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Selenoprotein dSelK in *Drosophila* Elevates Release of Ca^{2+} from Endoplasmic Reticulum by Upregulating Expression of Inositol 1,4,5-Tris-phosphate Receptor

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Abstract—dSelK (G-rich), a homolog of human and mouse SelK, is one of three selenoproteins in *Drosophila melanogaster*. It is the only trans-membrane selenoprotein in *D. melanogaster* integrated into both the endoplasmic reticulum (ER) membrane and the Golgi apparatus. The gene expression profile of *Drosophila* Schneider 2 (S2) cells after the dsRNA interference (dsRNAi) targeting of dSelK was examined with the GeneChip *Drosophila* Genome 2.0 Array (Affymetrix), a high-density oligonucleotide microarray encompassing nearly the full *Drosophila* genome. The results showed that the transcriptional expression of eight genes whose proteins are located on (or related to) the ER or the Golgi apparatus was highly induced or repressed by the dsRNAi treatment. The mRNA levels of the inositol 1,4,5-tris-phosphate receptor (IP3 receptor), whose gene product is integrated into the ER membrane and regulates the release of Ca^{2+} from the ER to the cytosol, were significantly downregulated. In contrast, the expression of inositol 1,4,5-tris-phosphate kinase 1, which is a cytosolic protein with opposing functions to the IP3 receptor, was significantly upregulated. Quantitative real-time PCR verified these results. The concentration of intracellular free Ca^{2+} of the *Drosophila* S2 cells was significantly decreased after the knockdown of dSelK, whereas overexpression of dSelK significantly increased the intracellular free Ca^{2+} concentration. These results indicate that dSelK in *D. melanogaster* is involved in regulating the release of Ca^{2+} from the ER to the cytosol and may play important roles in the signal transduction pathways involving Ca^{2+} mobilization.

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Key words: *Drosophila melanogaster*, selenoprotein, dSelK, dsRNAi, inositol 1,4,5-tris-phosphate receptor, Ca^{2+}

Selenium has important functions in human and other vertebrates in the prevention of cancers, cardiovascular diseases, infections, and skin diseases and the regulation of thyroid function, the immune response, and reproduction [1-10]. Selenoproteins acquire selenium in the form of selenocysteine. Selenocysteine is incorporated into selenoproteins in response to the terminating codon, UGA, through a special decoding mechanism during mRNA translation [11, 12]. Many physiological functions of selenium are closely related to selenoproteins, and selenocysteine is located in the active site of the selenoproteins [13].

There have been 25 selenoproteins identified in humans, and 24 have been identified in rodents [14, 15]. Mammalian selenoproteins can be classified into two groups based on whether they associate with the endoplasmic reticulum (ER). When mouse embryonic stem cells are treated with the oxidizing agent sodium arsenite,

Abbreviations: dsRNAi, dsRNA interference; ER, endoplasmic reticulum; FI, fluorescence intensity; Gpx1, -4, glutathione peroxidases 1 and 4; IP3 kinase 1, inositol 1,4,5-tris-phosphate kinase 1; IP3 receptor, inositol 1,4,5-tris-phosphate receptor; ROS, reactive oxygen species; RyR, ryanodine receptors; S2, Schneider 2 (cells); Sep15, 15-kDa selenoprotein; SelK, SelM, SelN, SelS, and SelT, selenoproteins K, M, N, S, and T; Tr1, thioredoxin reductase 1.

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the mRNA levels of the selenoproteins that are non-ER-associated and have antioxidant activity (Gpx1, Gpx4, and Tr1) are significantly increased, but the ER-associated selenoproteins (Sep15, SelK, SelM, and SelS) are significantly decreased [16]. This indicates that the selenoproteins associated with the ER have significantly different physiological functions from the antioxidant selenoproteins located mainly in the cytosol. The ER-associated selenoproteins, such as selenoprotein T (SelT) often participate in the regulation of calcium mobilization [17]. In addition, selenoprotein N (SelN) can regulate ryanodine receptor (RyR)-mediated calcium mobilization required during normal muscle development and differentiation [18].

