Copyright © 2000 by the Genetics Society of America

A Genetic Screen for Modifiers of a Kinase Suppressor of Ras-Dependent Rough Eye Phenotype in Drosophila

Marc Therrien,*,[1] Deborah K. Morrison,[†] Allan M. Wong* and Gerald M. Rubin*

**Howard Hughes Medical Institute and Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-3200 and [†]Molecular Basis of Carcinogenesis Laboratory, ABL-Basic Research Program, National Cancer Institute, Frederick Cancer Research and Development Center, Frederick, Maryland 21702*

Manuscript received May 3, 2000
Accepted for publication July 25, 2000

ABSTRACT

kinase suppressor of Ras (*ksr*) encodes a putative protein kinase that by genetic criteria appears to function downstream of RAS in multiple receptor tyrosine kinase (RTK) pathways. While biochemical evidence suggests that the role of KSR is closely linked to the signal transduction mechanism of the MAPK cascade, the precise molecular function of KSR remains unresolved. To further elucidate the role of KSR and to identify proteins that may be required for KSR function, we conducted a dominant modifier screen in Drosophila based on a KSR-dependent phenotype. Overexpression of the KSR kinase domain in a subset of cells during Drosophila eye development blocks photoreceptor cell differentiation and results in the external roughening of the adult eye. Therefore, mutations in genes functioning with KSR might modify the KSR-dependent phenotype. We screened ~185,000 mutagenized progeny for dominant modifiers of the KSR-dependent rough eye phenotype. A total of 15 complementation groups of Enhancers and four complementation groups of Suppressors were derived. Ten of these complementation groups correspond to mutations in known components of the Ras1 pathway, demonstrating the ability of the screen to specifically identify loci critical for Ras1 signaling and further confirming a role for KSR in Ras1 signaling. In addition, we have identified 4 additional complementation groups. One of them corresponds to the *kismet* locus, which encodes a putative chromatin remodeling factor. The relevance of these loci with respect to the function of KSR and the Ras1 pathway in general is discussed.

THE mitogen-activated protein kinase (MAPK) pathway is a central route through which the small GTPase RAS transmits signals promoting cell proliferation, cell differentiation, and cell survival (reviewed in Cobb 1999). At the core of this pathway are three kinases [RAF, MAPK kinase (MEK), and MAPK], which convey RAS-dependent signals in a sequential manner. RAS activation induces RAF activation at the plasma membrane, which then phosphorylates MEK, which in turn phosphorylates and activates MAPK. Activated MAPK then modulates the activity of a wide range of cytoplasmic and nuclear substrates via phosphorylation events, ultimately generating a specific cellular response.

Several other proteins have also been shown to contribute to the mechanism of signal propagation within the MAPK pathway (reviewed in Campbell *et al.* 1998; Sternberg and Alberola-Ila 1998; Garrington and Johnson 1999). Although the function of most of these molecules appears critical, their mode of action remains poorly understood. One such protein is kinase suppressor of RAS (KSR). Loss-of-function mutations in the *ksr* gene were originally isolated as dominant suppressors of a constitutively activated Ras allele in Drosophila and *Caenorhabditis elegans* (Kornfeld *et al.* 1995; Sundaram and Han 1995; Therrien *et al.* 1995). In genetic studies performed in Drosophila, KSR activity appeared to be required downstream of RAS, but upstream of or in a pathway parallel to RAF, thus indicating that KSR functions either in the RAS/MAPK pathway or in another essential parallel pathway (Therrien *et al.* 1995). These studies demonstrated the involvement of KSR in several receptor tyrosine kinase (RTK)-mediated pathways, suggesting that KSR is a general signaling component in RTK/RAS pathways. Biochemical characterization of the murine form of KSR (mKSR1) has corroborated a role for KSR in RAS-mediated signaling and has further demonstrated that KSR is directly involved in signal transmission within the MAPK pathway (Therrien *et al.* 1996; Michaud *et al.* 1997; Xing *et al.* 1997). Although the exact nature of its molecular function is currently unknown, a growing body of evidence suggests a scaffolding role for KSR in the MAPK pathway. For instance, protein interaction studies have demonstrated that KSR interacts directly or indirectly with RAF, MEK, MAPK, and 14-3-3 (Therrien *et al.* 1996; Michaud *et al.* 1997; Xing *et al.* 1997; Denouel-Galy *et al.* 1998; Yu *et al.* 1998; Cacace *et al.* 1999;

Corresponding author: Gerald M. Rubin, Howard Hughes Medical Institute, 545 Life Sciences addition #3200, University of California, Berkeley, CA 94720-3200. E-mail: gerry@fruitfly.BDGP.berkeley.edu

[1] *Present address:* Clinical Research Institute of Montreal, 110 Pine Ave. W., Montreal PQ, H2W 1R7, Canada.

Jacobs *et al.* 1999; Stewart *et al.* 1999). In addition, KSR was found to accelerate signal transmission within the MAPK pathway in a kinase-independent manner (Michaud *et al.* 1997; Stewart *et al.* 1999). Interestingly, the interactions between KSR and the components of the pathway, as well as the intracellular localization of KSR, were found to be modulated according to the activation state of the cell. It has been found that KSR is bound directly to MEK in the cytoplasm of quiescent cells; upon activation of the MAPK pathway by growth factors or activated RAS, KSR is relocalized to the plasma membrane in a complex containing RAF, MEK, and MAPK (Michaud *et al.* 1997; Denouel-Galy *et al.* 1998; Yu *et al.* 1998; Stewart *et al.* 1999). On the basis of these observations and the finding that MEK is not a substrate of KSR (Therrien *et al.* 1996; Denouel-Galy *et al.* 1998; Yu *et al.* 1998), it is possible that the role of KSR is to bring together MEK and its activator RAF in a favorable enzyme-substrate context at the plasma membrane. The role of KSR as a scaffolding protein is appealing given the genetic and biochemical evidence to date; however, KSR may act outside the MAPK signaling module and play other roles in cell signaling.

The identification of proteins that physically interact with KSR has provided a framework to define the function of KSR. However, the lack of an *in vitro* biochemical assay that depends on KSR activity has made it difficult to assess the significance of the protein interactions and has prevented the identification of other nonassociating proteins that may be required for KSR activity. To circumvent these limitations, we took a genetic approach to identify novel gene products potentially relevant for the function of KSR. Our strategy was to perform a dominant modifier screen in Drosophila based on a KSR-dependent phenotype. We reasoned that a twofold reduction in the gene dosage of a component (by mutating one of the two alleles) acting in concert with KSR might be sufficient to modify a KSR-induced phenotype and thus allow for its identification. Artificially induced phenotypes in Drosophila, by the overexpression of constitutively activated or dominant-negative molecules in the eye, have been successfully used in dominant modifier screens to identify signaling components of various pathways (Dickson *et al.* 1996; Karim *et al.* 1996; Verheyen *et al.* 1996; Barrett *et al.* 1997; Staehling-Hampton *et al.* 1999). To generate a KSR-dependent phenotype in Drosophila, we took advantage of the fact that photoreceptor cell differentiation in Drosophila is governed by RAS/MAPK-mediated signaling (reviewed in Zipursky and Rubin 1994). Previously, we have shown that overexpression of the mKSR1 kinase domain, which lacks the noncatalytic N-terminal domain, specifically blocks signal transmission within the MAPK pathway (Therrien *et al.* 1996). Therefore, we overexpressed the kinase domain of Drosophila KSR in a subset of cells during Drosophila eye development. The overexpression of this allele specifically antagonized photoreceptor cell differentiation in a dosage-sensitive manner and resulted in a visible roughening of the adult eye surface.

We screened ~185,000 mutagenized progeny for dominant enhancer and suppressor mutations of the dominant-negative KSR (KDN)-dependent rough eye phenotype. The results of the screen strongly support a role for KSR in the MAPK pathway, in that mutations in genes encoding known components of the pathway in Drosophila were isolated, including the *14-3-3ε*, *Dsor1/mek*, *rolled/mapk*, *pointed*, *yan*, and *ksr* loci. Furthermore, due to the ability of KDN to block RAS/MAPK-mediated signaling, mutations in genes expected to function upstream of *ksr* were also isolated. These included mutations in the *egfr*, *Star*, *Sos*, and *Ras1* loci. Here we present the identification of four of the loci isolated in this screen. The relationship between these loci and the function of KSR is discussed. In addition, the molecular characterization of one of these four loci, which corresponds to the *kismet* locus, is presented. Interestingly, the finding that *kismet* encodes a protein with domains found in chromatin remodeling factors raises the possibility that RAS-mediated signals are integrated by chromatin remodeling factors to control gene expression.

