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# Defective dorsal closure and loss of epidermal *decapentaplegic* expression in *Drosophila fos* mutants

**Julia Zeitlinger, Lutz Kockel, Fiorenzo A.Peverali[1], David B.Jackson, Marek Mlodzik[2] and Dirk Bohmann[2]**

EMBL, Meyerhofstr. 1, 69117 Heidelberg, Germany and [1]IGBE-CNR, via Abbiategrasso 207, I-27100 Pavia, Italy

[2]Corresponding authors
e-mail: mlodzik@embl-heidelberg.de and bohmann@embl-heidelberg.de

***Drosophila kayak*** **mutant embryos exhibit defects in dorsal closure, a morphogenetic cell sheet movement during embryogenesis. Here we show that *kayak* encodes D-Fos, the *Drosophila* homologue of the mammalian proto-oncogene product, c-Fos. D-Fos is shown to act in a similar manner to *Drosophila* Jun: in the cells of the leading edge it is required for the expression of the TGFβ-like Decapentaplegic (Dpp) protein, which is believed to control the cell shape changes that take place during dorsal closure. Defects observed in mutant embryos, and adults with reduced Fos expression, are reminiscent of phenotypes caused by 'loss of function' mutations in the *Drosophila* JNKK homologue, *hemipterous*. These results indicate that D-Fos is required downstream of the *Drosophila* JNK signal transduction pathway, consistent with a role in heterodimerization with D-Jun, to activate downstream targets such as *dpp*.**
*Keywords*: *Drosophila* embryogenesis/Fos/Jun/*kayak*/dorsal closure

## Introduction

The JNK signal transduction pathway was initially discovered in vertebrate tissue culture as a mediator of the cellular stress response (Hibi *et al*., 1993; Dérijard *et al*., 1994). JNK belongs to the superfamily of mitogen-activated protein kinases (MAPKs), which are activated by an upstream signaling cascade consisting of the so-called MAPK kinase (MAPKK, or JNKK in the case of the JNK pathway) and the MAPK kinase kinase (MAPKKK) (Marshall, 1994). In response to activation by this cascade, JNK can phosphorylate transcription factors such as Jun. While it has been shown that a variety of stress-related stimuli, as well as certain cytokines, can activate the JNK pathway, and that members of the Rac and Rho families of small GTP binding proteins appear to be involved in this process (Coso *et al*., 1995; Hill *et al*., 1995; Minden *et al*., 1995), the understanding of the pathway is still fragmentary. Recent advances have identified *Drosophila* as a suitable organism for the genetic dissection of JNK signaling (Glise *et al*., 1995; Riesgo-Escovar *et al*., 1996; Sluss *et al*., 1996).

During *Drosophila* embryogenesis, germ band elongation and retraction leave the embryo with an opening in the dorsal epidermis, uncovering a tissue termed amnioserosa (Campos-Ortega and Hartenstein, 1985). Subsequently, the process of dorsal closure leads to a spreading of the epidermal sheets over the amnioserosa, to cover the entire embryo (Young *et al*., 1991, 1993; Knust 1996). During this process, which occurs in the absence of mitosis, the cells of the epidermis elongate along the dorso-ventral axis. This concerted shape change initiates in the most dorsal row of epidermal cells, which form the so-called leading edge. A signal emanating from these leading-edge cells is thought to control and coordinate the progress of dorsal closure (Knust, 1996; Hou *et al*., 1997; Riesgo-Escovar and Hafen, 1997).

Dorsal closure is disrupted in a number of *Drosophila* mutants. Some of these mutations affect JNK signal transduction components such as *hemipterous* (*hep*) and *basket* (*bsk*), which encode a *Drosophila* JNK kinase (JNKK) and a JNK, respectively (Glise *et al*., 1995; Riesgo-Escovar *et al*., 1996; Sluss *et al*., 1996). Null alleles of *Drosophila jun* (D-*jun*) are also embryonic-lethal, and cause similar defects in dorsal closure (Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar and Hafen, 1997).

The phenotype resulting from all of these mutations is marked by the absence of cell elongation in the lateral epidermis, which concurs with a loss of *dpp* expression in the leading-edge. The signal transduction pathway, triggered by the *Drosophila* TGFβ-homologue, Dpp, had previously been shown to be essential for dorsal closure (Affolter *et al*., 1994; Letsou *et al*., 1995). It was concluded that the activation of Jun by Bsk/JNK in the leading edge upregulates *dpp*, possibly through direct transcriptional activation of the *dpp* gene by D-Jun (Riesgo-Escovar and Hafen, 1997).

To activate the transcription of target genes, Jun binds to specific promoter or enhancer elements (Bohmann *et al*., 1987; Curran and Franza, 1988). For this protein–DNA interaction to occur, Jun must dimerize through its leucine zipper domain, resulting in either a homodimer consisting of two Jun molecules, or a heterodimer formed between Jun and a different leucine zipper protein (Turner and Tjian, 1989). The most notable dimerization partner of Jun is Fos. Fos–Jun heterodimers are more stable than Jun homodimers (O'Shea *et al*., 1992), and are believed to be the prevalent, and biologically most relevant, forms of Jun *in vivo* (Kovary and Bravo, 1991, 1992). Whereas four *fos*-like genes have been identified in mammals (Angel and Karin, 1991), *Drosophila* seems to possess only one *fos* homologue, D-*fos* [also referred to as D-*fra* (Perkins *et al*., 1990)]. *Drosophila* Jun and Fos have been characterized biochemically, and have been shown to act similarly to their mammalian counterparts (Perkins *et al*., 1990; Zhang *et al*., 1990). In particular, it has been documented that D-Jun and D-Fos can form heterodimers,