Three selenoproteins, BthD (also called dSelH), dSelK (also called G-rich, SelG), and selenophosphate synthetase 2 (SPS2), have been identified in *Drosophila melanogaster* [19, 20]. SPS2 is located in the cytosol and is a selenoprotein related to the synthesis of selenoproteins [21]. BthD is located in the cytosol and functions in antioxidant regulation [22]. dSelK, a *Drosophila* homolog of the human selenoprotein SelK, whose molecular functions have yet to be determined, is a selenoprotein associated with the ER and the Golgi apparatus [23, 24]. Liu and Peng proposed that SelK may have anti-oxidant functions in humans [24], but these functions have not yet been discovered for dSelK in *Drosophila* [25].

In an attempt to elucidate the role of the *Drosophila* dSelK protein, the expression profile after dsRNA interference (dsRNAi) targeting of dSelK was obtained by microarray analysis. Differentially expressed genes, including ones encoding ER-associated proteins, were identified. We discovered that the concentration of intracellular free Ca^{2+} was dependent on the expression level of dSelK, suggesting that dSelK regulates Ca^{2+} mobilization.

MATERIALS AND METHODS

Cell line and reagents. The *D. melanogaster* Schneider 2 (S2) cell line was a gift from Prof. Xun Huang of the Institute of Genetics and Developmental Biology at the Chinese Academy of Sciences. Schneider's *Drosophila* Medium and Fluo-3 AM were purchased from Invitrogen (USA). The MEGAscript T7 Kit was purchased from Ambion (Japan). The GeneChip *Drosophila* Genome 2.0 Array was purchased from Affymetrix (USA). SYBR PrimeScript RT-PCR Kit II, RNAiso Reagent, Taq DNA polymerase, DNA marker, RNA marker, and restriction enzymes were purchased from TaKaRa Biotechnology Inc. (China). RNase was purchased from BBI (Germany). The plasmid pGM-T and T4 DNA ligase were purchased from Qiagen (USA). M-MLV reverse transcriptase was purchased from Super-Bio (USA). The primers used in this study were synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (China). Transfection reagent DDAB was purchased from Pharmacia (Sweden).

Synthesis of double-stranded RNA. To construct the plasmid pGM-T-dSelK used for the production of the template for the *in vitro* transcription of the selenoprotein dSelK, total RNA was extracted from adult flies using the RNAiso Reagent and was reverse transcribed using the M-MLV reverse transcriptase. The cDNA of dSelK (GenBank accession number AAK72981) was amplified by PCR using 5′-TGTAATACGACTCACTATAGGGAGAGTGTACATCGACCATAATGG-3′ and 5′-TGTAATACGACTCACTATAGGGAGAGGGTACTGTGACTTCGAATT-3′ as the forward and reverse primers, respectively. The amplified fragment was subcloned into the pGM-T plasmid to construct the plasmid pGM-T-dSelK.

For dSelK *in vitro* transcription, a dSelK region was amplified from pGM-T-dSelK by PCR with the forward and reverse primers, 5′-TGTAATACGACTCACTATAGGGAGAGTGTACATCGACCATAATGG-3′ and 5′-TGTAATACGACTCACTATAGGGAGAGGGTACTGTGACTTCGAATT-3′. The amplified DNA contains the T7 promoter sequence tag on both ends (underlined characters). To produce the dsRNA of selenoprotein dSelK, the amplified DNA fragments were employed as templates for the T7 MEGAscript kit (Ambion) to transcribe the sense and anti-sense RNA of dSelK in both directions according to the manufacturer's protocols. Both the sense and the anti-sense stranded RNA were annealed by resuspending the RNA products in 2× annealing buffer (40 mM Hepes-KOH, pH 7.6, 10 mM EDTA, pH 8.0) and incubating the reaction mixture for 5 min at 95°C, followed by slow cooling of the reaction to room temperature. The annealed products, 701 bp in length, were extracted with acidic phenol–chloroform–isoamyl alcohol followed by ethanol precipitation. The RNA pellets were resuspended in sterile water.

Double-stranded RNA interference. The *Drosophila* S2 cells were cultured in Schneider's *Drosophila* Medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, and 100 U/ml streptomycin. The cells were maintained at 25°C. Before dsRNA interference, the cells were diluted to a concentration of $1 \cdot 10^6$ cells/ml in serum-free medium according to Hannon's method [26]. One milliliter of the cell suspension was transferred to a 6-well plate and cultured at 25°C for 1 h to allow for cell attachment. To initiate RNAi, 70 μg of dsRNA of dSelK was directly added to the medium under constant agitation. After incubating the reaction for 2 h, 1 ml of Schneider's *Drosophila* Medium containing 10% fetal bovine serum was added to the cells, and the cells were continuously cultured for 48 h prior to the isolation of the RNA. Control cells were incubated with the same volume of sterile water.