MATERIALS AND METHODS

Drosophila maintenance and mutagenesis: Fly culture and crosses were performed according to standard procedures. For the mutagenesis, w^- males isogenic for the second and the third chromosomes were either irradiated (4000 rad) or fed a 25-mM ethyl methanesulfonate (EMS), 10-mM Tris-HCl pH 7.5, 1% sucrose solution. Mutagenized males were then mated *en masse* with either *CKDN* (*CyO*, *sE-KDN*)/*Adv* or *TKDN* (*TM3*, *sE-KDN*)/*e*, *ftz*, *ry* virgin females. The F_1 *CKDN* or *TKDN* progeny were scored, using a stereomicroscope, for alteration in eye roughness compared to the F_0 females.

Chromosomal linkage, complementation tests, and meiotic mapping: The modified F_1 progeny were backcrossed to *CKDN* and *TKDN* flies to verify the germline transmission of the modification. The progeny of this cross were also used to determine the chromosomal linkage of the modifiers, which were then balanced over either the *CKDN* or the *TKDN* balancers. *X*-linked modifiers were balanced over the *FM7c* balancer. Allelism was assessed by complementation tests based on recessive lethality associated with most of the modifier lines. Allelism was not established for the majority of the homozygous viable modifier lines. Lethal groups corresponding to previously known loci required for the RTK/MAPK pathway in flies were identified by lethal complementation tests using the following alleles: flb^{2C82}, $Sos^{l(2)k05224}$, $Ras1^{e2f}$, $14\text{-}3\text{-}3\varepsilon^{S\text{-}696}$, $ksr^{S\text{-}638}$, $Dsor1/mek^{XS\text{-}520}$, and $Src42A^{l(2)k10108}$, or alleles isolated in previous modifier screens in the laboratory, which included S^{BA651}, $rolled/mapk^{S\text{-}352}$, pnt^{AP553}, and $yan^{XE\text{-}2140}$. Novel complementation groups were meiotically mapped by using either a second chromosome mapping stock with the recessive markers b^1, pr^1, c^1, px^1, and sp^1 or a third chromosome mapping stock that included the recessive markers ru^1, h^1, th^1, st^1, cu^1, sr^1, e^1, and ca^1.

Histology: Scanning electron microscopy and apical sec-

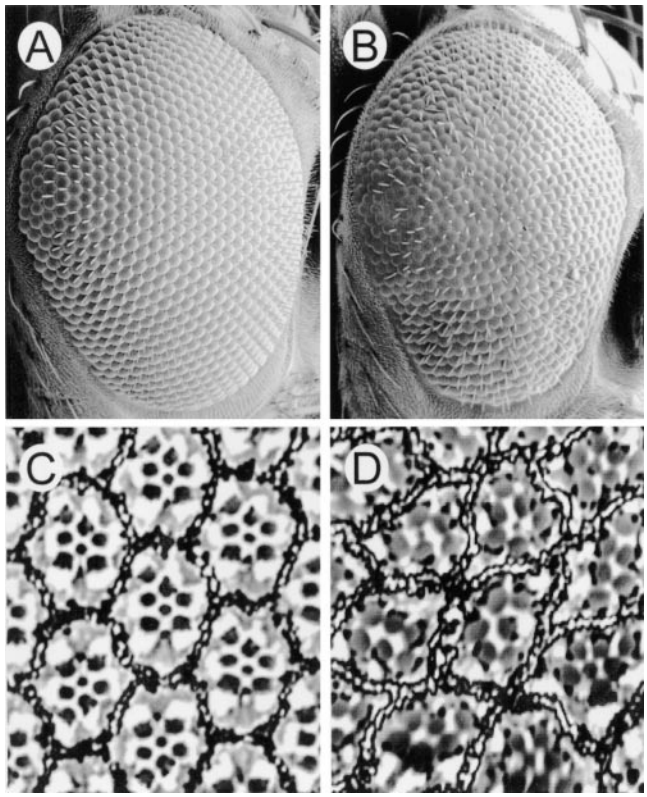


FIGURE 1.—Overexpression of a dominant-negative form of KSR (KDN) during Drosophila eye development blocks photoreceptor cell differentiation. (A and B) Scanning electron micrographs and (C and D) apical sections of adult eyes of the following genotypes: (A and C) wild type; (B and D) *CyO, P*[*sE-KDN*]/+. Analysis of *sE-KDN* apical eye sections reveals that 80% of the ommatidia are missing an R7 photoreceptor cell and 65% are also missing an outer photoreceptor cell. Anterior is to the right.

tions of adult eyes were performed as described by KIMMEL *et al.* (1990) and by TOMLINSON and READY (1987), respectively.

Isolation of *kismet* cDNA clones: Genomic DNA flanking the *l(2)07812 P* element was isolated by plasmid rescue and used to probe a Drosophila embryonic cDNA library (LD library; Berkeley Drosophila Genome Project). Three overlapping cDNA clones (*kis2*, *kis30*, and *kis40A*) were isolated and sequenced on both strands to assemble a 17.4-kb contig that contained a complete (15,966 bp) open reading frame.

RESULTS

Strategy for the KDN dominant modifier screen: To identify novel signaling components that may be relevant for the function of KSR, we conducted an F_1 genetic screen in Drosophila designed to isolate dominant enhancers and suppressors of a KSR-dependent rough eye. We expressed the kinase domain of Drosophila KSR (Δ*Nksr*; amino acids 484–966) fused to the amino terminus of Torso4021 (Tor^{4021}; DICKSON *et al.* 1992) under the control of the *sevenless* (*sev*) enhancer/*hsp70* promoter (*sE*; BASLER *et al.* 1991); this directs expression in a subset of cells in the eye (R3, R4, and R7 photoreceptor cells). As shown in Figure 1, overexpression of *tor*^{4021}Δ*Nksr* in the eye resulted in the roughening of the external eye surface (compare Figure 1A and 1B), which correlated with a block in photoreceptor cell differentiation (compare Figure 1C and 1D; see also THERRIEN *et al.* 1996). This phenotype appeared to be dependent on KSR activity since overexpression of a "kinase-defective" *tor*^{4021}Δ*Nksr* construct, where an invariant lysine residue within kinase subdomain II (amino acid 705) of KSR has been changed to a methionine residue, was unable to mediate this effect (THERRIEN *et al.* 1996). Because Drosophila photoreceptor cell differentiation is controlled by the Ras1 pathway (SIMON *et al.* 1991), the ability of *tor*^{4021}Δ*Nksr* to antagonize photoreceptor differentiation suggests that, like mKSR1, the isolated Drosophila kinase domain acts in a dominant-negative manner to interfere with Ras1 signaling. In agreement with this, we found that the ability of activated Ras1 (Ras1^{V12}) to transform cone cells into R7 cells is strongly suppressed by the coexpression of *tor*^{4021}Δ*Nksr* (data not shown). For simplicity, we rename the *tor*^{4021}Δ*Nksr* construct, *KDN*.

Our previous work characterizing mKSR1 function in the Xenopus oocyte system suggests that the mKSR1 kinase domain blocks RAS-mediated signal transmission at a step between RAF and MEK (THERRIEN *et al.* 1996). Given the high degree of sequence conservation between Drosophila and mammalian KSR (THERRIEN *et al.* 1995) and the finding that the mouse and Drosophila KSR kinase domains behave in a similar fashion, we suspect that these proteins may interfere with RAS signaling by a related if not identical mechanism. A schematic representation of the putative effect of KDN on RAS/MAPK signaling during photoreceptor cell differentiation is shown in Figure 2. In contrast to the effect of Ras1^{V12}, which induces the transformation of cone cells into additional R7 cells by increasing signaling through the MAPK pathway, KDN interferes with the differentiation of photoreceptor cells that express high levels of the transgene (R3, R4, and R7), presumably because it reduces signaling through the MAPK pathway.

