An LEA group 3 family member is involved in survival of *C. elegans* during exposure to stress

Tali Z. Gal[a], Itamar Glazer[a], Hinanit Koltai[b,*]

[a] *Department of Nematology, ARO, The Volcani Center, Bet Dagan 50250, Israel*
[b] *Department of Genomics, ARO, The Volcani Center, Bet Dagan 50250, Israel*

Received 18 August 2004; revised 21 September 2004; accepted 24 September 2004

Available online 6 October 2004

Edited by Takashi Gojobori

Abstract In order to establish a functional role for late embryogenesis abundant (LEA) proteins in response to stress conditions in *Caenorhabditis elegans*, we silenced the expression of an *LEA* (*Ce-lea-1*) gene and determined the survival of worms under stress conditions. *Ce-lea-1* transcription was induced during dehydration of *C. elegans* dauer juveniles. Following partial silencing of *Ce-lea-1* transcription, we demonstrated a specific and significant reduction in worm survival during induction of desiccation, osmotic and heat stress. Together, these results establish a functional role for *Ce-lea-1* in stress survival of *C. elegans* and suggest that *Ce-lea-1* may function as a component that is common to the responses to the examined stress conditions.

Keywords: *Ce-lea-1*; *Ce-lin-53*; *Ce-unc-120*; Desiccation stress; Heat stress; Osmotic stress

1. Introduction

The removal of water from cells, the survival of cells in an air-dried state, and the rewetting of air-dried cells impose considerable physiological constraints. Nematodes are among the most successful organisms in withstanding desiccation and viable specimens have been recovered from dry desert soils [1]. Only little is known about the behavioral, biochemical and molecular events in nematodes that lead to the physiological responses to dehydration stress conditions. Research in *Caenorhabditis elegans* offers a unique opportunity to understand the dehydration response better. More specifically, little is known of the mechanisms that enable *C. elegans* dauer juveniles (DJs) to survive exposure to dehydration, apart from their high desiccation tolerance, as recorded by Ohba and Ishibashi [2]. Dauer juveniles, which have evolved in several nematode families, are a relatively stress-resistant juvenile stage that is adapted to remain in the environment without feeding while searching for a new food source [3,4]. The genetic pathway of dauer formation has been extensively studied, especially in the nematode *C. elegans* (recently reviewed in [5,6]).

Late embryogenesis abundant (LEA) proteins are thought to be prominent in the stress response in various organisms including plants, algae, yeasts and bacteria. They are hydrophilic and accumulate in higher plants under conditions of extreme desiccation, during the last stage of seed formation, and during periods of water deficit in vegetative organs [7–20]. The biological role of LEA proteins was demonstrated in the bacterium *Deinococcus radiodurans*, in which disruption of the LEA76 locus resulted in reduction in the viability of desiccated cultures, which suggests that it is a critical component of a cell's overall strategy to tolerate extreme dryness [7]. In addition, a functional role for LEA proteins in *Chlorella vulgaris* was demonstrated when they were found to protect a freeze-labile enzyme against freeze inactivation [13].

LEA group 3 members are characterized by a motif of 11 amino acids that is predicted to form an amphipathic α-helix that is probably involved in structural interactions [21]. They are thought to be mainly involved in counteracting the irreversible damaging effects of increased ionic strength in the cytosol during desiccation, perhaps through the binding of both anions and cations to the helical region of the protein [8]. Ried and Walker-Simmons [9] demonstrated that the levels of LEA group 3 proteins correlated well with tissue dehydration tolerance. Distinctive roles in the protection of cells against cellular dehydration were demonstrated for proteins of LEA groups 2 (Le4) and 3 (HVA1) from tomato and barley, respectively, by means of a yeast heterologous expression system [12]. Moreover, under soil water deficit conditions, transgenic wheat that expressed the LEA group 3 barley *HVA1* gene had significantly greater mass and weight than the control, which further supports its role in the dehydration response [10].