Analysis of mRNA expression using oligonucleotide array. Gene expression analysis was performed using the

GeneChip *Drosophila* Genome 2.0 Array according to the manufacturer's recommended protocol. Briefly, total RNA was extracted from the RNAi-treated *Drosophila* S2 cells and control cells using the Trizol reagent (Invitrogen) and purified with the RNeasy Mini Kit (Qiagen). Poly(A)+mRNA was isolated from ~40 μg of total RNA using the Oligotex mRNA Kit (Qiagen). The first and second strands of cDNA were synthesized using the One-Cycle Synthesis Kit (Affymetrix) and purified by the GeneChip Sample Cleanup Module (Affymetrix). Biotin-labeled cRNA was transcribed using the GeneChip IVT Labeling Kit (Affymetrix) in the presence of biotin-labeled CTP and UTP. The biotin-labeled cRNA was fragmented and hybridized to the GeneChip *Drosophila* Genome 2.0 Array according to the manufacturer's protocol. The hybridized probe array was stained with streptavidin–phycoerythrin conjugate on a Fluidics Station (Affymetrix) and scanned by the GeneChip Scanner 3000 controlled by the Affymetrix GeneChip Operating Software (GCOS). Data analyses were performed using the GeneChip Expression Analysis Software (v.3.2, Affymetrix).

Quantitative real-time PCR verification of the results from the microarray. The dsRNAi treatment was similar to the above, and the total RNA of the dSelK dsRNAi-treated and control *Drosophila* S2 cells was extracted using the RNAiso Reagent according to manufacturer's protocol. Single-stranded cDNA was synthesized from the total RNA using the M-MLV reverse transcriptase according to the manufacturer's protocols. Quantitative RT-PCR was performed using the ABI 7500 Real-Time PCR system (Applied Biosystems, USA) and the SYBR PrimeScript RT-PCR Kit II according to manufacturer's protocols. The forward and reverse primers of detected gene IP3 receptor were 5′-GCTACCGTATCCTGAGGTTGTCG-3′ and 5′-TCTGCGGCTGTTATGTGCTTCT-3′, and the length of the product was 175 bp. The forward and reverse primers of the detected gene IP3 kinase 1 were 5′-CGAAGTACATGCCTACCGAC-3′ and 5′-CATTGCTGACCTCCGATT-3′, and the length of the product was 194 bp. RP49 mRNA, a constitutive ribosomal gene, was used for normalization. The forward and reverse primers for RP49 were 5′-GCCGCTTCAAGGGACAGT-3′ and 5′-TCTCGCCGCAGTAAACG-3′, and the length of the product was 156 bp. The PCR amplification protocol was 42°C for 5 min and 95°C for 10 sec, followed by 40 cycles of 95°C for 5 sec, 60°C for 31 sec, and 72°C for 30 sec. Amplification data were quantified by the standard curve method [27]. Values of the examined genes were normalized to that obtained with the primers of RP49. The dissociation curve consisting of a single peak confirmed the specificity of the detected signals. All of the samples were run in triplicate, and the results were averaged.

Detection of intracellular free Ca^{2+} concentration after dsRNAi of dSelK. The dsRNAi treatment was performed similarly as above except that the cells were transferred to glass-bottomed 35 mm cell culture dishes before treatment. Thirty hours after dsRNAi treatment, the free Ca^{2+} was stained by the fluorescent probe Fluo-3 AM (Invitrogen), which is a third-generation long wavelength fluorescent probe sensitive to the variations of intracellular free Ca^{2+} [28], the strength of fluorescence being directly proportional to the concentration of intracellular free Ca^{2+}. Fluo-3 AM was dissolved in dimethylsulfoxide at a concentration of 5 mM and stored at −20°C shielded from light. The cells were washed with phosphate-buffered saline two times before Fluo-3 AM was added to a final concentration of 5 μM and incubated at 25°C for at least 30 min. Cells in the glass-bottomed dishes were placed under a confocal microscope (MTC-600; Bio-Rad, USA) for fluorescence measurements with excitation by a 488 nm argon laser. The fluorescence strength was analyzed with Ca^{2+} fluorescence strength analyzing software. The fluorescence intensity (FI) of the cells from three dsRNAi dishes (15 cells from three fields for every dish) along with that of the cells from three control dishes (15 cells from three fields for every dish) was measured, and the average FI of every dish was taken to represent the levels of $[Ca^{2+}]_i$. The statistical analysis was based on the average FI of every dish.

