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# **FIBROAL**<br> **Fibroblast growth factor receptor-dependent**<br> **Proceptor-dependent morphogenesis of the** *Drosophila* **mesoderm**

### **Robert Wilson and Maria Leptin**\*

*Institute of Genetics, University of Cologne,Weyertal 121, D-50931 Cologne, Germany*

Institute of Genetics, University of Cologne, Weyertal 121, D-50931 Cologne, Germany<br>The *Drosophila* fibroblast growth factor (FGF) receptors Heartless and Breathless are required for the<br>morphogenesis of the mesoderm and The *Drosophila* fibroblast growth factor (FGF) receptors Heartless and Breathless are required for the morphogenesis of the mesoderm and the tracheal system. In this article we discuss a number of questions relating to th The *Drosophila* fibroblast growth factor (FGF) receptors Heartless and Breathless are required for the<br>morphogenesis of the mesoderm and the tracheal system. In this article we discuss a number of questions<br>relating to th morphogenesis of the mesoderm and the tracheal system. In this article we discuss a number of questions<br>relating to the morphogenesis of these tissues and speculate on poorly understood aspects of the<br>underlying mechanisms relating to the morphogenesis of these tissues and speculate on poorly understood aspects of the<br>underlying mechanisms. As yet a ligand has not been identified for Heartless, but in the case of Breathless<br>the ligand may in underlying mechanisms. As yet a ligand has not been identified for Heartless, but in the case of Breathless<br>the ligand may in some situations act as a chemotactic signal. We consider it unlikely that release of a<br>distant c the ligand may in some situations act as a chemotactic signal. We consider it unlikely that release of a distant chemotactic signal plays a role in the morphogenesis of the mesoderm. Instead we propose that the change in t distant chemotactic signal plays a role in the morphogenesis of the mesoderm. Instead we propose that the change in the mesoderm from an invaginated epithelium to a single layer of cells spread out on the ectoderm could be the change in the mesoderm from an invaginated epithelium to a single layer of cells spread out on the understood. The signal could simply be permissive, causing cells to become motile, or it could act as a Exactly how the activation of the FGF receptors affects cell behaviour and leads to cell movement is not understood. The signal could simply be permissive, causing cells to become motile, or it could act as a directional s understood. The signal could simply be permissive, causing cells to become motile, or it could act as a directional signal for cells that are already motile, or perhaps provide both functions. Furthermore, it is unclear ho directional signal for cells that are already motile, or perhaps provide both functions. Furthermore, it is unclear how signal transduction is coupled to morphological change. It seems unlikely that activation of transcrip unclear how signal transduction is coupled to morphological change. It seems unlikely that activation of transcription targets is essential for cell migration and it is possible that FGF signalling may have a direct effect transcription targets is essential for cell migration and it is possible that FGF signalling may have a direct<br>effect on the cytoskeleton independent of the activation of the mitogen-activated protein kinase cascade.<br>Analy effect on the cytoskeleton independent of<br>Analysis of the function of *dof*, which ence<br>may provide an insight into these issues. may provide an insight into these issues.<br> **Keywords:** fibroblast growth factor (FGF); Heartless; Dof; signalling; tyrosine kinase; migration

#### **1. INTRODUCTION**

**1. INTRODUCTION**<br>Cell rearrangement and migration are important<br>morphogenetic processes that shape the developing EMTRODUCTION<br>Cell rearrangement and migration are important<br>morphogenetic processes that shape the developing<br>organism and reshape parts of the adult during wound Cell rearrangement and migration are important<br>morphogenetic processes that shape the developing<br>organism and reshape parts of the adult during wound<br>healing angiogenesis and regeneration Cell movement is morphogenetic processes that shape the developing<br>organism and reshape parts of the adult during wound<br>healing, angiogenesis and regeneration. Cell movement is<br>controlled at various levels. The differentiation state of organism and reshape parts of the adult during wound<br>healing, angiogenesis and regeneration. Cell movement is<br>controlled at various levels. The differentiation state of<br>the cells and specifically the transcription factors healing, angiogenesis and regeneration. Cell movement is<br>controlled at various levels. The differentiation state of<br>the cells, and specifically the transcription factors present,<br>determines which recentors and components o controlled at various levels. The differentiation state of<br>the cells, and specifically the transcription factors present,<br>determines which receptors and components of signal<br>transduction pathways are expressed and hence ho the cells, and specifically the transcription factors present,<br>determines which receptors and components of signal<br>transduction pathways are expressed and hence how the<br>cells respond to their environment. The behaviour of determines which receptors and components of signal<br>transduction pathways are expressed and hence how the<br>cells respond to their environment. The behaviour of<br>differentiated cells is affected by different cues in the transduction pathways are expressed and hence how the cells respond to their environment. The behaviour of differentiated cells is affected by different cues in the cells respond to their environment. The behaviour of<br>differentiated cells is affected by different cues in the<br>environment. There is good evidence that the extra-<br>cellular matrix neighbouring cells growth factors and differentiated cells is affected by different cues in the<br>environment. There is good evidence that the extra-<br>cellular matrix, neighbouring cells, growth factors and<br>chemotactic factors all influence cell movement When a cellular matrix, neighbouring cells, growth factors and chemotactic factors all influence cell movement. When a cell receives a signal from the environment it is relayed to chemotactic factors all influence cell movement. When a<br>cell receives a signal from the environment it is relayed to<br>the actin cytoskeleton by the small GTP-binding proteins<br>R ho Cdc42 and Rac (Chant & Stowers 1995; Ridley cell receives a signal from the environment it is relayed to <br>the actin cytoskeleton by the small GTP-binding proteins of<br>Rho, Cdc42 and Rac (Chant & Stowers 1995; Ridley & re<br>Hall 1992) These molecules can modulate the ac the actin cytoskeleton by the small GTP-binding proteins<br>Rho, Cdc42 and Rac (Chant & Stowers 1995; Ridley &<br>Hall 1992). These molecules can modulate the actin cyto-<br>skeleton of cultured cells in different ways, resulting i Rho, Cdc42 and Rac (Chant & Stowers 1995; Ridley &<br>Hall 1992). These molecules can modulate the actin cyto-<br>skeleton of cultured cells in different ways, resulting in<br>the appearance of filopodia membrane ruffles or lamelli Hall 1992). These molecules can modulate the actin cyto-<br>skeleton of cultured cells in different ways, resulting in<br>the appearance of filopodia, membrane ruffles or lamelli-<br>podia. However little is known about how these s skeleton of cultured cells in different ways, resulting in<br>the appearance of filopodia, membrane ruffles or lamelli-<br>podia. However, little is known about how these structures<br>influence or are employed in viva in the morph the appearance of filopodia, membrane ruffles or lamelli-<br>podia. However, little is known about how these structures<br>influence or are employed *in vivo* in the morphogenic<br>processes of multicellular organisms podia. However, little is known about how these structures<br>influence or are employed *in vivo* in the morphogenic<br>processes of multicellular organisms.<br>One type of receptor for extracellular signals that has duence or are employed *in vivo* in the morphogenic<br>ocesses of multicellular organisms.<br>One type of receptor for extracellular signals that has<br>en shown to be necessary for cell migration in

