Chapter 26
An Emerging Picture of the Biological Roles of Selenoprotein K

Peter R. Hoffmann

Abstract Recent insight has been made regarding the biological role(s) for selenoprotein K (SelK) in humans and other organisms. Suggested functions for mammalian SelK include protection against oxidative stress in cardiomyocytes, regulation of endoplasmic reticulum (ER) stress in HepG2 cells, and facilitation of calcium flux in immune cells during receptor-mediated activation. The data supporting these functions as well as other aspects of SelK are summarized in this chapter.

26.1 Introduction

Selenoprotein K (SelK) was one of several novel human selenoproteins identified by the Gladyshev laboratory in the landmark 2003 study in which members of the human selenoproteome were revealed [1]. Since then, progress has been slow in elucidating the biological role or roles of SelK in humans or other species. However, some recent in vitro and in vivo data have provided key insights into SelK function. This chapter will cover various aspects of SelK in terms of expression patterns, structural features, and biological functions.

26.2 *Drosophila* SelK

Prior to the "discovery" of the mammalian SelK gene and protein in 2003, some experimental data had been obtained pertaining to the SelK ortholog in *Drosophila*, dSelK (also called dSelG or G-rich). The first published report involving dSelK

P.R. Hoffmann (✉)
Department of Cell and Molecular Biology, John A. Burns School of Medicine,
University of Hawaii, 651 Ilalo Street, Honolulu, HI 96816, USA
e-mail: peterh@pbrc.hawaii.edu

D.L. Hatfield et al. (eds.), *Selenium: Its Molecular Biology and Role in Human Health*,
DOI 10.1007/978-1-4614-1025-6_26, © Springer Science+Business Media, LLC 2012

identified the gene encoding this 110 amino acid protein [2]. Similar to its mammalian counterpart, dSelK contains one Sec residue near the C-terminus. In addition, this report described a cysteine-paralog encoded by a gene only 320 base pairs (bp) from the gene encoding the Sec-containing dSelK. Using in situ hybridization, the mRNA for dSelK was found to be expressed ubiquitously in embryos throughout development. It was unclear how much in situ signal was attributable to dSelK vs. its cysteine paralog. Still, these data are consistent with those presented in a subsequent study using RNAi to inhibit expression of dSelK in developing embryos, which found that dSelK expression was necessary for normal embryonic development in *Drosophila* [3]. Interestingly, dSelK was not as important as dSelH for maintaining antioxidant status in embryos. Topological studies in *Drosophila* demonstrated that the Sec residue in dSelK was located in the cytoplasm, making it a type III transmembrane protein (N-terminus in lumen with no N-terminal signal peptide) [4]. In addition to this important revelation, this study also identified dSelK as a Golgi-localized protein, although whether it also localized to adjacent endoplasmic reticulum (ER) is not clear from the data. Of course, there are fundamental differences between insect and mammalian biology and SelK may carry out quite different functions in these different species. More comparisons to features of mammalian SelK are described below.

26.3 Tissue Distribution and Subcellular Localization of SelK in Mice

Based on Northern blot analysis, expression of SelK mRNA was suggested to be relatively high in the heart [5]. However, real-time RT-PCR data published by our laboratory demonstrated that SelK mRNA expression levels are more widely distributed throughout tissues, with particularly high levels detected in spleen and testes [6]. Analyses of SelK protein expression in our laboratory subsequently demonstrated the highest expression in immune cells and lymphoid tissues [7], which is consistent with immunohistochemical survey of human tissues [8]. To our knowledge, this is the first selenoprotein exhibiting enriched expression in immune cells or tissues. Interestingly, increasing the dietary Se intake to above-adequate levels in mice (from 0.25 to 1.0 ppm Se) increased western blot detection of SelK in nearly all tissues examined, with the exception of heart [7]. The relative abundance of SelK in different tissues is shown in Fig. 26.1.

