The novel SAM domain protein Aveugle is required for Raf activation in the Drosophila EGF receptor signaling pathway

Jean-Yves Roignant, Sophie Hamel,1 Florence Janody,2 and Jessica E. Treisman3

Skirball Institute for Biomolecular Medicine and Department of Cell Biology, New York University School of Medicine, New York, New York 10016, USA

Activation of the Raf kinase by GTP-bound Ras is a poorly understood step in receptor tyrosine kinase signaling pathways. One such pathway, the epidermal growth factor receptor (EGFR) pathway, is critical for cell differentiation, survival, and cell cycle regulation in many systems, including the Drosophila eye. We have identified a mutation in a novel gene, aveugle, based on its requirement for normal photoreceptor differentiation. The phenotypes of aveugle mutant cells in the eye and wing imaginal discs resemble those caused by reduction of EGFR pathway function. We show that aveugle is required between ras and raf for EGFR signaling in the eye and for mitogen-activated protein kinase phosphorylation in cell culture. Aveugle encodes a small protein with a sterile/H9251 motif (SAM) domain that can physically interact with the scaffold protein connector enhancer of Ksr (Cnk). We propose that Aveugle acts together with Cnk to promote Raf activation, perhaps by recruiting an activating kinase.

[Keywords: Raf; MAP kinase; receptor tyrosine kinase; EGFR; photoreceptor; SAM]

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Many receptor tyrosine kinases (RTKs), including the epidermal growth factor receptor (EGFR) and fibroblast growth factor receptor (FGFR), signal through the Ras/mitogen-activated protein kinase (MAPK) pathway [Nishida and Gotoh 1993]. These receptors have important developmental functions and are also misregulated in a variety of cancers [Holbro and Hynes 2004], making it critical to understand their signaling mechanism. Genetic screens in Drosophila and Caenorhabditis elegans, coupled with biochemical analysis in cultured cells, have allowed the identification of numerous Ras/MAPK pathway components [Rubin et al. 1997; Moghal and Sternberg 2003].

The Drosophila eye is a particularly useful system for genetic analysis of the EGFR pathway, which has well-defined functions in photoreceptor development [Freeman 1997; Halfar et al. 2001; Yang and Baker 2003]. As the morphogen Hedgehog [Hh] drives progression of the morphogenetic furrow across the eye disc, it induces the expression of the transcription factor Atonal, which specifies the first photoreceptor to differentiate in each cluster, R8 [Jarman et al. 1995; Dominguez 1999]. Once the R8 photoreceptor has been specified, it sequentially recruits additional photoreceptors, cone cells, and pigment cells from the surrounding pool of undifferentiated cells. The signal for this recruitment is the EGFR ligand Spitz [Spi], which is secreted by R8 and subsequently by other photoreceptors as they differentiate [Freeman 1996]. Production of the downstream feedback inhibitor Argos [Aos], which binds to Spi and blocks its binding to the receptor [Klein et al. 2004], restricts the response to Spi to a small number of cells, allowing the stepwise recruitment of ommatidial cell types [Freeman 1997]. A second RTK, Sevenless, also contributes to R7 differentiation [Freeman 1996]. The precursors of the R2, R5, R3, and R4 photoreceptors remain arrested in the G1 phase of the cell cycle after leaving the morphogenetic furrow. This arrest also requires EGFR signaling, but occurs at a lower threshold than the differentiation response [Yang and Baker 2003]. Mitosis of the remaining cells in the second mitotic wave is likewise driven by EGFR signaling, through activation of the target gene string, which encodes a cdc25 phosphatase [Baonza et al. 2002; Yang and Baker 2003]. Finally, loss of EGFR signaling leads to...
apoptosis, as this pathway is required to down-regulate the expression and activity of the proapoptotic protein head involution defective [Hid] [Bergmann et al. 1998, Kurada and White 1998; Hallow et al. 2001; Yang and Baker 2003].

Ligand binding to the EGFR and other RTKs induces their dimerization and autophosphorylation [Schlessinger 2002]. The pathway downstream has been elucidated by both genetic and biochemical analysis [for review, see Nishida and Gotth 1993; Rubin et al. 1997; Moghal and Sternberg 2003]. Phosphorylated EGFR can interact with the adaptor protein downstream of receptor kinases [Drk], which links it to son of sevenless [Sos], a guanine nucleotide exchange factor for Ras. This stimulates conversion of Ras to an active GTP-bound form, while the reverse reaction leading to an inactive GDP-bound form is enhanced by Gap1. In a complex scaffolded by connector enhancer of ksr [Cnk], GTP-Ras activates the kinase Raf. This initiates a kinase cascade in which Raf, in the presence of the scaffold protein kinase suppressor of ras [Ksr], phosphorylates downstream of Raf1 [Drosor1 or MEK], which itself phosphorylates MAPK. Phosphorylated MAPK can enter the nucleus, where it phosphorylates two Ets transcription factors, Pointed P2 [PntP2] and Yan. EGFR target gene expression is activated by phosphorylated PntP2, while the transcriptional repressor Yan is exported to the cytoplasm and degraded upon phosphorylation. Two transcriptional targets of the pathway are another isoform of pointed, pntP1, and the feedback inhibitor aos [Gabay et al. 1996; Golembo et al. 1996].

One step in this pathway that remains unclear is the precise mechanism by which Ras activates Raf [Dhillon and Kolch 2002, Wellbrock et al. 2004]. Raf activation in mammalian cells requires its recruitment to the plasma membrane through binding to Ras [Herrmann et al. 1995; Marais et al. 1995], as well as dephosphorylation by protein phosphatase 2A of binding sites for the 14–3–3 protein in the N-terminal region of Raf [Jaumot and Hancock 2001; Kubicke et al. 2002; Dumaz and Marais 2003], and phosphorylation of sites upstream of the catalytic domain and within the activation segment by unidentified kinases [Fabian et al. 1993; Zhang and Guan 2000; Chong et al. 2001]. In Drosophila, the scaffold protein Cnk appears to have dual roles in the activation event; its N-terminal region, which contains essential sterile α motif [SAM] and conserved region in Cnk [CRIC] domains, promotes Raf activation downstream of Ras, while its C-terminal region, which contains a Raf-binding domain, plays an inhibitory role downstream of Raf [Therrien et al. 1999, Douziech et al. 2003]. It has been proposed that Src42 binding antagonizes this inhibition, allowing Cnk to integrate Ras and Src42 signals [Laberge et al. 2005]. In C. elegans, Cnk-1 acts downstream of Raf dephosphorylation, but upstream of the activating phosphorylation events [Rocheleau et al. 2005]. SAM domains have been shown to mediate homo- or hetero-oligomerization in both nuclear and membrane proteins [Stapleton et al. 1999, Kim et al. 2001, 2002; Ramachander et al. 2002], suggesting that the function of the SAM domain of Cnk might be to interact with another SAM domain protein; however, such a partner has not yet been identified.