*Caenorhabditis elegans* and *Drosophila* is the receptor for ¢broblast growth factor (FGF), which is a receptor

tyrosine kinase (RTK). Like other receptors of this class, tyrosine kinase (RTK). Like other receptors of this class,<br>the FGF receptor activates the mitogen-activated protein<br>kinase (MAPK), cascade via the adaptor Grb<sup>2</sup>/Drk and tyrosine kinase (RTK). Like other receptors of this class,<br>the FGF receptor activates the mitogen-activated protein<br>kinase (MAPK) cascade via the adaptor Grb2/Drk and<br>the small GTPase Ras (Wassarman *et al.* 1995). Howeve the FGF receptor activates the mitogen-activated protein<br>kinase (MAPK) cascade via the adaptor Grb2/Drk and<br>the small GTPase Ras (Wassarman *et al.* 1995). However,<br>unlike other RTKs, it does not bind directly to Grb<sup>2</sup> (s kinase (MAPK) cascade via the adaptor Grb2/Drk and<br>the small GTPase Ras (Wassarman *et al.* 1995). However,<br>unlike other RTKs, it does not bind directly to Grb2 (see<br>Klint *et al.* 1995: Koubara *et al.* 1997). One of the the small GTPase Ras (Wassarman *et al.* 1995). However, unlike other RTKs, it does not bind directly to Grb2 (see Klint *et al.* 1995; Kouhara *et al.* 1997). One of the unsolved problems is therefore how the signal from unlike other RTKs, it does not bind directly to Grb2 (see<br>Klint *et al.* 1995; Kouhara *et al.* 1997). One of the<br>unsolved problems is therefore how the signal from the<br>EGE receptor is transmitted to Grb2 and Ras. Other Klint *et al.* 1995; Kouhara *et al.* 1997). One of the unsolved problems is therefore how the signal from the FGF receptor is transmitted to Grb2 and Ras. Other proteins must exist that help in establishing a link unsolved problems is therefore how the signal from the FGF receptor is transmitted to Grb2 and Ras. Other proteins must exist that help in establishing a link between the FGF receptor and the MAPK module Two FGF receptor is transmitted to Grb2 and Ras. Other<br>proteins must exist that help in establishing a link<br>between the FGF receptor and the MAPK module. Two<br>candidates FRS2 and Dof have been found (Koubara et proteins must exist that help in establishing a link<br>between the FGF receptor and the MAPK module. Two<br>candidates, FRS2 and Dof, have been found (Kouhara *et*<br>al. 1997: Vincent et al. 1998: Wang et al. 1996) FRS2 was between the FGF receptor and the MAPK module. Two candidates, FRS2 and Dof, have been found (Kouhara *et al.* 1997; Vincent *et al.* 1998; Wang *et al.* 1996). FRS2 was candidates, FRS2 and Dof, have been found (Kouhara *et al.* 1997; Vincent *et al.* 1998; Wang *et al.* 1996). FRS2 was identified biochemically in vertebrates as a protein that is phosphorylated upon stimulation of cells al. 1997; Vincent *et al.* 1998; Wang *et al.* 1996). FRS2 was<br>identified biochemically in vertebrates as a protein that is<br>phosphorylated upon stimulation of cells by FGF, and<br>forms a complex with Grb2 and Sos. Dof was f identified biochemically in vertebrates as a protein that is<br>phosphorylated upon stimulation of cells by FGF, and<br>forms a complex with Grb2 and Sos. Dof was found in<br>*Drasobbila* and shown to be essential for the transmiss phosphorylated upon stimulation of cells by FGF, and<br>forms a complex with Grb2 and Sos. Dof was found in<br>*Drosophila* and shown to be essential for the transmission<br>of the EGF signal to MAPK acting downstream of the forms a complex with Grb2 and Sos. Dof was found in<br>*Drosophila* and shown to be essential for the transmission<br>of the FGF signal to MAPK, acting downstream of the<br>receptor but unstream of Ras (Imam *et al.* 1999) receptor, but upstream of Ras (Imam *et al*. 1999; of the FGF signal to MAPK, acting downstream of the

#### **2. FGF RECEPTOR SIGNALLING DURING** *DROSOPHILA* **DEVELOPMENT**

One type of receptor for extracellular signals that has and Breathless, are required for the morphogenesis of been shown to be necessary for cell migration in different tissues. The gene *breathless* encodes the FGF **EXECTEDE SIGNALLING DURING**<br> **CHOCOPHILA DEVELOPMENT**<br>
The two known FGF receptors in *Drosophila*, Heartless<br>
d Breathless are required for the morphogenesis of **EXECT SEVELOPMENT**<br>The two known FGF receptors in *Drosophila*, Heartless<br>and Breathless, are required for the morphogenesis of<br>different tissues. The gene *breathless* encodes the FGF The two known FGF receptors in *Drosophila*, Heartless<br>and Breathless, are required for the morphogenesis of<br>different tissues. The gene *breathless* encodes the FGF<br>receptor expressed in the respiratory system of the fly and Breathless, are required for the morphogenesis of<br>different tissues. The gene *breathless* encodes the FGF<br>receptor expressed in the respiratory system of the fly, the<br>trackease and is needed both for the establishment different tissues. The gene *breathless* encodes the FGF<br>receptor expressed in the respiratory system of the fly, the<br>tracheae, and is needed both for the establishment of the<br>tracheal tree and for its remodelling in respo receptor expressed in the respiratory system of the fly, the<br>tracheae, and is needed both for the establishment of the<br>tracheal tree and for its remodelling in response to<br>changes in overgn requirement (Jarecki *et al* 199 tracheae, and is needed both for the establishment of the tracheal tree and for its remodelling in response to changes in oxygen requirement (Jarecki *et al.* 1999;



