Current Biology, Vol. 12, 427–433, March 5, 2002, ©2002 Elsevier Science Ltd. All rights reserved. PII S0960-9822(02)00690-5

C. elegans ksr-1 and *ksr-2* Have Both Unique and Redundant Functions and Are Required for MPK-1 ERK Phosphorylation

Mitsue Ohmachi,[1] Christian E. Rocheleau,[2] Diane Church,[3] Eric Lambie,[3] Tim Schedl,[1] and Meera V. Sundaram[2,4]
[1]Department of Genetics
Washington University School of Medicine
St. Louis, Missouri 63110
[2]Department of Genetics
University of Pennsylvania School of Medicine
Philadelphia, Pennsylvania 19104
[3]Department of Biology
Dartmouth College
Hanover, New Hampshire 03755

Summary

Kinase Suppressor of Ras (KSR) is a conserved protein that positively regulates Ras signaling and may function as a scaffold for Raf, MEK, and ERK [1]. However, the precise role of KSR is not well understood, and some observations have suggested that KSR might act in a parallel pathway. In *C. elegans*, *ksr-1* is only required for a specific Ras-mediated process (sex myoblast migration) and is a nonessential positive regulator of other Ras-mediated developmental events [2, 3]. We report the existence of a second *C. elegans ksr* gene, *ksr-2*, which is required for Ras-mediated signaling during germline meiotic progression and functions redundantly with *ksr-1* during development of the excretory system, hermaphrodite vulva, and male spicules. Thus, while the *ksr-1* and *ksr-2* genes are individually required only for specific Ras-dependent processes, together these two genes appear necessary for most aspects of Ras-mediated signaling in *C. elegans*. The finding that *ksr-2; ksr-1* double mutants have strong *ras*-like phenotypes and severely reduced or absent levels of diphosphorylated MPK-1 ERK strongly supports models where KSR acts to promote the activation or maintenance of the Raf/MEK/ERK kinase cascade.

Results and Discussion

The Ras GTPase controls many aspects of normal animal development by stimulating the Raf/MEK/ERK kinase cascade and several other less well characterized signaling pathways [4]. Kinase Suppressor of Ras (KSR) is a positive regulator of Ras signaling that was identified by genetic studies in *Drosophila* and *C. elegans*, where epistasis analyses suggested that it functions at a step between (or in parallel to) Ras and Raf [2, 3, 5, 6]. KSR orthologs were subsequently identified in vertebrates as well [5]. KSR proteins are somewhat similar to Raf in sequence and overall structure in that they have an N-terminal cysteine-rich domain (CA3), a central serine/threonine-rich domain (CA4), and a C-terminal kinase-like domain (CA5). However, unlike Raf, KSR proteins also possess two unique N-terminal conserved areas (CA1 and CA2), lack a Ras binding domain, and may also lack kinase activity [1] (Figure 1). KSR interacts with Raf, MEK, ERK, 14-3-3, C-TAK1, and many other proteins in a large complex [7–15], and KSR overexpression can promote the translocation of MEK from cytosolic to membrane compartments [11, 12]. These observations are consistent with a model where KSR functions as a scaffold to assemble membrane-localized Raf/MEK/ERK signaling protein complexes [1]. However, KSR function is not well understood, and there have been many conflicting reports about the effects of KSR on Raf, MEK, and ERK kinase activities in vertebrate cells [10, 13, 14, 16–18]. Therefore, it has remained possible that KSR acts in an uncharacterized parallel pathway.

In *C. elegans*, Ras signaling is required for multiple developmental events, including excretory system development (and hence viability) [19], vulval development [20], sex myoblast migration [21], male spicule development [22], and germline meiotic progression (and hence fertility) [23]. Although *ksr-1* mutations suppress the vulval and spicule defects associated with hyperactive Ras and enhance several defects associated with reduced Ras signaling, *ksr-1* mutants themselves have very few defects other than abnormalities in sex myoblast migration [2, 3, 21]. Thus, the genetic analysis of *ksr-1* suggested either that *ksr-1* performs a nonessential function or acts redundantly with another gene.

Recently available sequences from *C. elegans* chromosome I reveal a second *ksr*-like gene, F58D5.4, hereafter called *ksr-2*. The cDNA clones yk343d6 and yk404b8 correspond to alternative splice forms of this gene (*ksr-2a* and *ksr-2b*) and encode proteins with moderate similarity to *C. elegans* KSR-1 and to KSR proteins from other species and with lesser similarity to Raf (Figure 1). Like other KSR proteins, both predicted KSR-2 isoforms possess a cysteine-rich domain (CA3) and a C-terminal kinase-like domain (CA5). The kinase-like domain lacks two residues normally required for catalytic activity: in subdomain I, it contains an arginine in place of the usual catalytic lysine, and, in subdomain VII, it contains an alanine in place of the normal invariant glycine. Interestingly, both KSR-2 isoforms lack the CA1 domain and part or all of the CA4 domain, including conserved sites for ERK docking and phosphorylation. Despite these differences, its overall sequence similarity to KSR proteins and its striking redundancy with KSR-1 (see below) argue that KSR-2 is a functional KSR family member.