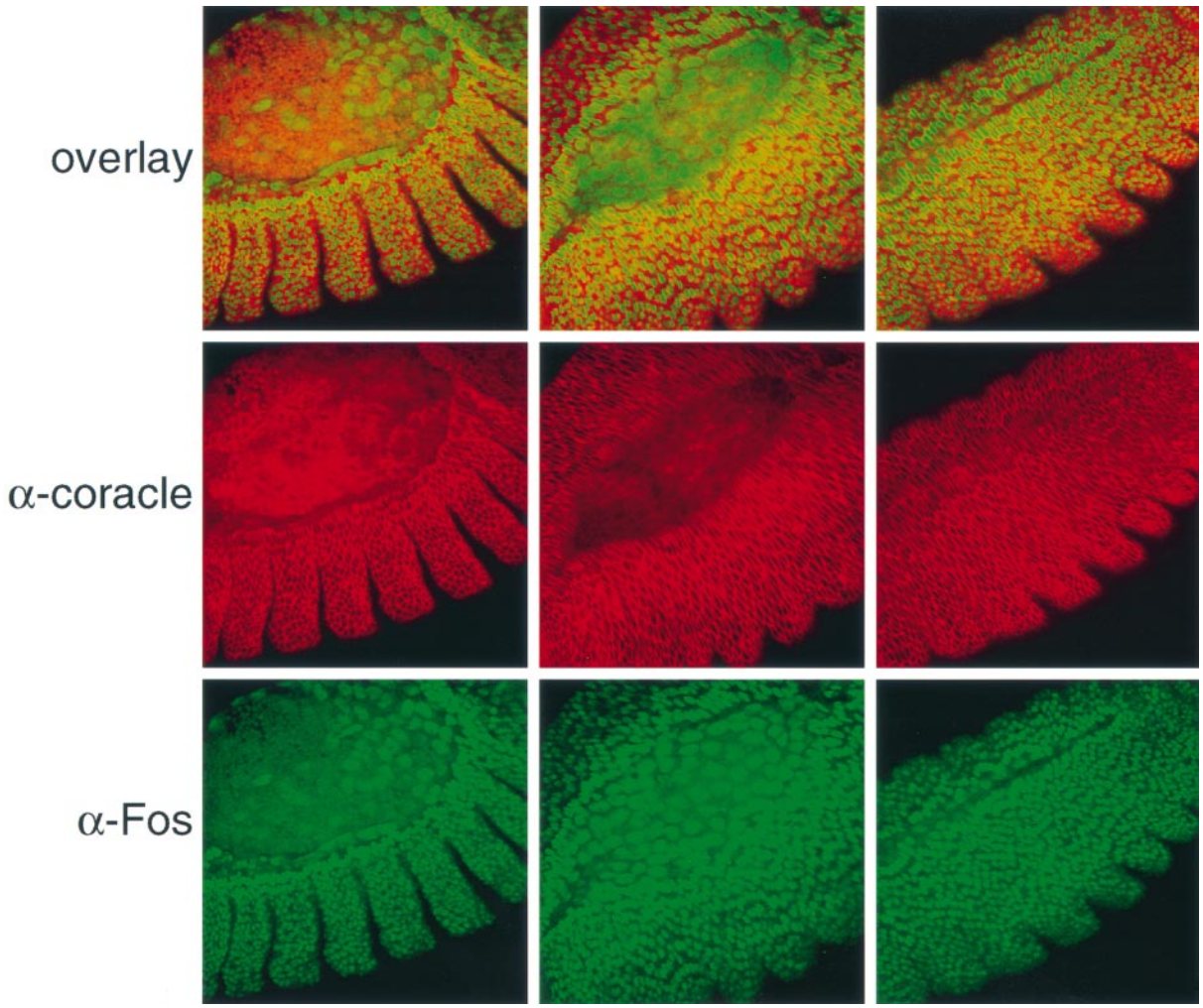


**Fig. 1.** D-Fos is expressed in epidermal cells during dorsal closure. Dorso-lateral views of wild-type embryos stained with anti-Coracle and anti-D-Fos are shown. Anterior is left. From left to right, the panels show three progressing stages of dorsal closure with its initiation, left, and its final stages, right. Anti-Coracle staining (in red) highlights the shape of the epidermal cells by staining the septate junctions (Fehon *et al*., 1994). Nuclear anti-D-Fos staining is shown in green. Note that during initiation of dorsal closure (right panels), the highest levels of D-Fos are detected in the most dorsal row of cells, the leading edge. These cells have started to elongate along the dorso-ventral axis, as visualized by Coracle staining. Subsequently, D-Fos levels increase in more lateral cells (middle panels) concomitant with their elongation. The right panels show an embryo that has almost completed dorsal closure: D-Fos is expressed at high levels throughout the epidermis and the lateral epidermal cells appear elongated. Note that the cells of the leading edge express the highest levels of D-Fos throughout the process and that it is also expressed in the amnioserosa.

which are more stable than the respective homodimers (Perkins *et al*., 1990).

Here we show that the previously identified gene *kayak* (*kay*) (Jürgens *et al*., 1984) encodes D-Fos. Genetic and phenotypic analyses indicate that D-*fos*, like D-*jun*, is required for dorsal closure and the elongation of the cells in the lateral epidermis, as well as for the expression of *dpp* in cells of the leading edge. These results suggest that D-Fos is a component of the JNK signaling pathway and provide the basis for detailed genetic and molecular analysis of the role of Fos in development and signal transduction *in vivo*.

## Results

### *Epidermal cells express D-Fos in a dynamic fashion during dorsal closure*

Since a requirement of D-Jun in dorsal closure has been documented (Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar and Hafen, 1997), we investigated a potential role for its dimerization partner, D-Fos, in this process. Thus, we raised polyclonal antibodies against bacterially expressed D-Fos and examined its expression during the relevant stages of embryogenesis by immunostaining.

Fos expression is widespread, with varying levels throughout embryogenesis. Strikingly, tissues with high Fos expression include the cells of the amnioserosa and the lateral epidermis during the process of dorsal closure (Figure 1). At the onset of dorsal closure, elevated levels of D-Fos (shown in green) can be detected in the nuclei of leading-edge cells as they initiate elongation. Subsequently, elevated expression of Fos can also be observed in more ventrally located epidermal cells. Concurrently, cell elongation spreads laterally (visualized by anti-Coracle staining, red channel in Figure 1), until the two edges meet at the dorsal midline. At this stage, Fos is strongly expressed throughout the embryonic epidermis, with the highest levels remaining in the cells of the leading edge (Figure 1). This expression pattern is very similar to that of D-Jun (Kockel *et al*., 1997 and unpublished data) and