In nematodes, several pieces of evidence suggest that LEA family members are involved in the desiccation response. Among the stress-related genes found to be highly expressed during dehydration of the entomopathogenic nematode *Steinernema feltiae*, IS-6 is an LEA group 3 protein [22]. In addition, the level of LEA-like protein desc47 was increased 10-fold during dehydration of *S. feltiae* IS-6 [23]. In the free-living nematode *Aphelenchus avenae*, LEA group 3 AavLEA1 transcription was upregulated following exposure to 90% relative humidity (RH) [24], and investigation of its structure demonstrated limited oligomerization, and configuration changes of AavLEA1 on drying [25].

* Corresponding author. Fax: +972-3-9669583.
E-mail address: hkoltai@volcani.agri.gov.il (H. Koltai).

Abbreviations: DJ, dauer juveniles; L1, first-stage larva; L4, fourth-stage larva; LEA, late embryogenesis abundant; RH, relative humidity; RNAi, RNA interference

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doi:10.1016/j.febslet.2004.09.049

In the present research, we conducted functional studies to determine the role of expression of an LEA group 3 gene (*Ce-lea-1*) in *C. elegans* DJ survival under stress. The induction of *Ce-lea-1* expression and the reduction in worm survival during desiccation suggested a role for Ce-lea-1 in dehydration survival of *C. elegans* DJs. In addition, the reduced survival rates of worms under osmotic and heat stresses, following *Ce-lea-1* silencing, suggested that *Ce-lea-1* may be a critical component of the worm's strategy to tolerate the dehydration associated with osmotic- and heat-stress conditions.

2. Materials and methods

2.1. Nematode culture and induction of dauer juveniles in F1 generation

Fifty wild-type *Caenorhabditis elegans* N2 (var. Bristol) fourth-stage juveniles (L4) were placed onto NGM plates containing 1 mM IPTG and 50 μg/ml of ampicillin (Amp), seeded with bacterial colonies containing a L4440 feeding vector without an insert, and were incubated for 72 h at 17 °C. This treatment of the worms is referred to below as "control". The worms were then washed and transferred onto new sets of similar plates for 24 h at 25 °C, to lay their eggs. For induction of DJs in the F1 generation, the egg-laying plates were washed with M9 buffer and incubated for 6 days at 25 °C, with agitation. The worm population was then filtered through a 20-μm sieve to remove dead and adult nematodes. Microscopic examination of a sample of the DJ population verified their percentage in the population before the worms were taken for desiccation bioassays and RNA extraction.

2.2. Induction of desiccation, osmotic and heat stresses

C. elegans DJs were dehydrated as described by Solomon et al. [26]: they were exposed to 97% RH at 23 °C, in a sealed desiccator with 60 ml of saturated K_2SO_4, for 8 or 24 h. Non-dehydrated worms were stored in M9 buffer at 23 °C. Survival scores were calculated from populations of 80–100 juveniles for each replication of the experiments. All data from the three repeats for a certain time point were pooled and the mean and standard deviation of DJ survival were calculated for the pooled data. Significance was determined by the Student–Newman–Keuls test ($\alpha = 0.05$; $P < 0.0001$).

Osmotic stress was induced as described by Lamitina et al. [27], with modifications. The F1 generation of *Ce-lea-1* RNA interference (RNAi)-treated and control worms was exposed, at the L4 larval stage, to several concentrations of sucrose (305, 400, 484 and 652 mM) on NGM plates at 25 °C for 24 h. The survival rates were determined following 24 h of recovery in M9 buffer at 25 °C. Survival scores were calculated from populations of 100–120 juveniles for each replication of the experiments. All data from the three repeats for each sucrose concentration were pooled and the mean and standard deviation of DJ survival were calculated for the pooled data. Significance was determined by *T* test ($P < 0.05$).