Overexpression of GFP-tagged SelK resulted in its localization to the ER [5], while other data have suggested that SelK may also localize to the plasma membrane [1]. Immunofluorescence data from our laboratory involving primary immune cells support the notion that SelK is predominantly localized to the ER [7], and we have not observed any pattern of SelK staining that reflects plasma membrane localization. However, it may be that only small amounts of SelK are transported to the plasma membrane or subcellular localization may differ between tissues or cell types. Also, regions of the ER come very close to the plasma membrane (within 10–25 nm) that

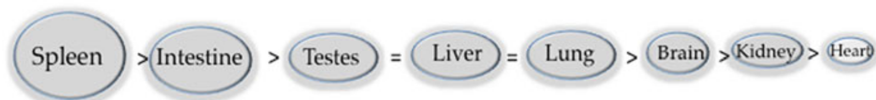

Fig. 26.1 Relative abundance of SelK in mouse tissues. Eight different mouse tissues were examined for assessing the relative amounts of SelK

are commonly referred to as puncta [9], and SelK may be localized to these regions. Puncta provide microenvironments in which key signaling events occur between the ER and the plasma membrane, particularly during Ca^{2+} influx and activation of T cells and other immune cells [10]. Whether SelK resides in puncta or is distributed throughout ER membranes proximal or distal to plasma membranes has yet to be determined. However, it is tempting to speculate that SelK may be integral in the ER membrane and enriched in puncta, providing it the opportunity to interact with plasma membrane-proximal proteins. This certainly is consistent with the functional data described in more detail below linking SelK with ER Ca^{2+} flux during immune cell activation.

SelK is localized to the ER, but how does it get there? The amino acid sequence of SelK contains a predicted transmembrane domain, a feature found only in four other selenoproteins: Dio2, SelI, SelN, and SelS [11]. Cellular fractionation of human Jurkat T cells confirmed the notion that SelK resides in the membrane fraction [7]. But the transmembrane domain is not sufficient for directing SelK into the ER membrane. Curiously, the SelK amino acid sequence contains no motifs corresponding to a signal peptide or localization signals, leaving the means by which SelK is inserted into the ER membrane a mystery. SelK could be bound by chaperones or other ER-localized proteins immediately upon translation, and guided to the ER membrane in this manner. Experimental confirmation of the means by which SelK is localized to the ER membrane is needed.

26.4 Structure of SelK

SelK is a small (94 amino acid) protein predicted to be an integral, single-spanning transmembrane protein (Fig. 26.2). SelK amino acid sequences from human and mouse share 91% identity, with the Sec residue located near the C-terminus for both species. As mentioned above, topological studies in *Drosophila* demonstrated that the Sec residue resides in the cytoplasm, making dSelK a type III transmembrane protein (N-terminus in lumen with no N-terminal signal peptide). Presumably, mammalian SelK is also situated with its Sec residue in the cytosol, although this has not been experimentally confirmed. The predicted molecular mass of SelK is 10.6 kDa, but we have found that it migrates on western blots closer to 15–16 kDa. This is most likely due to the abundance of positively charged residues (estimated $pI = 10.8$), because mutation of three or four positively charged amino acids to the

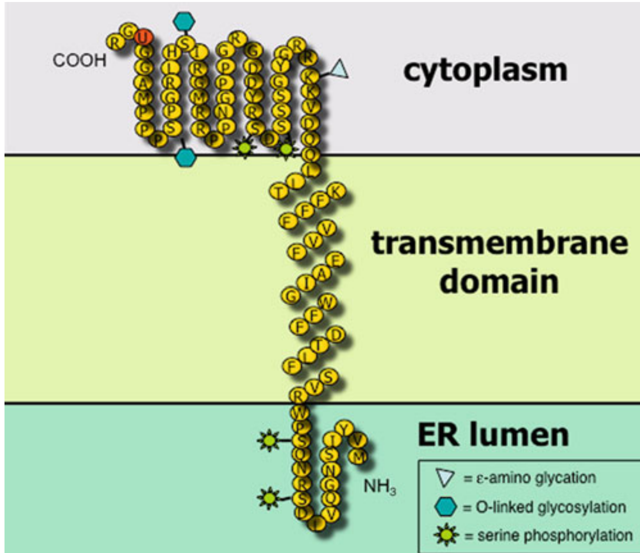

Fig. 26.2 Predicted structural features of SelK. NCBI amino acid sequence (NP_064363) was used to predict secondary structure and domains using SOSUI system software, Mitaku group, Nagoya University. Predicted transmembrane domain was confirmed using TMHMM2.0, glycosylation sites were identified using NetGlycate 1.0 and NetOGlyc 3.1, and phosphorylation sites identified using NetPhos 2.0. The Sec residue is represented by U