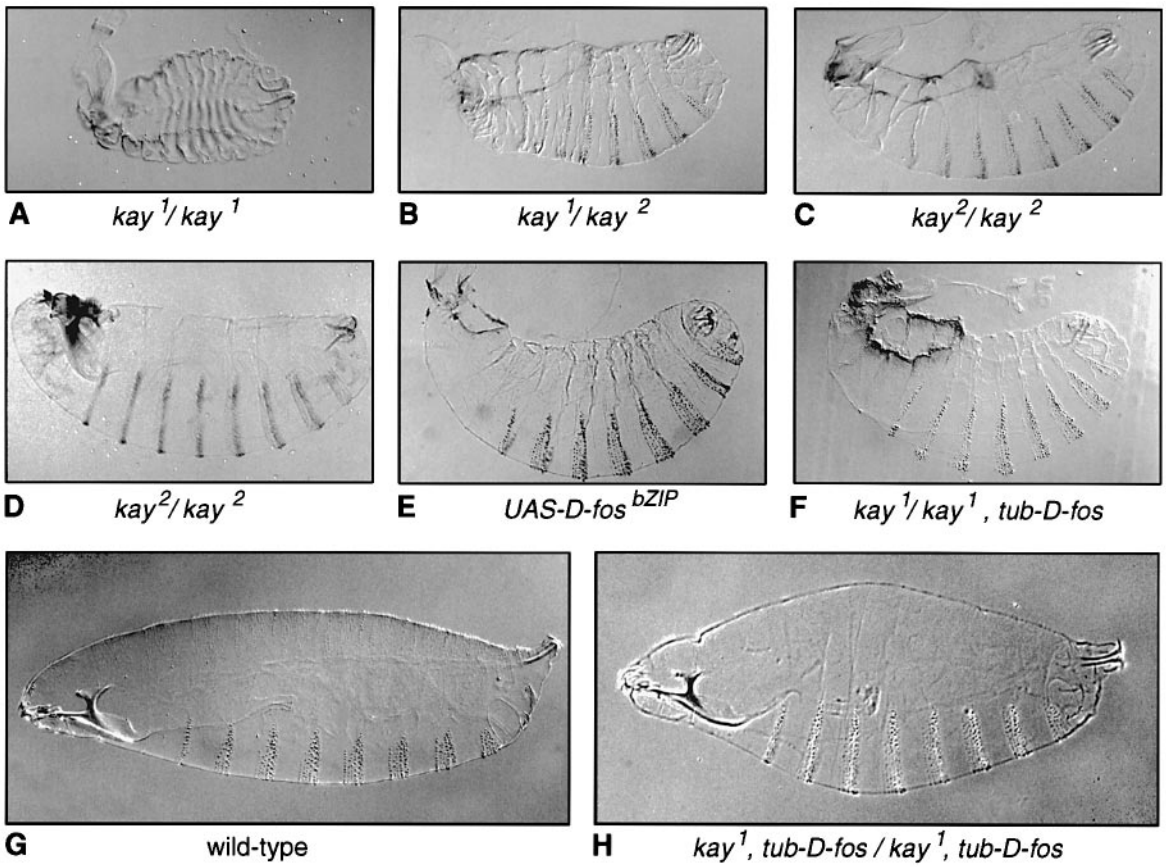


**Fig. 2.** Embryonic phenotype of *kayak* mutants. Cuticle preparations of the following genotypes are shown in scale [all cuticles are from animals raised at 25°C, except in (D) which was raised at 18°C, and are oriented anterior left and dorsal side up]: (**A**) kay$^1$/ kay$^1$; (**B**) kay$^1$/kay$^2$; (**C**) kay$^2$/kay$^2$; (**D**) kay$^2$/kay$^2$ (at 18°C); (**E**) *UAS*-D-*Fos*$^{bZIP}$*; C765-GAL4*; (**F**) *kay*$^1$/*kay*$^1$, *tub-D-fos*; (**G**) wild-type; (**H**) *kay*$^1$, *tub-D-fos/ kay*$^1$, *tub-D-fos*. Note the defects in dorsal closure that lead to an anteriorly and dorsally open cuticle in the mutant embryos (A–E). *kay*$^1$ always behaves as the stronger allele with a severe, highly penetrant phenotype, while the transheterozygotes (*kay*$^1$/*kay*$^2$) display an intermediate phenotype. Epidermal expression of dominant-negative D-Fos (E) causes a phenotype similar to the hypomorphic *kay*$^2$ allele. *tub-D-fos* rescues in a dosage sensitive manner. The *kay*$^1$, *tub-D-fos/ kay*$^1$, *tub-D-fos* mutants die only as fully developed embryos (H) and fail to hatch (possibly because the protein levels of D-Fos as expressed by the *tub-D-fos* transgene were not high enough, or due to a second site mutation near *kay*).

also correlates with the JNK-pathway-dependent stripe of *dpp* expression in the leading edge, which becomes apparent during the initiation phase of dorsal closure (St Johnston and Gelbart, 1987; Glise and Noselli, 1997; Hou *et al*., 1997; Riesgo-Escovar and Hafen, 1997). Therefore, it is conceivable that *Drosophila* Fos acts in conjunction with Jun to regulate dorsal closure and *dpp* expression.

### *The 'dorsal open' locus kayak*

Mutant alleles of D-*fos* have not yet been described. Based on the potential involvement of D-Fos in the process of dorsal closure, as suggested above, we examined known dorsal open mutants for defects in D-*fos*. We concentrated on *kayak* (*kay*), an EMS-induced embryonic lethal mutant (Jürgens *et al*., 1984).