To determine the functional requirements for *ksr-2*, we initially used RNA-mediated interference (RNAi [24]) to reduce endogenous *ksr-2* expression. *ksr-2(RNAi)* caused no apparent somatic defects but caused highly penetrant sterility in the F1 progeny of injected animals (Table 1). The sterile animals contained morphologically normal sperm but completely lacked oocytes. DAPI staining indicated that many meiotic germ cells were arrested in the pachytene stage of prophase I (data not shown). This pachytene arrest phenotype is similar to that caused by loss-of-function mutations in *let-60* Ras,

[4]Correspondence: sundaram@mail.med.upenn.edu

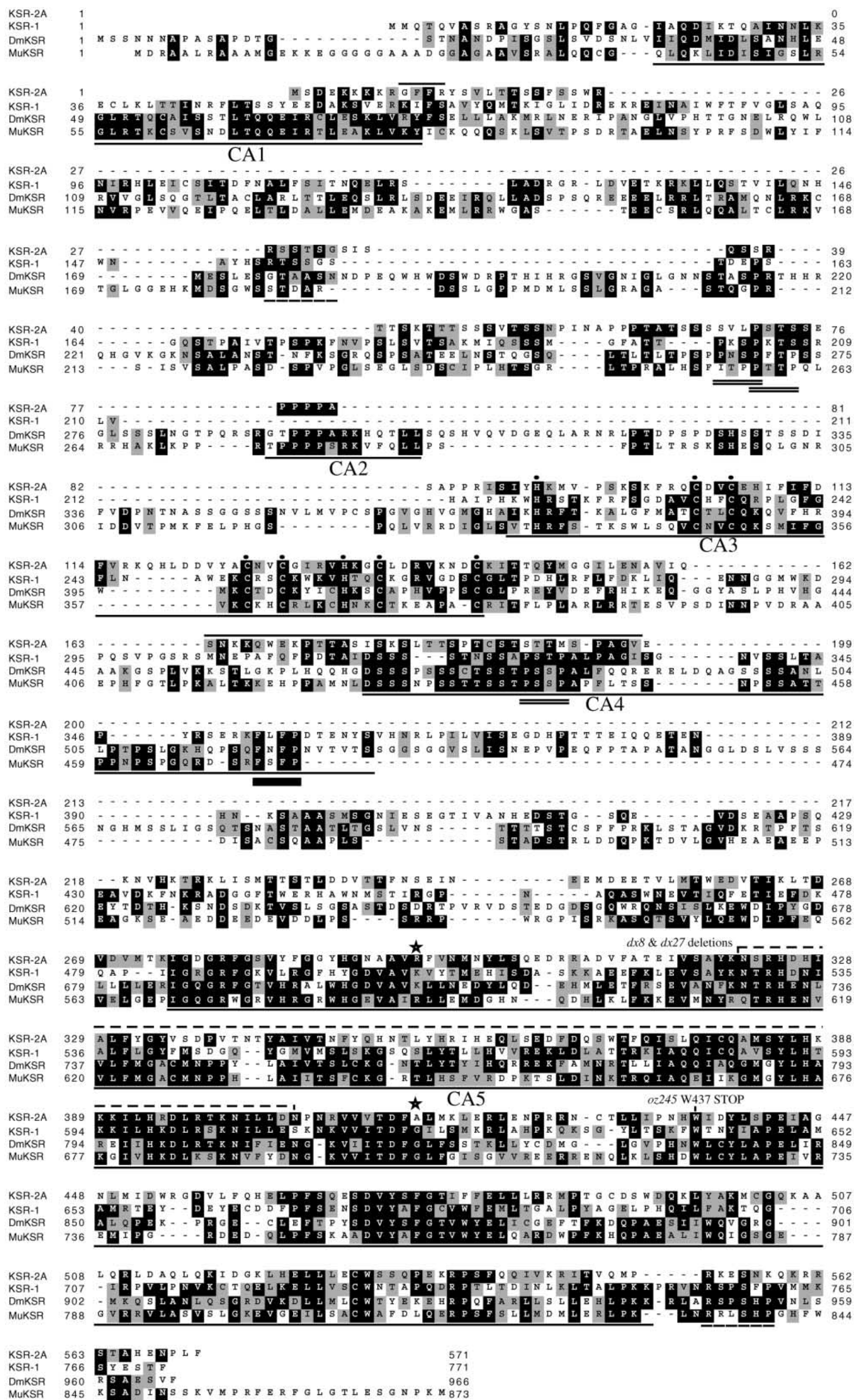

Figure 1. Sequence Alignment of KSR-2 versus Other KSR Proteins

KSR-2A is 35% identical to KSR-1, 32% identical to *Drosophila* KSR (DmKSR), 31% identical to murine KSR (MuKSR), and 27% identical to LIN-45 Raf (data not shown). KSR-2B differs from KSR-2A in that it lacks amino acids 10–13 and 163–198, indicated by the solid overlines. The KSR conserved areas (CA) [5] are labeled and underlined. Identical amino acid residues are boxed in black, and similar residues are shaded gray. Black dots mark the conserved cysteines and histidines of the CA3 domain. Stars indicate two residues in the KSR-2 kinase-like domain (CA5) that suggest this domain lacks catalytic activity. A double underline indicates ERK/MAPK consensus phosphorylation sites

Table 1. *ksr-2(RNAi)* Causes Sterility and Is Synthetic Lethal with *ksr-1*

Genotype	dsRNA	Percent Rod-like Larval Lethal (n)	Fertility
+	none	0 (220)	fertile
+	*ksr-2*	0 (570)	sterile
ksr-1(n2526)	none	0 (219)	fertile
ksr-1(n2526)	*ksr-2*	93 (524)	ND
lin-45(ku112)	none	0 (285)	fertile
lin-45(ku112)	*ksr-2*	$<$1 (441)	sterile
sur-8(ku167)	none	0 (81)	fertile
sur-8(ku167)	*ksr-2*	0 (591)	sterile

