Identification and Characterization of a Ste20-Like Kinase in Artemia and Its Role in the Developmental Regulation and Resistance to Environmental Stress

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Abstract

Background: To adapt to extreme environments, the crustacean Artemia has evolved two alternative reproductive pathways. During ovoviviparous (direct) development, nauplius larvae are produced. In contrast, Artemia females release encysted diapause embryos (cysts) via the oviparous pathway. To date, the cellular mechanisms that regulate stress resistance of Artemia remain largely unknown. Ste20-like kinase (SLK) participates in multiple biological processes, including stress responses, apoptosis, and cell cycle progression.

Principal Finding: We isolated and characterized a member of the SLK superfamily termed ArSLK from Artemia parthenogenetica. The ArSLK gene is transcribed throughout both ovoviviparous and oviparous development; however, the protein is located mainly in the nuclei of stress-resistant diapause cysts, unlike the nauplii and nauplius-destined embryos where it is cytoplasmic. Interestingly, exposure of nauplii to heat shock, acidic pH, and UV irradiation induced the translocation of ArSLK from cytoplasm to nucleus. This translocation was reversed following stress removal. Moreover, under physiologically-stressful conditions, the nauplius larvae produced by adults after gene knockdown of endogenous ArSLK by RNAi, lost the ability of free-swimming much earlier than those of control larvae from females injected with GFP dsRNA.

Conclusions/Significance: Taken together, this study demonstrated that trafficking of ArSLK between the cytoplasm and the nucleus participates in regulating the stress resistance of Artemia. Our findings may provide significant insight into the functions of members of the SLK superfamily.

Introduction

The brine shrimp Artemia parthenogenetica is widely distributed in hypersaline environments characterized by such things as anoxia, high salinity, pH extremes, high UV irradiation and temperature extremes. This primitive crustacean has evolved two independent reproductive pathways that enable it to adapt to these environments. Under certain conditions females produce and release nauplius larvae (the ovoviviparous pathway) [1,2]. By contrast, under harsh conditions Artemia releases encysted diapause embryos (cysts) in which development is arrested and metabolic activity is greatly depressed (the oviparous pathway) [1,2]. The striking stress resistance of Artemia makes it an ideal model system in which to study the adaptive mechanisms involved.

The accumulation of molecular chaperones in high levels has been implicated as one of these mechanisms in diapause embryos. Among these molecular chaperones, p26 and artemin have been well characterized. The small heat shock protein, p26, located in the nucleus and cytoplasm of diapause embryos, functions as a chaperone for proteins [2–6], while the ferritin-like protein artemin, located only in the cytoplasm, exhibits chaperone activity for proteins as well as RNA [7,8]. Mammalian cells expressing p26 or artemin display enhanced tolerance to heat stress [6,9] and those expressing p26 are also more resistant to desiccation [10]. Another abundant molecule is the disaccharide trehalose, which reaches about 15% of the diapause embryo’s dry weight, has been shown to be critical to desiccation tolerance [2,11]. Although these results represent progress in our understanding of stress tolerance in Artemia, the detailed regulatory mechanisms involved in this resistance remain to be described.

Sterile 20 (Ste20) kinase, of much significance in our study, was initially identified as a mitogen-activated protein kinase kinase kinase (also known as MAP3K) involved in the mating pathway of the yeast Saccharomyces cerevisiae [12,13]. Mammalian homologs of Ste20 kinase have since been identified and are classified into the p21-activated kinase and germinal center kinase subfamilies on the basis of the distinct locations of their kinase domains [14]. Ste20-like kinase (SLK) belongs to the germinal center kinase subfamily, containing a conserved N-terminal kinase domain and...
a variable C-terminal regulatory domain [14]. This protein kinase interacts with specific partners and participates in various signalling pathways, including those involved with the control of programmed cell death, cell proliferation and cell migration [15,16]. Overexpression of SLK induces apoptosis, which is mediated by its ability to activate #e-Jun N-terminal kinase# and p38 MAPK kinase activity [17,18]. Studies on *Xenopus laevis* oocytes and human somatic cells revealed that SLK activity is also required for the cell cycle transition from G2 to M phase [19,20].