As with the Ras1^{V12} rough eye phenotype (KARIM *et al.* 1996), the KDN eye phenotype is dosage-sensitive in that, when compared to the expression of a single copy, two copies of the KDN transgene produced a much rougher eye that is severely reduced in size (data not shown). This indicates that the KDN-modulated signaling is at a threshold and that the phenotype would be suitable for a dominant modifier screen. Thus, we predict (as is shown in Figure 2) that mutations in genes encoding components of the MAPK pathway should dominantly modify the KDN-dependent eye phenotype since a twofold decrease in their respective gene products would alter signaling through the MAPK pathway. In agreement with this prediction, we found that loss-of-function alleles of *mapk* and *yan* dominantly enhanced and suppressed the KDN rough eye phenotype,

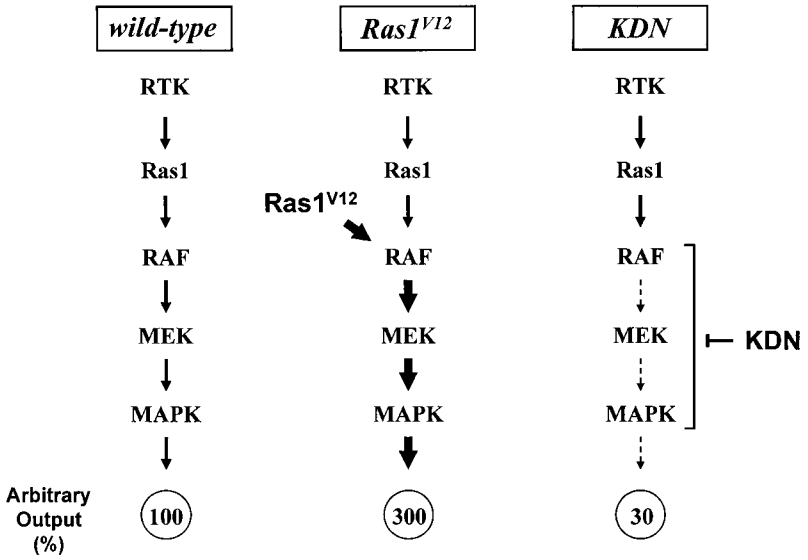


FIGURE 2.—Models depicting the effect of Ras1^{V12} and KDN on the RTK/MAPK pathway. Compared to normal (100%) signaling output generated by a wild-type MAPK pathway, overexpression of Ras1^{V12} in cells increases signaling output of the pathway (a threefold increase is provided as an example) probably by directly overstimulating RAF activity. Conversely, overexpression of KDN attenuates the signaling output of the pathway (a threefold decrease is shown as an example) probably by interfering with signal transmission within the MAPK cascade (THERRIEN *et al.* 1996).

respectively (data not shown). A second prediction of our model is that the KDN phenotype should allow us to isolate mutations in genes functioning either downstream or upstream of *Ras1* since both classes of mutants might have an effect on a crippled MAPK pathway. This is in contrast to the use of a gain-of-function molecule such as Ras1^{V12} where upstream inputs are more likely to be negligible (see Figure 2). Consistent with this interpretation, our previous Ras1^{V12} dominant modifier screen failed to identify mutations in signaling components functioning upstream of Ras1 (KARIM *et al.* 1996).

As depicted in Figure 3A, the screen was performed by crossing w^{1118} males, mutagenized with either EMS or X rays, to females carrying the *sE-KDN* construct in a *pW8 P*-element vector inserted onto either the *CyO* balancer (*CKDN* flies) or the *TM3* balancer (*TKDN* flies). To minimize the risk that a particular integration site prevents the detection of certain modifiers, two independent *P*-element lines (*CKDN* and *TKDN*) were used. Approximately 75,000 and 110,000 progeny were derived from EMS-treated and X-ray-treated males, respectively, and scored for modification of eye roughness. Adult flies having eyes with a smoother external appearance (more regular ommatidial array) were isolated as suppressors, while flies having a rougher eye appearance (a more disorganized ommatidial array that is often accompanied by a slight reduction in the size of the eye) were identified as enhancers.

The results of the screen: A total of 720 *Enhancers of KDN* (*EKDN*) and 111 *Suppressors of KDN* (*SKDN*) lines were established (Figure 3B). On the basis of our previous results with the Ras1^{V12} dominant modifier screen (KARIM *et al.* 1996), we predicted that at least two main classes of modifiers would be isolated. The first class consists of mutations in RAS/KSR signaling components, while the second class represents mutations in genes affecting the expression level of the *KDN* transgene (basic transcription machinery components, transcription factors specific for the *sevenless* enhancer, etc.). To identify and eliminate mutations of the second class, a simple test cross was performed. We reasoned that mutations in genes controlling the expression of the *KDN* transgene might similarly affect the expression level of the Ras1^{V12} transgene. For example, a mutation that increases the expression of KDN would be isolated as an enhancer. If the same mutation also increases the expression of Ras1^{V12}, it would likewise be scored as an enhancer of Ras1^{V12} even though KDN and Ras1^{V12} generate opposite cellular phenotypes. In contrast, a mutation that decreases RAS/KSR signaling should enhance the KDN phenotype, but suppress the Ras1^{V12} phenotype if it functions downstream of Ras1, or have no effect on the Ras1^{V12} phenotype if it functions upstream of Ras1 or in a parallel pathway (like a putative Ras1-independent KSR-specific pathway). It should be noted that some modifiers of KDN might not interact with *sev-Ras1^{V12}* if they function sufficiently far downstream of Ras1.

Therefore, to eliminate modifiers that most likely affect the expression level of KDN, we crossed the *EKDN* and *SKDN* lines to flies expressing Ras1^{V12} under the *sevenless* enhancer/promoter cassette (*sev-Ras1^{V12}*; KARIM *et al.* 1996). Of the 720 *EKDN*, 118 dominantly enhanced Ras1^{V12} rough eye phenotype and were not further characterized (Figure 3B). For the *SKDN*, 3 of the 111 lines suppressed Ras1^{V12} and were eliminated. Of the remaining 602 *EKDN*, 55 are homozygous viable mutations that show no apparent phenotype. We have not determined allelism for these viable enhancers and do not know whether they form viable complementation groups or are hypomorphic alleles of recessive lethal groups. By performing lethal complementation tests, allelism was determined for the remaining 547 recessive lethal *EKDN* lines. Fifteen lethal complementation

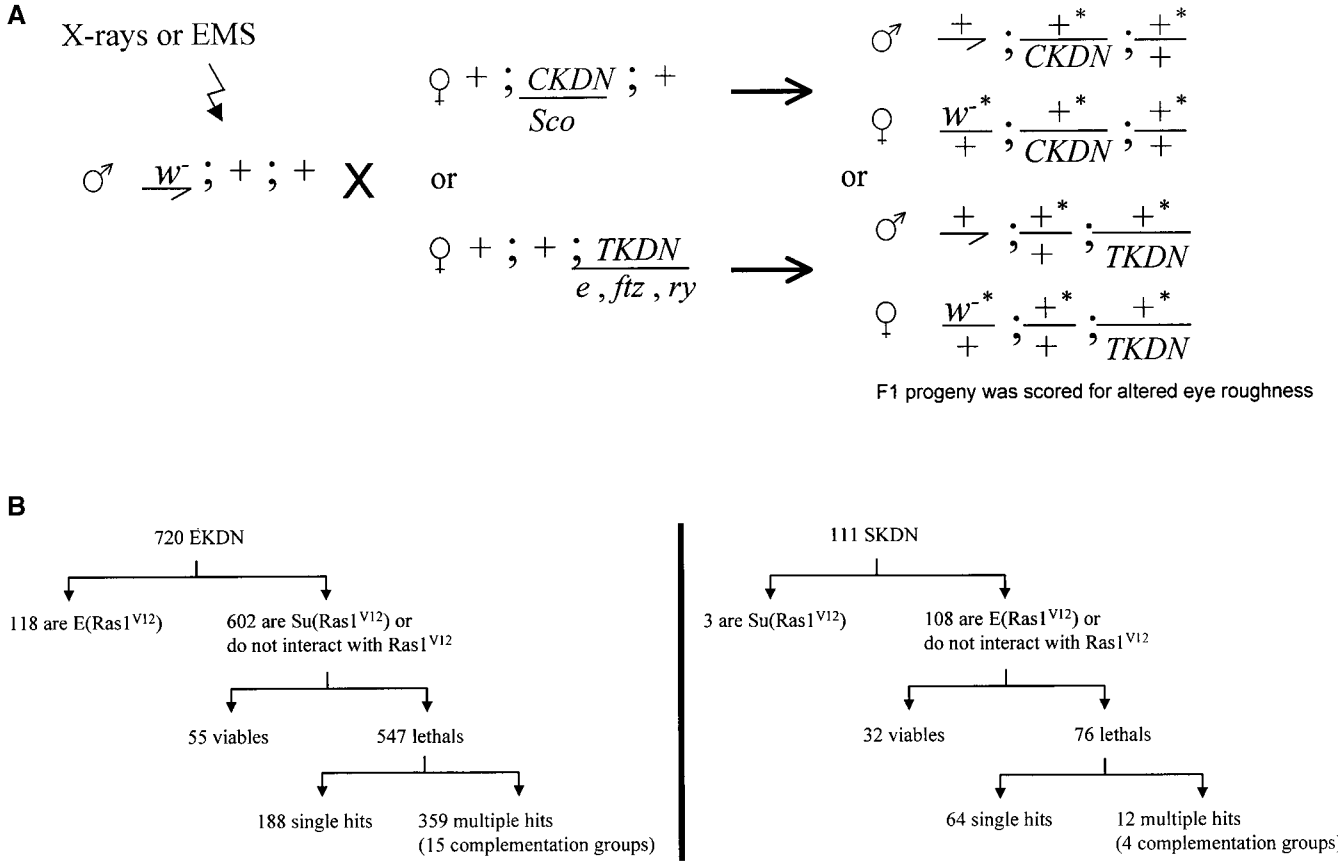


FIGURE 3.—Schematic representation of the KDN dominant modifier screen strategy and breakdown of the collection of modifiers isolated. (A) w^- males isogenic for the second and the third chromosomes were mutagenized (X ray or EMS) and crossed to *CKDN* (*CyO*, *sE-KDN*) or *TKDN* (*TM3*, *sE-KDN*) females. The F_1 progeny were scored for rougher (enhancers) or smoother (suppressors) looking eyes. The asterisks show the mutagenized chromosomes. (B) The numbers of *Enhancers of KDN* (*EKDN*) and *Suppressors of KDN* (*SKDN*), respectively, falling into the categories defined in the text.

groups that included 362 *EKDN* alleles were derived. The remaining 185 recessive lethal enhancers did not fall into any lethal complementation group and appear to be single-hit mutations. However, since these lines have not been further characterized, it is possible that they may be allelic to some of the viable enhancers mentioned above but are recessive lethal because they carry a second-site lethal mutation.