RNA interference experiments were performed essentially as described in [24]. Double stranded (ds) RNA ($\sim$1 mg/ml) corresponding to *ksr-2* was prepared by in vitro transcription with T3 and T7 polymerases, using a PCR-derived *ksr-2* genomic template. PCR primers used were oMS110 (AATTAACCCTCACTAAAGGGAATGTCTCCAG CTGGTGTTG) and oMS111 (GTAATACGACTCACTATAGGGTCACA GCCCGTTGGCATAC). Rod-like larval lethality and sterility were scored in the F1 self-progeny of dsRNA-injected mothers. *ksr-1(n2526)* is a putative null allele [2], *lin-45(ku112)* is a weak hypomorphic allele (C.E.R. et al., submitted), and *sur-8(ku167)* is a strong hypomorphic allele [39].

mek-2 MEK, and *mpk-1* ERK [23], suggesting that *ksr-2* is required for Ras-mediated signaling during germline meiotic progression.

The *dx8*, *dx27*, and *oz245* mutations are alleles of *ksr-2*. These recessive EMS-induced mutations were identified in a genetic screen for sterile mutants (D.C., E.L., and T.S., unpublished data) and cause a pachytene arrest phenotype identical to that seen with *ksr-2(RNAi)* (Figure 2). The mutations map to the *ksr-2* region on chromosome I (data not shown), and each contains a lesion within the *ksr-2* coding sequence (Figure 1). *dx8* and *dx27* contain identical 285 bp deletions that remove part of the kinase-like domain. *oz245* contains a nonsense mutation predicted to truncate the protein within the kinase-like domain. Thus, all three alleles are likely to severely reduce or eliminate *ksr-2* function. *ksr-2* mutants appear normal for viability, vulval development, sex myoblast migration, and male spicule development (Table 2 and data not shown), indicating that *ksr-2* is not required for Ras-mediated signaling events during somatic development.

To determine whether *ksr-2* functions redundantly with *ksr-1*, we performed *ksr-2* RNAi in a *ksr-1* mutant background. *ksr-2* RNAi will reduce both maternal and zygotic *ksr-2* activity. Whereas *ksr-1(n2526)* null mutants are viable, most *ksr-1(n2526); ksr-2(RNAi)* animals arrested as rod-like L1 larvae (Table 1). This lethal phenotype was indistinguishable from that of strong *let-60 ras* loss-of-function mutants, which fill up with fluid and die due to lack of an excretory duct cell [19]. This genetic interaction was specific for the *ksr-1* mutant background, as *ksr-2(RNAi)* caused no or only weakly penetrant larval lethality in several other sensitized genetic backgrounds tested (Table 1). Therefore, *ksr-2* functions redundantly with *ksr-1* to promote excretory system development and larval viability.

To test further for redundancy between *ksr-2* and *ksr-1*, we examined *ksr-2(dx27)*; *ksr-1(n2526)* double mutants. Such animals are maternally rescued for viability when segregating from *ksr-2/+; ksr-1* mothers, but hermaphrodites are Vulvaless, and males have crumpled spicules (Table 2 and data not shown). Therefore, *ksr-1* and *ksr-2* function redundantly to promote vulva and spicule development. *ksr-2; ksr-1* hermaphrodites have sex myoblast migration defects that are no more severe than those of *ksr-1* single mutants (Table 2); therefore, only *ksr-1* appears to function during sex myoblast migration (assuming no maternal *ksr-2* contribution). Finally, *ksr-2; ksr-1* mutants make sperm but no oocytes and exhibit a pachytene arrest phenotype that is no more severe than that of *ksr-2* single mutants (data not shown); therefore, only *ksr-2* appears to function in meiotic progression during oogenesis. Notably, the *ksr-2; ksr-1* Vulvaless defect is incompletely penetrant at 20°C but is strongly penetrant at 25°C (Table 2). We suggest that maternal *ksr-2* product may be depleted more rapidly at higher temperatures and that the 25°C phenotype therefore most closely resembles the *ksr* null phenotype.

We used the Vulvaless and sterile phenotypes of *ksr-2; ksr-1* double mutants to confirm the epistatic relationship between *ksr* and other Ras pathway components. Both phenotypes are still seen in the *let-60(n1046gf)* mutant background (Table 2), consistent with the prior genetic placement of *ksr-1* downstream or in parallel to *let-60* Ras [2, 3]. The Vulvaless phenotype is completely suppressed by joint overexpression of activated *Dmek* and *mpk-1* and by a *lin-1* null mutation (Table 2), suggesting that *ksr* functions upstream of *mek-2*, *mpk-1*, and the *lin-1* Ets transcriptional regulator. However, the sterile phenotype is not suppressed by the *Dmek/mpk-1* transgene, consistent with prior observations that transgenes are usually silenced in the germline [25], or by *lin-1*, consistent with the prior suggestion that *lin-1* is not a target of Ras signaling in the germline [23].