neutral amino acid, alanine, causes a shift toward the predicted 10.8 kDa (our unpublished data). Posttranslational modifications that contribute to a higher molecular mass cannot be ruled out, but no modifications have been experimentally confirmed. The cytosolic region of SelK is rich in both proline and glycines. The region of SelK protruding into the lumen has no identifiable features, with a distinct absence of any Ca^{2+}-binding motifs such as EF-hands, epidermal growth factor (EGF)-like repeats, cadherin repeats, and thrombospondin repeats. Structurally, the amino acid sequence of SelK provides very little clues regarding potential roles for either the cytosolic or luminal regions of SelK.

26.5 Phenotype of the SelK Knockout Mice

26.5.1 Development and Growth

As reported by our laboratory, SelK knockout (KO) mice are healthy and fertile with no apparent phenotype [7]. Preliminary tests involving behavior, anxiety, and motor skills suggested no differences between KO and wild-type (WT) mice (our unpublished data). Similarly, cardiac function was measured using ultrasound-based

echocardiography and no physiological differences were found between KO and WT mice. This is in contrast to the early in vitro studies suggesting an important protective, antioxidant function in cardiomyocytes [5]. Given the relatively high expression levels of SelK in immune cells and tissues, we examined the SelK KO mice for signs of immune system development. However, immune system development was not affected in SelK KO mice, as the numbers of total cells and cell subtypes were similar to WT in primary lymphoid tissues (bone marrow and thymus), secondary lymphoid tissues (spleen and lymph nodes), and nonlymphoid tissues (lung and liver) [7]. Overall, SelK does not appear to be required for growth or development of mice. However, studies are in progress for fully characterizing these mice regarding various aspects of health.

26.5.2 Immune System Challenges and Ca^{2+} Flux

Despite SelK-deletion having no effect on immune system development in mice, the impact on the immune system became apparent when the KO mice were challenged with inflammatory agents [7]. For example, treatment of the mice with a viral mimetic, poly(i:c), produced significantly lower levels of inflammatory chemokines, such as MCP-1 and KC, and lower infiltration of neutrophils compared to WT controls. KO macrophages secreted lower levels of inflammatory cytokines, IL-6 and TNFα, in response to poly(i:c)- or LPS-treatment. KO macrophages also exhibited decreased oxidative burst upon phagocytosis through the Fcγ receptors. T cells, neutrophils, and macrophages showed impaired migration in response to chemotactic agents. When infected with West Nile virus (WNV), SelK KO mice produced ineffective immune responses that failed to clear the virus, resulting in higher neuropathology and significantly higher mortality rates. These immune cell defects can be explained by the decreased Ca^{2+} flux induced in the SelK KO immune cells as described below.

26.6 Questions Regarding the Function of SelK

26.6.1 Is SelK an Antioxidant Enzyme?

One of the first studies to describe a potential function for mammalian SelK was that of Lu et al. in which an antioxidant, cardioprotective role was proposed [5]. In this study, overexpression of SelK in neonatal rat cardiomyocytes was shown to reduce endogenous ROS produced in the cells. Furthermore, overexpression of SelK protected these cells from a challenge with hydrogen peroxide. Based on these results, the authors suggested an antioxidant role for SelK in cardiomyocytes. However, all of these experiments involved comparisons of overexpressed SelK to

overexpressed GFP, and it is possible that overexpression of any Sec-containing protein may shift the redox balance toward a proreducing environment. In fact, overexpression of other ER-localized selenoproteins, such as SelM and SelS, also has shown to protect against hydrogen peroxide challenge [12, 13]. Does this indicate that all of these ER selenoproteins serve to mitigate oxidative stress? One must consider the possibility that an overabundance of nearly any selenoprotein may reduce ROS and this does not necessarily reflect on the biological role of that selenoprotein at physiological levels. Specific mechanisms by which SelK may act as an antioxidant have not yet been described and the *Drosophila* homolog of SelK (dSelK) was not found to contribute to overall antioxidant potential [5]. Furthermore, SelK lacks defined redox motifs such as Cys–X–X–Sec or Cys–X–X–Ser (X is any amino acid) found in antioxidant selenoproteins like the GPx enzymes [14]. Thus, the current data do not clearly support the notion that SelK is an antioxidant enzyme in vivo. However, it is possible that SelK forms a complex with other proteins that utilize its Sec residue for its reducing capacity.

