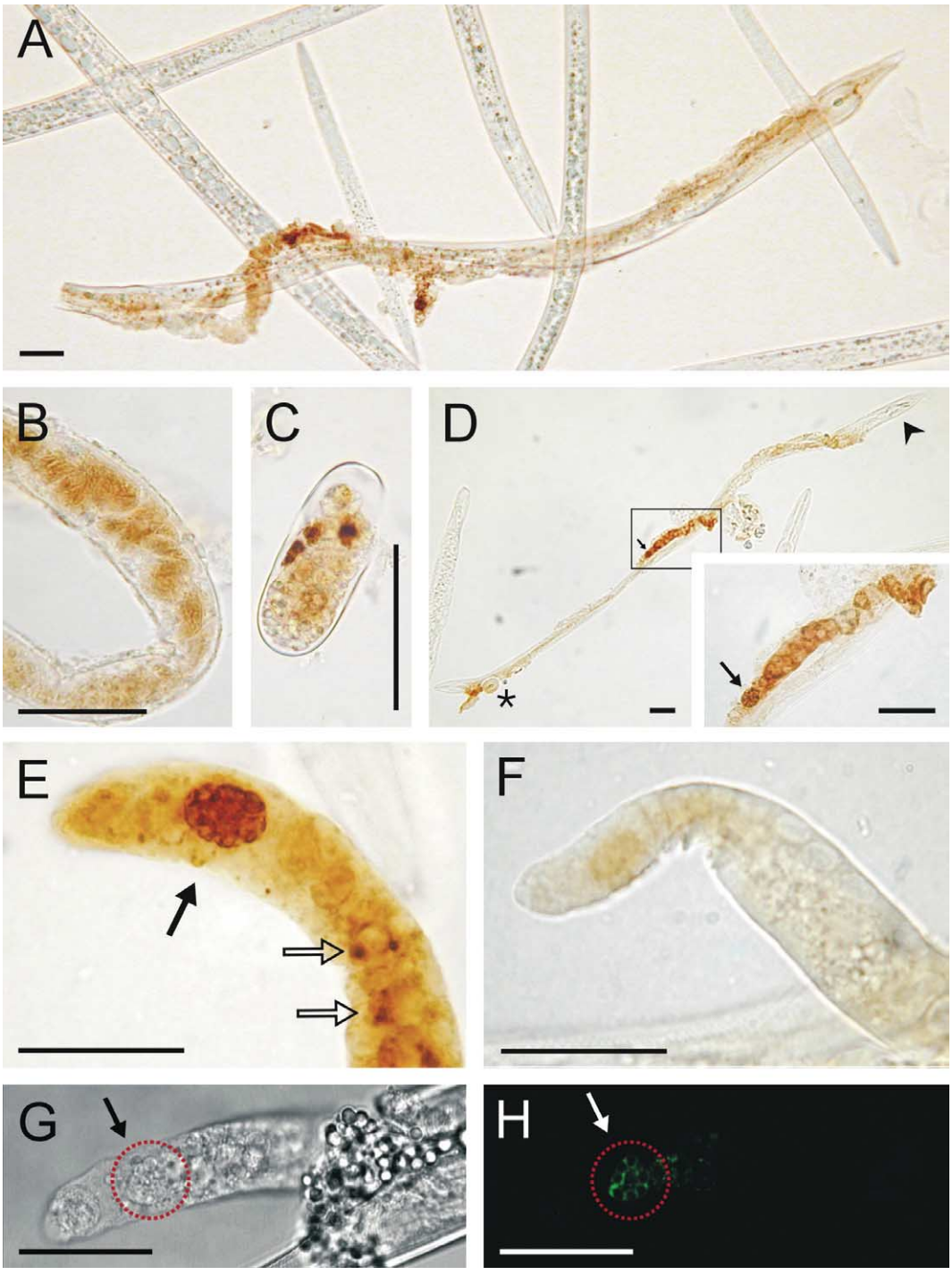

Fig. 1. Immunodetection of LEA protein in *A. avenae*. LEA protein immunoreaction (brown coloration) in (A) the body of a female worm (head at right); (B) embryonated eggs in the uterus; (C) an egg after laying; (D) a male worm (asterisk indicates head end, arrowhead posterior end). Gonadal immunoreactivity is strong as shown in the enlarged inset, where the arrow indicates probable germ cells. (E) A cluster of germ cells in the ovary showing strong LEA protein immunoreaction (black arrow); open arrows indicate immunoreactivity in the cytoplasm of growing oocytes. (F) Control experiment: lack of staining of ovary after preabsorbing antiserum with recombinant AavLEA1. Scale bar, A–F = 20 μm. (G) Nomarsky optics of extruded ovary, with strongly immunofluorescent stem cells in confocal image (H) circled and indicated with an arrow. Green fluorescence in the cytoplasm represents LEA protein. Scale bar, G, H = 16 μm.