Heat stress was induced according to Lithgow et al. [28], by exposure of the F1 generation of *Ce-lea-1* RNAi-treated and control worms, at the first-larval stage (L1), to 35 °C for 3, 6 or 8 h, on NGM plates. Heat-stressed cultures were returned to 22 °C to allow recovery and the worms were scored for survivals. Survival scores were calculated from populations of 80–100 juveniles for each replication of the experiments. All data from the three repeats were pooled and means and standard deviations of DJ survival were calculated for the pooled data. *T* Test was applied to determine significance ($P < 0.0001$).

2.3. RNA extraction

Total RNA was isolated from RNAi-treated and control *C. elegans* that had been dehydrated for 8 or 24 h, and also from the non-dehydrated worms, by using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The RNA was treated with RNase-Free DNase Set (Qiagen).

2.4. RNAi by feeding

Gene fragments for double-stranded RNA (dsRNA)-mediated interference were amplified by PCR from cDNA extracted from a mixed *C. elegans* population. The specific primers for *Ce-lea-1* were 5′-GGACAAGAAGCTTCCGACAG-3′ and 5′-CATCTCCGTGTTTCTGAGCA-3′, those for *Ce-unc-120* (Accession No. NM_059895) were 5′-TCAACTGGTCTATTGCCAAATGG-3′ and 5′-TGTGGAGAACAGTGGAGCCATTG-3′ and those for *Ce-lin-53* (Accession No. NM_060151) were 5′-ATTCATCGGCTCATTC- TTGG-3′ and 5′-TCAACGACTGACTCGTGACC-3′. Amplified fragments were sequenced and cloned into pGEM-T vector (Promega, Madison, WI), digested by *Eco*RI and *Sac*II (Fermentas, Foster City, CA) and recloned into L4440 feeding vector [29]. The resulting plasmids were transformed into the $HT_{115}(DE_3)$ RNase III-deficient *Escherichia coli* strain. Bacterial colonies containing L4440 were selected by PCR for those that were cloned with the gene fragments, and single colonies were picked and grown for 8–18 h in LB medium with 100 μg/ml of Amp. For control, bacterial colonies containing a L4440 vector without an insert were grown as described above. The bacterial cultures were seeded onto NGM plates with 1 mM IPTG [30] and 50 μg/ml of Amp. The seeded plates were allowed to dry at room temperature and dsRNA expression of the cloned gene was induced overnight at room temperature [30]. Three (for *Ce-lin-53*) and 50 (for *Ce-lin-53*, *Ce-lea-1* and *Ce-unc-120*) L4-stage juveniles were placed onto NGM plates containing seeded bacteria that expressed either dsRNA for each gene or, as a control, L4440 feeding vector without the insert. The plates were incubated at 17 C for 72 h, after which they were washed and transferred onto new sets of similar plates at 25 °C for 24 h, for egg laying. Induction of DJs in the F1 generation was performed as described above.

2.5. Real-time RT-PCR

Total RNA samples (2 μg), extracted from dehydrated or non-dehydrated *C. elegans*, RNAi treated or controls, were reverse-transcribed (RT) [22]. Real-time RT-PCR was performed with the qPCR Mastermix for Sybr Green (Eurogentec, Seraing, Belgium) according to the manufacturer's instructions, with modifications as described by Gal et al. [22]. The target gene expression level in dehydrated worms was compared with that of the non-dehydrated worms. Means and standard deviations of gene expression were calculated from three replications of the experiment. To minimize mRNA quantification errors and genomic DNA contamination biases, and to correct for inter-sample variations, we used 18SrRNAs of *C. elegans* as an internal control, and the relative expression ratio was based on the expression of a target gene relative to that of 18SrRNA. Primers used for detection of *Ce-lea-1* (Accession No. AF016513) transcripts were 5′-TGCTTCGGAAAGTGCAGAGTC-3′ and 5′-CAACAGAAGCAGCTCCCTCG-3′, and those for 18SrRNA (Accession No. X03680) were 5′-ACCGCTATGTGTCTCCTGGTG-3′ and 5′-CGAAACCGAACCACGATCAT-3′. Significance was determined with *T* test ($P < 0.05$).