When overexpressed in vertebrate cells, KSR proteins have been found to promote, interfere with, or have no effect on ERK phosphorylation [10, 13, 14, 16–18]. These conflicting reports may reflect the problems inherent in

(PXS/TP) conserved between two or more KSR proteins. A thick underline indicates the ERK/MAPK docking site (FXFP) [8]; note that this site is not present in KSR-2. Dashed underlines indicate a potential C-terminal 14-3-3 binding site (RSXSXP) [33] and an N-terminal degenerate site shared between KSR-2 and KSR-1. MuKSR sites of C-TAK1 phosphorylation and 14-3-3 binding (Ser297 and Ser392) [11] are not conserved in the *C. elegans* KSR proteins. The *oz245* nonsense mutation that changes W437 to a STOP (TGG to TGA) is labeled. The *dx8* and *dx27* mutations are identical 285 base deletions between the direct repeats ACAAT located at -38 bases from the 5′ end of exon 6 and 247 bases into exon 6. These deletions remove part of the kinase-like domain, indicated by dashed line above corresponding sequence. The KSR-2 protein sequences are based on our analyses of cDNA clones yk343d6 (KSR-2A) and yk404b8 (KSR-2B) and RT-PCR products and differ from those predicted by genefinder. RT-PCR analysis suggests that *ksr-2* is both SL1 and SL2 *trans*-spliced 16 nucleotides upstream of the initiating methionine in the cDNA clones and that *ksr-2* contains a 546 bp 3′UTR that is largely lacking in the cDNA clones because of priming off an internal polyA tract. A single band of $\sim$2.7 kb is detected on Northern blots of mixed-stage RNAs (data not shown), in good agreement with the size of the predicted cDNA.