Using a genetic mosaic screen to identify genes required for normal photoreceptor differentiation, we have isolated a mutation in a novel Drosophila gene, aveugle [ave], with phenotypes characteristic of components of the EGFR signaling pathway in both the eye and wing discs. Loss of ave appears to reduce but not abolish signaling through the pathway, and epistasis tests in vivo and in cell culture indicate that ave acts between ras and raf to promote MAPK phosphorylation. ave encodes a small protein consisting almost entirely of a SAM domain. Ave can directly bind to Cnk in a SAM-domain-dependent manner, and colocalizes and communoprecipitates with Cnk in S2 cells. We suggest that the interaction between Ave and Cnk recruits an activator to Raf.

**Results**

**aveugle is required for EGFR signaling during eye development**

In a mosaic genetic screen for genes required for photo-receptor differentiation [Janody et al. 2004], we isolated one ethyl methanesulfonate (EMS)-induced lethal allele of a new gene that we have called **aveugle** [ave]. In the wild-type eye disc, photoreceptors are recruited sequentially to clusters posterior to the morphogenetic furrow [Fig. 1A] and can be visualized by staining with the neuronal nuclear marker Elav [Fig. 1B,D]. In **ave** mutant clones, fewer Elav-expressing nuclei were present within each cluster [Fig. 1E,G]. However, expression of Senseless [Sens], a marker for the first photoreceptor to differentiate, R8, was largely normal in **ave** mutant clones [Fig. 1C,F]. Hedgehog produced by more posterior cells induces R8 differentiation by activating expression of the transcription factor Atonal [Jarman et al. 1995, Dominguez 1999], while recruitment of all the other cell types to the cluster is dependent on EGFR signaling [Freeman 1996]. We used markers for different ommatidial cell types [Fig. 1A] to determine which were missing in **ave** mutants. Staining for the cone cell marker Cut [Blochlinger et al. 1993] revealed that very few cone cells differentiated in **ave** mutant clones [Fig. 2A–C]. Expression of Bar, a marker for the R1 and R6 photoreceptors [Higashijima et al. 1992], was also almost absent from **ave** mutant clones [Fig. 2D–F]. However, Spalt, which labels R3 and R4 in anterior regions of the eye disc [Domíngos et al. 2004], was less affected, indicating that a reduced number of R3 and R4 cells were able to differentiate in **ave** mutant clones [Fig. 2G–I]. **ave** mutant clones also contained Elav-expressing cells not labeled by Spalt, most likely corresponding to R2 and R5 cells. The requirement for **ave** in the differentiation of cells other than R8 suggested that **ave** might be involved in EGFR signaling during eye development. However, the **ave** mutant phenotype appeared less severe than mutations in other components of the EGFR pathway, such as **cnk** [Fig. 1H–J; Therrien et al. 1998]. Most **ave** mutant
Ave is required for Raf activation

Figure 1. Ave is required for photoreceptor differentiation. (A) Diagram of the stages of photoreceptor recruitment in the larval eye disc. In the morphogenetic furrow [left], R8 differentiation is triggered by Hh. R8 then expresses Spi to recruit R2 and R5, followed by R3 and R4, R1 and R6, R7, and then the four cone cells. Expression of Spi in each cell as it differentiates contributes to the recruitment of subsequent cell types. The markers used in this study to recognize R8, R3/R4, R1/R6, and cone cells are indicated. (B–I) Third instar eye imaginal discs with anterior to the left. The arrows in D, G, and J indicate the morphogenetic furrow. (B–D) Wild-type eye disc. (E–G) Eye disc with a large ave mutant clone marked by lack of GFP [green in G]. (H–J) Eye disc with a large cnk mutant clone marked by lack of GFP [green in J]. Photoreceptors are stained with anti-Elav [B,E,H,I, blue in D,G,J], and R8 is stained with anti-Senseless [C,F,J; red in D,G,J]. Most cnk mutant ommatidia contain only R8, while most ave mutant ommatidia contain one or two photoreceptors in addition to R8.

Ave is required for EGFR activity in wing development

We next examined the effects of loss of ave on EGFR signaling outside the eye. EGFR signaling is required for wing vein formation, and activates expression of the target gene aos along the vein primordia in the wing disc [Gabay et al. 1997, Guichard et al. 1999]. Induction of ave mutant clones led to a loss of wing veins in the adult wing (Fig. 3B). Consistent with the lack of vein differentiation, aos-lacZ expression was autonomously lost from ave mutant clones in the wing disc (Fig. 3F,G). EGFR signaling has also been shown to define the notum primordium of the wing disc, marked by expression of Homothorax [Hth] [Azpiazu and Morata 2000, Wang et al. 2000, Zecca and Struhl 2002]. Homozygous ave mutants can survive until the third larval instar; at this stage, we found that their wing discs had greatly reduced notum primordia (Fig. 3D).

Ave encodes a novel SAM domain protein

We used complementation tests with the deficiency kit available from the Bloomington Drosophila stock center as well as recombination mapping relative to P[w+] elements to map ave to chromosome region 51C1–C5.
Since we found only a single allele of ave in a screen in which multiple alleles of genes of moderate size were obtained (Janody et al. 2004), we expected ave to be a small gene. We therefore sequenced the six smallest predicted genes contained in this region from ave homozygous larvae, and found a nonsense mutation in the predicted gene CG30476 that would truncate the encoded protein at amino acid 34 (Fig. 4B). This mutation was not present in the isogenic parental flies used for the screen.