Construction of dSelK overexpression plasmid. To express the recombinant protein of dSelK fused to the red fluorescent protein in S2 cells, the DsRed tag from the pAc-PDI-DsRed plasmid was inserted into the *Eco*RI and *Not*I sites of the plasmid, pAc-dSelK-GFP [23], and the constructed plasmid was designated pAc-dSelK-DsRed.

Detection of change of intracellular free Ca^{2+} concentration after overexpression of dSelK. Two milliliters of S2 cells in serum-free Schneider's *Drosophila* Medium was transferred to glass-bottomed 35 mm cell culture dishes at a concentration of $(2\text{-}4)\cdot10^6$ cells/dish. Two micrograms of DNA from the plasmid pAc-dSelK-DsRed, 200 μl medium and 100 μl (125 μg/ml) DDAB were vortexed for 30 sec and incubated for 30 min at room temperature. The mixture was added to the cells, and the cells were cultured at 25°C for 24 h. The cells were then changed into Schneider's *Drosophila* Medium containing 10% fetal bovine serum.

Thirty hours after the overexpression of the selenoprotein dSelK, the intracellular free Ca^{2+} was stained using the fluorescent probe, Fluo-3 AM, for 30 min as described above. The fluorescence intensity resulting from the intracellular free Ca^{2+} was observed by confocal microscopy and analyzed as described above. The FI of the cells from three overexpression dishes (21 cells from three fields for every dish) along with that of the cells from three control dishes (21 cells from three fields for every dish) was measured, and the average FI of every dish was taken to represent the levels of $[Ca^{2+}]_i$. Statistical analysis was based on the average FI of every dish.

Statistical analysis. The results of the quantitative real-time RT-PCR experiments and free Ca^{2+} concentrations were expressed as mean value ± standard deviations of the means. Statistical significance was determined using the Student's *t*-test method. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Microarray detection of mRNA expression after dsRNA interference of dSelK. Total RNA was extracted from the dsRNAi-treated cells where dSelK was targeted for knockdown and gene expression was analyzed using the GeneChip *Drosophila* Genome 2.0 Array with high-density oligonucleotide microarrays encompassing nearly the full *Drosophila* genome. The non-dsRNAi-treated cells were used as the negative control. When the expression of the selenoprotein dSelK was knocked down (table), the expression of approximately 406 other genes significantly changed (there were 18,953 genes on the chips). Of these, 241 genes were upregulated, and 165 genes were downregulated.

For the subsequent analysis, we focused on the genes that encode for proteins found on the ER or the Golgi apparatus because dSelK is associated with these organelles [23, 24]. It was found that the transcriptional expression of three genes was significantly upregulated and that of five genes was significantly downregulated (table). The mRNA level of the IP3 receptor (CG1063) was significantly downregulated after the knockdown of dSelK compared to the control cells. IP3 receptor is a protein integrated into the ER membrane and regulates the release of Ca^{2+} from the ER [29-32]. Conversely, the mRNA level of the IP3 kinase 1 (CG4026), which inhibits the release of Ca^{2+} from the ER (although it is not associated with the ER or the Golgi apparatus) [33], was significantly upregulated. Because both the IP3 receptor and IP3 kinase 1 play important roles in regulating Ca^{2+} release from the ER, the release of Ca^{2+} from the ER is possibly controlled by the expression of selenoprotein dSelK.

Sarco/endoplasmic-reticulum-Ca^{2+}-ATPases pump calcium against the Ca^{2+}-gradient into the ER. Ca^{2+}-release from the ER occurs by opening of the receptor channels, IP3 receptor and RyR. And the intracellular Ca^{2+} levels can be restored by plasma membrane calcium ATPase, the Na^{+}/Ca^{2+}-exchanger, and voltage-gated calcium complex [34]. Besides that of the IP3 receptor, the changes in the RNA levels of the other genes related to Ca^{2+} regulation were also analyzed according to the microarray data. It was found that the RNA levels of all of the above genes were not significantly increased or decreased after the knockdown of dSelK by dsRNAi. It could be concluded that the changes in intracellular free Ca^{2+} levels after the knockdown of dSelK are not resulting from other pathways.