Figure 1. (*a*) Stages of mesoderm morphogenesis in the *Drosophila* embryo. Drawings of cross-sections of embryos at three successive stages of mesoderm invagination. Cells in which specific changes are observed are marked in colour. The mesectodermal cells, which border the mesoderm on each side, are ¢lled in with grey. (i) The invagination begins with the successive stages of mesoderm invagination. Cells in which specific changes are observed are marked in colour. The<br>mesectodermal cells, which border the mesoderm on each side, are filled in with grey. (i) The invagination mesectodermal cells, which border the mesoderm on each side, are filled in with grey. (i) The invagination begins with the<br>formation of the ventral furrow by apical constriction of the most ventral cells (constricted apica formation of the ventral furrow by apical constriction of the most ventral cells (constricted apical sides of cells in blue). (ii) When<br>the mesoderm has invaginated, its central part, still an epithelial tube, begins to ma the mesoderm has invaginated, its central part, still an epithelial tube, begins to make contact with the ectoderm. Yellow: cells<br>expressing the FGF receptor Heartless and its downstream signalling mediator, Dof. MAPK acti expressing the FGF receptor Heartless and its downstream signalling mediator, Dof. MAPK activity begins to be detectable in<br>the first cells that have already contacted the ectoderm (not marked here). (iii) As the mesoderma against the ectoderm under the influence of FGF receptor signalling (dark green: cells in which the MAPK cascade has been<br>activated), cell division begins throughout the mesoderm primordium (light green). (*b*) Defects in against the ectoderm under the influence of FGF receptor signalling (dark green: cells in which the MAPK cascade has been<br>activated), cell division begins throughout the mesoderm primordium (light green). (*b*) Defects in activated), cell division begins throughout the mesoderm primordium (light green). (b) Defects in heartless and dof mutant<br>embryos. (i) The first steps of mesoderm morphogenesis are unaffected. The ventral furrow forms an embryos. (i) The first steps of mesoderm morphogenesis are unaffected. The ventral furrow forms and the mesoderm invaginates<br>as an epithelial tube. (ii) Once the tube is fully internalized, the establishment of the tight c as an epithelial tube. (ii) Once the tube is fully internalized, the establishment of the tight contact between the mesoderm and<br>the ectoderm is lost. No MAPK activity is detectable. (iii) The mesoderm loses its epithelial the ectoderm is lost. No MAPK activity is detectable. (iii) The mesoderm loses its epithelial structure and its cells divide. Now<br>some cells are in contact with the ectoderm but most remain in the centre near the site of i some cells are in contact with the ectoderm but most remain in the centre near the site of invagination. Activation of MAPK is<br>still not observed. (c) Possible mechanisms by which the mesodermal cell layer is established. still not observed. (c) Possible mechanisms by which the mesodermal cell layer is established. (i) The cells at the leading edge of<br>the mesoderm migrate towards a chemotactic signal at the dorsal edge of the ectoderm. (ii) the mesoderm migrate towards a chemotactic signal at the dorsal edge of the ectoderm. (ii) The cells of the mesoderm have a low<br>affinity for each other (pink) and a high affinity for the ectoderm (red) and seek to maximize extension to each other (pink) and a high affinity for the ectoderm (red) and seek to maximize their contact with the ectoderm.<br>(iii) Convergent extension in the mesoderm reduces the mesoderm to a single-cell layer and ext (iii) Convergent extension<br>extension to occur in the<br>and more internal cells.

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> and more internal cells.<br>Klämbt *et al.* 1992; Reichman-Fried *et al.* 1994). The gene<br>heartless encodes an EGE receptor expressed in the Klämbt *et al.* 1992; Reichman-Fried *et al.* 1994). The gene *heartless* encodes an FGF receptor expressed in the embryonic mesoderm and was first identified because of Klämbt *et al.* 1992; Reichman-Fried *et al.* 1994). The gene<br>*heartless* encodes an FGF receptor expressed in the<br>embryonic mesoderm and was first identified because of<br>its essential role in the development of one of the *heartless* encodes an FGF receptor expressed in the embryonic mesoderm and was first identified because of its essential role in the development of one of the mesodermal derivatives the heart (Beiman *et al* 1996; embryonic mesoderm and was first identified because of<br>its essential role in the development of one of the<br>mesodermal derivatives, the heart (Beiman *et al.* 1996;<br>Gisselbrecht *et al.* 1996; Shishido *et al.* 1993–1997) its essential role in the development of one<br>mesodermal derivatives, the heart (Beiman *et al.*<br>Gisselbrecht *et al.* 1996; Shishido *et al.* 1993, 1997).<br>The development of the tracheal system begins **w** expodermal derivatives, the heart (Beiman *et al.* 1996; sselbrecht *et al.* 1996; Shishido *et al.* 1993, 1997).<br>The development of the tracheal system begins with the vacination of the enithelial primordium. The anlage

Gisselbrecht *et al.* 1996; Shishido *et al.* 1993, 1997).<br>The development of the tracheal system begins with the invagination of the epithelial primordium. The anlage of The development of the tracheal system begins with the<br>invagination of the epithelial primordium. The anlage of<br>the tracheal tree consists of ten segmentally arranged pairs<br>of deen ectodermal invaginations of approximately invagination of the epithelial primordium. The anlage of<br>the tracheal tree consists of ten segmentally arranged pairs<br>of deep ectodermal invaginations of approximately 20 cells<br>each. Each of these invaginated sacks undergo the tracheal tree consists of ten segmentally arranged pairs<br>of deep ectodermal invaginations of approximately 20 cells<br>each. Each of these invaginated sacks undergoes a stereo-<br>typed sequence of cell rearrangements that c of deep ectodermal invaginations of approximately 20 cells<br>each. Each of these invaginated sacks undergoes a stereoeach. Each of these invaginated sacks undergoes a stereotyped sequence of cell rearrangements that converts the epithelial invagination into a branched structure (Sama-<br>kovlis et al. 1996). Further cell rearrangements exte typed sequence of cell rearrangements that converts the epithelial invagination into a branched structure (Sama-<br>kovlis *et al.* 1996). Further cell rearrangements extend and<br>bifurcate the branches a subset of which eventu epithelial invagination into a branched structure (Sama-kovlis *et al.* 1996). Further cell rearrangements extend and bifurcate the branches, a subset of which eventually fuse to kovlis *et al.* 1996). Further cell rearrangements extend and<br>bifurcate the branches, a subset of which eventually fuse to<br>create a continuous tracheal system. Finally, fine tertiary<br>branches grow into tissues, directed by bifurcate the branches, a subset of which eventually fuse to<br>create a continuous tracheal system. Finally, fine tertiary<br>branches grow into tissues, directed by the oxygen require-<br>ment of the target tissues (Jarecki *et a* create a continuous tracheal system. Finally, fine tertiary<br>branches grow into tissues, directed by the oxygen require-<br>ment of the target tissues (Jarecki *et al.* 1999). In *breathless*<br>mutants the epithelial invaginatio branches grow into tissues, directed by the oxygen requirement of the target tissues (Jarecki *et al.* 1999). In *breathless* mutants, the epithelial invagination of the tracheal

primordium is unaffected, but the later steps of tracheal<br>morphogenesis fail to occur. However, at least part of the primordium is unaffected, but the later steps of tracheal<br>morphogenesis fail to occur. However, at least part of the<br>differentiation programme of the cells in the unbranched primordium is unaffected, but the later steps of tracheal<br>morphogenesis fail to occur. However, at least part of the<br>differentiation programme of the cells in the unbranched<br>invaginations continues, as judged by the expres morphogenesis fail to occur. However, at least part of the differentiation programme of the cells in the unbranched invaginations continues, as judged by the expression of several late tracheal differentiation markers (Klä 1992; Reichman-Fried et al. 1994; Samakovlis et al. 1996). veral late tracheal differentiation markers (Klämbt *et al.*)<br>92; Reichman-Fried *et al.* 1994; Samakovlis *et al.* 1996).<br>The ligand for the tracheal FGF receptor has been<br>entified as the product of the *branchless* gene