We also found that ave failed to complement the deficiency Df(Rpn6)2F, which completely removes CG30476 and partially deletes the adjacent genes Rpn6 and CG10151 (Fig. 4A). Finally, we generated transgenic fly lines expressing an HA-tagged full-length CG30476 protein under the control of UAS sequences. Expression of this transgene in ave mutant clones was sufficient to rescue the photoreceptor differentiation defect (Fig. 5C,D). Overexpressing CG30476 ubiquitously throughout development using daughterless-GAL4 had no apparent effect on wild-type flies. Based on the stop codon present in the mutant allele, the failure to complement Df(Rpn6)2F, and the ability of CG30476 cDNA to rescue the ave mutant phenotype, we conclude that ave corresponds to the predicted gene CG30476.

ave encodes a small protein of 106 amino acids that contains a SAM domain (Fig. 4B). This domain is a protein module ∼70 residues long that has been implicated in protein–protein interactions (Stapleton et al. 1999; Kim et al. 2001, 2002; Ramachander et al. 2002). Our allele of ave is likely to be a functional null, as the truncated protein would contain only 11 amino acids of the SAM domain. Homologs of ave exist in other species, including humans (Fig. 4B), but their functions are still unknown. Using in situ hybridization, we found that ave transcripts were present ubiquitously throughout development (Fig. 4C), as might be expected for a component required for transduction of a common developmental signal.

Figure 2. ave is required for EGFR signaling during eye development. ave mutant clones generated in third instar eye discs are marked by loss of GFP [B,E,H,K,N,Q,T, green in C,F,I,L,O,R,U]. (A–C) Cone cells were stained with anti-Cut (A, red in C) and photoreceptors with anti-Elav (blue in C). Very few cone cells differentiate in ave mutant clones. (D–F) R1 and R6 photoreceptors were stained with anti-Bar (D, red in F) and all photoreceptors with anti-Elav (blue in F). Most ave mutant clusters lack R1 and R6 photoreceptors. (G–I) R3 and R4 photoreceptors were labeled with anti-Spalt (G, red in I) and all photoreceptors with anti-Elav (blue in I). Some ave mutant cells express Spalt, indicating that some R3 and R4 photoreceptors can differentiate in the absence of Ave. In the posterior of the eye disc, Spalt stains R7, R8, and cone cells. The arrows in C, F, and I indicate ave mutant cells that do express Cut, Bar, and Spalt respectively. (J–L) Staining for Cyclin B ([J, red in L] and Elav (blue in L). Cyclin B expression is increased in ave mutant clones, indicating that more cells reenter the cell cycle posterior to the morphogenetic furrow. However, some cells that do not differentiate as photoreceptors also do not express Cyclin B [arrow in magnification in L]. (M–O) Cell death was monitored with an antibody to activated Caspase ([M, red in O]). An increase in activated Caspase staining was observed in ave mutant cells posterior to the morphogenetic furrow [arrow], indicating that some cells undergo apoptosis. (P–R) Expression of PntP1 ([P, red in R], a positive target of EGFR signaling, is reduced in ave mutant cells but not completely lost [arrows]. (S–U) Activation of MAPK was assessed with an antibody to phosphorylated MAPK ([S, red in U]). Phospho-MAPK staining is autonomously missing in ave mutant cells [arrow].
Figure 3. Ave is required for EGFR signaling in wing development. (A) Wild-type adult wing. (B) Wing containing large unmarked ave mutant clones. Wing vein formation is disrupted. (C) Wild-type wing disc. (D) Homozygous ave mutant wing disc. Wg is stained in magenta and Homothorax in green. The wing pouch of homozygous ave mutant discs is largely normal in size, but most of the notum is missing. (E–G) Third instar wing discs stained with anti-β-galactosidase reflecting argos-lacZ expression. (E,F) magenta in G1. (E) Wild type. (F,G) Wing disc with ave mutant clone positively marked by GFP expression (green in G). argos is not expressed in vein primordia in the absence of ave.

Ave functions downstream of Ras but upstream of Raf

To determine the point at which Ave acts in the EGFR signaling pathway, we attempted to rescue the photoreceptor differentiation defect in ave mutant clones by expressing activated forms of components of the pathway. RasV12 is a constitutively activated form of Drosophila Ras1, and RafF179 encodes a gain-of-function version of Raf in which the N-terminal domain of Raf is deleted. Expressing either of these gain-of-function alleles in wild-type eye discs caused ectopic photoreceptor differentiation [Fig. 5E,F, data not shown]. Ave mutant clones expressing RasV12 displayed the photoreceptor differentiation defect typical of ave mutant clones [Fig. 5G,H]. In contrast, RafF179 expressed in ave mutant clones was still able to induce excessive photoreceptor differentiation [Fig. 5I,J]. These results strongly suggest that Ave acts downstream of Ras but upstream of Raf in the EGFR signaling pathway that controls photoreceptor differentiation. In this case, ave should be required for down-stream events such as MAPK activation. We tested this prediction using an antibody directed specifically against phosphorylated MAPK (Gabay et al. 1997). Phospho-MAPK is present at high levels in the group of cells that strongly express PntP1 within the morphogenetic furrow. As expected, based on our epistasis experiments, we found that phospho-MAPK was lost from ave mutant clones [Fig. 2S–U]. Together, these results confirm that Ave acts upstream of the Raf/MEK/MAPK kinase cascade in the eye disc.

We next examined whether the requirement for Ave at this position in the EGFR pathway is conserved in other cell types. The embryonically derived Drosophila S2 cell line expresses most components of the EGFR pathway [Roy et al. 2002]. We first showed by RT-PCR that ave is also endogenously expressed in these cells and that it could be strongly depleted by RNA interference [RNAi] [Supplementary Fig. 1A]. To test the requirement for Ave in Ras/MAPK signaling, we removed endogenous Ave by RNAi in S2 cells expressing RasV12 and used MAPK phosphorylation as a readout of pathway activation. As shown in Figure 5K, RNAi directed against Ras or against the downstream components Cnk or Ksr specifically blocked MAPK phosphorylation [lanes 5–7]. Ave depletion gave an identical result [Fig. 5K, lane 8], indicating that Ave is required downstream of Ras for MAPK activation in S2 cells. We next examined the effect of removing Ave on MAPK activation induced by an activated form of Raf [Tor4021RAFc] (Douziech et al. 2003); activated Ras and activated Raf induced equivalent levels of MAPK phosphorylation [data not shown]. As predicted by the epistasis experiments in photoreceptors, Ave was not required for MAPK phosphorylation induced by activated Raf [Fig. 5L, lane 5]. Cnk depletion likewise had no effect; as a control, we showed that depletion of MEK, the kinase directly downstream of Raf, abrogated MAPK activation [Fig. 5L, lanes 4,6]. These results indicate that Ave acts between Ras and Raf in S2 cells, suggesting a general requirement for Ave at this position in the EGFR signaling pathway.