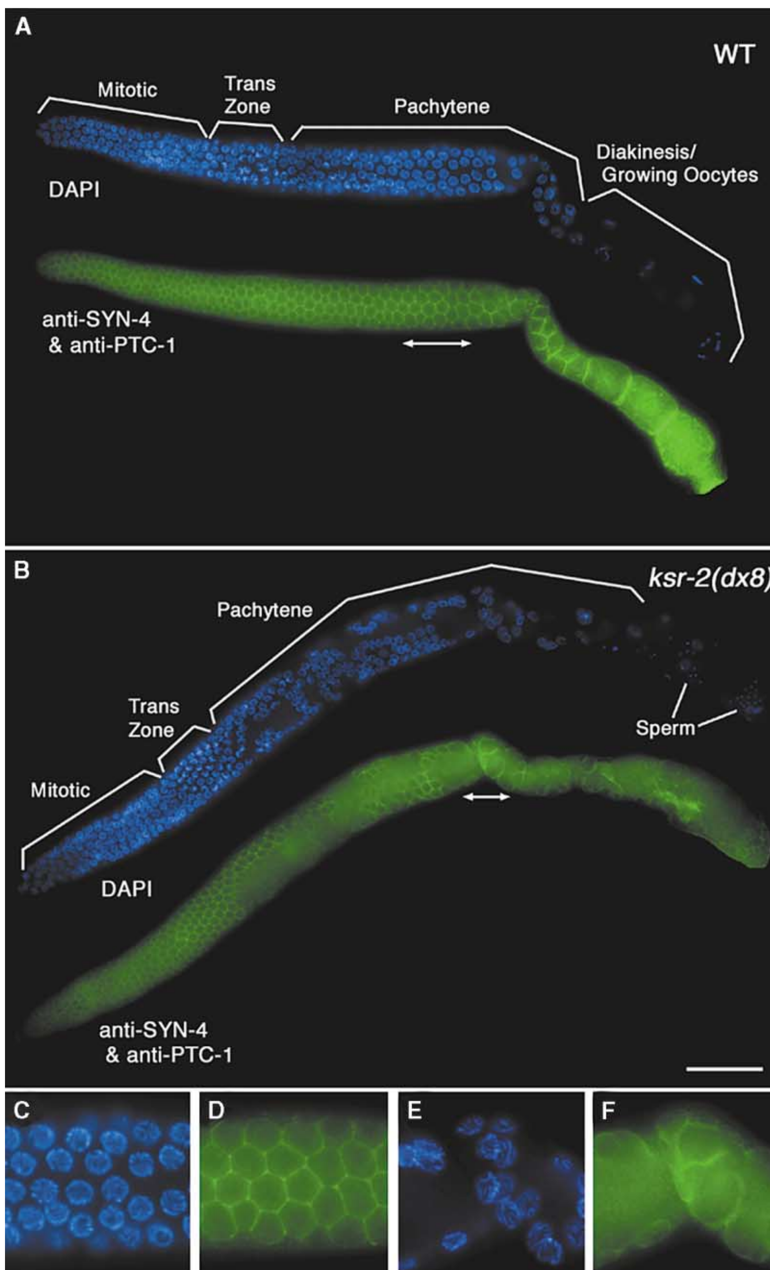

Figure 2. *ksr-2* Mutants Exhibit a Germline Pachytene Arrest Phenotype

(A) WT or (B) *ksr-2(dx8)* adult hermphrodite gonads were dissected and stained with DAPI (blue) to visualize DNA organization and anti-SYN-4 plus anti-PTC-1 antibodies (green) to visualize membranes (gifts of M. Glotzer and P. Kuwabara, respectively [34]). At the distal end of the wild-type gonad (A), germ cells proliferate mitotically, enter meiotic prophase in the transition zone, and then progress through an extended pachytene stage. These nuclei are found primarily on the surface of the gonadal tube, packed in a "honeycomb" arrangement. Each nucleus is surrounded by membranes on all sides but the interior, which is incomplete, having a cytoplasmic passageway to the acellular cytoplasmic core or rachis [35, 36]. Although this is a syncitium, each nucleus and its surrounding cytoplasm and membranes is called a germ cell. At the loop and into the proximal gonad, nuclei progress from pachytene to diplotene and then diakinesis, with contemporaneous oocyte cellularization and growth. By contrast, in *ksr-2(dx8)* hermaphrodites (B), nuclei fail to progress past pachytene or form oocytes in the proximal gonad. Furthermore, instead of pachytene nuclei being found on the surface of the gonadal tube, pachytene nuclei are in clumps, with regions of the surface devoid of nuclei and membranes. Enlargements of surface regions bounded by arrowheads in (A) and (B) are presented in (C) and (D) (wild-type) and (E) and (F) [*ksr-2(dx8)*], showing DAPI and SYN-4/PTC-1 staining, respectively. In wild-type, pachytene germ cells are arranged in a hexagonal pattern, while, in *ksr-2(dx8)* mutants, pachytene nuclei are in clumps surrounded by disorganized membranes. Scale bar, 20 μm.

overexpressing a protein that can bind to multiple other signaling components. To test if removing *ksr-1* and *ksr-2* affects MPK-1 ERK phosphorylation in *C. elegans*, we used a monoclonal antibody to assess levels of active diphosphorylated MPK-1 in lysates from *ksr-1* and *ksr-2* single and double mutants [26, 27] (Figure 3). *C. elegans* has two forms of MPK-1 [28], a 55 Kd isoform that is germline specific and a 45 Kd isoform that is predominantly somatic (M.H. Lee et al., unpublished data). Phosphorylation of both isoforms is readily detected in wild-type adult lysates and is absolutely dependent on *mek-2* (Figure 3; M.H. Lee et al., unpublished data). The situation is most straightforward in the soma; the phosphorylated 45 Kd MPK-1 isoform is present at normal levels in *ksr-1* and *ksr-2* single mutants but is undetectable in both young and old *ksr-2; ksr-1* double mutants. Therefore, *ksr-1* and *ksr-2* function redundantly to promote MPK-1 phosphorylation in the soma. The phosphorylated 55 Kd germline-specific MPK-1 isoform is present at normal levels in *ksr-1* single mutants but is severely reduced or undetectable in young (24 hr time point) *ksr-2* or *ksr-2; ksr-1* adults, indicating that *ksr-2* promotes MPK-1 phosphorylation in the germline. Surprisingly, the phosphorylated 55 Kd MPK-1 isoform reappears in older (48 hr time point) *ksr-2* or *ksr-2; ksr-1* adults, suggesting a delayed KSR-independent mechanism for MPK-1 phosphorylation in the germline. However, this late pool of phosphorylated MPK-1 appears nonfunctional, since old *ksr-2* adults continue to show the pachytene arrest phenotype, and removal of *mek-2*

Table 2. *ksr-2*; *ksr-1* Double Mutant Analysis and Epistasis

Genotype[a]	Temperature	Muv (%)	WT (%)	Vul (%)	Average Number VPCs Induced (n)	SM mig (%)	(n)	Fertility
krs-2	20°	0	100	0	3.0 (22)	0	(14)	sterile
ksr-2	25°	0	100	0	3.0 (25)	0	(36)	sterile
ksr-2/+; *ksr-1*	20°	0	90	10	2.94 (21)	42	(45)	fertile
ksr-2/+; *ksr-1*	25°	0	100	0	3.0 (13)	24	(70)	fertile
ksr-2; *ksr-1*	20°	0	28	72	1.62 (18)	37	(19)	sterile
ksr-2; *ksr-1*	25°	0	0	100	0.13 (27)	27	(26)	sterile
ksr-2/+; *let-60(gf)*; *ksr-1*	25°	6	94	0	3.03 (16)	ND		fertile
ksr-2; *let-60(gf)*; *ksr-1*	25°	0	0	100	0.0 (13)	ND		sterile
ksr-2/+; *gaIs36*; *ksr-1*	25°	100	0	0	5.18 (11)	ND		fertile
ksr-2; *gaIs36*; *ksr-1*	25°	86	14	0	5.07 (7)	ND		sterile[b]
ksr-2/+; *lin-1*; *ksr-1*	25°	100	0	0	5.54 (13)	ND		fertile
ksr-2; *lin-1*; *ksr-1*	25°	100	0	0	5.33 (6)	ND		sterile

Vulval and sex myoblast migration (SM mig) phenotypes were scored in L4 or L3 stage larvae, respectively, using differential interference contrast microscopy (DIC) as described in [3] and [21]. In wild-type (WT) animals, three vulval precursor cells (VPCs) are induced to adopt a vulval fate [20]. Vulvaless (Vul) animals had fewer than three VPCs induced, while Multivulva (Muv) animals had greater than three VPCs induced. All *ksr-2* mutant adults and no *ksr-1* mutant adults are sterile. n, number of animals scored for vulval defects or number of SM cells scored for migration defects. ND, not determined.