26.6.2 Is SelK Involved in the ER-Stress Response?

A recent report demonstrated a potential role for SelK in both being regulated by and regulating ER-stress in the HepG2 cell line [15]. SelK expression was increased in a dose- and time-dependent manner in response to ER-stress reagents. Decreasing SelK levels with siRNA induced the ER-stress marker, GRP78, with or without the addition of ER-stress reagents. Cell viability was slightly lower in cells with diminished SelK expression when exposed to ER-stress. Overall, in HepG2 cells there appears to be a relationship between SelK and ER-stress. However, cells and tissues from SelK KO mice show no signs of ER stress [7]. This discrepancy may be due to a difference of cell type or species. Alternatively, in vivo effects of deleting SelK on ER-stress may be alleviated by redundant or compensating systems (perhaps SelS?), and this redundant system may not exist in the HepG2 cells. At this point in time, it appears that SelK may play a role in regulating ER-stress, but its specific role in this process remains unknown and the lack of apparent ER-stress in the SelK KO must be explained.

26.6.3 Is SelK Involved in Calcium Flux from the ER?

Based on data from cells purified from the SelK knockout mouse, there appears to be a specific role for SelK in promoting receptor-mediated Ca^{2+} flux [7]. In three different types of immune cells (T cells, neutrophils, and macrophages), SelK deletion was shown to significantly reduce receptor-mediated Ca^{2+} flux. To illustrate the step at which SelK may influence or regulate Ca^{2+} from the ER, T cell stimulation through the T cell receptor (TCR) receptor is shown in Fig. 26.3. Upon TCR crosslinking

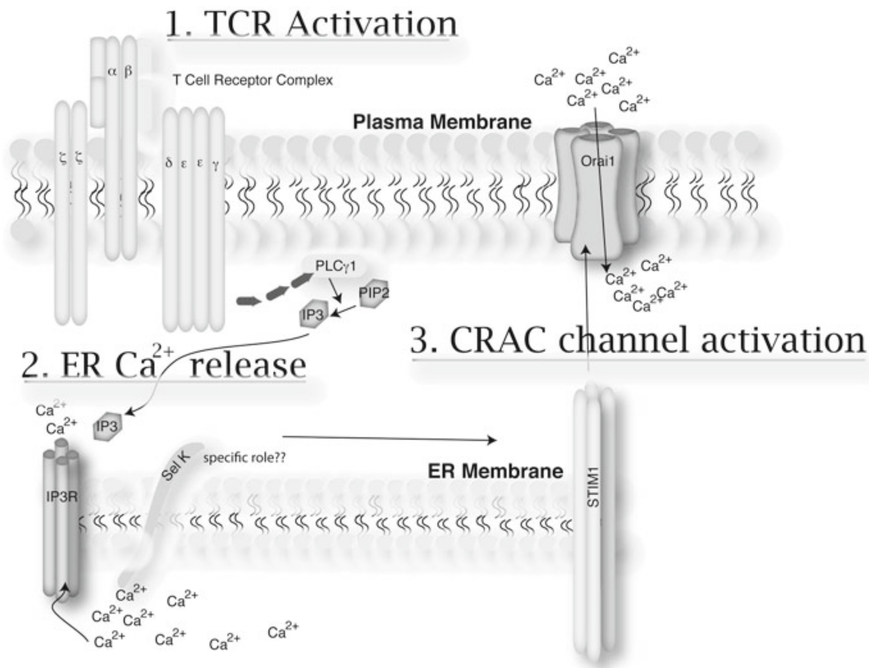

Fig. 26.3 Diagram of signaling events occurring during activation of T cells. Engagement of the T cell receptor (TCR) activates phosphoinositide-specific phospholipase C (PLCγ1), which catalyzes the degradation of phosphatidylinositol-4,5-bisphosphate to generate inositol-1,4,5-triphosphate (IP3) and diacylglycerol. IP3 binds to its receptor, located on the surface of internal Ca^{2+} stores, primarily the ER. Binding of IP3 with the IP3 receptor results in release of Ca^{2+} from ER lumen to the cytosol, which causes oligomerization of STIM1 and subsequent translocation of STIM1 oligomers to the plasma membrane where they interact with the pore-forming unit of Ca^{2+} release-activated Ca^{2+} (CRAC) channels. CRAC channel activation results in an influx of extracellular Ca^{2+}, which leads to activation of multiple signaling pathways indispensable for cellular proliferation and differentiation. SelK modulates the effects of IP3 receptor stimulation