In this study, we examined the role of SLK (ArSLK) in the stress resistance of *Artemia*. We found that accumulation of ArSLK protein in the nucleus of diapause cysts suggests that it might play an important role in developmental arrest to resistant stress. Furthermore, gene knockdown of ArSLK verified that a decrease in the amount of ArSLK reduced the degree of stress resistance. The results to be presented here indicate that the nuclear translocation of ArSLK is involved in *Artemia* stress-resistance and sheds further light on additional functions of the SLK superfamily.

**Materials and Methods**

**Animals**

*Artemia parthenogenetica* cysts from Gahai were a kind gift from Prof. Feng-Qi Liu of Nankai University, Tianjin, China. Animals were raised in artificial sea water (Blue Starfish, Hangzhou, Zhejiang, China) at 25°C and were fed once every 2 days with *Chlorella* powder. Ooviviparous *Artemia* adults, releasing nauplius larvae, were raised at 25°C seawater at 25°C. The second round of PCR was then performed using the ArSLK–3′F primers, producing a 2.5 kb fragment. To verify the full-length ArSLK cDNA sequence, a pair of gene-specific primers (ArSLK–F and ArSLK–R) (Table 1) was used to amplify a 4020 bp open reading frame fragment. The PCR product was subcloned into the pUCm-T vector (Sangon, Shanghai, China) and then sequenced. The nucleotide sequence of this ArSLK-encoding cDNA was submitted to GenBank and the accession number was KC818632.

**Immunoblotting**

Proteins extracted from tissues of *Artemia* at different developmental stages were prepared using TRIzol (Invitrogen, Carlsbad, CA, USA) reagent, as per the manufacturer’s instructions, and then quantified by the Bradford method [21]. Forty micrograms of each total protein sample were separated on 10% SDS-PAGE gels and then transferred to PVDF membranes (Roche, Indianapolis, IN, USA). After incubation of membranes with primary antibodies at 4°C overnight, specific proteins were detected using the BM Chemiluminescence Western Blotting Kit (Roche, Indianapolis, IN, USA). To obtain the anti-ArSLK antibody (HuaAn, Hangzhou, Zhejiang, China), rabbits were immunised with a peptide that was based on amino acids 391–405 of ArSLK. The anti-α-tubulin antibody (Sigma, St. Louis, MO, USA) was purchased.

**Cell fractionation**

Cell fractionation was performed as previously described [22]. In brief, specimens were homogenized in a Dounce homogenizer on ice in 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer (10 mM HEPES, 4 mM MgCl₂, 1 mM phenylmethylsulfonyl fluoride (PMSF), pH 7.5). To obtain the cytoplasmic fraction, homogenates were centrifuged at 3300 g for 15 min at 4°C and the supernatant was retained. To obtain the nuclear fraction, the pellet was re-suspended and re-homogenized in fractionation buffer (10 mM Tris, 10 mM NaCl, 10 mM EDTA, 0.5 mM EGTA, 4 mM MgCl₂, pH 7.4), loaded onto 45% (w/v) sucrose buffer drop by drop, and then centrifuged at 13000 g for 15 min at 4°C. The pellets were washed once and then dissolved in EBC buffer (50 mM Tris, 120 mM MgCl₂, 1 mM EDTA, 0.5% NP-40, 1 mM PMSF, pH 7.5). Proteins were quantified by the Bradford method [21]. Equal amounts of protein were loaded for immunoblotting. Anti-α-tubulin (1:5000) (Sigma, St. Louis, MO, USA), anti-histone H3 (1:5000) (Epitomics, Burlingame, CA, USA), and anti-ArSLK (1:1000) (HuaAn, Hangzhou, Zhejiang, China) antibodies were used for detection.

**Real time PCR**

Specimens from different developmental stages were snap-frozen in liquid nitrogen and then total RNA was prepared from homogenized specimens using TRIZol reagent (Invitrogen, Carlsbad, CA, USA). Extracted RNA was quantified by measuring absorbance at 260 nm with a Genova UV/visible spectrophotometer. First-strand cDNAs were prepared from the total RNA specimens as described above. After reverse transcription, all real time PCR reactions were performed on the Bio-Rad MiniOpticon™ Real-Time PCR System using SYBR Premix Ex Taq™ (TaqKaRa, Shiga, Japan) with gene-specific primers to amplify ArSLK and tubulin as an internal control (ArSLK–3′F and ArSLK–R for ArSLK; Tubulin–F and Tubulin–R for tubulin) (Table 1). The relative mRNA amounts were analysed by the comparative CT method as described by Schmittgen and Livak [23]. All data were expressed as means ± SE from three independent repetitions. All statistical analyses were performed by a two-tailed, paired Student’s t test, and the differences were considered significant for P<0.01.
Table 1. Nucleotide sequences and positions of primers used in polymerase chain reactions.