1992; Reichman-Fried *et al.* 1994; Samakovlis *et al.* 1996).<br>The ligand for the tracheal FGF receptor has been<br>identified as the product of the *branchless* gene (Sutherland<br> $et$  al. 1996). The *branchless* gene, which c The ligand for the tracheal FGF receptor has been<br>identified as the product of the *branchless* gene, (Sutherland<br>*et al.* 1996). The *branchless* gene, which codes for an FGF<br>homologue shows the same mutant phenotyne as identified as the product of the *branchless* gene (Sutherland *et al.* 1996). The *branchless* gene, which codes for an FGF homologue, shows the same mutant phenotype as *breathless*. Interestingly, *branchless* is expres et al. 1996). The branchless gene, which codes for an FGF growing tracheal branches, fading in the region which a Interestingly, *branchless* is expressed near the tips of the growing tracheal branches, fading in the region which a branch has just reached, and being activated at the next point towards which the branch is destined to e growing tracheal branches, fading in the region which a<br>branch has just reached, and being activated at the next<br>point towards which the branch is destined to extend. This<br>has led to the suggestion that Branchless acts as branch has just reached, and being activated at the next<br>point towards which the branch is destined to extend. This<br>has led to the suggestion that Branchless acts as a chemo-<br>tactic signal (Sutherland *et al.* 1996). The point towards which the branch is destined to extend. This<br>has led to the suggestion that Branchless acts as a chemo-<br>tactic signal (Sutherland *et al.* 1996). The chemotactic<br>properties of Branchless, are best demonstrate has led to the suggestion that Branchless acts as a chemotactic signal (Sutherland *et al.* 1996). The chemotactic properties of Branchless are best demonstrated by its ability to attract the growth of the fine tertiary br tactic signal (Sutherland *et al.* 1996). The chemotactic properties of Branchless are best demonstrated by its ability to attract the growth of the fine tertiary branches in larvae towards oxygen-starved cells (Iarecki properties of Branchless are best demonstrated by its ability to attract the growth of the fine tertiary branches in larvae towards oxygen-starved cells (Jarecki *et al.* 1999).



Figure 2. The consequence of changing ectodermal cell fates upon the migration of the mesoderm. Embryos in which the fates of ectodermal cells are changed due to mutations in zygotic ventralizing genes were used to test w Figure 2. The consequence of changing ectodermal cell fates upon the migration of the mesoderm. Embryos in which the fates of ectodermal cells are changed due to mutations in zygotic ventralizing genes were used to test w ectodermal cells are changed due to mutations in zygotic ventralizing genes were used to test whether the fates of the underlyin<br>ectoderm cells influence the migration of mesodermal cells (*a*) *wt*; (*b*) *sog*; (*c*) *t* about 50% egg length of embryos at the fully extended germ-band stage are shown, which have been stained with antibodies<br>directed against Twist to visualize the mesoderm. The phenotypes of the mutant embryos are variable, phenotypes (i.e. where migration was furthest towards the dorsal midline) are shown.

The mesoderm also invaginates as an epithelial layer, The mesoderm also invaginates as an epithelial layer,<br>driven by cell shape changes under the control of two<br>transcription factors Twist and Spail, which also control The mesoderm also invaginates as an epithelial layer,<br>driven by cell shape changes under the control of two<br>transcription factors, Twist and Snail, which also control<br>all subsequent steps of mesoderm development including driven by cell shape changes under the control of two<br>transcription factors, Twist and Snail, which also control<br>all subsequent steps of mesoderm development, including<br>the expression of the EGF recentor Heartless and Dof transcription factors, Twist and Snail, which also control<br>all subsequent steps of mesoderm development, including<br>the expression of the FGF receptor Heartless and Dof.<br>When the central part of the mesoderm is fully intern all subsequent steps of mesoderm development, including<br>the expression of the FGF receptor Heartless and Dof.<br>When the central part of the mesoderm is fully internal-<br>ized its cells make contact with the ectoderm (figure l the expression of the FGF receptor Heartless and Dof.<br>When the central part of the mesoderm is fully internal-<br>ized, its cells make contact with the ectoderm (figure 1*a*).<br>Adherens iunctions that had been established in t When the central part of the mesoderm is fully internal-<br>ized, its cells make contact with the ectoderm (figure 1*a*).<br>Adherens junctions that had been established in the<br>blastoderm are now lost (Oda & Tsukita 1999) and t ized, its cells make contact with the ectoderm (figure 1*a*).<br>Adherens junctions that had been established in the<br>blastoderm are now lost (Oda & Tsukita 1999) and the<br>enithelium dissociates into single cells which spread o Adherens junctions that had been established in the blastoderm are now lost (Oda & Tsukita 1999) and the epithelium dissociates into single cells which spread out to blastoderm are now lost (Oda & Tsukita 1999) and the<br>epithelium dissociates into single-cell swhich spread out to<br>cover the ectoderm as a single-cell layer (figure 1*a*). The<br>loss of the epithelial state does not depend on epithelium dissociates into single cells which spread out to cover the ectoderm as a single-cell layer (figure la). The loss of the epithelial state does not depend on cell division although the cells divide during this pr cover the ectoderm as a single-cell layer (figure la). The<br>loss of the epithelial state does not depend on cell<br>division, although the cells divide during this process,<br>since it also occurs in *string* mutants in which no loss of the epithelial state does not depend on cell<br>division, although the cells divide during this process,<br>since it also occurs in *string* mutants, in which no division<br>cytoplasmic protein that unlike most intracellula division, although the cells divide during this process,<br>since it also occurs in *string* mutants, in which no division<br>takes place (Leptin & Grunewald 1990). One possibility<br>is that this state is caused or facilitated by since it also occurs in *string* mutants, in which no division<br>takes place (Leptin & Grunewald 1990). One possibility<br>is that this state is caused or facilitated by mesodermal<br>cells switching from expression of ectodermal takes place (Leptin & Grunewald 1990). One possibility<br>is that this state is caused or facilitated by mesodermal<br>cells switching from expression of ectodermal E-cadherin<br>to N-cadherin. The change from E- to N-cadherin is is that this state is caused or facilitated by mesodermal<br>cells switching from expression of ectodermal E-cadherin<br>to N-cadherin. The change from E- to N-cadherin is<br>under the control of the two key transcription factors t cells switching from expression of ectodermal E-cadherin<br>to N-cadherin. The change from E- to N-cadherin is<br>under the control of the two key transcription factors that<br>regulate development of the mesoderm. Spail represses to N-cadherin. The change from E- to N-cadherin is<br>under the control of the two key transcription factors that<br>regulate development of the mesoderm. Snail represses<br>E-cadherin in the mesoderm while Twist activates the under the control of the two key transcription factors that regulate development of the mesoderm. Snail represses E-cadherin in the mesoderm, while Twist activates the expression of N-cadherin (Oda *et al.* 1998). regulate development of the mesoderm. Snail represses cadherin in the mesoderm, while Twist activates the<br>pression of N-cadherin (Oda *et al.* 1998).<br>The function of the FGF signalling pathway is needed<br>mediately after mesoderm invagination. In heartless and