Ave colocalizes and interacts with Cnk

Cnk is a scaffolding protein that is required downstream of Ras for Raf activation and, like Ave, contains a SAM domain. This prompted us to ask whether Cnk and Ave might function in the same complex. We first determined whether Ave and Cnk showed the same subcellular localization. Flag-tagged Cnk has been shown to be predominantly located in the cytoplasm of S2 cells, with some accumulation at the plasma membrane (Therrien et al. 1998). We cotransfected adherent S2R+ cells with Flag-Cnk, HA-tagged Ave, and a nuclear form of GFP. As expected, Cnk was found in the cytoplasm with some accumulation at the plasma membrane [Therrien et al. 1998]. We cotransfected adherent S2R+ cells with Flag-Cnk, HA-tagged Ave, and a nuclear form of GFP. As expected, Cnk was found in the cytoplasm with some enrichment at the plasma membrane [Fig. 6B]. Ave and Cnk clearly colocalized in both locations [Fig. 6A–C], supporting a possible interaction between these two proteins. We confirmed this interaction using a yeast two-hybrid assay. Ave strongly interacted with the N-terminal domain of Cnk, consisting of the SAM, CRIC, and
PDZ domains (Fig. 6D). Deletion of the SAM domain from this Cnk construct abolished the interaction with Ave (Fig. 6D), suggesting that the interaction is mediated by SAM domain heterodimerization. We did not detect any direct interaction between Ave and either the N-terminal or C-terminal domains of Raf; as a control, we confirmed that the C-terminal domain of Raf could interact with MEK (Fig. 6D; Therrien et al. 1996). Ave was also unable to homodimerize (Fig. 6D). To determine whether Ave also interacts with Cnk in vivo, cell lysates from S2R+ cells expressing Flag-Cnk and HA-Ave were immunoprecipitated with anti-HA antibody and probed with anti-Cnk antibody. Cnk was coimmunoprecipitated with HA-Ave, and the association was specific since no Cnk was immunoprecipitated from cells not transfected with HA-Ave (Fig. 6D). Since Cnk and Ave act at the same position in the pathway, share a SAM domain, colocalize within the cell, and physically interact, it is likely that they function together to promote Raf activation.

Discussion

Although Raf activation is a critical step in RTK signaling pathways, its mechanism is still not fully understood. Key steps include Raf translocation to the plasma membrane and release of its protein kinase domain from an intramolecular inhibitory domain through changes in the phosphorylation state of specific residues. These processes occur in the context of the essential scaffolding proteins Cnk and Ksr. Here we identify the novel SAM domain protein Ave as another component required for Raf activation. Ave is required between Ras and Raf for EGFR signaling in differentiating photoreceptors and in S2 cells, and is present in the same complex as Cnk. We discuss below how Ave may contribute to Raf activation.

ave is required for maximal levels of EGFR signaling

Loss of ave in the eye disc disrupts normal photoreceptor differentiation, while R8 cells differentiate correctly, most of the other photoreceptors are missing. Although
the mutation we isolated is likely to be a null allele of 
ave, its phenotype is weaker than loss of function of core 
components of the EGFR pathway, including cnk. R8 is 
still able to recruit a few photoreceptors in the absence of 
ave, and only a small proportion of ave mutant cells die 
during the third larval instar. The reduced expression in 
ave mutant cells of PntP1, a direct target of the pathway, 
suggests that ave is required to increase the overall level 
of EGFR signaling. We note that MAPK phosphorylation 
is undetectable in the absence of ave in both eye disc 
cells and S2 cells, suggesting that examination of EGFR 
responses in vivo is more sensitive than detection of 
phospho-MAPK.

If loss of ave simply reduces the level of EGFR signal-
ing, it would imply that distinct thresholds of EGFR sig-
Journal of Cell Biology. 1996;133:1229–1246. naling recruit different subclasses of ommatidial cells, 
since ave has a stronger effect on recruitment of R1, R6, 
and cone cells than on R2–R5. The dependence of many 
different ommatidial cell fates on EGFR signaling has 
been taken to imply that the response of an undifferen-
tiated cell to the EGFR signal changes over time [Freem-
an 1996]. This change in cellular competence may be 
due to changes in transcription factor expression in sig-
Journal of Cell Biology. 1996;133:1229–1246. nal-receiving cells [Flores et al. 2000; Xu et al. 2000]. The 
intermediate phenotype of ave mutants suggests that 
specification of early differentiating photoreceptors such 
as R3 and R4 requires a lower level of EGFR signaling 
than specification of later differentiating cells such as 
R1, R6, and cone cells. Interestingly, phosphorylated 
MAPK levels are lower in the region of the eye disc in 
which R2–R5 differentiate than in more posterior re-
gions [Yang and Baker 2003]. In addition, R7 differentia-
tion has been shown to require both EGFR and Sevenless 
to signal through the Ras/MAPK module, suggesting 
that an elevated amount of signal is required for its speci-
fication [Freeman 1996]. An alternative means of tempo-
ral control is the induction by EGFR activity of signaling 
molecules required to recruit later cell types; for in-
stance, EGFR recruits cone cells in part by activating 
expression in photoreceptors of the Notch ligand Delta 
[Tsuda et al. 2002]. ave might be required for the expres-
sion of specific EGFR target genes such as Delta that promote 
sequential induction of late-differentiating cell types.

In addition to photoreceptor differentiation, EGFR sig-
naling in the eye is required for cell survival and cell 
cycle arrest; these two functions have been proposed to 
require a lower level of EGFR activity than differentia-
tion of R1–R7 [Haller et al. 2001; Yang and Baker 2003].
Our results support this conclusion, since we found that some *ave* mutant cells that do not differentiate as photoreceptors are still able to arrest in G1. However, we found an increase in apoptosis in *ave* mutant clones, despite their ability to differentiate some photoreceptors in addition to R8. This result suggests that there may not be a sharp threshold between the differentiation and survival responses; the level of EGFR signaling achieved in the absence of *ave* can allow differentiation of some photoreceptors without preventing all apoptosis.