Of the 108 remaining *SKDN*, 32 are homozygous viable. Although they have not been further analyzed, some of these viable alleles (all on the second chromosome) display a slight recessive rough eye phenotype. Complementation tests for the recessive rough eye phenotype and genetic mapping will be required to establish allelism between these viable enhancers. For the remaining 76 recessive lethal *SKDN* lines, allelism was determined by performing lethal complementation tests. Four lethal complementation groups that included 12 *SKDN* alleles were identified. The remaining 64 recessive lethal suppressors did not fall into any lethal complementation group and appear to be single-hit mutations. It is possible, however, that they are homozygous viable enhancers with a second-site recessive lethal mutation. Genetic mapping will be required to determine possible allelism among themselves and the viable *SKDN* alleles mentioned above.

Tables 1 and 2 summarize the enhancer and suppressor groups, respectively. The ability of a particular group to modify the $Ras1^{V12}$ eye phenotype is shown as are the genetic interactions between representative alleles of the modifier groups and a temperature-sensitive hypomorphic allele of Raf, Raf^{HM7}. Hemizygous Raf^{HM7} males die as pharate adults at 25°, but eclose as viable adults at 18° and have a mild rough eye phenotype (MELNICK *et al.* 1993). Heterozygous mutations in *bona fide* components of the Ras1 pathway have been shown to interact genetically with Raf^{HM7} (KARIM *et al.* 1996). Mutations in genes that mediate Ras1 signals enhance the lethality of Raf^{HM7} at 18°, while mutations in negative regulators suppress the lethality at 25°. These interactions occur most likely because the Ras1/MAPK pathway is severely attenuated in Raf^{HM7} hemizygous males; therefore, a slight variation in signaling strength caused by mutations in other components of the pathway can significantly alter the overall signaling output.

To determine the identity of the suppressor and en-

TABLE 1

Groups of lethal enhancers of KDN

Chromosome	Groups	Genes	Map position	No. of alleles	*Ras1*^{V12}	*Raf*^{HM7}
X	*EK1-1*	*Dsor1/mek*	8D3–9	5/8	S	ND
II	*EK2-1*	*rolled/mapk*	h41	70/53	S	E
	EK2-2	*Star*	21E2	18/29	No	E
	EK2-3	*cnk*	54B10–13	12/23	S	E
	EK2-4	*kismet*	21B7	2/15	S	E[a]
	EK2-5		Second	—/5	S	No
	EK2-6	*egfr*	57F1	4/17	No	E
	EK2-7		Second	—/4	S	No
	EK2-8	*sos*	34D	—/2	No	E
	EK2-9	*spen*	21B7	—/4	No	E
III	*EK3-1*	*ksr*	83A3–5	6/20	S	E
	EK3-2	*pnt*	92F1–2	14/29	S	E
	EK3-3[b]		Between *cu* and *sr*	8/8	S	E
	EK3-4	*Ras1*	85D11–27	1/4	No	E
	EK3-5	*14-3-3ε*	90F6–7	—/1	S	E

List of the enhancer groups found on the *X*, the second (*II*), and the third (*III*) chromosomes. The number of X-ray-/EMS-induced alleles isolated for each group is shown. The ability of representative alleles for each group to either suppress (S) or enhance (E) the *sev-Ras1*^{V12} external rough eye phenotype (Karim *et al.* 1996) or *Raf*^{HM7} hemizygous male lethality is indicated. No, no genetic interaction has been observed; ND, not determined.

[a] *EK2-4* alleles enhance eye roughness of *Raf*^{HM7} at 18°, but do not enhance the lethality.

[b] *EK3-3* alleles might define a haploinsufficient locus since all the alleles show reduced viability and have a slight external rough eye phenotype as heterozygotes.

hancer groups, lethal complementation tests were conducted using loss-of-function mutations in known components of the RAS pathway (see MATERIALS AND METHODS). This analysis clearly indicated that the screen was specific for the Ras1 pathway and revealed that 9 of the 15 enhancer groups and one of the four suppressor groups corresponded to loss-of-function mutations in known components of the Ras pathway in Drosophila (Wassarman and Therrien 1997). The loci identified in the enhancer groups correspond to the *egf receptor (egfr)*, *Star*, *Son-of-sevenless* (*Sos*), *Ras1*, *ksr*, *14-3-3ε*, *Dsor1/mek*, *rolled/mapk*, and *pointed* (*pnt*) loci, while the locus corresponding to the suppressor group is *yan* (Tables 1 and 2). The two genetic tests used to classify the groups further corroborated the identity of the known components. As expected, all of the enhancer groups enhanced *Raf*^{HM7} lethality at 18°. The groups corresponding to genes functioning downstream of *Ras1* (*ksr*, *14-3-3ε*, *Dsor1/mek*, *rolled/mapk*, and *pnt*) suppressed Ras1^{V12} rough eye phenotype, while the ones corresponding to *Ras1* itself or functioning upstream of *Ras1* (*egfr*, *Star*, and *Sos*) did not interact with Ras1^{V12} (Table 1). In addition, *yan* alleles enhanced Ras1^{V12} rough eye phenotype and suppressed *Raf*^{HM7} lethality at 25° (Table 2).

Since loci that interact genetically with *Raf*^{HM7} are

TABLE 2

Groups of lethal suppressors of KDN

Chromosome	Groups	Genes	Map position	No. of alleles	*Ras1*^{V12}	*Raf*^{HM7}
II	*SK2-1*	*yan*	22D1–2	1/2	E	S
	SK2-3		Second	—/2	E	No
	SK2-4	*Src42A*	42A1–2	—/3	E	S
III	*SK3-1*		99A–B	1/3	E	S

List of the suppressor groups found on the second (*II*) and the third (*III*) chromosomes. The number of X-ray-/EMS-induced alleles isolated for each group is shown. The ability of representative alleles for each group to either suppress (S) or enhance (E) the *sev-Ras1*^{V12} external rough eye phenotype (Karim *et al.* 1996) or *Raf*^{HM7} hemizygous male lethality is indicated. No, no genetic interaction has been observed.

more likely to encode general signaling molecules functioning in the Ras1 pathway, meiotic and deficiency mapping was conducted on the remaining modifier groups that altered the Raf^{HM7} phenotype. They included four enhancer groups, *EK2-3*, *EK2-4*, *EK2-9*, and *EK3-3*, and two suppressor groups, *SK2-4* and *SK3-1* (Tables 1 and 2). Recently, we reported the identification of *EK2-3* and *SK2-4*, which encode a putative multiadaptor protein named *c*onnector e*n*hancer of *K*SR (CNK) and a Drosophila homologue of Src tyrosine kinase named Src42A, respectively (Therrien *et al.* 1998). The identification of *EK2-9* and *EK2-4* is presented below, while *EK3-3* and *SK3-1* remain to be characterized.

The *EK3-3* locus is recessive lethal and has been meiotically mapped between *cu* and *sr* on *3*R. No deficiencies have been found to uncover this locus. As heterozygotes, all the *EK3-3* alleles display a slight external rough eye phenotype and wing vein defects, and their viability appears to be reduced (data not shown). The *SK3-1* group includes four alleles that have been meiotically mapped to ~3–100 on *3*R. Two of the alleles ($SK3\text{-}1^{XS\text{-}88}$ and $SK3\text{-}1^{S\text{-}331}$) are homozygous viable with no apparent phenotype, while the two others ($SK3\text{-}1^{S\text{-}292}$ and $SK3\text{-}1^{S\text{-}355}$) are recessive lethal. The $SK3\text{-}1^{S\text{-}292}$ and $SK3\text{-}1^{S\text{-}355}$ alleles were further mapped to 99A1–B11, which corresponds to the breakpoints of *Df(3R)Dr-rv1*, a deficiency that uncovers their lethality.