on the surface of T cells, the organization of puncta allows rapid association of ER and plasma membrane proteins, providing interactions during the Ca^{2+}-dependent signaling crucial for their activation. While many of the early steps of T cell activation have been elucidated, other puncta-associated proteins participating in this process have yet to be identified. The predominant pathway of Ca^{2+} entry in T cells involves inositol-1,4,5-triphosphate (IP3)-receptor mediated Ca^{2+} release from the ER Ca^{2+} store, which subsequently induces the opening of plasma membrane-expressed store-operated Ca^{2+} channels, also known as calcium release-activated Ca^{2+} (CRAC) channels. The overall process is known as store operated Ca^{2+} entry (SOCE) [16]. During SOCE, TCR engagement activates phosphoinositide-specific phospholipase C, which catalyzes the degradation of phosphatidylinositol-4,5-bisphosphate to generate IP3 and diacylglycerol (DAG). IP3 binds to the IP3 receptor,

located on the surface of internal Ca^{2+} stores, primarily the ER [17]. Binding of IP3 with the IP3 receptor results in release of Ca^{2+} from ER lumen to the cytosol. The loss of Ca^{2+} from ER causes entry of Ca^{2+} through CRAC channels on the plasma membrane, which leads to activation of multiple signaling pathways indispensable for cellular proliferation and differentiation [18, 19]. SOCE is crucial not only for T cell activation, but also for IgE-dependent mast cell activation [20] and for specific aspects of macrophage activation [21].

Our data suggest that SelK deletion impairs SOCE in immune cells, but is this merely due to ER-stress? If SelK deletion caused ER stress or dysfunctional storage of Ca^{2+} in the ER, one would expect that a Ca^{2+}-mobilizing reagent such as thapsigargin or ionomycin would result in decreased Ca^{2+} flux in KO cells. However, these reagents produced no differences in Ca^{2+} flux in KO compared to WT cells. Similarly, the defects in Ca^{2+} were not mediated by ER stress in SelK KO cells due to the fact that ER stress markers did not differ between KO and WT cells or tissues. In fact, no evidence of ER stress has been detected in the KO mice. Thus, SelK deletion impairs Ca^{2+} from ER stores through a receptor-dependent mechanism, not by disrupting ER function in a general manner. This suggests SelK is an important component of the signaling network operating between cell surface receptors and ER membrane receptors. Effective Ca^{2+} flux is crucial for proper cellular responses to stimulation induced through a number of receptor systems including the TCR, chemokine receptors, Toll-like receptors, and Fcγ receptors. Our data clearly show that Ca^{2+} flux induced by these receptors was impaired in KO cells compared to WT controls. The role of SelK in regulating immune cell activation and in vivo immune responses via Ca^{2+} flux is not completely understood, but a clearer picture is emerging for SelK in this important process and is discussed in more detail below.

26.7 Similarities and Differences Between SelK and SelS

There are some important similarities between SelK and SelS. Both are transmembrane proteins localized to the ER membrane. Expression of both selenoproteins is increased in cells treated with reagents that cause ER stress [13, 15]. This may suggest similar roles for SelK and SelS in mitigating ER stress. The role of SelS in retrotranslocation of misfolded proteins has been experimentally demonstrated [22], whereas any data showing a similar function for SelK have yet to be published. Other than the transmembrane domain and the Sec residue, SelK and SelS share no similar structural features. Also, SelK is a type III transmembrane with the Sec residue in the cytosol, but SelS has been suggested to be a type II transmembrane protein with the Sec residue in the ER lumen [4, 23]. The latter requires experimental confirmation and, if indeed this proves to be true, it would be an important difference that may be related to the functional roles of these proteins. Perhaps the most important difference between SelK and SelS is the effect on inflammation in the absence of either of these two selenoproteins. Diminished SelS expression increases inflammatory cytokines, whereas SelK KO mice show no signs of increased inflammation [7, 24].