The requirement for Ave in other EGFR-dependent processes appears to be variable. In the wing disc, *ave* is essential for notum growth and for expression of the EGFR target gene *aos*; *aos* is likely to be a high-threshold target, as it is expressed in cells containing high levels of phosphorylated MAPK (Gabay et al. 1997). However, *ave* is not required for all signaling by EGFR or the RTK Torso during embryogenesis. Embryos lacking both the maternal and zygotic contribution of *ave* did not show any detectable change in midline *aos-lacZ* or terminal *tailless* expression (data not shown). As in the wing disc, *aos* is thought to be activated by high levels of EGFR signaling, due to its overlap with phospho-MAPK staining (Gabay et al. 1997). *ave* might be redundant with another molecule expressed at this stage of development, although no close sequence homolog is present in the *Drosophila* genome. Alternatively, the Ras/MAPK module may use a distinct mechanism for signal transduction during embryogenesis. In this regard, it will be interesting to test whether *cnk* is required for EGFR signaling in the embryo.

### Ave interacts with Cnk to promote Raf activation

Genetic and biochemical studies have shown that the scaffolding protein Cnk is required for RTK signaling downstream of Ras but upstream of Raf (Therrien et al. 1998; Douziech et al. 2003). Its N-terminal SAM and CRIC domains are essential for its function in promoting Raf activity (Douziech et al. 2003). SAM domains frequently act as homo- or heterodimerization motifs. The SAM domains of Ave and Cnk can directly interact in yeast, suggesting that the essential function of the SAM domain of Cnk may be to interact with Ave (Fig. 6F).

How might the interaction of Ave with Cnk promote Raf activation? Since Cnk binds to Raf through a C-terminal Raf-interacting motif (RIM) (Therrien et al. 1998), this binding is unlikely to require Ave. In addition, the RIM is dispensable for the transduction of Ras signaling and, in fact, seems to have an inhibitory effect on Ras signaling (Douziech et al. 2003). We have not observed any change in the strength of the interaction between Raf and Cnk when *ave* is removed by RNAi (Supplementary Fig. 1A, B). A more likely possibility is that association of Ave with Cnk helps to bring an activator kinase into proximity with Raf. Raf activation in mammalian cells involves dephosphorylation of inhibitory sites followed by phosphorylation of activating sites (for review, see Dhillon and Kolch 2002; Chong et al. 2003). However, the identity of the activating kinases is still unclear; Ksr was a candidate, but the current view is that it acts as a scaffolding protein rather than an active kinase (Morrison 2001). In *C. elegans*, epistasis tests suggest...
that Cnk promotes Raf activation after dephosphorylation but before the activating phosphorylation events (Rocheleau et al. 2005), consistent with a model in which Cnk in association with Ave attracts an activator kinase to Raf. Certain SAM domains have been shown to act as kinase-docking sites; for example, the SAM domain of ETS-1 provides a docking site for the ERK-2 MAPK, promoting phosphorylation of and transcriptional activation by ETS-1 (Seidel and Graves 2002). Likewise, the ETS-2 SAM domain serves as a docking site for the Cdc2 family kinase Cdk10 [Kasten and Giordano 2001]. A search for other binding partners of Ave may lead to the identification of the activating kinase for Raf.

An alternative possibility is that association of Ave with Cnk could help to recruit Raf to the plasma membrane. In S2 cells, Cnk is required for recruitment of Raf [Anselmo et al. 2002], but it may not be sufficient for this function, since overexpression in CHO cells of MAGUIN-1, the closest mammalian homolog of Drosophila Cnk, does not recruit Raf-1 to the plasma membrane (Yao et al. 2000). The SAM domain of human p73 has been shown to directly bind lipid membranes [Barrera et al. 2003], suggesting the possibility that Ave links Cnk or Raf directly to the plasma membrane. However, we have not seen a clear change in the subcellular localization of tagged Cnk when Ave is knocked down by RNAi (Supplementary Fig. 1C–F).

Another well-described property of SAM domains is their ability to polymerize, promoting the formation of homo- or hetero-oligomers. This mechanism underlies long-range transcriptional repression by the SAM domain proteins TEL and Polyhomeotic [Kim et al. 2001, 2002]. In the context of Raf activation, it is possible that polymerization of Ave, together with Cnk and perhaps other SAM domain-containing proteins, leads to the formation of large scaffolding complexes in which the local concentration of Raf and/or its activators is increased. Interestingly, the yeast adaptor protein Ste50, which is required for the activation of a MAPKKK, Ste11 [Ramezani-Rad 2003], induces polymerization of Ste11 through interactions between the SAM domains of the two molecules [Bhattachariya et al. 2005]. This may stabilize a complex in which the Ste20 kinase can phosphorylate Ste11 [Ramezani-Rad 2003]. A stabilizing function might explain why Ave is not essential in all contexts in Drosophila, as high concentrations of the molecules it recruits could lead to Ave-independent signaling. The evolutionary conservation of Ave suggests that it is likely to regulate the Ras/Raf/MAPK module in other species.

Materials and methods

Fly stocks, genetics, and molecular biology

A single ave allele (ave108V) was isolated from a mosaic screen for mutations affecting photoreceptor differentiation [Janody et al. 2004]. This mutation failed to complement Dif[Rpn6]FR [Lier and Paululat 2002]. The fly stocks used were da-GAL4, argosW, UAS-rasV12 [FlyBase], UAS-raFR70 [Martin-Blanco et al. 1999], and cnk14AC [Janody et al. 2004]. ave108V mutant clones were generated by crossing FRT42D, ave/Cyo, y' males to FRT42D, ubi-GFP; hsFLP122 females. Large clones were generated by crossing FRT42D, ave/Cyo, y' males to FRT42D, ubi-GFP, M(2)S8F/Cyo, y'; eyFLP1 females, or to FRT42D, ubi-GFP, M(2)S8F/Cyo, y'; hsFLP122 females. Epistasis experiments were done using the MARCM system. For example, RasV12 was overexpressed in ave mutant clones by crossing FRT42D, ave, UAS-rasV12/SM6-TM6B males to eyFLP1, UAS-GFP, FRT42D, tub-GAL80, tub-GAL4/TM6B females. Germline clones were made by crossing hsFLP122; FRT42D, ubi-GFP to FRT42D, ave/Cyo. Larvae were heat shocked for 1 h at 38.5°C in both first and second instar. The resulting hsFLP122, FRT42D, ave, ubi-GFP females were crossed to FRT42D, ave, argosW/S6-TM6B males, and embryos were selected for the absence of GFP staining.