[a] Alleles used were *ksr-2(dx27)*, *ksr-1(n2526)*, *let-60(n1046gf)*, *gaIs36*, and *lin-1(n304)*. *let-60(n1046gf)* is a hypermorphic allele [40]. *gaIs36* is an integrated transgene consisting of *Dmek(gf)* and *hs-mpk-1(+)* [28]. *gaIs36* was linked to *him-5(e1490)*. *lin-1(n304)* is a null allele [41]. All animals were obtained from *ksr-2/hT2[qIs48]* mothers. *qIs48* is a recessive lethal insertion of the *pes-10::*GFP, *myo-2::*GFP, and *F22B7.9::*GFP reporters into the *hT2* balancer chromosome [42, 43] and allowed *ksr-2* homozygotes and heterozygotes to be recognized as GFP(−) and GFP(+) segregants, respectively.

[b] Since transgenes are generally not expressed in the germline [25], *gaIs36* would not be expected to rescue *ksr-2* sterility.

in the *mek-2 ksr-2* double mutant while eliminating the delayed phosphorylation does not alter the pachytene arrest phenotype.

The discovery of a second *C. elegans ksr* gene, *ksr-2*, which has both unique and redundant functions with *ksr-1* explains the surprisingly mild phenotypic defects previously described for *ksr-1* mutants and substantiates observations from *Drosophila* [5] that *ksr* is required for Ras-mediated signaling. Since the vulval and germline phenotypes of *ksr-2; ksr-1* double mutants are slightly less penetrant and/or expressive than those described for *let-60 ras* or *mpk-1* null mutants and since *ksr-2; ksr-1* double mutants still have delayed phosphorylation of MPK-1 in the germline, Ras may have some limited ability to stimulate ERK in the absence of KSR. However, the finding that *ksr-2; ksr-1* double mutants have strong *let-60* Ras-like phenotypes and severely reduced or absent levels of diphosphorylated MPK-1 ERK in the soma clearly indicates that KSR plays a critical role in the activation or maintenance of the Raf/MEK/ERK kinase cascade.

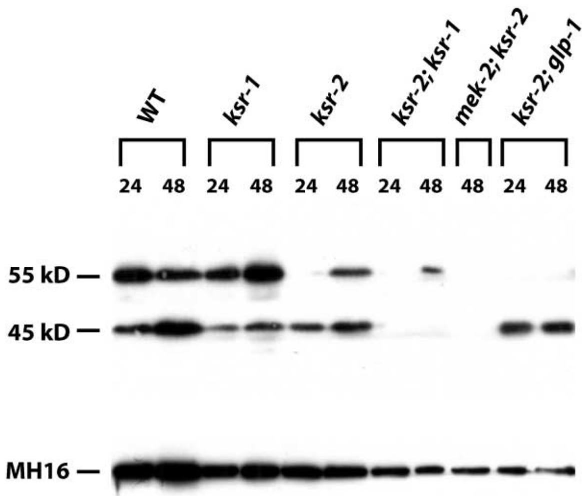

Figure 3. *ksr-1* and *ksr-2* Are Jointly Required for MPK-1 ERK Phosphorylation in the Soma

Western blot probed with diphospho-specific anti-ERK antibody (MAPKYT, Sigma). Each lane contains total extract from 100 adult hermaphrodites that were collected at the L4 stage and harvested either 24 hr or 48 hr later as indicated. As a control for loading, the blot was reprobed with monoclonal antibody MH16 that detects paramyosin [37]. Mutant alleles used were *ksr-1(n2526)*, *ksr-2(dx27)*, *mek-2(h294)*, and *glp-1(q175)*. *glp-1* animals lack a germline [38] and thus demonstrate that the delayed appearing 55 kDa phosphorylated MPK-1 isoform in *ksr-2* mutants is specific to the germline (M.H. Lee et al., unpublished data).

The precise mechanisms by which KSR proteins promote Raf/MEK/ERK activity remain obscure. Mammalian KSR has been suggested to phosphorylate and help activate Raf [18, 29]. However, we do not think that *C. elegans* KSR is likely to function as a Raf kinase because the putative phosphorylation site on Raf is not conserved in LIN-45 RAF, predicted kinase-dead forms of KSR-1 have wild-type function in transgenic rescue assays [12], and the KSR-2 kinase domain lacks two critical residues that are usually required for kinase activity. We do think that KSR function is closely related to that of Raf, based on epistasis analysis [5, 6] and our observations that, like *ksr-2* but unlike *mek-2*, *lin-45* Raf mutants also display delayed phosphorylation of MPK-1 ERK in the germline (M.H. Lee et al., unpublished data). Direct interactions between KSR and Raf have not been demonstrated, but KSR function in vivo correlates with its ability to bind to MEK [12, 30]. KSR-2 also interacts with MEK-2 but not LIN-45 in yeast two-hybrid assays (L. Girard and M.V.S., unpublished data). Therefore, we propose that, via interactions with MEK and other un-

known components, KSR is recruited to Raf and acts directly or indirectly to promote or sustain Raf and/or MEK activity.

KSR-2 is the most divergent of the KSR proteins described to date and lacks several hallmarks of the family, including the CA1 domain and conserved sites for ERK binding and phosphorylation. Therefore, it is possible that KSR-2 could function or be regulated somewhat differently from other KSR proteins. However, the ability of KSR-2 to functionally compensate for a lack of KSR-1 in multiple somatic tissues argues against any fundamental difference in function. Furthermore, the inability of KSR-1 to compensate for the lack of KSR-2 in the germline could simply be due to the fact that most X-linked genes in *C. elegans*, including *ksr-1*, are either poorly/not expressed in the germline [31] or expressed at the very end of pachytene [32], too late to function in pachytene progression. Since the *ksr-1* gene is not well positioned to function during germline meiosis, the autosomal *ksr-2* gene may have evolved to serve this function.