Meiotic recombination mapping placed the *kay* locus, which has not been characterized at the molecular level yet, at the chromosomal region between positions 99A and 100A on chromosome arm 3R (Jürgens *et al*., 1984). This spans the cytological location of D-*fos*, which was mapped at 99B9–99B10 (Perkins *et al*., 1990). *kay*$^1$ mutant embryos all die during embryogenesis with large dorsal and anterior holes, indicating failed dorsal closure and head involution (Jürgens *et al*., 1984; Figure 2A). In *kay*$^2$ embryos, dorsal holes are also observed, but at lower penetrance (Figure 2C and D). Depending on the temperature and genetic background, up to ~1% of *kay*$^2$ homozygotes even develop to adulthood, as seen after recessive markers had been removed from the *kay*$^2$ mutant chromosome by recombination (see Materials and methods). The transheterozygous *kay*$^1$/*kay*$^2$ allelic combination displays an intermediate phenotype and embryonic, or early larval, lethality (Figure 2B). These observations indicate that *kay*$^2$ is a weaker allele than *kay*$^1$, and thus is a hypomorph. To examine the cause of the *kay* defect at the cellular level, mutant and wild-type embryos were stained with an anti-Coracle serum (Fehon *et al*., 1994), which outlines the epidermal cells (Figure 3). This analysis indicates that the *kay* mutant phenotype is caused by a failure of the lateral epidermal cells to elongate. As previously observed in the case of D-*jun* (Kockel *et al*., 1997), leading-edge cells of zygotic *kay*$^1$ mutants initiate elongation transiently, but fail to maintain it and subsequently resume the unelongated shape (Figure 3). The more lateral epidermal cells elongate to a very minor extent and resume the typical polygonal shape after the process has been terminated prematurely (Figure 3F). Thus, the *kay* mutant phenotype closely resembles those described for *hep*, *bsk* and D-*jun* mutant embryos, also at the cellular level (Hou *et al*., 1997; Kockel *et al*., 1997;

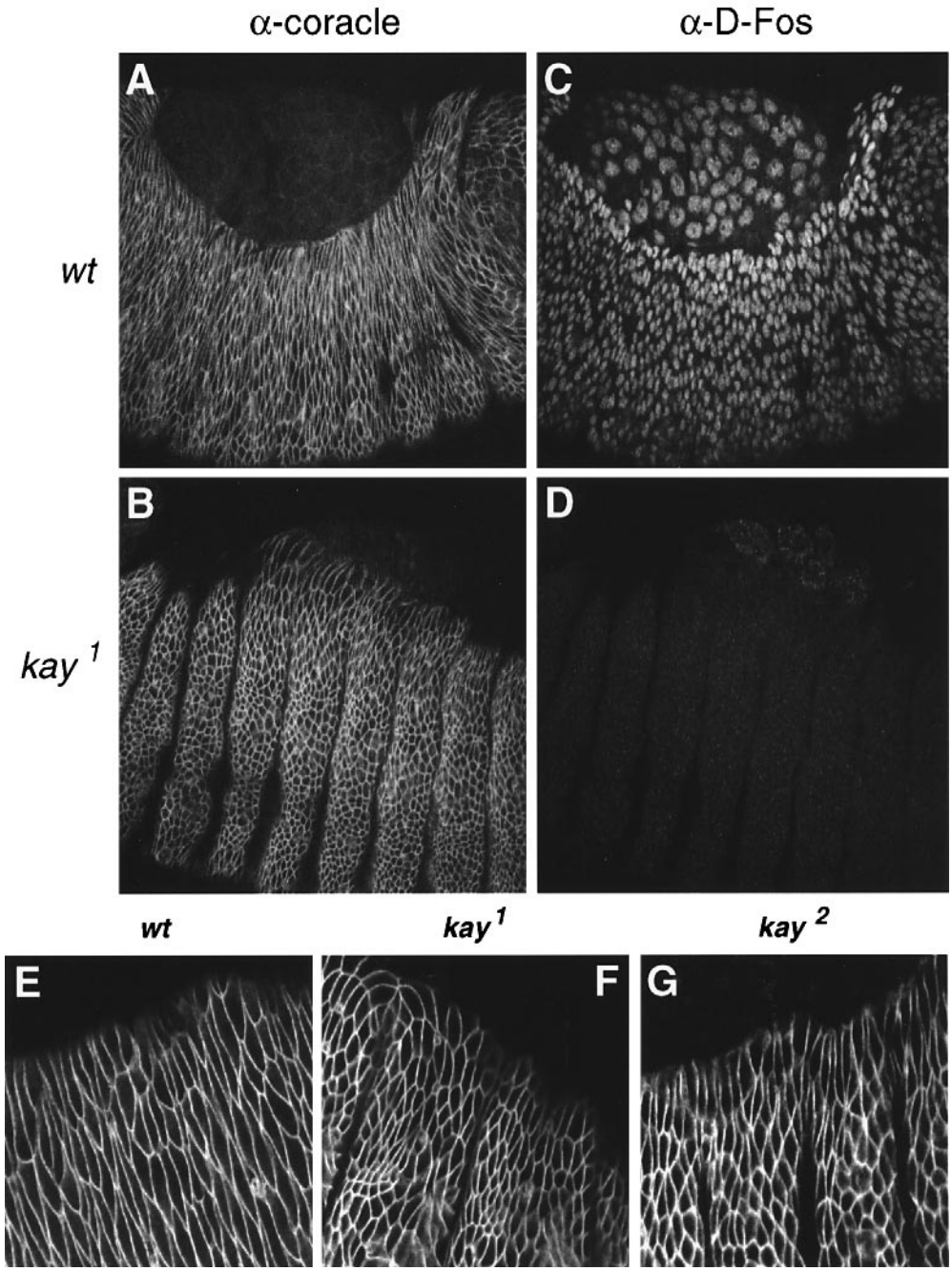


**Fig. 3**. Lateral epidermal cells do not elongate and D-Fos expression is lost in *kay*$^1$ mutants. Cell shape changes during dorsal closure in wild-type and *kay* mutants are visualized with anti-Coracle staining in panels (**A**), (**C**), (**E**), (**F**) and (**G**). Nuclear anti-D-Fos staining is shown in the panels (**B**) and (**D**). All panels are oriented anterior left and dorsal up. Panels (A) and (C) show a wild-type late stage 13 embryo, and (B) and (D) show a corresponding *kay*$^1$ embryo at the same stage. (A) In wild-type embryos, all epidermal cells elongate along the dorso-ventral axis, starting from the leading edge, and progressively include more lateral cells. (B) In *kay*$^1$ mutants, only a transient and incomplete elongation of a few leading-edge cells is seen. (C) and (D) show the same embryos as in (A) and (B), immuno-stained for D-Fos expression. Note that the strong nuclear staining seen in wild type embryos is lost in the *kay*$^1$ mutant. The bottom panels (E–G) show a higher magnification of the lateral epidermis, including the leading edge of slightly older (stage 14) embryos, than shown in panels (A/C) and (B/D): in wild-type (E), lateral cells elongate, while in *kay*$^1$ mutants (F) no elongation of lateral cells is detectable at this stage. Although some leading-edge cells initiate elongation transiently, they never reach their fully elongated state. After the premature termination of dorsal closure, they resume their unelongated shape. The *kay*$^2$ mutant (G) displays a generally weaker phenotype, consistent with the cuticle analysis (see Figure 2).