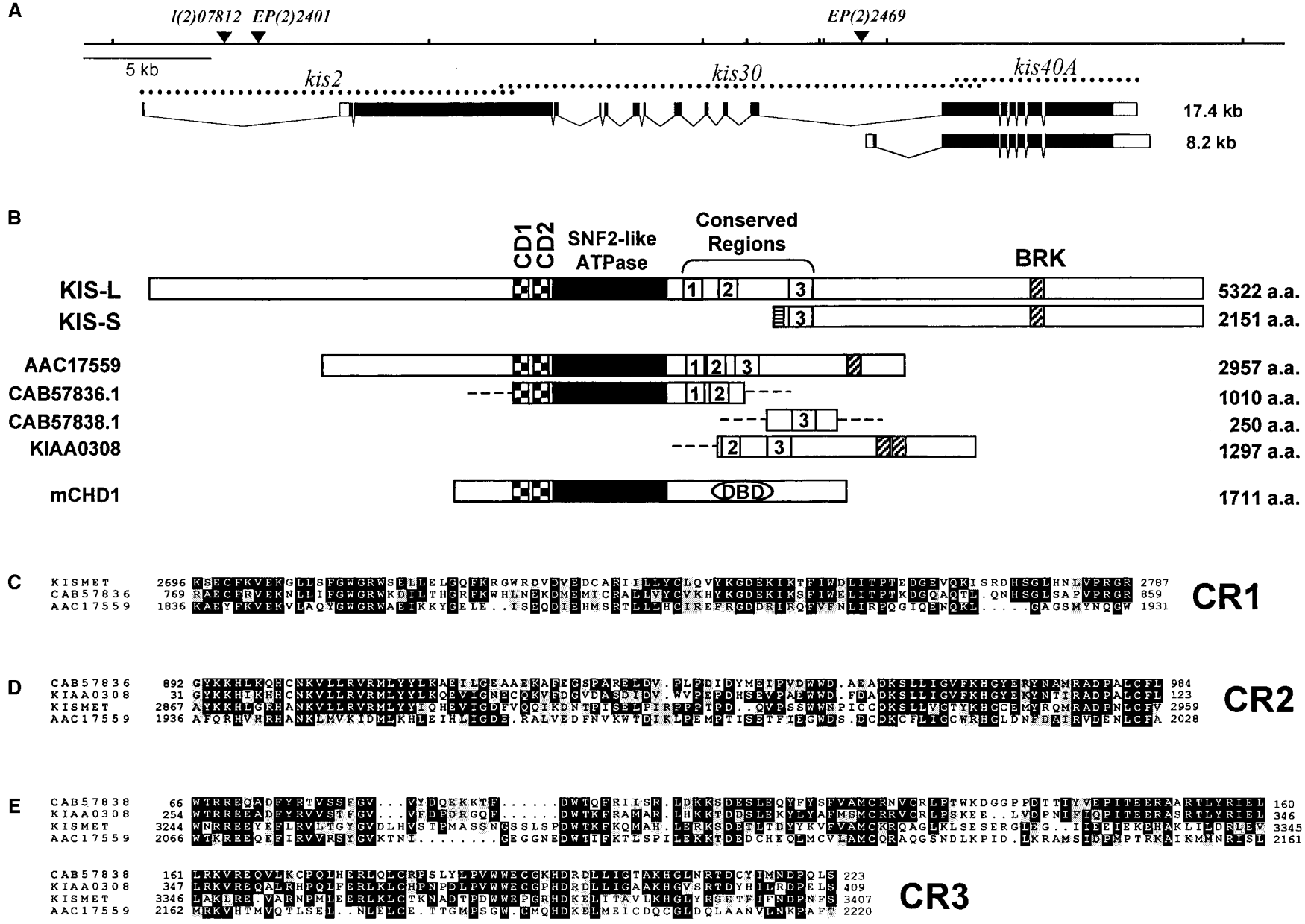
The *SK3-1* viable alleles were grouped with the lethal alleles on the basis of the following criteria: first, the ability of all *SK3-1* alleles to suppress *KDN* was mapped to the same location. Second, all alleles enhanced the $Ras1^{V12}$ eye roughness and efficiently suppressed Raf^{HM7} lethality at 25°. Third, although the viable alleles are also viable over the *Df(3R)Dr-rv1* chromosome, they are subviable when placed over the lethal $SK3\text{-}1^{S\text{-}355}$ allele and display a clear external rough eye phenotype and wing defects (missing veins and atrophied wings; data not shown). No *P* element disrupting this novel locus has yet been identified.

The *EK2-9* group comprises four recessive lethal EMS alleles (Table 1) that do not dominantly suppress the $Ras1^{V12}$ rough eye phenotype but do enhance Raf^{HM7} lethality at 18°. These data suggest that *EK2-9* might encode a general signaling component required either upstream or in a parallel pathway to *Ras1*. *EK2-9* alleles were meiotically mapped to the distal end of the left arm of chromosome *2*. Consistent with this, the lethality was uncovered by *Df(2L)net-PMF* (21A1–B8), but was not uncovered by *Df(2L)net62* (21A1–B4), thus placing the locus at 21B4–B8 in the nonoverlapping interval between the two deficiencies. *P* elements mapping to this region were tested for noncomplementation of the lethality of the *EK2-9* alleles. Two independent lethal *P* elements [*l(2)03350* and *l(2)k10325*] failed to complement the recessive lethality associated with *EK2-9*. These *P* elements have recently been shown to disrupt a gene, *split ends* (*spen*), which encodes an RNA recognition motif (RRM)-containing protein (Wiellette *et al.* 1999; Rebay *et al.* 2000). Therefore, on the basis of the complementation test results, we conclude that *EK2-9* is allelic to *spen*.

Molecular characterization of *EK2-4*: The *EK2-4* alleles are recessive lethal and they dominantly suppress the $Ras1^{V12}$ rough eye phenotype. The *EK2-4* alleles, however, do not enhance Raf^{HM7} lethality at 18°, but do strikingly enhance the rough eye phenotype seen in hemizygous Raf^{HM7} males (data not shown). These observations suggest that the protein encoded by *EK2-4* is not a general Ras1 signaling component, but rather that its function is required for some specific aspects of Ras1 signaling during eye development. As with *EK2-9*, the *EK2-4* alleles were meiotically mapped to the distal end of *2*L. The recessive lethality associated with the *EK2-4* alleles is also uncovered by *Df(2L)net-PMF*, but not by *Df(2L)net62*, placing the locus at 21B4–8. The *EK2-4* group, however, is not allelic to *EK2-9*. Several *P* elements that disrupt the *kismet* locus fail to complement the lethality of all the *EK2-4* alleles tested. In addition, an unrelated *kismet* allele, kis^{1}, isolated as a dominant suppressor of PC^{4} (Kennison and Tamkun 1988), also fails to complement the lethality of the *EK2-4* alleles. Together, these results strongly suggest that the *EK2-4* locus corresponds to the *kismet* gene.

To identify a cDNA encoding the kismet/EK2-4 gene, we first isolated genomic DNA that flanked *l(2)07812* (a *P* element that is allelic to *kismet/EK2-4*). When this DNA was used to screen a Drosophila embryonic cDNA library, one class of transcripts was isolated. The sequence of the longest cDNA (*kis2*; Figure 4A) contains a single long open reading frame (ORF) that lacks a STOP codon. We then used the *kis2* cDNA to isolate overlapping cDNAs extending the sequence in the 3′ direction until a STOP codon was reached. As shown in Figure 4A, two additional cDNAs (*kis30* and *kis40A*) were sequentially isolated and, together with *kis2*, formed an ~17.4-kb cDNA contig. Alignment of the putative *kismet* transcript sequence to the genomic sequence [Berkeley Drosophila Genome Project (BDGP)], revealed a genomic organization of 18 exons spread over ~40 kb (Figure 4A). In addition, three *P* elements that fail to complement the *EK2-4* alleles are found to be inserted in either the first or the twelfth introns, strongly suggesting that the relevant cDNAs had been isolated. Although the assembled *kismet* cDNA contig is artificial, two naturally occurring *kismet* transcripts of ~8.5 and 17 kb have been recently reported (Daubresse *et al.* 1999). Daubresse *et al.* (1999) also reported the isolation and the complete sequence of the shorter form (8.2 kb), which shares the last six exons with our 17.4-kb contig (Figure 4A). It is not clear whether these two types of transcript are generated by alternative spicing or by the use of different promoters.