Acknowledgments

We thank Y. Kohara for cDNA clones; J. Kimble, S. Kim, and the Caenorhabditis Genetics Center for strains; and members of our laboratories for helpful discussions and comments on the manuscript. This work was supported by National Institutes of Health grants GM49785 (to E.L.), GM63310 (to T.S.), and GM58540 (to M.V.S.). C.E.R. is a postdoctoral fellow of the Jane Coffin Childs Memorial Fund for Medical Research.

Received: December 10, 2001
Accepted: January 9, 2002
Published: March 5, 2002

References

1. Morrison, D.K. (2001). KSR: a MAPK scaffold of the Ras pathway? J. Cell Sci. *114*, 1609–1612.
2. Kornfeld, K., Hom, D.B., and Horvitz, H.R. (1995). The *ksr-1* gene encodes a novel protein kinase involved in ras-mediated signaling in *Caenorhabditis elegans.* Cell *83*, 903–913.
3. Sundaram, M., and Han, M. (1995). The *C. elegans ksr-1* gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. Cell *83*, 889–901.
4. Vojtek, A.B., and Der, C.J. (1998). Increasing complexity of the Ras signaling pathway. J. Biol. Chem. *273*, 19925–19928.
5. Therrien, M., Chang, H.C., Solomon, N.M., Karim, F.D., Wassarman, D.A., and Rubin, G.M. (1995). KSR, a novel protein kinase required for Ras signal transduction. Cell *83*, 879–888.
6. Sieburth, D.S., Sundaram, M., Howard, R.M., and Han, M. (1999). A PP2A regulatory subunit positively regulates Ras-mediated signaling during *Caenorhabditis elegans* vulval induction. Genes Dev. *13*, 2562–2569.
7. Denouel-Galy, A., Douville, E.M., Warne, P.H., Papin, C., Laugier, D., Calothy, G., Downward, J., and Eychene, A. (1998). Murine Ksr interacts with MEK and inhibits Ras-induced transformation. Curr. Biol. *8*, 46–55.
8. Jacobs, D., Glossip, D., Xing, H., Muslin, A.J., and Kornfeld, K. (1999). Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. Genes Dev. *13*, 163–175.
9. Joneson, T., Fulton, J.A., Volle, D.J., Chaika, O., Bar-Sagi, D., and Lewis, R.E. (1998). Kinase Suppressor of Ras inhibits the activation of extracellular ligand-regulated (ERK) mitogen-activated protein (MAP) kinase by growth factors, activated Ras, and Ras effectors. J. Biol. Chem. *273*, 7743–7748.
10. Michaud, N.R., Therrien, M., Cacace, A., Edsall, L.C., Spiegel, S., Rubin, G.M., and Morrison, D.K. (1997). KSR stimulates Raf-1 activity in a kinase-independent manner. Proc. Natl. Acad. Sci. USA *94*, 12792–12796.
11. Muller, J., Ory, S., Copeland, T., Piwnica-Worms, H., and Morrison, D.K. (2001). C-TAK1 regulates Ras signaling by phosphorylating the MAPK scaffold KSR1. Mol. Cell *8*, 983–993.
12. Stewart, S., Sundaram, M., Zhang, Y., Lee, J., Han, M., and Guan, K.L. (1999). Kinase Suppressor of Ras (KSR) forms a multi-protein signaling complex and modulates MEK localization. Mol. Cell. Biol. *19*, 5523–5534.
13. Therrien, M., Michaud, N.R., Rubin, G., and Morrison, D.K. (1996). KSR modulates signal propagation within the MAPK cascade. Genes Dev. *10*, 2684–2695.
14. Yu, W., Fantl, W.J., Harrowe, G., and Williams, L.T. (1997). Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK. Curr. Biol. *8*, 56–64.
15. Xing, H., Kornfeld, K., and Muslin, A.J. (1997). The protein kinase KSR interacts with 14-3-3 protein and Raf. Curr. Biol. *7*, 294–300.
16. Cacace, A.M., Michaud, N.R., Therrien, M., Mathes, K., Copeland, T., Rubin, G.M., and Morrison, D.K. (1999). Identification of constitutive and Ras-inducible phosphorylation sites of KSR: Implications for 14-3-3 binding, mitogen-activated protein kinase binding, and KSR overexpression. Mol. Cell. Biol. *19*, 229–240.
17. Sugimoto, T., Stewart, S., Han, M., and Guan, K.L. (1998). The kinase suppressor of Ras (KSR) modulates growth factor and Ras signaling by uncoupling Elk-1 phosphorylation from MAP kinase activation. EMBO J. *17*, 1717–1727.
18. Zhang, Y., Yao, B., Delikat, S., Bayoumy, S., Lin, X.H., Basu, S., McGinley, M., Chan-Hui, P.Y., Lichenstein, H., and Kolesnick, R. (1997). Kinase suppressor of Ras is ceramide-activated protein kinase. Cell *89*, 63–72.
19. Yochem, J., Sundaram, M., and Han, M. (1997). Ras is required for a limited number of cell fates and not for general proliferation in *Caenorhabditis elegans.* Mol. Cell. Biol. *17*, 2716–2722.
20. Sternberg, P.W., and Han, M. (1998). Genetics of RAS signaling in *C. elegans.* Trends Genet. *14*, 466–472.
21. Sundaram, M., Yochem, J., and Han, M. (1996). A Ras-mediated signal transduction pathway is involved in the control of sex myoblast migration in *Caenorhabditis elegans.* Development *122*, 2823–2833.
22. Chamberlin, H.M., and Sternberg, P.W. (1994). The lin-3/let-23 pathway mediates inductive signalling during male spicule development in *Caenorhabditis elegans.* Development *120*, 2713–2721.
23. Church, D., Guan, K.L., and Lambie, E.J. (1995). Three genes of the MAP kinase cascade, *mek-2, mpk-1/sur-1* and *let-60 ras*, are required for meiotic cell cycle progression in *Caenorhabditis elegans.* Development *121*, 2525–2535.
24. Fire, A., Xu, S., Montgomery, M.K., Kostas, S.A., Driver, S.E., and Mello, C.C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans.* Nature *391*, 806–811.
25. Kelly, W.G., and Fire, A. (1998). Chromatin silencing and the maintenance of a functional germline in *Caenorhabditis elegans.* Development *125*, 2451–2456.
26. Miller, M.A., Nguyen, V.Q., Lee, M.H., Kosinski, M., Schedl, T., Caprioli, R.M., and Greenstein, D. (2001). A sperm cytoskeletal protein that signals oocyte meiotic maturation and ovulation. Science *291*, 2144–2147.
27. Page, B.D., Guedes, S., Waring, D., and Priess, J.R. (2001). The *C. elegans* E2F- and DP-related proteins are required for embryonic asymmetry and negatively regulate Ras/MAPK signaling. Mol. Cell *7*, 451–460.
28. Lackner, M.R., and Kim, S.K. (1998). Genetic analysis of the *Caenorhabditis elegans* MAP kinase gene *mpk-1.* Genetics *150*, 103–117.
29. Xing, H.R., and Kolesnick, R. (2001). Kinase suppressor of Ras signals through Thr269 of c-Raf-1. J. Biol. Chem. *276*, 9733–9741.
30. Muller, J., Cacace, A.M., Lyons, W.E., McGill, C.B., and Morrison, D.K. (2000). Identification of B-KSR1, a novel brain-specific isoform of KSR1 that functions in neuronal signaling. Mol. Cell. Biol. *20*, 5529–5539.
31. Reinke, V., Smith, H.E., Nance, J., Wang, J., Van Doren, C.,