| Primer     | Length (bp) | Position | Direction | Sequence (5' -3')                  |
|------------|-------------|----------|-----------|------------------------------------|
| ArSLK-F1   | 23          | 253–275  | F         | GCNTTYGGAARGTNTAYAARGC             |
| ArSLK-F2   | 23          | 244–266  | F         | GGGAYGGYGGCNYTYYGNAARGT            |
| ArSLK-R1   | 23          | 691–713  | R         | ATCCARTANGGNATCDDATRA              |
| ArSLK-R2   | 23          | 700–722  | R         | TCNGNNGGCCAATCARTANGGNT            |
| ArSLK-S'R1 | 21          | 636–656  | R         | GCAGACACACCAAAAAATCGCT             |
| ArSLK-S'R2 | 21          | 285–305  | R         | GCAGCTAATGCTGGTCTG                |
| ArSLK-3F1  | 21          | 595–615  | F         | GCTGGAATGTTCTACGAGC               |
| ArSLK-3F2  | 21          | 664–684  | F         | AAGTCACACCTTCAGAAACGA             |
| ArSLK-3F3  | 20          | 1760–1779| F         | TTATGCTGGCTATGAACT                |
| ArSLK-3F4  | 20          | 1981–2000| F         | GGAATGGCATAGACAAAGG               |
| ArSLK-F    | 22          | 109–130  | F         | ATGTGTTTTTCTCAAGGAAGAG            |
| ArSLK-R    | 23          | 4106–4128| R         | TTACGAATGTCGATATGTCGCA            |
| ArSLK-RTR  | 20          | 2128–2147| R         | TCTCTCAATGCTATCTG                 |
| Tubulin-F  | 20          | 446–465  | F         | GCAGTGGTCTACAAAGGTTTC             |
| Tubulin-R  | 22          | 774–795  | R         | ATCAAAAGCAAGGCTGGCCTG             |
| ArSLK-dsF  | 29          | 1160–1179| R         | GCTTAGACGTTCCCTACACCTACA          |
| ArSLK-dsR  | 28          | 1720–1739| R         | GGAATGGTCCCGAGGTGGTCATATTCC       |

F and R indicate the forward and reverse directions, respectively. doi:10.1371/journal.pone.0092234.t001

Stress treatments

The post-diapause cysts were rehydrated and hatched as described above. The fresh hatched nauplius larvae, which are incubated at 25°C, were used for the following stress treatments. For heat shock, nauplius larvae were directly exposed in 28 g/L artificial seawater at 42°C in a water bath for 30 min and 60 min. For acidic pH stress, larvae were cultured in 28 g/L artificial seawater at pH 3.0 for 30 min and 60 min. For UV irradiation, nauplius larvae were exposed directly to a UV lamp (310 nm) at 0.15 joules/cm² for 15 min and 30 min according to the previous study [24]. Larvae collected after exposure to heat shock (60 min), acidic pH exposure (60 min), and UV irradiation (30 min) were incubated in 28 g/L artificial seawater at pH 3.0 for 30 min and 60 min. For UV irradiation, nauplius larvae were exposed directly to a UV lamp (310 nm) at 0.15 joules/cm² for 15 min and 30 min according to the previous study [24]. Larvae collected after exposure to heat shock (60 min), acidic pH exposure (60 min), and UV irradiation (30 min) were incubated in 28 g/L artificial seawater for 7 h under laboratory conditions. About 90 percent nauplius larvae resumed their swimming abilities of free-swimming and the live larvae were obtained as the stress released specimens.

RNA interference (RNAi)

For double-strand RNA (dsRNA) preparation, plasmid PET-T7 that contained two inverted T7 polymerase sites flanking the cloning region was used as the dsRNA expression vector [25]. To obtain the reconstructed plasmid expressing the dsRNA of ArSLK, a 580 bp fragment in the coding region of ArSLK gene was amplified with specific primers (ArSLK-dsF and ArSLK-dsR) (Table 1), excised and subcloned into PET-T7 at XbaI and EcoRI sites. The recombinant plasmidtransformed into E. coli DH5α firstly for confirming the inserted nucleotide sequence by DNA sequencing, and then into E. coli HT115 for expressing dsRNA. The plasmid expressing dsRNA targeting GFP had been constructed as previously described and used as the negative control [26]. The dsRNA was produced and purified according to the methods reported by Yodmuang et al. [27].