expression of N-cadherin (Oda *et al.* 1998).<br>The function of the FGF signalling pathway is needed<br>immediately after mesoderm invagination. In *heartless* and<br>*dof* mutants the mesoderm primordium fails to make The function of the FGF signalling pathway is needed<br>immediately after mesoderm invagination. In *heartless* and<br>*dof* mutants the mesoderm primordium fails to make<br>contact with the ectoderm and spread out as a single-cell immediately after mesoderm invagination. In *heartless* and *dof* mutants the mesoderm primordium fails to make contact with the ectoderm and spread out as a single-cell layer and initially remains near the site of invagin *dof* mutants the mesoderm primordium fails to make<br>contact with the ectoderm and spread out as a single-cell<br>layer, and initially remains near the site of invagination<br>(figure 1b) Mesodermal cells only begin to distribut contact with the ectoderm and spread out as a single-cell<br>layer, and initially remains near the site of invagination<br>(figure 1*b*). Mesodermal cells only begin to distribute over layer, and initially remains near the site of invagination<br>(figure 1b). Mesodermal cells only begin to distribute over<br>the ectodermal surface after a long delay. The resulting<br>mesoderm varies in thickness and is narrower t (figure  $1b$ ). Mesodermal cells only begin to distribute over<br>the ectodermal surface after a long delay. The resulting<br>mesoderm varies in thickness and is narrower than in the<br>wild-type. One effect of this is that cells a the ectodermal surface after a long delay. The resulting<br>mesoderm varies in thickness and is narrower than in the<br>wild-type. One effect of this is that cells at the edge of the<br>mesoderm, which are normally induced by a sig mesoderm varies in thickness and is narrower than in the wild-type. One effect of this is that cells at the edge of the mesoderm, which are normally induced by a signal from

the ectoderm to develop as visceral musculature and<br>heart fail to reach the regions in which they can receive the ectoderm to develop as visceral musculature and<br>heart, fail to reach the regions in which they can receive<br>this signal, and the primordia of these tissues are therethe ectoderm to develop as visceral musculature and<br>heart, fail to reach the regions in which they can receive<br>this signal, and the primordia of these tissues are there-<br>fore reduced. In wild-type embryos the mesodermal ce heart, fail to reach the regions in which they can receive<br>this signal, and the primordia of these tissues are there-<br>fore reduced. In wild-type embryos the mesodermal cells<br>that establish the initial contact between the m this signal, and the primordia of these tissues are there-<br>fore reduced. In wild-type embryos the mesodermal cells<br>that establish the initial contact between the mesoderm<br>and the ectoderm accumulate the diphosphorylated fo that establish the initial contact between the mesoderm<br>and the ectoderm accumulate the diphosphorylated form that establish the initial contact between the mesoderm<br>and the ectoderm accumulate the diphosphorylated form<br>of extracellular signal-regulated kinase (Erk) (Gabay<br> $et$ <sup>al</sup> 1997). The activation of the MAPK cascade in these and the ectoderm accumulate the diphosphorylated form<br>of extracellular signal-regulated kinase (Erk) (Gabay<br>*et al.* 1997). The activation of the MAPK cascade in these<br>cells is absolutely dependent upon EGF-mediated signal of extracellular signal-regulated kinase (Erk) (Gabay *et al.* 1997). The activation of the MAPK cascade in these cells is absolutely dependent upon FGF-mediated signal-<br>ling since activation of Erk does not occur in eithe et al. 1997). The activation of the MAPK cascade in these cells is absolutely dependent upon FGF-mediated signal-<br>ling, since activation of Erk does not occur in either *heartless* or *dof* mutants (Gabay *et al.* 1997; Vincent *et al.* 1998).<br>*less or dof* mutants (Gabay *et al.* 1997; Vincent *et al.* 1998).<br>It is not exactly clear how Dof acts within the signalling ling, since activation of Erk does not occur in either *heart-*<br>*less* or *dof* mutants (Gabay *et al.* 1997; Vincent *et al.* 1998).<br>It is not exactly clear how Dof acts within the signalling<br>pathway or with which other p *less* or *dof* mutants (Gabay *et al.* 1997; Vincent *et al.* 1998).<br>It is not exactly clear how Dof acts within the signalling<br>pathway or with which other proteins it interacts. Dof is a<br>cytoplasmic protein that unlike m It is not exactly clear how Dof acts within the signalling pathway or with which other proteins it interacts. Dof is a<br>cytoplasmic protein that unlike most intracellular signal-<br>ling components, which are expressed ubiquitously, is<br>only expressed in cells that express the EGE rece cytoplasmic protein that unlike most intracellular signal-<br>ling components, which are expressed ubiquitously, is<br>only expressed in cells that express the FGF receptors.<br>The protein contains an ankyrin repeat a coiled-coil ling components, which are expressed ubiquitously, is<br>only expressed in cells that express the FGF receptors.<br>The protein contains an ankyrin repeat, a coiled-coil<br>structure and many tyrosines within environments that only expressed in cells that express the FGF receptors.<br>The protein contains an ankyrin repeat, a coiled-coil<br>structure and many tyrosines within environments that<br>suggest that if phosphorylated they could act as hinding The protein contains an ankyrin repeat, a coiled-coil<br>structure and many tyrosines within environments that<br>suggest that if phosphorylated they could act as binding<br>sites for the SH2 domains of proteins such as Grb2/drk structure and many tyrosines within environments that<br>suggest that if phosphorylated they could act as binding<br>sites for the SH2 domains of proteins such as Grb2/drk,<br> $Csw/Shn2$ ,  $Ras-GAP$  and PI3-kinase (Vincent et al. suggest that if phosphorylated they could act as binding<br>sites for the SH2 domains of proteins such as Grb2/drk,<br>Csw/Shp2, Ras-GAP and PI3-kinase (Vincent *et al.* 1998). Hence, Dof may be needed to assemble the FGF Csw/Shp2, Ras-GAP and PI3-kinase (Vincent *et al.* 1998). Hence, Dof may be needed to assemble the FGF receptor or allow it to autophosphorylate, it may be an adaptor that links the receptor to Ras, or it may perhaps receptor or allow it to autophosphorylate, it may be an receptor or allow it to autophosphorylate, it may be an adaptor that links the receptor to Ras, or it may perhaps<br>be involved in distributing the signal to different down-<br>stream targets adaptor that linl<br>be involved in c<br>stream targets. **3. HOW DOES FGF SIGNALLING CONTROL CELL**