Riesgo-Escovar and Hafen, 1997; J.Zeitlinger and L.Kockel, unpublished data).

### *kayak represents mutations in D-fos*

To examine a possible role for D-Fos in the regulation of dorsal closure, a truncated derivative of the protein, encompassing only the basic region and leucine zipper (D-Fos$^{bZIP}$), was expressed in epidermal cells during embryogenesis, using the GAL4/UAS system (Brand and Perrimon, 1993). Equivalent constructs of Jun have previously been shown to act as dominant-negative mutants and to cause dorsal open phenotypes when similarly expressed (Bohmann *et al*., 1994; Kockel *et al*., 1997). Flies carrying a *UAS*-D-*fos*$^{bZIP}$ transgene and the epidermal

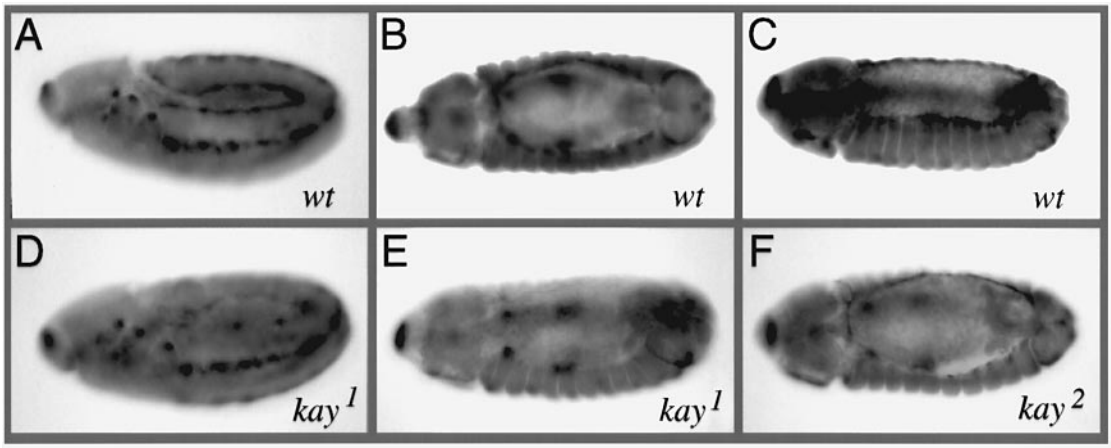


**Fig. 4.** *dpp* expression in cells of the leading edge is regulated by D-*fos/kayak*. All panels show whole mount embryos that have been hybridized with an anti-sense *dpp* probe. Anterior is left and dorsal up, except in (B), (E) and (F) which show a dorsal view. Panels (A) and (E) show germ band extended-stage embryos, and all others show embryos following germ band retraction at the onset of dorsal closure. (**A**–**C**) are wild-type, (**D**) and (**E**) are *kay*$^1$, and (**F**) is *kay*$^2$. The *dpp* expression in the cells of the leading edge is absent in *kay*$^1$ and reduced in *kay*$^2$ embryos. Note that other pattern elements of *dpp* expression, e.g. midgut specific expression and the second more ventral stripe (arrows in panels D and E), that are not related to dorsal closure, are not affected.

GAL4 driver line C765 (see materials and methods) were analyzed for cuticle phenotypes (Figure 2E). Such animals develop to late embryonic stages and die with a dorsal anterior hole, reminiscent of the phenotypes of the hypomorphic *kay*$^2$ allele (Figure 2E). Although D-Fos$^{bZIP}$ could theoretically interfere with both Fos and Jun function, the observations that both endogenous proteins are similarly expressed in the lateral epidermis, and that D-Jun$^{bZIP}$ and D-Fos$^{bZIP}$ cause indistinguishable dorsal open phenotypes, are consistent with the notion that D-Fos, like D-Jun, is required for dorsal closure.

Next, *kay* homozygous embryos were immuno-stained for D-Fos expression. The strong nuclear D-Fos signal observed in the wild-type (Figure 3C) is undetectable in homozygous mutant *kay*$^1$ embryos (Figure 3D). In agreement with this observation, Fos expression levels are significantly reduced in heterozygous *kay*$^1$ third instar larvae and pupae, as judged by Western blot analysis (data not shown). The loss of D-Fos expression in *kay*$^1$ mutants, together with the phenotypes of the dominant-negative D-Fos$^{bZIP}$ expression, suggests that the *kay* phenotype results from a 'loss of function' mutation in D-*fos*. In order to corroborate this hypothesis, we attempted to rescue the mutant phenotype of *kay*$^1$ embryos by expression of a D-*fos* cDNA from a heterologous promoter. *Drosophila* stocks carrying a *tubulin* driven D-*fos* transgene (*tub-D-fos*) were combined with homozygous *kay*$^1$ mutants (*kay*$^1$, *tub-D-fos/kay*$^1$, *tub-D-fos*). Such embryos underwent normal dorsal closure and no longer displayed the dorsal open phenotype typical of *kay*$^1$ mutants (Figure 2H). This rescue is dependent on the dose of the transgene, suggesting that higher D-Fos protein levels are critical for full rescue (compare Figure 2F and H). Moreover, the strict lethality of *kay*$^1$/*kay*$^2$ transheterozygotes can be rescued to adulthood by the *tub-D-fos* transgene. Interestingly, such rescued flies display certain phenotypic abnormalities which are reminiscent of strains carrying hypomorphic alleles of *hep/JNKK* (see below). If a similar rescue experiment is attempted with the D-*fos* cDNA under the control of an appropriately stimulated heat shock promoter, adult flies are recovered, some of which resemble wild-type controls (Figure 5, see also Materials and methods). In conclusion, expression of the D-*fos* cDNA is sufficient to alleviate the *kay* mutant phenotype.