Artemia adults were injected with 1 µg dsRNA targeting ArSLK or GFP, respectively, using the Ultra-MicroPump II equipped with the Micro4TM MicroSyringe pump controller (World Precision Instruments, Sarasota, FL, USA). After injection, animals were cultured in 55 g/L artificial seawater with 16 h light per day. Eight ovisac specimens were isolated from adults at the early embryo stage according to the method of Liu et al. [26] to extract RNA and protein, followed by RT-PCR and Western blotting for assessing the RNAi efficiency. Three independent groups were repeated for analysis.

Analysis of the stress resistance of larvae after RNAi-treatment

Nauplius larvae, released by ArSLK or GFP RNAi-treated Artemia adults, were collected for stress treatment as described above. Fifty larvae were used for each group. The number of free-swimming nauplius larvae was recorded every 5 min in the heat shock, acidic pH and UV irradiation stress groups.

All data are given as means ± SE from three independent repetitions. All statistical analyses were performed by a two-tailed, paired Student’s t test, and the differences were considered significant for P<0.01.

Results

Identification and characterization of ArSLK cDNA

The full-length cDNA encoding ArSLK was isolated from Artemia parthenogenetica. A sequence analysis revealed that the 4396 bp cDNA encodes a 1340 amino acid protein with a predicted molecular mass of 153.1 kD (Fig. 1).

Alignment of the N-terminal amino acid sequence of ArSLK was identified. The kinase domain is conserved across multiple species (Fig. 2A). The consensus sequence G46-X-G48-X-X-G51, which is essential for ATP binding, and the adjacent V53 residue, which is necessary for the correct positioning and interaction of the conserved glycine residues with ATP, were identified. A conserved lysine residue within the ATP-binding site was identified at
The nucleotide sequence and deduced amino acid sequence of ArSLK.

The start and stop codons are indicated by bold and underlined text. The conserved N-terminal kinase domain (residues 1–349) is boxed. The coiled-coil domain at the C-terminus (residues 879–1279) is indicated by grey highlighting.

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Expression of ArSLK in Artemia during development

Real-time PCR was used to analyze the amounts of the AbSLK mRNA. There were no significant differences between AbSLK mRNA at different stages.
mRNA amounts in any stage of the two developmental pathways (Fig. 3A). But amounts of \textit{ArSLK} mRNA were higher in the late oocyte stage (LO) than in all other stages of both pathways (Fig. 3A). Amounts of \textit{ArSLK} mRNA were highest during earlier development (4–16 h) compared to later periods (20–48 h) (Fig. 3B).

Immunoblotting was used to estimate the amounts of ArSLK protein, which were very low in oocytes of the ovoviviparous pathway with modest increases in later stages (Fig. 4A). The amounts of ArSLK were higher in the oviparous pathway with a dramatic increase in diapause cysts (Fig. 4A). Amounts of ArSLK protein remained about the same during the initial 4 h of development of post-diapause cysts, but then increased substantially, peaking at about 12 h then falling to much lower levels at the later developmental times (Fig. 4B).

**Figure. 2. Sequence analysis of ArSLK.** (A) Alignment of the amino acid sequence of the N-terminal kinase domain of ArSLK with those of SLKs from other species. The Ste20 signature sequence is highlighted and conserved amino acid residues are indicated by asterisks. The black shaded areas represent identical amino acid residues. (B) Analysis of the coiled-coil region of the ArSLK. The probability of formation of a coiled-coil structure was calculated for each residue with a window of 28 amino acids using the ‘COILS’ program. (C) Phylogenetic analysis of the amino acid sequences of SLKs from multiple species. GenBank accession numbers of the sequences used in the amino acid alignment and phylogenetic analysis are as follows: Artemia parthenogenetica, KC818632; Drosophila melanogaster, NP_726441; Aedes aegypti, XP_001649074; Daphnia pulex, EF576394; Danio rerio, NP_001139073; Homo sapiens, NP_055355; Xenopus laevis, NP_001079164; Cluex quinquefasciatus, XP_0016846707; Nasonia vitripennis, XP_001603525; Xenopus tropicalis, NP_001072623; Mus musculus, NP_033315; Schistosoma mansoni, AAN72832; Clonorchis sinensis, GAA300009. doi:10.1371/journal.pone.0092234.g002
Subcellular location of ArSLK during Artemia development process