2.6. Determination of uncoordinated movement and embryonic lethality phenotypes of RNAi-treated worms

For scoring of uncoordinated (or paralyzed) phenotypes, F1 of RNAi-*Ce-unc-120* treated and control populations were grown to adulthood on NGM plates, and scored for uncoordinated (or paralyzed) phenotypes [30]. To determine the lethality of the RNAi-*Ce-lin-53* treatment to the F1 nematodes, RNAi-treated and control L4s were placed on NGM plates and incubated for 24 and 48 h at 25 °C for egg laying. The plates were then scored for hatched larvae [30].

3. Results

3.1. Survival and induction of Ce-lea-1 gene expression in dehydrated C. elegans

We induced DJ formation in the *C. elegans* F1 generation and the appearance of DJs in the population was confirmed by microscopic examination (not shown); following filtration, DJs formed $90 \pm 5\%$ of the F1 population. After 8 and 24 h of dehydration, 78 ± 3.4 and $69.7 \pm 5.6\%$ ($n = 3$), respectively, of the F1 population survived (control, Fig. 1). Despite the differences in desiccation conditions, which prevents direct comparison, these results are in conceptual agreement with Ohba and Ishibashi's [2] findings concerning *C. elegans* DJ survival under desiccation stress. In addition, the coiling response reported by Solomon et al. [26] for *S. feltiae* IS-6 infective juveniles (IJs) was not detected for *C. elegans* DJs. In

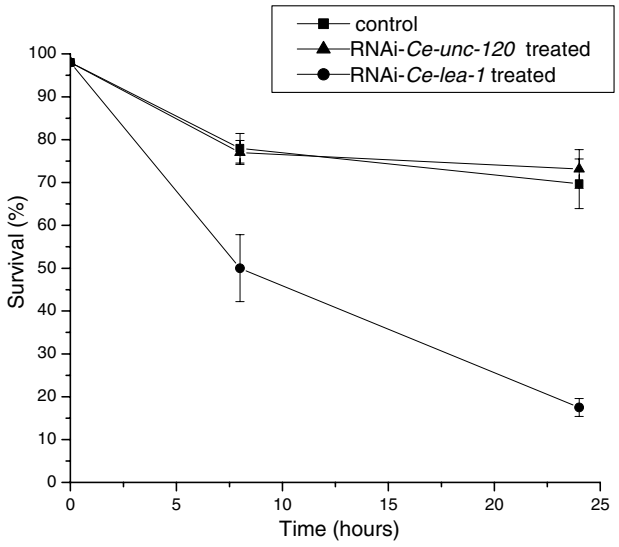

Fig. 1. Survival (%) of *C. elegans* dauer juveniles, F1 generation of control, and RNAi-*Ce-lea-1*- and RNAi-*Ce-unc-120*-treated worms following 8 and 24 h exposure to desiccation stress (97% RH at 23 °C). Bars represent standard deviations calculated from three repeats of each experiment.