## **MOVEMENT?**

How cell behaviour is affected by the inability of the mesoderm to receive an FGF signal in *heartless* and *dof*

**BIOLOGICAL**<br>SCIENCES

THE ROYAL

**PHILOSOPHICAL**<br>TRANSACTIONS

PHILOSOPHICAL<br>TRANSACTIONS

**BIOLOGICAL** 

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凹 **CELL** 

**PHILOSOPHICAL**<br>TRANSACTIONS

mutants is not clear. Activation of the FGF receptor may<br>simply play a permissive role in migration of the mesomutants is not clear. Activation of the FGF receptor may<br>simply play a permissive role in migration of the meso-<br>derm causing cells to become motile, so that they can mutants is not clear. Activation of the FGF receptor may<br>simply play a permissive role in migration of the meso-<br>derm causing cells to become motile, so that they can<br>move towards a second directional signal. Alternatively simply play a permissive role in migration of the meso-<br>derm causing cells to become motile, so that they can<br>move towards a second, directional signal. Alternatively,<br>it might function in an instructive fashion as a direc derm causing cells to become motile, so that they can<br>move towards a second, directional signal. Alternatively,<br>it might function in an instructive fashion as a directional<br>signal for cells that are already motile: or it c move towards a second, directional signal. Alternatively,<br>it might function in an instructive fashion as a directional<br>signal for cells that are already motile; or it could even<br>provide both functions, induction of motilit it might function in an instructive fashion as a directional<br>signal for cells that are already motile; or it could even<br>provide both functions, induction of motility and spatial<br>cues When Branchless, the ligand for the EGE signal for cells that are already motile; or it could even<br>provide both functions, induction of motility and spatial<br>cues. When Branchless, the ligand for the FGF receptor<br>Breathless is secreted by oxygen-staryed cells it provide both functions, induction of motility and spatial<br>cues. When Branchless, the ligand for the FGF receptor<br>Breathless, is secreted by oxygen-starved cells it clearly<br>has the ability to attract the extensions of trach cues. When Branchless, the ligand for the FGF receptor<br>Breathless, is secreted by oxygen-starved cells it clearly<br>has the ability to attract the extensions of tracheal cells<br>(Jarecki *et al.* 1999) However, it is less clea Breathless, is secreted by oxygen-starved cells it clearly<br>has the ability to attract the extensions of tracheal cells<br>(Jarecki *et al.* 1999). However, it is less clear that this<br>situation is relevant to cell migration in (Jarecki *et al.* 1999). However, it is less clear that this situation is relevant to cell migration in the mesoderm, situation is relevant to cell migration in the mesoderm, or recognition molecules are used to guide movement ince a ligand for Heartless has not yet been identified toward the correct target. situation is relevant to cell migration in the mesoderm,<br>since a ligand for Heartless has not yet been identified<br>and it is not clear that a ligand must act as a chemo-<br>attractant for mesodermal cells since a ligand for Heartless has<br>and it is not clear that a ligand<br>attractant for mesodermal cells.<br>There are several reasons that d it is not clear that a ligand must act as a chemo-<br>tractant for mesodermal cells.<br>There are several reasons that make it unlikely the<br>realward spreading of the mesoderm is directed by a

attractant for mesodermal cells.<br>There are several reasons that make it unlikely the<br>dorsalward spreading of the mesoderm is directed by a<br>distant chemotactic signal originating from the dorsal There are several reasons that make it unlikely the<br>dorsalward spreading of the mesoderm is directed by a<br>distant chemotactic signal originating from the dorsal<br>part of the ectoderm (figure  $|c\rangle$ ) First, when the width o dorsalward spreading of the mesoderm is directed by a distant chemotactic signal originating from the dorsal part of the ectoderm (figure *lc*). First, when the width of the mesoderm primordium is reduced and fewer mesodistant chemotactic signal originating from the dorsal<br>part of the ectoderm (figure  $lc$ ). First, when the width of<br>the mesoderm primordium is reduced and fewer meso-<br>dermal cells invaginate, these cells spread out only un part of the ectoderm (figure *lc*). First, when the width of<br>the mesoderm primordium is reduced and fewer meso-<br>dermal cells invaginate, these cells spread out only until<br>they have flattened into a single-cell layer (Magg the mesoderm primordium is reduced and fewer meso-<br>dermal cells invaginate, these cells spread out only until<br>they have flattened into a single-cell layer (Maggert *et al*.<br>1995). If there was directed cell movement toward they have flattened into a single-cell layer (Maggert *et al.* 1995). If there was directed cell movement towards a chemotactic signal, one would expect cells to migrate to the dorsal edge even when the mesodermal primordi 1995). If there was directed cell movement towards a m<br>chemotactic signal, one would expect cells to migrate to m<br>the dorsal edge even when the mesodermal primordium<br>consisted of a small number of cells. However the leadin chemotactic signal, one would expect cells to migrate to<br>the dorsal edge even when the mesodermal primordium<br>consisted of a small number of cells. However, the leading<br>edge of the mesodermal cell layer does not continue to the dorsal edge even when the mesodermal primordium<br>consisted of a small number of cells. However, the leading<br>edge of the mesodermal cell layer does not continue to consisted of a small number of cells. However, the leading<br>edge of the mesodermal cell layer does not continue to<br>move to the region at the dorsal edge of the ectoderm and<br>no gaps appear in the mesodermal cell layer. Secon edge of the mesodermal cell layer does not continue to<br>move to the region at the dorsal edge of the ectoderm and<br>no gaps appear in the mesodermal cell layer. Second, we<br>have analysed mesoderm migration in mutant embryos in move to the region at the dorsal edge of the ectoderm and<br>no gaps appear in the mesodermal cell layer. Second, we<br>have analysed mesoderm migration in mutant embryos in<br>which the fates of ectodermal cells had been changed. no gaps appear in the mesodermal cell layer. Second, we<br>have analysed mesoderm migration in mutant embryos in<br>which the fates of ectodermal cells had been changed. No<br>matter what changes in the fate of the ectoderm were have analysed mesoderm migration in mutant embryos in<br>which the fates of ectodermal cells had been changed. No<br>matter what changes in the fate of the ectoderm were<br>introduced, the mesodermal cells always spread dorsally which the fates of ectodermal cells had been changed. No matter what changes in the fate of the ectoderm were introduced, the mesodermal cells always spread dorsally matter what changes in the fate of the ectoderm were<br>introduced, the mesodermal cells always spread dorsally<br>(figure 2). Therefore, there is no specific ectodermal cell<br>population required to direct the movement of mesointroduced, the mesodermal cells always spread dorsally<br>(figure 2). Therefore, there is no specific ectodermal cell<br>population required to direct the movement of meso-<br>dermal cells dorsally (figure 2). Therefore, t<br>population required t<br>dermal cells dorsally.<br>There are two other population required to direct the movement of meso-<br>dermal cells dorsally.<br>There are two other mechanisms which might create a