Quantitative real-time PCR verification of mRNA levels after dsRNAi treatment. To confirm the GeneChip expression data of the IP3 receptor and IP3 kinase 1 after the knockdown of dSelK, quantitative real-time PCR of the IP3 receptor and the IP3 kinase 1 was performed. Both PCR signals were normalizing against the control RP49, a constitutive ribosomal gene. As shown in Fig. 1, when the expression of the selenoprotein dSelK was knocked down by dsRNAi, the relative RNA levels of the IP3 receptor were decreased from 0.16 ± 0.02 to 0.10 ± 0.01, normalized with RP49 ($P > 0.05$ by Student's *t*-test); the relative RNA levels of IP3 kinase 1 were increased from 1.17 ± 0.13 to 2.10 ± 0.15, normalized with RP49 ($P > 0.05$ by Student's *t*-test) (table). The expression levels of the genes obtained by quantitative real-time PCR were similar to those obtained by the microarray experiment, although both of the differences were determined to be not significant.

Knockdown of dSelK inhibits release of Ca^{2+} from ER. As the mRNA levels of the IP3 receptor were decreased and that of the IP3 kinase 1 were increased, we hypothesized that the level of intracellular free calcium might be decreased by dSelK knockdown. To determine this, intracellular free Ca^{2+} levels in the dsRNAi-treated *Drosophila* S2 cells were measured by the fluorescent probe Fluo-3 AM. The average fluorescence intensity (FI) was taken to represent the levels of $[Ca^{2+}]_i$. Figures 2 (see color insert) and 4a showed that the average FI of intracellular free Ca^{2+} of the dsRNAi-treated *Drosophila* S2 cells was significantly decreased. Free Ca^{2+} mainly existed in the cytosol of the cells (Fig. 2). The average FI of free Ca^{2+} significantly decreased from an average of 1616.58 ±

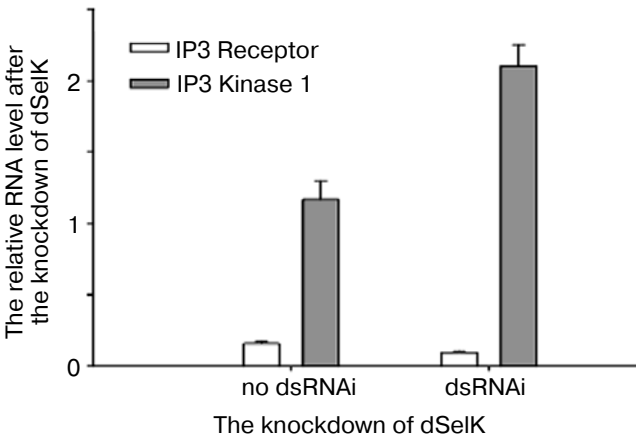

Fig. 1. Quantitative real-time PCR verification of the variation of the mRNA levels of the IP3 receptor and IP3 kinase 1 by RNAi-mediated knockdown of selenoprotein dSelK. The relative RNA levels of the IP3 receptor were decreased from 0.16 ± 0.02 to 0.10 ± 0.01, normalized with RP49, after the RNAi-mediated knockdown of dSelK ($P = 0.518$ by Student's *t*-test). The relative RNA levels of IP3 kinase 1 were increased from 1.17 ± 0.13 to 2.10 ± 0.15, normalized with RP49 ($P = 0.343$ by Student's *t*-test).

dSelK RNAi regulated genes located on (or related to) the ER or the Golgi apparatus*