The coding regions of CG30476, CG12859, CG10153, CG12854, CG12856, and CG10205 were amplified by PCR from genomic DNA obtained from homozygous ave mutant first instar larvae, and the PCR products were sequenced. The full-length ave coding region was amplified by PCR from the RE67675 clone (Drosophila Genomics Resource Center) using Pfu Turbo and cloned into HA-pUASt as an EcoRI/XhoI fragment to generate UAS-HA-ave. Transgenic flies were generated by standard methods.

PCR fragments encoding full-length Ave, the Raf N-terminal domain [amino acids 1–418], and the Raf C-terminal domain [amino acids 419–739] were subcloned into the bait vector pEG202 [Gyuris et al. 1993]. PCR fragments corresponding to full-length Ave, the Cnk N-terminal domain [amino acids 1–378], the Cnk N-terminal domain without the SAM domain [amino acids 86–378], the Raf C-terminal domain [amino acids 419–739], and full-length MEK were subcloned into the prey vector pG4-5 [Gyuris et al. 1993]. All constructs were confirmed by sequencing.

Immunostaining, histology, and in situ hybridization

The primary antibodies used were rat anti-Elav (1:5; Developmental Studies Hybridrism Bank), guinea pig anti-Sens (1:1000) [Frankfort et al. 2001], rabbit anti-BarS12 (1:50) [Higashijima et al. 1992], mouse anti-2B10 (1:10) [Bleichinger et al. 1993], mouse anti-Cyclin B (1:20; Developmental Studies Hybridrism Bank), rabbit anti-CM1 (1:500), BD Pharmingen), rabbit anti-Salmon (1:100) [Domíngos et al. 2004], mouse anti-Wingless (1:20, Developmental Studies Hybridrism Bank), rabbit anti-Pnt1 (1:500) [Alvarez et al. 2003], mouse anti-dpERK (phospho-MAPK; 1:50, Sigma), rabbit anti-GFP (1:2,500; Molecular Probes), mouse anti-GFP (1:2,500, Roche), mouse anti-β-galactosidase (1:200, Promega), rabbit anti-Homothorax (1:500) [Kurant et al. 1998], rat anti-HA 3F10 (1:1,000, Roche), and rabbit anti-Flag (1:500, Sigma). TSA enhancement was used for the anti-dpERK antibody. Eye and wing imaginal discs were dissected in 0.1 M sodium phosphate buffer (pH 7.2) and then fixed in PEM (0.1 M PIPES at pH 7, 2 mM MgSO4, 1 mM EGTA) containing 0.1 M sodium phosphate buffer (pH 7.2), and then fixed in PEM (0.1 M PIPES at pH 7, 2 mM MgSO4, 1 mM EGTA) containing 0.1 M sodium phosphate buffer (pH 7.2). Washes were done in 0.1 M phosphate buffer with 0.2% Triton X-100. Appropriate fluorescent-conjugated secondary antibodies were used (1:200, Jackson ImmunoResearch Laboratories). Fluorescent images were collected on a Leica TCS NT confocal microscope. S2R+ cells were fixed in PBS containing 4% formaldehyde and stained with appropriate antibodies. Adult wings were mounted in methyl salicylate. Canada balsam (1:2). Digoxigenin-UTP-labeled RNA probes homologous to the ave coding region were used for in situ hybridization. Embryos were hybridized as described [Lehmann and Tautz 1994], and eye discs were hybridized as described [Bach et al. 2003].
Cell culture
S2 and S2R+ cells were maintained in Schneider's medium (GIBCO) supplemented with 10% fetal calf serum and 50 U of penicillin–streptomycin (GIBCO). Cells were transfected with Effectene [Qiagen] according to the manufacturer’s instructions. pMet FL-CNK, pMet-HARasV12, pMet-pyoTor2001RAFc, and pMet-HAMAPK constructs were kindly provided by Marc Therrien (Université de Montreal, Montreal, Quebec, Canada) and pMet FL-CNK, pMet-HARasV12, pMet-pyoTor4021RAFc, s instruc-
sions. Three micrograms of total RNA were used per reaction. The PCR primers used for β-actin were 5’-GGCCGTTGAATACCGGTTA-3’ and 3’-GGATCCACAGCAAGAATGCTG-5’. RT-PCR
Total RNA was extracted from S2 cells using Trizol (Invitro-
gen). RT-PCR was performed using the Invitrogen SuperScript First-Strand kit in accordance with the manufacturer’s instructions. Three micrograms of total RNA were used per reaction. The PCR products for β-actin were 5’-TTAAATTTAATCTAGAATTTTGCGCTC-5’ and 3’-TTAAATTATCTAGAATTTTGCGCTC-3’.

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References
Alvarez, A.D., Shi, W., Wilson, B.A., and Skeath, J.B. 2003. pan-
nier and pointedP2 act sequentially to regulate Drosophila heart development. Development 130: 3015–3026.
Anselmo, A.N., Bumeister, R., Thomas, J.M., and White, M.A. 2002. Critical contribution of linker proteins to Raf kinase activation. J. Biol. Chem. 277: 5940–5943.
Azpiazu, N. and Morata, G. 2000. Function and regulation of homothorax in the wing imaginal disc of Drosophila. Develop-
ment 127: 2685–2693.
Bach, E.A., Vincent, S., Zeidler, M.P., and Perrimon, N. 2003. A sensitized genetic screen to identify novel regulators and components of the Drosophila janus kinase/signal trans-
ducer and activator of transcription pathway. Genetics 165: 1149–1166.
Baonza, A., Murawsky, C.M., Travers, A.A., and Freeman, M. 2002. Pointed and Tramtrack69 establish an EGF/Depen-
dential transcriptional switch to regulate mitosis. Nat. Cell
Biol. 4: 976–980.
Barrera, F.N., Poveda, J.A., Gonzalez-Ros, J.M., and Neira, J.L. 2003. Binding of the C-terminal sterile α motif [SAM] do-
main of human p73 to lipid membranes. J. Biol. Chem. 278: 46878–46885.
Blochlinger, K., Jan, L.Y., and Jan, Y.N. 1993. Postembryonic
Bhattacharjya, S., Xu, P., Chakrapani, M., Johnston, L., and Ni, F. 2005. Polymerization of the SAM domain of MAPKKK
Ste11 from the budding yeast: Implications for efficient sig-
naling through the MAPK cascades. Protein Sci. 14: 828–835.
Blochinger, K., Jan, L.Y., and Jan, Y.N. 1993. Postembryonic

Domingos, P.M., Brown, S., Barrio, R., Ratnakumar, K., Frank-
hedgehog

Flores, G.V., Duan, H., Yan, H., Nagaraj, R., Fu, W., Zou, Y.,

Domingos, P.M., Brown, S., Barrio, R., Ratnakumar, K., Frank-
fort, B.J., Mardon, G., Steller, H., and Mollereau, B. 2004. Regulation of R7 and R8 differentiation by the spalt genes.
Dev. Biol. 273: 121–133.