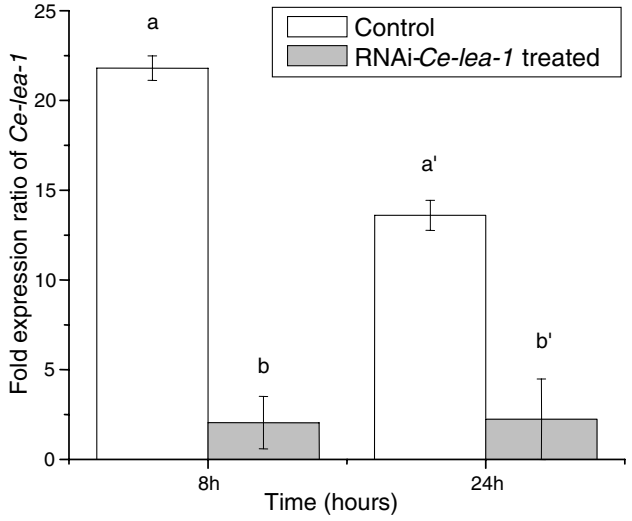

Fig. 2. Expression levels of *Ce-lea-1* in *C. elegans* dauer juveniles, F1 generation of control and of RNAi-*Ce-lea-1*-treated worms, following 8 and 24 h exposure to desiccation stress (97% RH at 23 °C). Expression ratios were calculated as the ratio between the means of the steady-state mRNA levels of dehydrated and of non-dehydrated worms (expression ratio $= 2^{-(\Delta \text{ dehydrated} - \Delta \text{ non-dehydrated})}$; Δ dehydrated and Δ non-dehydrated are the differences in threshold cycles for target and reference). Bars represent standard deviations calculated from three repeats of each experiment. a, b denote significant differences between expression values for 8 h of dehydration; a′, b′ denote significant differences between expression values for 24 h of dehydration.

order to determine the expression pattern of the *Ce-lea-1* gene during desiccation of *C. elegans* DJs, we determined the steady-state level of its transcripts. Following 8 and 24 h of *C. elegans* DJ dehydration, the steady-state level of *Ce-lea-1* transcripts was significantly increased ($P < 0.05$) by factors of 21.8 ± 0.7 ($n = 3$) and 13.6 ± 0.8 ($n = 3$), respectively, compared with that in non-dehydrated DJs (control, Fig. 2).

3.2. Silencing of Ce-lea-1 significantly reduced the survival of C. elegans during desiccation

In order to define the role of the *Ce-lea-1* gene in the desiccation tolerance of *C. elegans* DJs, we determined the effect of RNAi-mediated silencing of *Ce-lea-1* on the desiccation tolerance of the F1 DJ population. Specifically, DJs were induced as early as F1 in RNAi-treated and control worms, in order to prevent the reversion to the wild-type phenotype that occurs in subsequent generations [31]. Only 50 ± 8 ($n = 3$) and $17.5 \pm 2\%$ ($n = 3$) of the *Ce-lea-1* RNAi-treated F1 DJ population survived after exposure to 8 and 24 h, respectively, of desiccation (Fig. 1). This is a significant reduction in survival compared with the control ($P < 0.0001$).

Notably, RNAi-*Ce-lea-1*-treated worms exhibited the wild-type phenotype, as evaluated by visual inspection assay, which is consistent with the findings of Ahringer's group ([32]; WormBase release WS126, Gene Model K08H10.1), whose work did not include stress studies.

To confirm that the observed reductions in the survival of RNAi-*Ce-lea-1*-treated DJs following 8 and 24 h of dehydration (Fig. 1) were due to silencing of *Ce-lea-1* transcription, we quantitatively determined the steady-state level of *Ce-lea-1* transcripts in RNAi-*Ce-lea-1*-treated worms. In F1 of RNAi-*Ce-lea-1*-treated worms *Ce-lea-1* transcription was reduced by a factor of 11.7 ± 0.2 compared to the control ($n = 3$).

Following 8 h of dehydration of worms, the ratio between *Ce-lea-1* transcription level in dehydrated to that in non-dehydrated worms was only 2.05 ± 1.46 ($n = 3$), i.e., about one-tenth of the corresponding ratio in control worms. Following 24 h of desiccation, the corresponding ratio for RNAi-treated worms was only 2.24 ± 2.25 ($n = 3$), a mean of one-sixth of that observed for control worms (Fig. 2). These expression values in the RNAi-*Ce-lea-1*-treated worms were significantly lower than those in the controls ($P < 0.05$).