To assess the physical nature of the mutations in *kay*$^1$ and *kay*$^2$, the coding regions within the relatively large D-*fos* transcription unit (>20 kb) were amplified for sequence-analysis both from homozygous *kay*$^1$ and *kay*$^2$ embryos and from heterozygous adults (see Materials and methods). Within these regions, several polymorphisms, but no point mutations, were detected (see Note added in proof).

### *D-fos/kay regulates leading-edge specific dpp expression*

The phenotypic similarity between D-*jun* and *kay* mutants suggests that D-Fos and D-Jun act in concert to mediate dorsal closure. Thus, one may predict that D-*fos/kay*, like D-*jun* and the upstream signaling components *bsk/JNK* and *hep/JNKK*, is required for the expression of *dpp* in the leading edge cells. To test this idea, the expression of *dpp* mRNA was monitored in wild-type and *kay*-mutant backgrounds. Expression of *dpp* in the cells of the future leading edge is normally initiated when the germ band is fully extended (Figure 4A) and is maintained throughout dorsal closure (Figure 4B and C). In contrast, in *kay*$^1$ homozygous embryos, *dpp* expression is absent (or reduced in *kay*$^2$ mutants) in cells of the leading edge (Figure 4D–F). Significantly, other pattern elements of *dpp* expression, including a more ventral stripe and midgut-specific expression (arrows in Figure 4), which have previously been shown to be independent of JNK signaling (Glise and Noselli, 1997; Riesgo-Escovar and Hafen, 1997), are still present in the *kay/*D-*fos* mutants (Figure 4). Another downstream effect of Bsk signaling, the transcriptional activation of the *puckered* gene (*puc*) in the cells of the leading edge (Ring and Martinez-Arias, 1993; Glise *et al*., 1995) is also abrogated in *kay*$^1$ mutant embryos, as measured by a *puc*-lacZ enhancer trap (data not shown). Taken together, the requirement of both D-Fos and D-Jun for *dpp* and *puc* expression in leading-edge cells suggests

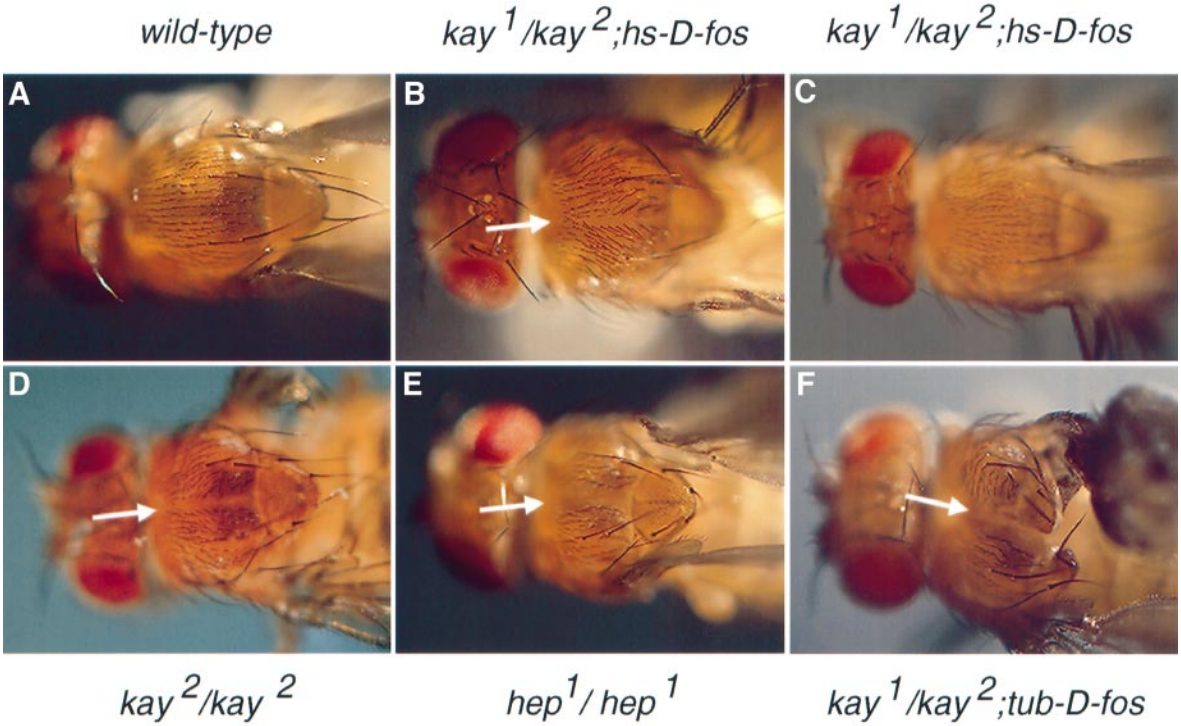


**Fig. 5.** Similarities in adult phenotypes of *kay* and *hep* hypomorphic alleles. All panels show a dorsal view of the thorax, anterior is left. The following genotypes are shown: (**A**) wild-type; (**B**) *kay*$^1$/*kay*$^2$*; hs*-D-*fos* (mild defect); (**C**) *kay*$^1$/*kay*$^2$*; hs*-D-*fos* (showing wild-type phenotype); (**D**) kay$^2$/kay$^2$ escapers; (**E**) *hep*$^1$/*hep*$^1$; (**F**) *kay*$^2$/ *kay*$^1$, *tub-D-fos*. Note that in the phenotypes shown in B, D, E and F, a cleft is visible along the dorsal midline of the thorax (indicated by arrows), where in wild-type animals, imaginal tissue originating from the right and left imaginal discs has fused to give rise to a normal thorax, as shown in wild-type (A) or the fully rescued *kay*$^1$/*kay*$^2$*; hs*-D-*fos* animals (C).

that the JNK signal is relayed by a heterodimeric transcription factor composed of D-Jun and D-Fos.