dermal cells dorsally.<br>There are two other mechanisms which might create a<br>single-cell layer from an epithelial tube. The mesodermal<br>cells could either seek to maximize their contact with the There are two other mechanisms which might create a<br>single-cell layer from an epithelial tube. The mesodermal<br>cells could either seek to maximize their contact with the<br>ectoderm or they could seek to intercalate between ea single-cell layer from an epithelial tube. The mesodermal<br>cells could either seek to maximize their contact with the<br>ectoderm, or they could seek to intercalate between each<br>other (figure lc). If mesodermal cells had a pro cells could either seek to maximize their contact with the ectoderm, or they could seek to intercalate between each other (figure 1*c*). If mesodermal cells had a propensity to ectoderm, or they could seek to intercalate between each<br>other (figure lc). If mesodermal cells had a propensity to<br>try to increase their contact with the ectoderm, this would<br>initially produce overall directional movement other (figure  $l\dot{c}$ ). If mesodermal cells had a propensity to<br>try to increase their contact with the ectoderm, this would<br>initially produce overall directional movement towards<br>the ectoderm. Cells already in contact wi try to increase their contact with the ectoderm, this would<br>initially produce overall directional movement towards<br>the ectoderm. Cells already in contact with the ectoderm<br>would not move much at all but the other mesoderma initially produce overall directional movement towards<br>the ectoderm. Cells already in contact with the ectoderm<br>would not move much at all, but the other mesodermal<br>cells would have to insert themselves between these cells the ectoderm. Cells already in contact with the ectoderm perhaps by using filopodia to find the ectoderm and pull perhaps by using filopodia to find the ectoderm and pull<br>themselves towards it, pushing the cells that made the<br>initial contact with the mesoderm aside. Eventually this<br>would result in a net movement of the mesodermal cell themselves towards it, pushing the cells that made the themselves towards it, pushing the cells that made the<br>initial contact with the mesoderm aside. Eventually this<br>would result in a net movement of the mesodermal cell<br>mass away from the site of invagination until the cells initial contact with the mesoderm aside. Eventually this<br>would result in a net movement of the mesodermal cell<br>mass away from the site of invagination until the cells<br>covered the area of the mesoderm required for each one would result in a net movement of the mesodermal cell  $\cup$ mass away from the site of invagination until the cells targets to cause reorganization of the cytoskeleton.<br>
covered the area of the mesoderm required for each one to<br>
adhere to the ectoderm. This is precisely the situati covered the area of the mesoderm required for each one to adhere to the ectoderm. This is precisely the situation seen when the number of mesodermal cells is reduced. While there is no evidence for such a process in the me  $\mathbf S$ adhere to the ectoderm. This is precisely the situation seen when the number of mesodermal cells is reduced. While<br>there is no evidence for such a process in the mesoderm,<br>long filopodial extensions of imaginal disc cells towards<br>sources of EGE have indeed been observed (Ramirezthere is no evidence for such a process in the mesoderm,<br>long filopodial extensions of imaginal disc cells towards<br>sources of FGF have indeed been observed (Ramirez-<br>Weber & Kornberg 1999) although at the moment their long filopodial extensions of imaginal disc cells towards<br>sources of FGF have indeed been observed (Ramirez-<br>Weber & Kornberg 1999), although at the moment their<br>significance is not clear. It is possible that EGF might act sources of FGF have indeed been observed (Ramirez-Weber & Kornberg 1999), although at the moment their significance is not clear. It is possible that FGF might act as Weber & Kornberg 1999), although at the moment their<br>significance is not clear. It is possible that FGF might act as<br>an instructive signal, emanating from the ectoderm and<br>serving as an attractant towards the underlying ec significance is not clear. It is possible that FGF might act as<br>an instructive signal, emanating from the ectoderm and<br>serving as an attractant towards the underlying ecto-<br>dermal cells, rather than towards a specific part an instructive signal, emanating from the ectoderm and<br>serving as an attractant towards the underlying ecto-<br>dermal cells, rather than towards a specific part of the<br>ectoderm lying some distance from the cells. A process o serving as an attractant towards the underlying ecto-<br>dermal cells, rather than towards a specific part of the<br>ectoderm lying some distance from the cells. A process of

convergent extension or cell intercalation among mesodermal cells, which would result in a single-cell layer apposed to the ectoderm, is more difficult to envisage. In dermal cells, which would result in a single-cell layer<br>apposed to the ectoderm, is more difficult to envisage. In<br>this instance FGF might alter the properties of cells<br>already in contact with the ectoderm cells and hence apposed to the ectoderm, is more difficult to envisage. In<br>this instance FGF might alter the properties of cells<br>already in contact with the ectoderm cells and hence<br>provide the spatial information pecessary for convergent this instance FGF might alter the properties of cells<br>already in contact with the ectoderm cells and hence<br>provide the spatial information necessary for convergent<br>extension or cell intercalation to form a single-cell laye already in contact with the ectoderm cells and hence<br>provide the spatial information necessary for convergent<br>extension or cell intercalation to form a single-cell layer<br>apposed to the ectoderm. However, it is equally poss provide the spatial information necessary for convergent<br>extension or cell intercalation to form a single-cell layer<br>apposed to the ectoderm. However, it is equally possible<br>that in either situation, the EGF signal might a extension or cell intercalation to form a single-cell layer<br>apposed to the ectoderm. However, it is equally possible<br>that in either situation, the FGF signal might act simply as<br>a nermissive signal stimulating motile activ that in either situation, the FGF signal might act simply as a permissive signal, stimulating motile activity or extenthat in either situation, the FGF signal might act simply as<br>a permissive signal, stimulating motile activity or exten-<br>sion of filopodia or lamellipodia, while other cell adhesion<br>or recognition molecules are used to guid a permissive signal, stimulating motile activity or extension of filopodia or lamellipodia, while other cell adhesion<br>or recognition molecules are used to guide movement<br>toward the correct target sion of filopodia or lamellip<br>or recognition molecules<br>toward the correct target.<br>It is debatable whether