Begley, R., Jones, S.J., Davis, E.B., Scherer, S., Ward, S., et al. (2000). A global profile of germline gene expression in *C. elegans.* Mol. Cell *6*, 605–616.
32. Kelly, W.G., Schaner, C.E., Dernburg, A.F., Lee, M.-H., Kim, S.K., Villeneuve, A.M., and Reinke, V. (2002). X-chromosome silencing in the germline of *C. elegans.* Development *129*, 479–492.
33. Muslin, A.J., Tanner, J.W., Allen, P.M., and Shaw, A.S. (1996). Interaction of 14-3-3 with signaling proteins is mediated by recognition of phosphoserine. Cell *84*, 889–897.
34. Kuwabara, P.E., Lee, M.H., Schedl, T., and Jefferis, G.S. (2000). A *C. elegans* patched gene, *ptc-1*, functions in germ-line cytokinesis. Genes Dev. *14*, 1933–1944.
35. Hirsh, D., Oppenheim, D., and Klass, M. (1976). Development of the reproductive system of *Caenorhabditis elegans.* Dev. Biol. *49*, 200–219.
36. Hall, D.H., Winfrey, V.P., Blaeuer, G., Hoffman, L.H., Furuta, T., Rose, K.L., Hobert, O., and Greenstein, D. (1999). Ultrastructural features of the adult hermaphrodite gonad of *Caenorhabditis elegans*: relations between the germ line and soma. Dev. Biol. *212*, 101–123.
37. Francis, G.R., and Waterston, R.H. (1985). Muscle organization in *Caenorhabditis elegans*: localization of proteins implicated in thin filament attachment and I-band organization. J. Cell Biol. *101*, 1532–1549.
38. Austin, J., and Kimble, J. (1987). *glp-1* is required in the germ line for regulation of the decision between mitosis and meiosis in *C. elegans.* Cell *51*, 589–599.
39. Sieburth, D.S., Sun, Q., and Han, M. (1998). SUR-8, a conserved Ras-binding protein with leucine-rich repeats, positively regulates Ras-mediated signaling in *C. elegans.* Cell *94*, 119–130.
40. Beitel, G.J., Clark, S.G., and Horvitz, H.R. (1990). *Caenorhabditis elegans ras* gene *let-60* acts as a switch in the pathway of vulval induction. Nature *348*, 503–509.
41. Beitel, G.J., Tuck, S., Greenwald, I., and Horvitz, H.R. (1995). The *Caenorhabditis elegans* gene *lin-1* encodes an ETS-domain protein and defines a branch in the vulval induction pathway. Genes Dev. *9*, 3149–3162.
42. McKim, K.S., and Rose, A.M. (1990). Chromosome I duplications in *Caenorhabditis elegans.* Genetics *124*, 115–132.
43. Wang, S., and Kimble, J. (2001). The TRA-1 transcription factor binds TRA-2 to regulate sexual fates in *Caenorhabditis elegans.* EMBO J. *20*, 1363–1372.