### *Phenotypic similarities between kay and hep mutants*

The rescued *kay*$^1$/*kay*$^2$*; tub-D-fos* adults display abnormalities which are reminiscent of the phenotypes observed in hypomorphic alleles of *hep* (Figure 5). The original *hep*$^1$ allele is viable and, in addition to poorly penetrant, unilateral deletions of adult structures such as the wing (which gave the gene its name) or the dorsal mesothorax (Glise *et al*., 1995), *hep*$^1$ flies often display a cleft in the middle of the dorsal thorax where the tissues derived from the right and left imaginal discs normally fuse (Figure 5E). The rescued *kay*$^1$/*kay*$^2$*; tub-D-fos* adults display very similar defects on the dorsal thorax (compare Figure 5E and F). Moreover, a comparable phenotypic appearance is observed in homozygous escapers that are found in the hypomorphic *kay*$^2$ stock at low frequency (Figure 5D). These adult defects point to additional functions of *kay*/D-*fos* in *Drosophila* after embryogenesis. The resemblance of the *hep* and *kay* phenotypes in adults and in embryos suggests that D-*fos* is generally required for the relay of Hep–Bsk pathway signals.

## Discussion

In this report we present strong evidence that the *kayak* mutants that were isolated in the Nüsslein-Volhard and Wieschaus screen (Nüsslein-Volhard and Wieschaus, 1980; Jürgens *et al*., 1984), arose from a defect in the gene encoding the *Drosophila* homologue of the mammalian proto-oncogene c-*fos*. The identification of *kay* as a mutant D-*fos* allele is based on several independent lines of evidence: (i) the chromosomal co-localization of the *kay* mutants and the D-*fos* gene; (ii) the lack of D-Fos expression in *kay*$^1$ homozygous embryos; (iii) the reconstitution of dorsal closure in *kay* homozygous mutant embryos after transgenic expression of a D-*fos* cDNA; and (iv) the rescue of the *kay*$^1$/*kay*$^2$ lethality by the expression of D-*fos* cDNA. Finally, since submission of the manuscript a lesion in exon 1 was identified which confirms the identity of *kay*$^1$ as a D-*fos* null allele (see Note added in proof).

The characterization of the D-*fos/kay* phenotype indicates that, at least in the context of dorsal closure, D-Fos acts in a very similar manner to that of its partner molecule, D-Jun. The dorsal closure defect associated with the loss of D-*fos* is indistinguishable from that observed in D-*jun* mutants (Hou *et al*., 1997; Kockel *et al*., 1997; Riesgo-Escovar and Hafen, 1997). In both D-*fos* and D-*jun* mutants, *dpp* expression in the leading edge of the lateral epidermis is lost. This parallel requirement of Fos and Jun for *dpp* expression and dorsal closure cannot be explained by an epistatic relationship of the two transcription factors, i.e. one directing the expression of the other, as no loss of D-Jun expression in a zygotic D-*fos* mutant background was observed, and vice versa (J.Zeitlinger, unpublished data). Similarly, neither D-Fos nor D-Jun expression is affected in zygotic *bsk*/*JNK* (*bsk*$^1$) mutants (J.Zeitlinger, unpublished data). The simplest interpretation of these observations is that D-Jun and D-Fos act together as an archetypal AP-1 heterodimer, to initiate

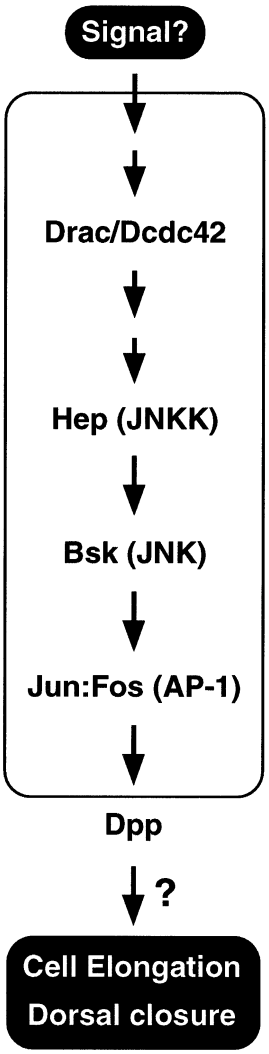


**Fig. 6**. Model for D-Fos function in dorsal closure. Since both D-Fos and D-Jun are required for dorsal closure, it is likely that they function as an archetypal AP-1 heterodimer in this context. See text for details.

dorsal closure by the transcriptional activation of appropriate target genes such as *dpp* and *puc*.

We conclude that a D-Jun–D-Fos heterodimer acts downstream of the *Drosophila* JNK pathway (Figure 6). This hypothesis is supported by the similar adult phenotypes elicited by hypomorphic *hep* and *kay* alleles. Whether D-Fos serves as a JNK substrate in its own right, or is merely required to support a JNK response mediated through the phosphorylation of D-Jun by Bsk, awaits biochemical and genetic clarification.

What is the purpose of having two separate molecules, if Jun and Fos act in such an indistinguishable fashion? It is possible that situations other than dorsal closure exist in which D-Fos and D-Jun play more distinct regulatory roles. In this context, it is worth noting that the expression patterns of the two proteins, though largely similar, are not identical throughout *Drosophila* development. In the embryonic CNS, for example, D-Fos expression is undetectable, whereas D-Jun is expressed at high levels (J.Zeitlinger, unpublished data). Hence, the two proteins appear not to act exclusively as D-Jun–D-Fos heterodimers, but may either form homodimers or have alternative dimerization partners, depending on the developmental and cellular context. Moreover, it has been shown that D-Fos is required in conjunction with Dpp signaling during midgut development, while D-Jun seems not to be involved in this process (Riese *et al*., 1997). Interestingly, in this particular context D-Fos appears to act downstream of Dpp signaling, rather than being required for the activation of *dpp* expression [(Riese *et al*., 1997), see also Figure 4 showing that *dpp* expression in the midgut is not affected in *kay*/D-*fos* mutants].

It is interesting to speculate that, in addition to the molecular conservation of the signal transduction cascade inducing AP-1 dependent transcription, the biological processes initiated by such signaling pathways may be similar as well. Morphogenesis during dorsal closure in the *Drosophila* embryo bears striking similarities to the processes of epiboly and wound healing in vertebrates. In each case, a tissue is encircled by an ectodermal cell sheet. Yolk is incorporated into the embryo during epiboly, and exposed mesodermal tissue is sealed again in wound healing. In some cases, these morphogenetic processes have been attributed to guidance by the ectodermal margin, a phenomenon characterized by unique gene expression patterns in this region (Ring and Martinez-Arias, 1993). In addition to dorsal closure in *Drosophila*, another example of this phenomenon is embryonic wound healing in vertebrates which, unlike adult wound healing, occurs without scar formation (Martin, 1997). Interestingly, both c-*fos* and TGFβ-1 are rapidly induced at the embryonic wound margin after skin excision from rat and mouse embryos (Martin and Nobes, 1992; Martin *et al*., 1993). Therefore, it is tempting to speculate that the molecular program underlying dorsal closure has been conserved or reused during evolution.