Conceptual translation of the longest ORF (15,966 bp) in the 17.4-kb *kismet* cDNA contig predicts a protein


A
l(2)07812
EP(2)2401
EP(2)2469
5 kb
kis2
kis30
kis40A
17.4 kb
8.2 kb
B
CD1
CD2
SNF2-like ATPase
Conserved Regions
BRK
KIS-L
KIS-S
AAC17559
CAB57836.1
CAB57838.1
KIAA0308
mCHD1
DBD
5322 a.a.
2151 a.a.
2957 a.a.
1010 a.a.
250 a.a.
1297 a.a.
1711 a.a.
C
CR1
D
CR2
E
CR3

of 5322 amino acids, named KISMET long isoform (KIS-L; Figure 4B). The shorter form, named KISMET short isoform (KIS-S), is 2151 amino acids long and is derived from the 8.2-kb contig described by Daubresse *et al.* (1999). The first 46 amino acids of KIS-S are unique, while the remaining 2105 amino acids are shared with KIS-L (Figure 4B). BLASTP searches of the GenBank database using the KIS-L amino acid sequence revealed the presence of two chromodomains followed by a SNF2-like ATPase domain; when compared to known proteins, this domain organization is most similar in sequence and in structure to the chromodomain-helicase domain (CHD) proteins (Woodage *et al.* 1997; Figure 4B). The homology with the CHD proteins, however, does not extend to other regions of the KIS proteins. One additional region of homology was previously identified in KIS-S by Daubresse *et al.* (1999). This region is named the Brahma (BRM)-KIS (BRK) domain and corresponds to a short amino acid sequence that exhibits homology between the KIS proteins and proteins of the Brahma family.

Database searches also identified several putative proteins in other phyla with high homology to KIS-L (Figure 4B). Most of them correspond to partial sequences, so it is not clear whether they are completely related to KIS-L. Nonetheless, the high degree of homology with KIS-L is a strong indication that KISMET homologues exist in other species. Interestingly, in addition to the known domains mentioned above, sequence comparison between KIS-L and some of the homologues has identified three novel *c*onserved *r*egions (CR1, CR2, and CR3; Figure 4B). Except KIS-S, which also contains the CR3 domain, database searches failed to identify known proteins containing any of these three CR domains.

DISCUSSION

ksr has been genetically identified as an important mediator of RAS-dependent signals. Although biochemical evidence suggests that KSR is directly involved in the MAPK pathway, its molecular function remains unclear. In this report, we present the results of a dominant modifier screen performed in Drosophila that was based on a KSR-dependent rough eye phenotype. We reasoned that some of the loci identified in such a screen might encode novel signaling molecules that could shed light on the function of KSR and/or might provide new clues regarding other aspects of RAS-dependent signaling mechanisms.

Since KSR activity appears to be required for the MAPK pathway (see Introduction), we expected that the KDN screen would uncover mutations in several known loci involved in RAS/MAPK signaling during Drosophila eye development (Wassarman and Therrien 1997). As predicted, 9 (*egfr*, *Star*, *Sos*, *Ras1*, *14-3-3ε*, *ksr*, *Dsor1/mek*, *rolled/mapk*, and *pointed*), of the 15 complementation groups of *Enhancer of KDN*, and one (*yan*) of the four groups of *Suppressor of KDN*, correspond to such critical genes (Tables 1 and 2). This result not only strongly correlates with the biochemical findings that KSR is required for signaling through the MAPK pathway but also demonstrates the effectiveness of the screen in identifying signaling components relevant for RTK/RAS-dependent signaling. Thus, it would be pre-

Figure 4.—Molecular characterization of the *kismet* locus and identification of its gene products. (A) Genomic organization of the *kismet* locus. The top line represents genomic DNA. The inverted triangles and the ticks on top of the line mark the position of *P*-element insertion sites and the *Eco*RI restriction sites, respectively. The position of the *P* elements was provided by aligning the flanking genomic DNA sequence available for each *P*-element insertion (Berkeley Drosophila Genome Project) to the genomic sequence of the *kismet* locus derived from two overlapping P1 genomic clones (see below). The three dotted lines represent the extent of the longest overlapping cDNAs isolated (*kis2*, *kis30*, and *kis40A*), which together form a 17.4-kb contig (GenBank accession no. AF215703). The exon/intron arrangement of the *kismet* gene was determined by aligning the 17.4-kb cDNA sequence with the *kismet* genomic sequence contained within two overlapping P1 genomic clones (GenBank accession nos. AC004274 and AC005334). Solid and open boxes denote the coding and noncoding sequences, respectively. The structure of the short 8.2-kb *kismet* transcript (Daubresse *et al.* 1999) is shown as a reference (GenBank accession no. AF113847). (B) Schematic structure of the long (KIS-L) and the short (KIS-S) KISMET protein isoforms. Database searches identified two chromodomains (checkered boxes: CD1, amino acid positions 1793–1921, and CD2, amino acid positions 1939–1996) and a SNF2-like ATPase domain (solid box: amino acid positions 2029–2626) specifically found in the KIS-L isoform. The two KIS isoforms, however, share a BRK domain (hatched box: amino acid positions 4376–4416 in KIS-L) recently defined as a 41-amino-acid motif conserved between KIS-S (amino acid positions 1205–1245) and the Brahma proteins (Daubresse *et al.* 1999). The schematic structure of mouse CHD1 (GenBank accession no. L10410) is provided as an example. In addition to the chromodomains and the SNF2-like ATPase domain, the CHD proteins also include a putative DNA-binding domain (DBD) that is not found in KIS-L. Database searches also identified a partial human expressed sequence tag (KIAA0308) and three predicted ORFs deduced from genomic sequences [a *C. elegans* sequence (protein ID, AAC17559), and two partial human sequences (protein IDs, CAB57836.1 and CAB57838.1)] that represent better KISMET homologues than the CHD proteins. For instance, the potentially full-length AAC17559 *C. elegans* sequence and the partial CAB57836.1 human sequence have a higher homology in the chromo-ATPase region (~65% identity) compared to the CHD proteins (~44% identity) and have additional conserved regions (1, 2, and 3) that are not found in the CHD proteins. Furthermore, the *C. elegans* sequence also contains a BRK domain. The two other partial human sequences (CAB57838.1 and KIAA0308) also contain some of the conserved regions found only in KISMET. KIAA0308 also contains two BRK domains. (C–E) Amino acid sequence comparison of the conserved regions (CR1, CR2, and CR3) found between KIS-L and the putative ORFs. Identical and conserved residues are highlighted in black and gray, respectively.

dicted that some of the novel loci may also encode important components of this signaling route.

Six (*EK2-3*, *EK2-4*, *EK2-9*, *EK3-3*, *SK2-4*, and *SK3-1*) of the nine novel complementation groups genetically interact with Raf^{HM7} (Tables 1 and 2). Since loci encoding *bona fide* components of the Ras1 pathway show an interaction with Raf^{HM7}, it is likely that these six new loci may also encode proteins involved in Ras1 signaling. In contrast, the three complementation groups (*EK2-5*, *EK2-7*, and *SK2-3*) that did not interact with Raf^{HM7} (Tables 1 and 2) are less likely to encode critical mediators of the pathway. However, we cannot exclude the possibility that there may be alternative explanations as to why these groups fail to interact with Raf^{HM7}. For instance, it is possible that the mutations for each of these three groups represent weak loss-of-function alleles that do not significantly alter signaling in the Raf^{HM7} background. It is also possible that these groups encode molecules required in other Ras1-dependent but MAPK-independent pathways and that the Raf^{HM7} assay is unable to detect genetic interactions with such signaling components. The ability of these three groups to interact with $sev\text{-}Ras1^{V12}$ (Tables 1 and 2) is consistent with this possibility. Their identification awaits further mapping and molecular characterization.

The identity of four (*EK2-3*, *EK2-4*, *EK2-9*, and *SK2-4*) of the six novel complementation groups that interact with Raf^{HM7} has been determined (Tables 1 and 2). All of the groups except one (*EK2-9*) are able to modify the $sev\text{-}Ras1^{V12}$ rough eye phenotype, indicating that their activity is required downstream of Ras1. From this data alone, we cannot unambiguously determine whether *EK2-9* is required upstream or downstream of Ras1, since it is possible that a 50% reduction in *EK2-9* gene dosage is not sufficient to alter $Ras1^{V12}$ signaling strength. The identity of *EK2-3* and *SK2-4* as *cnk* and *Src42A*, respectively, readily suggests a link to Ras1 signaling, while the proteins encoded by *EK2-4* and *EK2-9* point to novel classes of RAS signal mediators. *EK2-3* encodes a novel 1557-amino-acids long protein, named CNK. CNK contains several protein-protein interaction domains and may function as a multiadaptor molecule (Therrien *et al.* 1998). Consistent with a role in Ras1 signal transduction, *cnk* loss-of-function alleles not only suppress the $Ras1^{V12}$ rough eye phenotype but prevent photoreceptor cell differentiation and impair cell proliferation (Therrien *et al.* 1998). Like *ksr* loss-of-function alleles, *cnk* mutations do not suppress the rough eye phenotype induced by the overexpression of an activated form of Raf, $Tor^{4021}\Delta NRaf$, suggesting that CNK activity is required upstream of or in a parallel pathway to Raf (Therrien *et al.* 1998). In agreement with this position and with the possibility that CNK is required for the MAPK pathway, CNK directly interacts with RAF (Therrien *et al.* 1998). The fact that both CNK (directly) and KSR (indirectly) interact with Raf suggests that some aspects of their function converge on the regulation of Raf. These findings also suggest that KSR and CNK might coexist in a complex, which would reinforce the possibility of a functional relationship between the two molecules. Finally, we have recently presented genetic evidence suggesting that CNK activity is also required for a Ras1-dependent/MAPK-independent pathway (Therrien *et al.* 1999).