3.3. Silencing of a Ce-unc-120 had no significant effect on the survival of C. elegans during desiccation

To demonstrate that the effect of silencing *Ce-lea-1* on dehydration survival in *C. elegans* was specific to the LEA gene, and not a result of silencing of any non-lethal gene, we determined the effect of RNAi-*Ce-unc-120* treatment on the desiccation tolerance of the F1 DJ population. *Ce-unc-120* encodes a member of the MADS-box family of transcription factors; it is necessary for locomotion and muscle development and for formation of the normal number of muscle bands and proteins (WormBase release WS126, Gene Model D.1081.2). Kamath et al. [30] demonstrated that silencing of *Ce-unc-120* resulted in uncoordinated and paralyzed phenotype in at least 10% of the progeny. In our present experiments, $28 \pm 7\%$ ($n = 3$) of the RNAi-*Ce-unc-120*-treated F1 adults displayed the uncoordinated and paralyzed phenotype reported by Kamath et al. [30]. It is important that no significant changes ($P < 0.0001$), relative to the control, were recorded in the level of DJ survival during desiccation stress following RNAi-*Ce-unc-120* treatment (values of $77 \pm 3\%$ and $73 \pm 2\%$ during desiccation for 8 and 24 h, respectively; $n = 3$) (Fig. 1).

3.4. Silencing of Ce-lea-1 significantly reduced the survival of C. elegans during osmotic stress

To determine whether the effect of *Ce-lea-1* silencing on *C. elegans* survival was specific to dehydration stress or whether it

may also be observed during osmotic stress, we determined the effect of RNAi-*Ce-lea-1* treatment on the osmotic-stress survival of the late L4 and young adult stage of *C. elegans*. When sucrose was used at a concentration of 484 mM, survival in RNAi-*Ce-lea-1*-treated worms was significantly less than that in the control ($n = 3$; $P < 0.05$; Table 1). Nevertheless, exposing worms to concentrations of 305, 400 and 652 mM of sucrose did not result in significant differences between RNAi-*Ce-lea-1*-treated and control worms ($n = 3$; $P < 0.05$; Table 1). It should be noted that the survival rates of the controls that were recorded in the present study were higher than those reported by Lamitina et al. [27] except with the 652 mM concentration. With a 652 mM sucrose solution, there were no survivors in our present study (Table 1), whereas Lamitina et al. [27] observed a $21 \pm 3\%$ survival rate. In conclusion, RNAi-*Ce-lea-1* treatment reduced worms survival under the osmotic stress imposed by the sucrose concentration of 484 mM.

3.5. *Silencing of Ce-lea-1 significantly reduced the survival of* C. elegans *during heat stress*

In order to define whether the effects of *Ce-lea-1* gene silencing on desiccation and osmotic tolerance of *C. elegans* are specific to the pathway(s) shared by the two stresses, or whether *Ce-lea-1* is involved in heat-stress survival also, we determined the effect of RNAi-mediated silencing of *Ce-lea-1* on the heat survival of the L1 of *C. elegans*. In agreement with Lithgow et al. [28] and Solomon et al. [33], $100 \pm 0\%$ of the control L1 survived during 3 h of heat shock, whereas during 8 and 6 h of heat shock $84 \pm 4\%$ ($n = 3$) and $82 \pm 5\%$ ($n = 3$) of the control L1 survived (Fig. 3). In the RNAi-*Ce-lea-1*-treated population, a significant reduction ($n = 3$; $P < 0.0001$) in L1 survival was recorded: $88 \pm 2\%$, $70 \pm 5\%$ and $16 \pm 5\%$ survived during 3, 6 and 8 h, respectively, of heat stress (Fig. 3).

3.6. *RNAi treatment on large population resulted in partial penetrance of the RNAi phenotype*

Only partial reductions in *Ce-lea-1* transcription and worm survival were observed for RNAi-*Ce-lea-1*-treated worm populations during induction of desiccation, osmotic and heat stresses (described above). This may be a general outcome of RNAi treatment of a relatively large number of worms. Since Kamath et al. [30] found that RNAi-*Ce-lin-53* treatment of a small worm population (three L4 individuals) results in full penetrance of embryonic lethality, we determined the efficiency of RNAi-*Ce-lin-53* treatment on large worm population. *Ce-lin-53* is a member of the abnormal cell LINeage gene class and a homolog of a retinablastoma-binding protein (WormBase release WS126, Gene Model K07A1.12). In agreement with the findings of Kamath et al. [30], in the present study none of the F1 eggs from the batches of three treated worms hatched ($n = 3$), whereas an average of 80 ± 15 ($n = 3$) hatched from batches of three worms in the control. Nevertheless, when batches of 50 worms were treated with RNAi-*Ce-lin-53*, an average of 45 ± 15 ($n = 3$) of the F1 progeny hatched, representing about 4% of the number that hatched in the control batch of 50 worms (1050 ± 212; $n = 3$).