Dominguez, M. 1999. Dual role for hedgehog in the regulation of the proneural gene atonal during ommatidia development. Development 126: 2345–2353.

Douziech, M., Roy, F., Laberge, G., Lefrancois, M., Arnegond, A.V., and Therrien, M. 2003. Bimodal regulation of RAf by CNK in Drosophila. EMBO J. 22: 5068–5078.

Dumaz, N. and Marais, R. 2003. Protein kinase A blocks RAf-1 activity by stimulating 14–3–3 binding and blocking RAf-1 interaction with Ras. J. Biol. Chem. 278: 29819–29823.

Fabian, J.R., Daar, I.O., and Morrison, D.R. 1993. Critical tyro-
sine residues regulate the enzymatic and biological activity of RAf-1 kinase. Mol. Cell. Biol. 13: 7170–7179.

Flores, G.V., Duan, H., Yan, H., Nagaraj, R., Fu, W., Zou, Y., Noll, M., and Banerjee, U. 2000. Combinatorial signaling in the specification of unique cell fates. Cell 103: 75–85.

Frankort, B.J., Nolo, R., Zhang, Z., Bellen, H., and Mardon, G. 2001. senseless repression of rough is required for R8 photo-

Freeman, M. 1996. Reiterative use of the EGF receptor triggers differentiation of all cell types in the Drosophila eye. Cell 87: 651–660.

———. 1997. Cell determination strategies in the Drosophila eye. Development 124: 261–270.

Gabay, L., Scholz, H., Golembio, M., klaes, A., Shilo, B.Z., and Klambt, C. 1996. EGF receptor signaling induces pointed P1 transcription and inactivates Yan protein in the Drosophila embryonic ventral ectoderm. Development 122: 3355–3362.

Ghayur, L.A., Seger, R., and Shilo, B.Z. 1997. In situ activation pattern of Drosophila EGF receptor pathway during develop-
ment. Science 277: 1103–1106.

Golembio, M., Schweitzer, R., Freeman, M., and Shilo, B.Z. 1996. argos transcription is induced by the Drosophila EGF receptor pathway to form an inhibitory feedback loop. Development 122: 223–230.

Guichard, A., Bichs, B., Sturtevant, M.A., Wickline, L., Chacko, J., Howard, K., and Bier, E. 1999. rhomboid and Star interact synergistically to promote EGF/MAPK signaling during Drosophila wing vein development. Development 126: 2653–2676.

Gyuris, J., Golemis, E.A., Chertkov, H., and Brent, R. 1993. Cd11, a human G1 and S phase protein phosphatase that associates with Cdk2. Cell 75: 791–803.

Hafalr, K., Rommel, C., Stocker, H., and Hafen, E. 2001. Ras controls growth, survival and differentiation in the Dro-
sophila eye by different thresholds of MAP kinase activity. Development 128: 1687–1696.

Herrmann, C., Martin, G.A., and Wittinghofer, A. 1995. Quan-
titative analysis of the complex between p21ras and the Ras-

Higashijima, S.-I., Kojima, T., Michiupe, T., Ishimaru, S., Emori, Y., and Saigo, K. 1992. Dual Bar homo box genes of Drosophila required in two photoreceptor cells, R1 and R6, and primary pigment cells for normal eye development. Genes & Dev. 6: 50–60.

Holbro, T. and Hynes, N.E. 2004. ErbB receptors: Directing key signaling networks throughout life. Annu. Rev. Pharmacol. Toxicol. 44: 195–217.

Janody, F., Lee, J.D., Jahren, N., Hazelett, D.J., Benlali, A., Miura, G.I., Draskovic, I., and Treisman, J.E. 2004. A mosaic genetic screen reveals distinct roles for trithorax and Polycomb group genes in Drosophila eye development. Genetics 166: 187–200.

Jarman, A.P., Sun, Y., Jan, L.Y., and Jan, Y.N. 1995. Role of the proneural gene, atonal, in formation of Drosophila chor-
dotinal organs and photoreceptors. Development 121: 2019–2030.

Jaumot, M. and Hancock, J.F. 2001. Protein phosphatases 1 and 2A promote RAf-1 activation by regulating 14–3–3 interac-
tions. Oncogene 20: 3949–3958.

Kasten, M. and Giordano, A. 2001. Cdk10, a Cdc2-related ki-

Kim, C.A., Phillips, M.L., Kim, W., Gengery, M., Tran, H.H., Robinson, M.A., Faham, S., and Bowie, J.U. 2001. Polymer-
ization of the SAM domain of TEL in leukemogenesis and transcriptional repression. EMBO J. 20: 4173–4182.

Kim, C.A., Gengery, M., Pilpa, R.M., and Bowie, J.U. 2002. The SAM domain of polyhomeotic forms a helical polymer. Nat.
Struct. Biol. 9: 453–457.

Klein, D.E., Nappi, V.M., Reeves, G.T., Shvartsman, S.Y., and Lemmon, M.A. 2004. Argos inhibits epidermal growth factor receptor signalling by ligand sequestration. Nature 430: 1040–1044.

Kubicek, M., Pacher, M., Abraham, D., Podar, K., Eulitz, M., and Baccarini, M. 2002. Dephosphorylation of Ser-259 regulates RAf-1 membrane association. J. Biol. Chem. 277: 7913–7919.

Kurada, P. and White, K. 1998. Ras promotes cell survival in Drosophila by downregulating hid expression. Cell 95: 319–329.