The subcellular distribution of proteins is often related to their specific functions. To examine the subcellular location of ArSLK, cell fractionation was performed as described in the methods section. Successful fractionation was confirmed using anti-α-tubulin and anti-histone H3 antibodies as cytoplasmic and nuclear markers, respectively (Fig. 5A and 5B). The results revealed that ArSLK was detected mainly in the nuclear fractions from diapause and post-diapause embryos, and in the cytoplasmic fraction from nauplius larvae (Fig. 5A), while maintained in the cytoplasm during the whole ovoviviparous developmental process (from oocytes to embryos) (Fig. 5B).

The nuclear localization of ArSLK is required for the regulation of the stress response

Since ArSLK was differentially localized during Artemia development, experiments were performed to determine whether nuclear ArSLK is important to stress resistance. A cell fractionation assay was used to examine the location of ArSLK after exposure of nauplius larvae to the following stress treatments: heat shock (42°C) for 30 min and 60 min, acidic pH (pH 3.0) for 30 min and 60 min, UV irradiation (0.15 joules/cm²) for 15 min and 30 min. The results show that the ArSLK protein translocated from cytoplasm to nucleus after exposure to stresses. Moreover, as the exposure time extended, almost all the ArSLK translocated into the nucleus (Fig. 6A, 6B and 6C).

Following the treatments, a stress-release process was performed (R in all parts of Fig. 6) in which nauplius larvae were transferred to normal environmental culture conditions. Cell fractionation analysis revealed that the levels of ArSLK protein in the nuclear and cytoplasmic fractions of all stress-released larvae were similar to those of the unstressed control groups (C in all parts of Fig. 6).

The subcellular location of p26 in the both processes mentioned above was also observed. Cell fractionation analysis revealed that p26 was distributed mainly in the cytoplasm of control nauplius (C in all parts of Fig. 6), and then p26 in the nucleus increased during
the stress process (Fig. 6A, 6B and 6C). It returned to the cytoplasm after stress released (R in all parts of Fig. 6).

Decrease in ArSLK reduces the stress tolerance of nauplius larvae

To address further the function of ArSLK during stress resistance, gene knockdown by RNAi with Artemia adults was performed. The efficiency of knockdown was evaluated by the

Discussion

It has been very well documented that Artemia have an outstanding ability to withstand extremely adverse condition [1,2]. However, the intracellular signal transduction mechanisms that respond to environmental stresses are largely unknown. In the present study a homolog of mammalian SLK, called ArSLK, was identified and its role in stress resistance of Artemia was investigated.

Homologs of SLK have previously been identified in various species. SLK family members contain characteristic conserved N-terminal kinase domains and variable C-terminal non-catalytic domains [14], both of which were identified in ArSLK (Figs. 1 and 2). The coiled-coil structure in the C-terminal region can mediate protein-protein interactions and contribute to enhancing the kinase activity of human SLK via homodimerization [28,31]. Therefore, we speculate that ArSLK might also possess a kinase activity that is regulated in the same manner as that of human SLK.

Artemia diapause cysts with programmed metabolic and developmental arrest were formed and released under extremely stressful conditions. It is precisely because of this arrest state helping to ensure survival of this species in stress resistance [32]. In this study, we investigated the subcellular distribution of ArSLK in diapause embryos and the nauplius-destined developmental process. We found the nuclear ArSLK accumulated in diapause embryos (Fig. 5A), in which cell division was restricted and development was arrested at the gastrulae stage. In the nauplius and nauplius-destined embryos with cell division and continued embryonic development, ArSLK was mainly distributed in the cytoplasmic fraction (Fig. 5A and B). Thus, we proposed that the accumulation of ArSLK in the nucleus plays a role in developmental arrest to resistant environmental stress in diapause cysts.