Complementation test results reveal that *SK2-4* is allelic to *Src42A* (Therrien *et al.* 1998 and data not shown), the closest Drosophila homolog of the Src kinase family (Takahashi *et al.* 1996). Intriguingly, our genetic data (suppression of *sE-KDN*, enhancement of $sev\text{-}Ras1^{V12}$, and suppression of Raf^{HM7}) suggest an inhibitory role for Src42A in Ras1 signaling, which is opposite to the described function of Src-like kinases in vertebrates (Belsches *et al.* 1997; Hanks and Polte 1997). During the course of this work, *Src42A* was also identified by another laboratory as being a dominant suppressor of a hypomorphic allele of *Raf* (Raf^{C110}; Lu and Li 1999). The genetic and molecular characterization of different *Src42A* alleles by Lu and Li clearly supports an inhibitory function for Src42A in different RTK-dependent signaling pathways. Therefore, the further elucidation of the molecular function of Src42A in Drosophila may unveil a novel mechanism of action for this family of nonreceptor tyrosine kinases.

Since alleles of *EK2-9* failed to complement two independent *P* elements recently shown to disrupt the *split ends* locus (*spen*; Wiellette *et al.* 1999; Rebay *et al.* 2000), we conclude that EK2-9 is allelic to *spen*. This gene encodes at least three large (~5500 amino acids) and closely related protein isoforms (Wiellette *et al.* 1999; Rebay *et al.* 2000). The Spen proteins contain three RRMs at the N terminus and a conserved region of unknown function at the C terminus (Wiellette *et al.* 1999; Rebay *et al.* 2000). The presence of three RRMs suggests that the Spen proteins mediate their effect via a RNA binding-dependent mechanism such as RNA processing or transport. Interestingly, mutant alleles of *spen* have been isolated in several independent genetic screens in Drosophila. They were initially recovered in a screen for mutations affecting peripheral nervous system development (Kolodziej *et al.* 1995). Subsequently, they were isolated in two related screens that, like the KDN screen, were designed to identify novel components of the Ras1 pathway. Mutations in *spen* were isolated as dominant enhancers of a Raf-induced [*E*(*Raf*) *2A* complementation group; Dickson *et al.* 1996] and a Yan-induced rough eye phenotype (Rebay *et al.* 2000). The fact that three separate screens targeting Ras1 signaling (KSR-, Raf-, and Yan-dependent) recovered mutant alleles of *spen* suggests that this locus is relevant for Ras1-mediated signal transduction. Examination of the genetic interactions, however, indicates that the role of *spen* in Ras1 signaling may not be straightforward. For example, the ability of the *E*(*Raf*) *2A*/*spen* alleles to dominantly enhance an activated Raf-dependent phenotype (Dickson *et al.* 1996) suggests that *spen* is a negative regulator of the pathway. However, alleles of *spen* also

enhance Raf^{HM7} lethality at 18° (Dickson *et al.* 1996; Rebay *et al.* 2000; this study), and homozygous mutant clones in the eye are often missing R7 and outer photoreceptor cells, although extra photoreceptor cells are occasionally found (Dickson *et al.* 1996). These results are more consistent with a positive role for *spen* during Ras1 signaling and would agree with the fact that *spen* mutations were recovered as enhancers of *sE-KDN* and $gmr\text{-}yan^{act}$ (Table 1 and Rebay *et al.* 2000). Alleles of *spen* were also recovered as enhancers of a loss-of-function phenotype for the Hox gene *Deformed* (*Dfd*; Gellon *et al.* 1997). In that context, the genetic analysis of *spen* suggests that it functions in parallel to *Dfd* for the specification of head cuticular structures. Finally, mutations in *spen* have been identified as dominant enhancers of an E2F/Dp-induced rough eye phenotype and as dominant suppressors of $p21^{CIP1}$-induced phenotypes (Staehling-Hampton *et al.* 1999). The fact that S-phase entry is stimulated by the overexpression of E2F and Dp proteins in the eye but inhibited by $p21^{CIP1}$ overexpression suggests that *spen* may be involved in the negative regulation of cell cycle progression.

How *spen* activity links Ras1-dependent cell differentiation, Hox-dependent segment specification, and E2F-dependent cell cycle control is unknown. Nonetheless, their common requirement for *spen* function suggests that they are interconnected. In agreement with this idea, other loci have been found to be in common in the screens mentioned above, as well as in other related screens (see below). One of these loci corresponds to the *kismet* (*kis*) gene. In addition to the *EK2-4/kis* alleles identified in this KDN screen, mutations in *kis* were recovered as dominant enhancers in the *Dfd* screen (Gellon *et al.* 1997) and as dominant suppressors in a *Polycomb* (*Pc*) loss-of-function screen (Kennison and Tamkun 1988). Alleles of *kis* have also been identified as dominant suppressors of the synthetic lethality generated by the coexpression of activated Sevenless (SevS11) and $Ras1^{V12}$ (Maixner *et al.* 1998).

The ability of *kis* mutations to suppress the homeotic transformations observed in a *Pc* mutant background and to suppress the expression of the Hox gene *Sex combs reduced* suggests that *kis* is a member of the trithorax group (trxG) of genes (Kennison and Tamkun 1988; Daubresse *et al.* 1999). In Drosophila, trxG genes are essential for Hox gene expression, where they appear to counteract the repressive effect of the Polycomb group (PcG) of genes on Hox gene expression (reviewed in Pirrota 1998). Interestingly, a number of trxG proteins are similar to components of the large (2 MD) ATPase-dependent SWI/SNF complex that facilitates the access of transcription factors to their DNA binding sites by destabilizing the nucleosomes (Muchardt and Yaniv 1999). It is thus possible that trxG proteins control Hox gene expression by directly altering the chromatin at specific Hox loci. Strikingly, sequence analysis of the KIS proteins reveals strong homologies with the CHD proteins, which represent a novel class of putative ATPase subunits of chromatin remodeling complexes (Woodage *et al.* 1997), and with Brahma, a trxG protein that is the closest fly homologue to the yeast SWI2/SNF2 ATPase subunit (Tamkun *et al.* 1992). These observations strongly suggest that the KIS proteins are also involved in chromatin remodeling. Interestingly, Brahma and KIS proteins do not appear to coexist in a common complex (Daubresse *et al.* 1999). This raises the possibility that KIS is part of a different chromatin remodeling complex that might regulate the transcription of a distinct group of genes. Consistent with this hypothesis, *brahma* mutations have not been isolated in the Ras1 pathway-dependent screens mentioned above. On the basis of the presumed function for KIS and the observation that *kis* loss-of-function mutations suppress $sev\text{-}Ras1^{V12}$, and enhance *sE-KDN* and Raf^{HM7} rough eye phenotypes (Table 1), it is tempting to speculate that KIS is part of a complex that, in response to Ras1-dependent signals, directly alters the transcription of a specific group of genes required for Ras1-dependent cell differentiation during Drosophila eye development.

In summary, the KDN screen has enabled us to identify novel players in the intricate network of proteins that defines the RAS signal transduction pathway. The goal remaining is to elucidate the function of these proteins with respect to KSR and with respect to other components of the pathway. The functional characterizations of CNK and Src42A certainly represent promising avenues to unravel novel features of the Ras1/MAPK pathway, which may also provide important clues to deciphering the molecular function of KSR. Although a direct link may exist between KSR and Spen or KIS, the fact that *spen* and *kis* have been identified in multiple screens and that they encode nuclear proteins (Daubresse *et al.* 1999; Wiellette *et al.* 1999) suggests that they more likely function further downstream in the pathway. Still remaining to be characterized are five additional loci. Based on the genes that have been identified by the KDN screen so far, it is hoped that the identification of the remaining loci will provide new entries into a well-studied pathway.

We are grateful to Audrey Huang for critical reading of the manuscript. We thank Kevin Cook and Kathy Matthews (Bloomington Stock Center) for fly stocks, and the Berkeley Drosophila Genome Project group for the *kismet* genomic sequence. This work was supported by the National Cancer Institute of Canada and the Medical Research Council of Canada (M.T.); the National Cancer Institute, Department of Health and Human Services, under contract with ABL (D.K.M.); and the Howard Hughes Medical Institute (G.M.R.).

LITERATURE CITED

Barrett, K., M. Leptin and J. Settleman, 1997 The Rho GTPase and a putative RhoGEF mediate a signaling pathway for the cell shape changes in Drosophila gastrulation. Cell **91:** 905–915.

Basler, K., B. Christen and E. Hafen, 1991 Ligand-independent activation of the sevenless receptor tyrosine kinase changes the fate of cells in the developing Drosophila eye. Cell **64:** 1069–1081.