Kurant, E., Pai, C.Y., Sharif, R., Halachmi, N., Sun, Y.H., and Salzberg, A. 1998. Dorsotomal/homothorax, the Drosophila homologue of meis1, interacts with extradenticle in patterning of the embryonic PNS. Development 125: 1037–1048.

Laberge, C., Douziech, M., and Therrien, M. 2005. Src42 bind-
ing activity regulates Drosophila RAf by a novel CNK-de-
pendent derepression mechanism. EMBO J. 24: 487–498.

Lehmann, R. and Tautz, D. 1994. In situ hybridization to RNA. Methods Cell Biol. 44: 575–598.

Lier, S. and Paululat, A. 2002. The proteasome regulatory particle subunit Rpn6 is required for Drosophila development and interacts physically with signalosome subunit Alien/CSN2. Gene 298: 109–119.

Marais, R., Light, Y., Paterson, H.F., and Marshall, C.J. 1995. Ras recruits RAf-1 to the plasma membrane for activation by tyrosine phosphorylation. EMBO J. 14: 3136–3145.

Martin-Blanco, E., Roch, F., Mimola, A., Baonza, A., and Perrimon, N. 1999. A temporal switch in DER signaling controls the specification and differentiation of veins and inter-
vessels in the Drosophila wing. Development 126: 5739–5747.
Moghal, N. and Sternberg, P.W. 2003. The epidermal growth factor system in Caenorhabditis elegans. Exp. Cell Res. 284: 150–159.
Morrison, D.K. 2001. KSR: A MAPK scaffold of the Ras pathway? J. Cell Sci. 114: 1609–1612.
Nishida, E. and Gotoh, Y. 1993. The MAP kinase cascade is essential for diverse signal transduction pathways. Trends Biochem. Sci. 18: 128–131.
Ramachander, R., Kim, C.A., Phillips, M.L., Mackereth, C.D., Thanos, C.D., McIntosh, L.P., and Bowie, J.U. 2002. Oligomerization-dependent association of the SAM domains from Schizosaccharomyces pombe Byr2 and Ste4. J. Biol. Chem. 277: 39585–39593.
Ramezani-Rad, M. 2003. The role of adaptor protein Ste50-dependent regulation of the MAPKKK Ste11 in multiple signalling pathways of yeast. Curr. Genet. 43: 161–170.
Rocheleau, C.E., Ronnlund, A., Tuck, S., and Sundaram, M.V. 2005. Caenorhabditis elegans CNK-1 promotes Raf activation but is not essential for Ras/Raf signaling. Proc. Natl. Acad. Sci. 102: 11757–11762.
Roy, F., Laberge, G., Douziech, M., Ferland-McCollough, D., and Therrien, M. 2002. KSR is a scaffold required for activation of the ERK/MAPK module. Genes & Dev. 16: 427–438.
Rubin, G.M., Chang, H.C., Karim, F., Laverty, T., Michaud, N.R., Morrison, D.K., Rebay, I., Tang, A., Therrien, M., and Wassarman, D.A. 1997. Signal transduction downstream from Ras in Drosophila. Cold Spring Harb. Symp. Quant. Biol. 62: 347–352.
Schlessinger, J. 2002. Ligand-induced, receptor-mediated dimerization and activation of EGF receptor. Cell 110: 669–672.
Seidel, J.J. and Graves, B.J. 2002. An ERK2 docking site in the Pointed domain distinguishes a subset of ETS transcription factors. Genes & Dev. 16: 127–137.
Srinivasan, A., Roth, K.A., Sayers, R.O., Shindler, K.S., Wong, A.M., Fritz, L.C., and Tomaselli, K.J. 1998. In situ immunodetection of activated caspase-3 in apoptotic neurons in the developing nervous system. Cell Death Differ. 5: 1004–1016.
Stapleton, D., Balan, I., Pawson, T., and Sicheri, F. 1999. The crystal structure of an Eph receptor SAM domain reveals a mechanism for modular dimerization. Nat. Struct. Biol. 6: 44–49.
Therrien, M., Michaud, N.R., Rubin, G.M., and Morrison, D.K. 1996. KSR modulates signal propagation within the MAPK cascade. Genes & Dev. 10: 2684–2695.
Therrien, M., Wong, A.M., and Rubin, G.M. 1998. CNK, a RAF-binding multidomain protein required for RAS signaling. Cell 95: 343–353.
Therrien, M., Wong, A.M., Kwan, E., and Rubin, G.M. 1999. Functional analysis of CNK in RAS signaling. Proc. Natl. Acad. Sci. 96: 13259–13263.
Tsuda, L., Nagaraj, R., Zipursky, S.L., and Banerjee, U. 2002. An EGFR/Ebi/Sno pathway promotes Delta expression by inactivating Su(H)/SMRTER repression during inductive Notch signaling. Cell 110: 625–637.
Wang, S.H., Simcox, A., and Campbell, G. 2000. Dual role for Drosophila epidermal growth factor receptor signaling in early wing disc development. Genes & Dev. 14: 2271–2276.
Wellbrock, C., Karasarides, M., and Marais, R. 2004. The RAF proteins take centre stage. Nat. Rev. Mol. Cell Biol. 5: 875–885.
Xu, C., Kauffmann, R.C., Zhang, J., Kladny, S., and Cardew, R.W. 2000. Overlapping activators and repressors delimit transcriptional response to receptor tyrosine kinase signals in the Drosophila eye. Cell 103: 87–97.
Yang, L. and Baker, N.E. 2003. Cell cycle withdrawal, progression, and cell survival regulation by EGFR and its effectors in the differentiating Drosophila eye. Dev. Cell 4: 359–369.
Yao, I., Ohtsuka, T., Kawabe, H., Matsuura, Y., Takai, Y., and Hata, Y. 2000. Association of membrane-associated guanylate kinase-interacting protein-1 with Raf-1. Biochem. Biophys. Res. Commun. 270: 538–542.
Zecca, M. and Struhl, G. 2002. Subdivision of the Drosophila wing imaginal disc by EGFR-mediated signaling. Development 129: 1357–1368.
Zhang, B.H. and Guan, K.L. 2000. Activation of B-Raf kinase requires phosphorylation of the conserved residues Thr598 and Ser601. EMBO J. 19: 5429–5439.
The novel SAM domain protein Aveugle is required for Raf activation in the *Drosophila* EGF receptor signaling pathway

Jean-Yves Roignant, Sophie Hamel, Florence Janody, et al.

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