Trafficking of an activated signal component or its substrates from the cytoplasmic to nuclear compartment is required for the transduction of extracellular signals. Studies in rabbit brain showed that the stress-induced expression and nuclear translocation of hsp70 are characteristic features of the response to heat shock [33]. A previous study in Artemia also found that p26, a small heat shock protein, reversibly moves to the nucleus in response to stresses such as heat shock, acidic pH and anoxia during early development of activated (post-diapause) cysts [34]. To prove the role of ArSLK in stress resistance, the stress treatment assay of nauplius was performed. After stress-treated, both p26 and ArSLK in the nucleus were increased, while they translocated to the

Figure 6. Immunoblot analysis of the subcellular locations of ArSLK and p26 after exposure to 42°C heat shock (A), acidic pH stress (B), and UV irradiation (C). Tubulin and H3 were used as markers of the cytoplasmic and nuclear fractions, respectively. C, control group with no stress treatment; R, nauplius larvae after stress that underwent subsequent stress-free incubation for 7 h. doi:10.1371/journal.pone.0092234.g006
cytoplasm after stress releasing. Moreover, the result of gene knockdown revealed that the nauplius larvae from ArSLK RNAi group lost the ability of free-swimming much earlier than those from the GFP RNAi group when suffered the same stress. Based on these results, we suggested that nuclear translocation of ArSLK may also be a response mechanism to involve in the stress resistance via cell cycle regulation to development. However, the molecular mechanisms involved in this resistance are not well understood.

Previous studies have been proved that there are a number of links between the stress-response and cell cycle checkpoint pathway [35]. SLK was reported to play a role in cell proliferation. This regulation is achieved by phosphorylating Plk1 [20]. Although the in vitro activity of SLK phosphorylated on Plk1 has been proved in the previous studies [19,20], there is still controversy regarding the relationship between these two kinases in vivo. The variations in the cytoplasmic ArSLK levels during development described in our present study (Fig. 5) parallel the levels of Plk1 activation described previously [36]. For example, cytoplasmic ArSLK and activated Plk1 levels were both high in nauplius larvae and lower in diapause cysts [36], suggesting that cytoplasmic levels of ArSLK may be correlated with the level of Plk1 activation. However, the results of RNAi-mediated knockdown of Plk1 and ArSLK were confusing. Pseudo-diapause cysts were released following knockdown of Plk1 [36], while nauplius larvae were produced after knockdown of ArSLK. Therefore, ArSLK may not be the only kinase responsible for phosphorylating Plk1. Similar result has been obtained in the Drosophila S2 cells, in which the depleted

Figure 7. The effects of knockdown of the ArSLK gene on nauplius stress tolerance. (A) Real-time PCR analysis of relative amounts of ArSLK mRNA in Artemia adults treated with GFP-specific or ArSLK-specific RNAi. The mRNA amounts were normalized to those of tubulin mRNA. (B) Immunoblot analysis of ArSLK protein in the two RNAi-treated groups. Tubulin was used as a loading control. (C–E) The rates curves of free-swimming nauplius larvae after 42°C heat shock (C), acidic pH stress (D), and UV irradiation (E). (F) The median free-swimming times of nauplius larvae from RNAi-treated adults under three mentioned stresses were exhibited by histograms. Data are represented as mean ± SE of n = 3 independent repeats. Statistical analyses of the differences between the GFP-RNAi and ArSLK-RNAi groups were performed by two-tailed, paired Student’s t tests. ** P < 0.01.

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dPlkk has not impaired the activation of Plk1 [37]. The limited phosphorylation of Plk1 in the diapause cysts also might be correlated with the massive drop of ATP concentration for the global reduced metabolic activity, which induced the arrest of the enzymatic activity.

The stress kinases p38MAPK and JNK have also been reported to be the downstream of SLK signalling pathway and play a role in the linkage of stress and cell cycle checkpoint [17,18,35]. Thus, we speculate that p38MAPK and JNK mediate the regulation of ArSLK in stress resistance via cell cycle and developmental arrest.

In conclusion, the results of this study showed that ArSLK is translocated to the nucleus in both diapause cysts and also in nauplius larvae following exposure to environmental stresses. In addition we found that this translocation was involved in the regulation of stress tolerance in nauplii. We suggest that the trafficking of ArSLK is also involved in the stress resistance of diapause cysts via regulating developmental arrest, but we accept that more evidence is needed in that regard. These findings shed light on the functions of members of the SLK family and may provide a new regulatory strategy for stress responses.

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Author Contributions

Conceived and designed the experiments: RZ WJY FY. Performed the experiments: RZ YXS. Analyzed the data: RZ WJY FY. Contributed reagents/materials/analysis tools: RZ YXS FY. Wrote the paper: RZ WJY FY.

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