Probe set	Gene symbol	Gene title	Transcript ID	Fold of change	Cellular component	Molecular functions	Biological process
1633639_at	Cyp28d1	Cyp28d1	CG10833-RA	2.6	ER-resident	Electron carrier activity; heme binding; iron ion binding; monooxygenase activity	Unknown
1634619_at	IP3K1	Inositol 1,4,5-triphosphate kinase 1 (IP3K1)	CG4026-RA	2.5	Cytoplasm (functions opposite to IP3 receptor)	Inositol trisphosphate 3-kinase activity; calmodulin binding	Response to oxidative stress
1624101_at	Cyp6a23	Cyp6a23	CG10242-RA	2.4	ER-resident	Electron carrier activity; heme binding; iron ion binding; monooxygenase activity	Unknown
1625491_at	bwa	Brain washing	CG13969-RA	2.1	Golgi-resident	Ceramidase activity	Brain development; sphingolipid metabolic process; regulation of lipid metabolic process; ceramide catabolic process
1631674_at	O-fut1	Neurotic	CG12366-RA	–2.0	ER-resident	Notch binding; fucosyl transferase activity	Negative regulation of Notch signaling pathway; endocytosis; protein catabolic process; protein amino acid O-linked glycosylation; nervous system development; regulation of Notch signaling pathway; cell–cell adhesion
1627111_at	NHP2	NHP2	CG5258-RA	–2.1	–"–	rRNA pseudo-uridylation guide activity; snoRNA binding	rRNA pseudouridine synthesis; rRNA processing
1638890_at	Tapdelta	Translocon-associated protein delta	CG9035-RA	–2.1	–"–	Signal sequence binding	Protein retention in ER lumen
1639461_a_at	Itp-r83A	IP3 receptor	CG1063-RA	–2.2	–"–	Inositol-1,4,5-triphosphate receptor activity; calcium channel activity; inositol 1,4,5-triphosphate-sensitive calcium-release channel activity	Flight behavior; molting cycle, chitin-based cuticle; response to oxidative stress; mushroom body development; nuclear division; stabilization of membrane potential; release of sequestered calcium ion into cytosol; calcium ion transport
1640122_at	SelG (dSelK)	Selenoprotein G	CG1844-RA	–2.3	ER- and Golgi-resident	Unknown	Embryonic development; determination of adult life span; cell redox homeostasis
1630955_at	CG9870	CG9870	CG9870-RA	–3.3	ER-resident	Transferase activity, transferring acyl groups	GPI anchor biosynthetic process

* Molecular functions and biological process of genes were according to www.flybase.com.

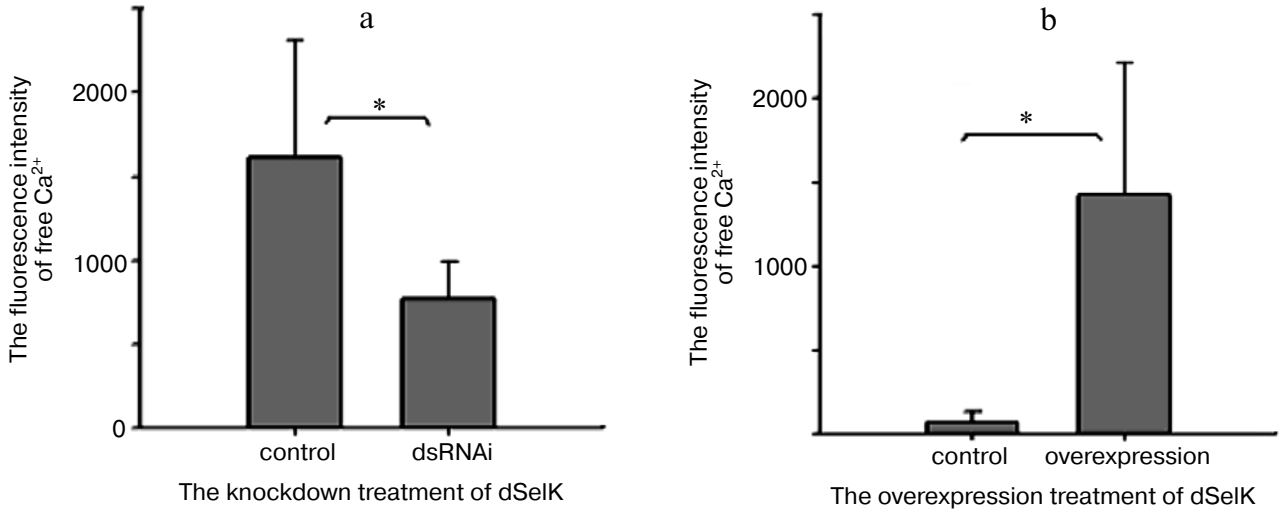

Fig. 4. Free Ca^{2+} concentration after RNAi-mediated knockdown and overexpression of dSelK. Average fluorescence intensity was taken to represent the levels of $[Ca^{2+}]_i$. a) Fluorescence intensity of free Ca^{2+} significantly decreased from an average of 1616.58 ± 694.06 to an average 774.70 ± 220.55 after the knockdown of dSelK (* $P = 0.02$ by Student's *t*-test). b) Fluorescence intensity of free Ca^{2+} significantly increased from an average of 69.52 ± 63.85 to an average of 1422.10 ± 793.21 after the overexpression of dSelK (* $P = 0.001$ by Student's *t*-test).