Belsches, A. P., M. D. Haskell and S. J. Parsons, 1997 Role of

c-Src tyrosine kinase in EGF-induced mitogenesis. Front Biosci. **2:** 501–518.

Cacace, A. M., N. R. Michaud, M. Therrien, K. Mathes, T. Copeland *et al.*, 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.

Campbell, S. L., R. Khosravi-Far, K. L. Rossman, G. J. Clark and C. J. Der, 1998 Increasing complexity of Ras signaling. Oncogene **17:** 1395–1413.

Cobb, M. H., 1999 MAP kinase pathways. Prog. Biophys. Mol. Biol. **71:** 479–500.

Daubresse, G., R. Deuring, L. Moore, O. Papoulas, I. Zakrajsek *et al.*, 1999 The Drosophila kismet gene is related to chromatin-remodeling factors and is required for both segmentation and segment. Development **126:** 1175–1187.

Denouel-Galy, A., E. M. Douville, P. H. Warne, C. Papin, D. Laugier *et al.*, 1998 Murine Ksr interacts with MEK and inhibits Ras-induced transformation. Curr. Biol. **8:** 46–55.

Dickson, B., F. Sprenger, D. Morrison and E. Hafen, 1992 Raf functions downstream of Ras1 in the Sevenless signal transduction pathway. Nature **360:** 600–603.

Dickson, B. J., A. Van Der Straten, M. Dominguez and E. Hafen, 1996 Mutations modulating Raf signaling in Drosophila eye development. Genetics **142:** 163–171.

Garrington, T. P., and G. L. Johnson, 1999 Organization and regulation of mitogen-activated protein kinase signaling pathways. Curr. Opin. Cell. Biol. **11:** 211–218.

Gellon, G., K. W. Harding, N. McGinnis, M. M. Matrin and W. McGinnis, 1997 A genetic screen for modifiers of Deformed homeotic function identifies novel genes required for head development. Development **124:** 3321–3331.

Hanks, S. K., and T. R. Polte, 1997 Signaling through focal adhesion kinase. Bioessays **19:** 137–145.

Jacobs, D., D. Glossip, H. Xing, A. J. Muslin and K. Kornfeld, 1999 Multiple docking sites on substrate proteins form a modular system that mediates recognition by ERK MAP kinase. Genes Dev. **13:** 163–175.

Karim, F. D., H. C. Chang, M. Therrien, D. A. Wassarman, T. Laverty *et al.*, 1996 A screen for genes that function downstream of Ras1 during Drosophila eye development. Genetics **143:** 315–329.

Kennison, J. A., and J. W. Tamkun, 1988 Dosage-dependent modifiers of polycomb and antennapedia mutations in Drosophila. Proc. Natl. Acad. Sci. USA **85:** 8136–8140.

Kimmel, B. E., U. Heberlein and G. M. Rubin, 1990 The homeo domain protein rough is expressed in a subset of cells in the developing Drosophila eye where it can specify photoreceptor cell subtype. Genes Dev. **4:** 712–727.

Kolodziej, P. A., L. Y. Jan and Y. N. Jan, 1995 Mutations that affect the length, fasciculation, or ventral orientation of specific sensory axons in the Drosophila embryo. Neuron **15:** 273–286.

Kornfeld, K., D. B. Hom and H. R. Horvitz, 1995 The ksr-1 gene encodes a novel protein kinase involved in Ras-mediated signaling in C. elegans. Cell **83:** 903–913.

Lu, X., and Y. Li, 1999 Drosophila Src42A is a negative regulator of RTK signaling. Dev. Biol. **208:** 233–243.

Maixner, A., T. P. Hecker, Q. N. Phan and D. A. Wassarman, 1998 A screen for mutations that prevent lethality caused by expression of activated sevenless and Ras1 in the Drosophila embryo. Dev. Genet. **23:** 347–361.

Melnick, M. B., L. A. Perkins, M. Lee, L. Ambrosio and N. Perrimon, 1993 Developmental and molecular characterization of mutations in the Drosophila-raf serine/threonine protein kinase. Development **118:** 127–138.

Michaud, N. R., M. Therrien, A. Cacace, L. C. Edsall, S. Spiegel *et al.*, 1997 KSR stimulates Raf-1 activity in a kinase-independent manner. Proc. Natl. Acad. Sci. USA **94:** 12792–12796.

Muchardt, C., and M. Yaniv, 1999 ATP-dependent chromatin remodelling: SWI/SNF and Co. are on the job. J. Mol. Biol. **293:** 187–198.

Pirrotta, V., 1998 Polycombing the genome: PcG, trxG, and chromatin silencing. Cell **93:** 333–336.

Rebay, I., F. Chen, F. Hsiao, P. A. Kolodziej, B. H. Kuang *et al.*, 2000 A genetic screen for novel components of the Ras/MAPK signaling pathway that interact with the *yan* gene of Drosophila identifies *split ends*, a new RRM motif containing protein. Genetics **154:** 695–712.

Simon, M. A., D. D. Bowtell, G. S. Dodson, T. R. Laverty and G. M. Rubin, 1991 Ras1 and a putative guanine nucleotide exchange factor perform crucial steps in signaling by the sevenless protein tyrosine kinase. Cell **67:** 701–716.

Staehling-Hampton, K., P. J. Ciampa, A. Brook and N. Dyson, 1999 A genetic screen for modifiers of E2F in *Drosophila melanogaster*. Genetics **153:** 275–287.

Sternberg, P. W., and J. Alberola-Ila, 1998 Conspiracy theory: RAS and RAF do not act alone. Cell **95:** 447–450.

Stewart, S., M. Sundaram, Y. Zhang, J. Lee, M. Han *et al.*, 1999 Kinase suppressor of Ras forms a multiprotein signaling complex and modulates MEK localization. Mol. Cell. Biol. **19:** 5523–5534.

Sundaram, M., and M. Han, 1995 The C. elegans ksr-1 gene encodes a novel Raf-related kinase involved in Ras-mediated signal transduction. Cell **83:** 889–901.

Takahashi, F., S. Endo, T. Kojima and K. Saigo, 1996 Regulation of cell-cell contacts in developing Drosophila eyes by Dsrc41, a new, close relative of vertebrate c-src. Genes Dev. **10:** 1645–1656.

Tamkun, J. W., R. Deuring, M. P. Scott, M. Kissinger, A. M. Pattatucci *et al.*, 1992 brahma: a regulator of Drosophila homeotic genes structurally related to the yeast transcriptional activator SNF2/SWI2. Cell **68:** 561–572.

Therrien, M., H. C. Chang, N. M. Solomon, F. D. Karim, D. A. Wassarman *et al.*, 1995 KSR, a novel protein kinase required for RAS signal transduction. Cell **83:** 879–888.

Therrien, M., N. R. Michaud, G. M. Rubin and D. K. Morrison, 1996 KSR modulates signal propagation within the MAPK cascade. Genes Dev. **10:** 2684–2695.

Therrien, M., A. M. Wong and G. M. Rubin, 1998 CNK, a RAF-binding multidomain protein required for RAS signaling. Cell **95:** 343–353.

Therrien, M., A. M. Wong, E. Kwan and G. M. Rubin, 1999 Functional analysis of CNK in RAS signaling. Proc. Natl. Acad. Sci. USA **96:** 13259–13263.

Tomlinson, A., and D. F. Ready, 1987 Neuronal differentiation in the Drosophila ommatidium. Dev. Biol. **120:** 366–376.

Verheyen, E. M., K. J. Purcell, M. E. Fortini and S. Artavanis-Tsakonas, 1996 Analysis of dominant enhancers and suppressors of activated Notch in Drosophila. Genetics **144:** 1127–1141.

Wassarman, D. A., and M. Therrien, 1997 Ras-mediated photoreceptor development in Drosophila. Adv. Dev. Biol. **5:** 1–41.

Wiellette, E. L., K. W. Harding, K. A. Mace, M. R. Ronshaugen, F. Y. Wang *et al.*, 1999 *spen* encodes an RNP motif protein that interacts with Hox pathways to repress the development of head-like sclerites in the Drosophila trunk. Development **126:** 5373–5385.

Woodage, T., M. A. Basrai, A. D. Baxevanis, P. Hieter and F. S. Collins, 1997 Characterization of the CHD family of proteins. Proc. Natl. Acad. Sci. USA **94:** 11472–11477.

Xing, H., K. Kornfeld and A. J. Muslin, 1997 The protein kinase KSR interacts with 14-3-3 protein and Raf. Curr. Biol. **7:** 294–300.

Yu, W., W. J. Fantl, G. Harrowe and L. T. Williams, 1998 Regulation of the MAP kinase pathway by mammalian Ksr through direct interaction with MEK and ERK. Curr. Biol. **8:** 56–64.

Zipursky, S. L., and G. M. Rubin, 1994 Determination of neuronal cell fate: lessons from the R7 neuron of Drosophila. Annu. Rev. Neurosci. **17:** 373–397.

Communicating editor: R. S. Hawley