Table 26.1 Comparison of Sel K and Sel S

Similarities	Differences
Both localized to ER membrane	Sel K is type III protein with Sec in cytosol, Sel S is suggested to be a type II protein with Sec in lumen
Both exhibit increased expression with ER stress	Sel S is also induced with glucose-deprivation, glucose-dependent expression of Sel K has not been determined
Decreased expression of either increases ER stress	Sel K-knockdown in HepG2 cells produces increased ER stress, but no apparent ER stress in cells or tissues from Sel K KO mice
Lowered expression affects inflammatory cytokines	Sel K KO mice and cells secrete *decreased* inflammatory cytokines but Sel S knockdown cells secrete *increased* inflammatory cytokines compared to WT controls

In fact, SelK KO macrophages secrete lower levels of proinflammatory cytokines upon stimulation with various inflammatory agents. Overall, it remains to be determined whether SelK and SelS are functionally similar and/or if they have biological roles completely independent of each other (Table 26.1).

26.8 Concluding Remarks

Overall, many questions remain pertaining to the biological role(s) of SelK. The low abundance of SelK throughout most tissues together with relatively higher expression in immune cells suggests to this author that SelK may play multiple roles. For example, in most tissues SelK may act to mitigate ER stress that may arise from misfolded proteins, viral infection, Ca^{2+} imbalance, or other conditions. SelK is unlikely the sole protein involved in this function, as the corresponding KO mice and cells appear to function normally with no apparent ER stress. This is in contrast to the siRNA studies in HepG2 cells described above, but this must be repeated in other cell types and in vivo to better define the role of SelK in ER stress. In addition to this ER stress-related function, SelK appears to serve an important role in immune cells to promote activation of these cells during receptor-mediated Ca^{2+} flux. The data from KO mice need to be corroborated in human cells and tissues, but the immune system in mice clearly exhibits a dependence on SelK for optimal function.

References

1. Kryukov GV, Castellano S, Novoselov SV et al (2003) Science 300:1439
2. Castellano S, Morozova N, Morey M et al (2001) EMBO Rep 2:697
3. Morozova N, Forry EP, Shahid E et al (2003) Genes Cells 8:963
4. Chen CL, Shim MS, Chung J et al (2006) Biochem Biophys Res Commun 348:1296
5. Lu C, Qiu F, Zhou H et al (2006) FEBS Lett 580:5189
6. Hoffmann PR, Hoge SC, Li PA et al (2007) Nucleic Acids Res 35:3963

7. Verma C, Hoffmann FW, Kumar M et al (2011) J Immunol 186(4):2127
8. Berglund LE, Björling P, Oksvold L et al (2008) Mol Cell Proteomics 10:2019
9. Wu MM, Buchanan J, Luik RM et al (2006) J Cell Biol 174:803
10. Luik RM, Wu MM, Buchanan J et al (2006) J Cell Biol 174:815
11. Reeves MA, Hoffmann PR (2009) Cell Mol Life Sci 66:2457
12. Reeves MA, Bellinger FP, Berry MJ (2010) Antioxid Redox Signal 12:809
13. Gao Y, Feng HC, Walder K et al (2004) FEBS Lett 563:185
14. Lobanov AV, Hatfield DL, Gladyshev VN (2009) Biochim Biophys Acta 1790:1424
15. Du S, Zhou J, Jia Y et al (2010) Arch Biochem Biophys 502:137
16. Lewis RS (2001) Annu Rev Immunol 19:497
17. Parekh AB, Penner R (1997) Physiol Rev 77:901
18. Tsien RY, Pozzan T, Rink TJ (1982) Nature 295:68
19. Quintana A, Griesemer D, Schwarz EC et al (2005) Pflugers Arch 450:1
20. Baba Y, Nishida K, Fujii Y et al (2008) Nat Immunol 9:81
21. Braun A, Gessner JE, Varga-Szabo D et al (2009) Blood 113:1097
22. Ye Y, Shibata Y, Yun C et al (2004) Nature 429:841
23. Gao Y, Walder K, Sunderland T et al (2003) Diabetes 52:929
24. Curran JE, Jowett JB, Elliott KS et al (2005) Nat Genet 37:1234