## Materials and methods

### *Drosophila strains, molecular and genetic methods*

The original *kay* alleles (Jürgens *et al*., 1984) on *rucuca* chromosomes, from C.Nüsslein-Volhard's collection in Tübingen, were recombined on $w^{1118}$; +; + chromosomes, with only *ca* left as a marker. All experiments produced essentially the same results whether they were performed with the recombined or the original chromosomes, with the notable exception that the original $kay^2$ chromosome was strictly homozygous lethal and had a slightly stronger phenotype before the removal of the recessive markers and potential modifiers.

The *kay* alleles on the recombined chromosomes displayed the following lethality: among $kay^2/kay^2$ embryos, 50% died at 25°C and 10% at 18°C. $kay^2/kay^1$ transheterozygotes are fully lethal as embryos at 25°C and 35% lethal at 18°C. The remaining 65% died as first instar larvae. The lethality also reflected the strength of phenotypes displayed. $kay^1/kay^1$ embryos always exhibited a severe dorsal open phenotype, regardless of the temperature.

The UAS-D-$Fos^{bZIP}$ *Drosophila* strain was generated by cloning the basic region and leucine zipper of *Drosophila* Fos into the P element transformation vector pUAST (Brand and Perrimon, 1993), followed by germline transformation using standard methods (Spradling and Rubin, 1982). The C765-GAL4 driver was a kind gift from Konrad Basler.

The *tubulin* rescue construct was cloned using the pCaSpeR 4 vector, a 2.4 kb *tubulin* promoter fragment (kindly provided by S.Cohen), D-*fos* cDNA and an SV40 poly (A) tail. Heat-shock inducible expression constructs of D-Fos were constructed by inserting the D-*fos cDNA* with a 0.5 kb *Ubx* leader sequence into the pKB267 p-element vector (Basler *et al*., 1991).

For the heat shock rescue experiment, heat shocks of 37° C were applied for 20 min every 8 h. Stocks were otherwise reared at 25° C. *hs*-D-*fos*, $kay^1$/TM6×*hs*-D-*fos*, $kay^2$ /TM3 resulted in >45% rescue (comparing 24 *hs*-D-*fos*, $kay^1$/ *hs*-D-*fos*, $kay^2$ to 52 *hs*-D-*fos*, $kay^2$/TM6

flies). With the tubulin construct, rescue efficiency was lower and more variable.

The *hep*$^{1}$ stock was kindly provided by Stéphane Noselli (Glise *et al*., 1995).

### Antibody production and histological techniques

For antibody production, the D-*fos* open reading frame was cloned into a His-tag bacterial expression vector, processed and purified as described previously for D-Jun (Bohmann *et al*., 1994).

Antibody staining was carried out according to standard methods, using 0.1% Tween. The affinity purified antibody against D-Fos was diluted 1:500. The anti-Coracle antibody (Fehon *et al*., 1994) was used at a 1:5000 dilution. To identify homozygous mutant embryos, *kay/*D-*fos* alleles were balanced over a *hb-lacZ*-marked TM3 balancer chromosome, and embryos were co-stained with a commercial anti-β-galactosidase antibody (Cappel). Staining was detected using a Zeiss confocal microscope (LSM 410) with an Uniphase HeNe 543 nm laser (0.7 mW). Comparison of D-Fos expression levels in wild-type and *kay*$^{1}$ backgrounds was performed on embryos from the same staining reaction by using identical parameter settings.

To detect *puc* expression in a *kay* mutant background, *puc*$^{E69}$ (a kind gift of A.Martinez-Arias) was recombined on the *kay*$^{1}$ chromosome and crossed with *kay*$^{1}$/TM3 *hb-lacZ*.

For whole mount *in situ* hybridization, embryos were treated as previously described (Tautz and Pfeifle, 1989) and hybridized with digoxigenin labeled *dpp* RNA probe. Cuticle preparations and analysis were performed as previously described (Nüsslein-Volhard *et al*., 1984).

### Molecular characterization of kay alleles

Several primer sets were designed to amplify the coding sequences of the *kay*$^{1}$ and *kay*$^{2}$ alleles as independent and overlapping PCR products (the physical characterization of the D-*fos* locus will be published elsewhere). To obtain homozygous genomic DNA, we collected 50–100 embryos that were laid by *kay/+* parents (from the original unrecombined stock) and showed the typical mutant phenotype. PCR products were cloned and sequenced by the EMBL sequencing facility. Both alleles share several identical silent mutations and two amino acid substitutions in the third exon, as compared with the published sequence from the wild-type CantonS strain (Perkins *et al*., 1990) (Ala>Pro at position 453 and Asp>Glu at position 298). These polymorphisms are, however, not allele-specific, as they were also found in the genomic D-*fos* region from *rucuca*, the parental strain used for the Nüsslein-Volhard and Wieschaus screen. PCR amplification of exon 1 sequences (which is devoid of sequence polymorphisms) proved difficult in the case of *kay*$^{1}$. In retrospect, we have to assume that the PCR product was amplified from contaminating maternal or balancer DNA (see Note added in proof).

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## Note added in proof

Since submission of this manuscript similar findings were reported by Riesgo-Escovar,J.R. and Hafen,E. (*Science*, **278**, 669–672). These authors describe a *Hinc*II restriction site polymorphism in the $kay^1$ allele that, according to their analysis, results in a nonsense mutation of codon 92 of the open reading frame and premature termination of the gene product. Our subsequent PCR and genomic Southern analysis of $kay^1$ stocks obtained both from the Tübingen stock center and also from Drs Hafen and Riesgo-Escovar reveals that the sequence of codon 92 remains unaltered in $kay^1$. We find, however, that the *Hinc*II polymorphism is caused by a deletion in the 5′ area of the gene that removes exon 1 (including the translation start site). This mutation supports our and Riesgo-Escovar and Hafen's classification of $kay^1$ as a D-*fos* null allele.