It is debatable whether all aspects of cell behaviour toward the correct target.<br>It is debatable whether all aspects of cell behaviour<br>controlled by FGF signalling are dependent upon one<br>another Migration might be regulated via the transcrip-It is debatable whether all aspects of cell behaviour<br>controlled by FGF signalling are dependent upon one<br>another. Migration might be regulated via the transcrip-<br>tional activation of genes by FGF signalling or by a controlled by FGF signalling are dependent upon one<br>another. Migration might be regulated via the transcrip-<br>tional activation of genes by FGF signalling, or by a<br>direct effect of EGF signalling on the cytoskeleton. One of another. Migration might be regulated via the transcriptional activation of genes by FGF signalling, or by a direct effect of FGF signalling on the cytoskeleton. One of the transcriptional targets in the tracheae is the tional activation of genes by FGF signalling, or by a<br>direct effect of FGF signalling on the cytoskeleton. One of<br>the transcriptional targets in the tracheae is the *Drosophila*<br>homologue of the serum response factor (SRF) direct effect of FGF signalling on the cytoskeleton. One of<br>the transcriptional targets in the tracheae is the *Drosophila*<br>homologue of the serum response factor (SRF), which<br>indeed controls the development of the fine te the transcriptional targets in the tracheae is the *Drosophila*<br>homologue of the serum response factor (SRF), which<br>indeed controls the development of the fine tertiary<br>tracheal branches (Montagne *et al* 1996). In this c homologue of the serum response factor (SRF), which<br>indeed controls the development of the fine tertiary<br>tracheal branches (Montagne *et al.* 1996). In this case the<br>morphogenetic signal of FGF therefore seems to be indeed controls the development of the fine tertiary tracheal branches (Montagne *et al.* 1996). In this case the morphogenetic signal of FGF therefore seems to be tracheal branches (Montagne *et al.* 1996). In this case the morphogenetic signal of FGF therefore seems to be mediated by transcriptional activation of SRF. However, recent experiments in tissue culture cells indicate tha morphogenetic signal of FGF therefore seems to be<br>mediated by transcriptional activation of SRF. However,<br>recent experiments in tissue culture cells indicate that the<br>activation of SRF in the trachea could be a consequence mediated by transcriptional activation of SRF. However,<br>recent experiments in tissue culture cells indicate that the<br>activation of SRF in the trachea could be a consequence<br>of depletion of G-actin within these cells (Sotir recent experiments in tissue culture cells indicate that the activation of SRF in the trachea could be a consequence of depletion of G-actin within these cells (Sotiropoulos *et* activation of SRF in the trachea could be a consequence<br>of depletion of G-actin within these cells (Sotiropoulos *et*<br>*al.* 1999). At earlier stages of development of the tracheal<br>tree, no such links are known, and it is t of depletion of G-actin within these cells (Sotiropoulos *et al.* 1999). At earlier stages of development of the tracheal tree, no such links are known, and it is therefore conceivable that  $FGF$  signalling might affect ce tree, no such links are known, and it is therefore conceivable that FGF signalling might affect cell behaviour tree, no such links are known, and it is therefore conceivable that FGF signalling might affect cell behaviour<br>not via transcription of genes but by a more direct<br>mechanism. Also in the mesoderm a morphogenetic ceivable that FGF signalling might affect cell behaviour<br>not via transcription of genes but by a more direct<br>mechanism. Also in the mesoderm a morphogenetic<br>mechanism independent of transcription appears more not via transcription of genes but by a more direct<br>mechanism. Also in the mesoderm a morphogenetic<br>mechanism independent of transcription appears more<br>likely since there is not sufficient time between the activamechanism. Also in the mesoderm a morphogenetic<br>mechanism independent of transcription appears more<br>likely since there is not sufficient time between the activamechanism independent of transcription appears more<br>likely since there is not sufficient time between the activa-<br>tion of the MAPK cascade via the FGF receptor and<br>detectable morphogenetic activity to allow transcription likely since there is not sufficient time between the activation of the MAPK cascade via the FGF receptor and detectable morphogenetic activity to allow transcription of new genes. In fact, it is not clear that the observe tion of the MAPK cascade via the FGF receptor and<br>detectable morphogenetic activity to allow transcription<br>of new genes. In fact, it is not clear that the observed<br>activation of MAPK is a prerequisite for or a consedetectable morphogenetic activity to allow transcription<br>of new genes. In fact, it is not clear that the observed<br>activation of MAPK is a prerequisite for, or a conse-<br>quence of establishing contact between ectoderm and of new genes. In fact, it is not clear that the observed<br>activation of MAPK is a prerequisite for, or a conse-<br>quence of, establishing contact between ectoderm and<br>mesoderm It may be required for the spreading of the activation of MAPK is a prerequisite for, or a consequence of, establishing contact between ectoderm and<br>mesoderm. It may be required for the spreading of the<br>mesoderm on the ectoderm or a parallel process quence of, establishing contact between ectoderm and<br>mesoderm. It may be required for the spreading of the<br>mesoderm on the ectoderm, or a parallel process<br>necessary for the activation of genes required at a later mesoderm. It may be required for the spreading of the<br>mesoderm on the ectoderm, or a parallel process<br>necessary for the activation of genes required at a later<br>stage MAPK activation can only be demonstrated by the mesoderm on the ectoderm, or a parallel process<br>necessary for the activation of genes required at a later<br>stage. MAPK activation can only be demonstrated by the<br>time the mesodermal cells have already contacted the necessary for the activation of genes required at a later<br>stage. MAPK activation can only be demonstrated by the<br>time the mesodermal cells have already contacted the stage. MAPK activation can only be demonstrated by the<br>time the mesodermal cells have already contacted the<br>ectoderm. An important question to address to under-<br>stand how EGE signalling influences cell migration will time the mesodermal cells have already contacted the<br>ectoderm. An important question to address to under-<br>stand how FGF signalling influences cell migration will<br>be whether the signalling pathway downstream of the ectoderm. An important question to address to understand how FGF signalling influences cell migration will<br>be whether the signalling pathway downstream of the<br>FGF receptor branches or is directed only to Ras and stand how FGF signalling influences cell migration will<br>be whether the signalling pathway downstream of the<br>FGF receptor branches or is directed only to Ras and be whether the signalling pathway downstream of the<br>FGF receptor branches or is directed only to Ras and<br>from there to the MAPK cascade, and at what level an<br>incoming signal might be directed to different subcellular FGF receptor branches or is directed only to Ras and<br>from there to the MAPK cascade, and at what level an<br>incoming signal might be directed to different subcellular<br>targets to cause reorganization of the cytoskeleton from there to the MAPK cascade, and at what level<br>incoming signal might be directed to different subce<br>targets to cause reorganization of the cytoskeleton.

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