Table 1
Survival (%) of *C. elegans* L4 and young adult stages, which are F1 generation of control and of RNAi-*Ce-lea-1*-treated worms, following 24 h of exposure to sucrose concentrations of 305, 400, 484, and 652 mM

	Sucrose concentration (mM)			
	305	400	484	652
Control	$100 \pm 1\%$	$90.3 \pm 4.5\%$	$90.0 \pm 2.6^*$	0 ± 0
RNAi-*Ce-lea-1* treated	$100 \pm 1\%$	$86.7 \pm 5.8\%$	$78.0 \pm 9.5^*$	0 ± 0

$n = 3$.
* The values are significantly different $P < 0.05$.

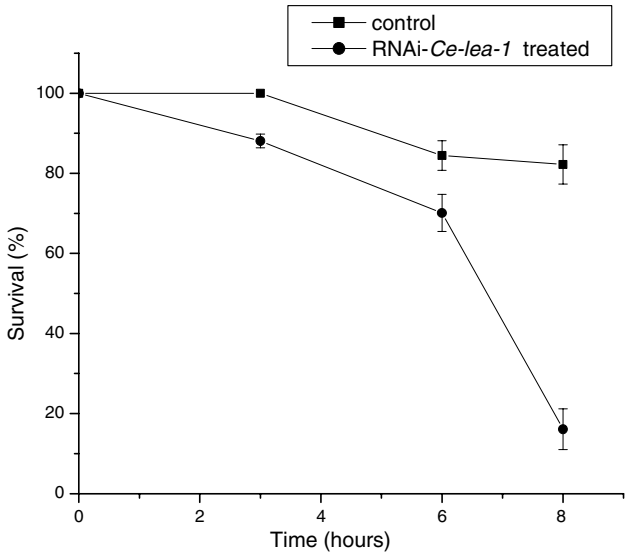

Fig. 3. Survival (%) of *C. elegans* L1, F1 generation of control and of RNAi-*Ce-lea-1*-treated worms following 3, 6 and 8 h of exposure to heat-shock stress (35 °C). Bars represent standard deviations calculated from three repeats of each experiment.

4. Discussion

In the present study, we focused on the role of *Ce-lea-1* expression in *C. elegans* survival during stress induction. Several LEA Group 3 proteins were previously demonstrated to be upregulated during nematodes' dehydration response [22–24]. Among them is *Sf-lea-1*, which is highly expressed in *S. feltiae* IS-6 IJs during dehydration [22]. A close homolog to *Sf-lea-1* in *C. elegans* is *Ce-lea-1* [22]. To examine the role of *Ce-lea-1* gene in the desiccation tolerance of *C. elegans*, we studied its expression pattern in worms and the effect of its being silenced on the survival of *C. elegans* during stress induction.

The steady-state level of *Ce-lea-1* transcripts increased during dehydration of *C. elegans* DJs by 8 and 24 h of desiccation. In addition, the partial silencing of *Ce-lea-1* transcription, which was verified at the level of gene transcription, reduced DJ survival under desiccation. No significant changes in the level of DJ survival, compared with that in the controls, were recorded following RNAi-*Ce-unc-120* treatment, thus suggesting that the effect of *Ce-lea-1* silencing on *C. elegans* dehydration survival was not a result of silencing of a non-lethal gene per se; rather, it was a specific effect of the silencing of the LEA gene. Collectively, these findings suggest the involvement of Ce-lea-1 in desiccation survival of *C. elegans* DJs and, especially, a silencing-based functional role for LEA proteins in nematode desiccation.