694.06 to an average of 774.70 ± 220.55 after the knockdown of dSelK ($P < 0.05$ by Student's *t*-test), suggesting that the knockdown of dSelK decreases the release of Ca^{2+} from the ER (Fig. 4a).

Overexpression of dSelK increases release of Ca^{2+} from ER. To examine the effect of overexpression of dSelK on Ca^{2+} release, *Drosophila* S2 cells were transfected with the plasmid pAc-dSelK-DsRed, which expresses dSelK fused to the red fluorescent protein (RFP). Free Ca^{2+} concentrations were determined using the green Ca^{2+} fluorescent probe Fluo-3 AM. The average FI was taken to represent the levels of $[Ca^{2+}]_i$. As shown in Figs. 3 (see color insert) and 4b, the average FI of free Ca^{2+} significantly increased from an average of 69.52 ± 63.85 to an average of 1422.10 ± 793.21 after the overexpression of dSelK ($P < 0.05$ by Student's *t*-test), suggesting that the release of Ca^{2+} from the ER was increased significantly by the overexpression of dSelK.

DISCUSSION

The overexpression of human selenoprotein SelK has been previously shown to decrease the levels of ROS in cardiomyocytes [24]. Whether this is due to SelK or other selenoproteins induced by sodium selenite is not clear because sodium selenite was added to the cell culture medium. *Drosophila* dSelK, the homolog of SelK, has not been found to contribute to antioxidant activity in *Drosophila* cells [25]. SelK is located on the ER, similarly to other selenoproteins such as SelT and SelN. SelT is a PACAP (pituitary adenylate cyclase-activating peptide)-regulated gene involved in intracellular Ca^{2+} mobilization and in neuroendocrine secretion [17]. The selenoprotein SelN can regulate the RyR-mediated calcium mobilization required for normal muscle development and differentiation [18]. It seems possible that the *Drosophila* selenoprotein dSelK can also regulate calcium mobilization. By performing microarray analyses, we have demonstrated that when the selenoprotein dSelK was knocked down, the transcriptional levels of the IP3 receptor decreased while IP3 kinase 1 increased. When dSelK was knocked down, the average FI of intracellular free Ca^{2+} was decreased to 50% of that of the control. However, when selenoprotein dSelK was overexpressed in S2 cells, the average FI of intracellular free Ca^{2+} increased to 20.45 times that of the control. The RNA levels of other genes related to Ca^{2+} regulation were not significantly increased or decreased after the knockdown of dSelK by dsRNAi. Because the IP3 receptor elevates Ca^{2+} release from the ER and IP3 kinase 1 has an opposing activity [29-33], our results suggest that the *Drosophila* selenoprotein dSelK regulates the concentration of intracellular free Ca^{2+}, possibly through the regulation of the expression of the IP3 receptor and IP3 kinase 1.

The expression of SelK has been suggested to be relatively high in the human heart [24]. Proper intracellular calcium signaling is essential for normal cardiac looping, gene expression, and organ development [35]. The IP3 receptors are essential for the proper generation of the pacemaker activity during early cardiogenesis and in fetal life [36]. In the past three decades, a number of observational studies have found an association between selenium status and the risk of cardiovascular disease, especial-

ly coronary heart disease [8-10]. However, a large clinical trial of more than 1300 older Americans who took selenium supplements every day for seven and a half years showed that there was no beneficial effect from selenium supplementation on the primary prevention of cardiovascular disease [37]. Therefore, selenium possibly influences early cardiogenesis in embryo development by controlling the production of the selenoprotein dSelK, which could then regulate the expression of the IP3 receptor.

In conclusion, the knockdown of selenoprotein dSelK by dsRNAi in *Drosophila* S2 cells downregulated the expression of the IP3 receptor and upregulated the expression of IP3 kinase 1. *Drosophila* dSelK may help liberate Ca^{2+} from the ER by regulating the expression of the IP3 receptor and IP3 kinase 1.

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