We have demonstrated that silencing of *Ce-lea-1* gene transcription also leads to reduced level of survival during osmotic

stress. The effect of *Ce-lea-1* silencing on worm survival was significant only at the intermediate sucrose concentration, of those tested. Perhaps, lower concentrations of sucrose did not significantly induce LEA expression in *C. elegans*, whereas the higher sucrose concentration may have imposed stress conditions that were too severe for worm survival.

Several findings in other research studies suggest a commonality in response to various stress conditions. Osmotic stress, similar to dehydration stress, leads to fluctuations of internal osmolarities in various organisms [14,34–36], and in human cells the molecular mechanisms of response to desiccation and to hyperosmotic stress partially overlap, involving MAPK activation [37]. Several studies suggested that in yeasts, bacteria, cyanobacterium and murines, dehydration that occurs during heating or during osmosis appears to have similar effects, including expression of heat shock proteins and involvement of trehalose [38–42]. In addition, some stress-induced heat shock proteins are LEA-like [23,43,44]. LEA proteins are heat stable (reviewed by [45]) and Glazer and Salame [46] demonstrated that evaporatively and osmotically desiccated nematodes were able to withstand heat stress.

Our results, which indicate that worm survival decreased during dehydration, osmotic or heat stresses following LEA silencing, suggest that *Ce-lea-1* is a common component needed for survival during the examined stresses and further support the notion of commonality of their molecular mechanisms. Conceivably, since LEA proteins are thought to have a role in the dehydration response [7–20], *Ce-lea-1* may be a critical component of the worm's strategy for tolerating the water discrepancies associated with dehydration-, osmotic- and heat-stress conditions.

dsRNA-mediated gene silencing in *C. elegans* is usually applied to individual worms, in order to study individual traits (e.g., proliferation and/or differentiation; recently reviewed in [47,48]). In the present study, we examined the effect of RNAi on dehydration survival, which is a DJ population trait. One of the major difficulties in the assessment of the effect of RNAi treatment on the DJ population arose from the necessity to examine the F1 generation, since a subsequent (F2) generation might revert to the wild-type phenotype [31]. By imposing severe starvation conditions, we induced the formation of DJs as early as the F1 generation. Treating a large F1 population with RNAi for gene silencing resulted in incomplete silencing of *Ce-lea-1* expression, and with only a restricted effect on the phenotype of the population, i.e., limited reduction in worm survival during stress induction and limited appearance of the embryonic lethality phenotype (for *Ce-lea-1* and *Ce-lin-53*, respectively). However, treating small batches of worms with RNAi-*Ce-lin-53* resulted in a total absence of F1 hatching ([30]; the present study). These results suggest that RNAi treatment of large populations of worms may achieve only a partial effect on the progeny population.

In conclusion, the present study demonstrated the importance of *Ce-lea-1* expression in the survival of *C. elegans* under exposure to dehydration, osmotic or heat stresses. The mechanism of stress response is undoubtedly complex: *Ce-lea-1* is just one member of an *LEA* gene family and other family members might exhibit similar activity. In addition, other components are likely to be involved. Further studies should lead to the comprehensive and detailed understanding of the molecular mechanisms of stress response.

Acknowledgements: We thank Liora Salame for technical assistance, the Caenorhabditis Genetics Center at the University of Minnesota in St. Paul, MN, USA, for the supply of N2 worms, Yosef Gruenbaum, of the Department of Genetics in the Institute of Life Sciences at the Hebrew University of Jerusalem, Israel, for the supply of L4440 feeding vector, and Miri Zarhi of the Department of Statistics at the Volcani Center for statistical analysis. The work was supported by the US–Israel Binational Agricultural Research and Development Fund (BARD) (US-2858-97R) and by Research Grant No. 116/3-1 from the German Ministry of Science.

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