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## **patterning Roles of Eph receptors and ephrins in segmental**

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Qiling Xu, Georg Mellitzer and David G. Wilkinson

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doi: 10.1098/rstb.2000.0635 Phil. Trans. R. Soc. Lond. B 2000 **355**, 993-1002

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# **ROIAL**<br> **Roles of Eph receptors and ephrins**<br> **in segmental patterning in Example 20 and Sephranger**<br> **in segmental patterning**

**Qiling Xu, Georg Mellitzer and David G. Wilkinson**\* *Division of Developmental Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, UK*

on of Developmental Neurobiology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW71AA, U<br>Eph receptor tyrosine kinases and their membrane-bound ligands, ephrins, have key roles in patterning<br>and Eph receptor tyrosine kinases and their membrane-bound ligands, ephrins, have key roles in patterning<br>and morphogenesis. Interactions between these molecules are promiscuous, but largely fall into two<br>groups: EphA receptor Eph receptor tyrosine kinases and their membrane-bound ligands, ephrins, have key roles in patterning<br>and morphogenesis. Interactions between these molecules are promiscuous, but largely fall into two<br>groups: EphA receptor and morphogenesis. Interactions between these molecules are promiscuous, but largely fall into two<br>groups: EphA receptors bind to glycosylphosphatidyl inositol-anchored ephrin-A ligands, and EphB<br>receptors bind to transmem groups: EphA receptors bind to glycosylphosphatidyl inositol-anchored ephrin-A ligands, and EphB<br>receptors bind to transmembrane ephrin-B proteins. Ephrin-B proteins transduce signals, such that<br>bidirectional signalling ca receptors bind to transmembrane ephrin-B proteins. Ephrin-B proteins transduce signals, such that<br>bidirectional signalling can occur upon interaction with the Eph receptor. In many tissues, there are<br>complementary and over bidirectional signalling can occur upon interaction with the Eph receptor. In many tissues, there are complementary and overlapping expression domains of interacting Eph receptors and ephrins. An important role of Eph rece complementary and overlapping expression domains of interacting Eph receptors and ephrins. An<br>important role of Eph receptors and ephrins is to mediate cell contact-dependent repulsion, and this has<br>been implicated in the important role of Eph receptors and ephrins is to mediate cell contact-dependent repulsion, and this has<br>been implicated in the pathfinding of axons and neural crest cells, and the restriction of cell intermingling<br>between been implicated in the pathfinding of axons and neural crest cells, and the restriction of cell intermingling<br>between hindbrain segments. Studies in an *in vitro* system show that bidirectional activation is required to<br>pr between hindbrain segments. Studies in an *in vitro* system show that bidirectional activation is required to prevent intermingling between cell populations, whereas unidirectional activation can restrict cell communicatio prevent intermingling between cell populations, whereas unidirectional activation can restrict cell<br>communication via gap junctions. Recent work indicates that Eph receptors can also upregulate cell<br>adhesion, but the bioch communication via gap junctions. Recent work indicates that Eph receptors can also upregulate cell<br>adhesion, but the biochemical basis of repulsion versus adhesion responses is unclear. Eph receptors and<br>ephrins have thus adhesion, but the biochemical basis of repulsion versus adhesion responses is unclear. Eph receptors and ephrins have thus emerged as key regulators that, in parallel with cell adhesion molecules, underlie the establishmen

**Keywords:** segmentation; cell signalling; receptor tyrosine kinase; cell movement

#### **1. INTRODUCTION**

One major aim of developmental biology is to identify the mechanisms that generate specific organized patterns of<br>mechanisms that generate specific organized patterns of<br>distinct cell types during embryogenesis. There has been One major aim of developmental biology is to identify the<br>mechanisms that generate specific organized patterns of<br>distinct cell types during embryogenesis. There has been<br>much progress in the identification and analysis of mechanisms that generate specific organized patterns of<br>distinct cell types during embryogenesis. There has been<br>much progress in the identification and analysis of inter-<br>cellular signals and transcription factors involve distinct cell types during embryogenesis. There has been<br>much progress in the identification and analysis of intermuch progress in the identification and analysis of inter-<br>cellular signals and transcription factors involved in the<br>induction of specific tissues or cell types at appropriate<br>locations in the developing embryo. However l cellular signals and transcription factors involved in the<br>induction of specific tissues or cell types at appropriate<br>locations in the developing embryo. However, less is known<br>regarding the mechanisms that control cell mo induction of specific tissues or cell types at appropriate<br>locations in the developing embryo. However, less is known<br>regarding the mechanisms that control cell movements locations in the developing embryo. However, less is known<br>regarding the mechanisms that control cell movements<br>crucial for patterning and morphogenesis. For example,<br>stereotyped movements such as convergent extension, and regarding the mechanisms that control cell movements<br>crucial for patterning and morphogenesis. For example,<br>stereotyped movements such as convergent extension, and<br>the migration of mesenchymal cells to specific destination crucial for patterning and morphogenesis. For example,<br>stereotyped movements such as convergent extension, and<br>the migration of mesenchymal cells to specific destinations,<br>are crucial for the morphogenesis and patterning o stereotyped movements such as convergent extension, and<br>the migration of mesenchymal cells to specific destinations,<br>are crucial for the morphogenesis and patterning of a number of tissues. In addition, there can be much moveare crucial for the morphogenesis and patterning of a<br>mumber of tissues. In addition, there can be much move-<br>ment and dispersal of clonally related cells, due to repeated (Steinberg 1970). Similar *in vitro* cell sorting number of tissues. In addition, there can be much move-<br>ment and dispersal of clonally related cells, due to repeated<br>rounds of division and the intercalation of adjacent cells<br>(see for example Kimmel *et al* 1994) This ra ment and dispersal of clonally related cells, due to repeated<br>rounds of division and the intercalation of adjacent cells<br>(see, for example, Kimmel *et al.* 1994). This raises the ques-<br>tion as to how organized patterns are rounds of division and the intercalation of adjacent cells<br>(see, for example, Kimmel *et al.* 1994). This raises the ques-<br>tion as to how organized patterns are maintained despite<br>such interminating that has the potential (see, for example, Kimmel *et al.* 1994). This raises the question as to how organized patterns are maintained despite such intermingling that has the potential to scramble distinct tissues or domains within a tissue that tion as to how organized patterns are maintained despite<br>such intermingling that has the potential to scramble<br>distinct tissues, or domains within a tissue that will later<br>form different derivatives. Similarly, how are pat such intermingling that has the potential to scramble<br>distinct tissues, or domains within a tissue that will later<br>form different derivatives. Similarly, how are patterns<br>maintained in distinct populations of mesenchymal c distinct tissues, or domains within a tissue that will later<br>form different derivatives. Similarly, how are patterns<br>maintained in distinct populations of mesenchymal cells<br>that have the potential to intermingle as cells m form different derivatives. Similarly, how are pattermaintained in distinct populations of mesenchymal cells that have the potential to intermingle as cells migrate?<br>Two general mechanisms can be envisaged to underly maintained in distinct populations of mesenchymal cells<br>that have the potential to intermingle as cells migrate?<br>Two general mechanisms can be envisaged to underlie

that have the potential to intermingle as cells migrate?<br>Two general mechanisms can be envisaged to underlie<br>the maintenance of organized patterns despite cell inter-<br>mingling. One mechanism involves a plasticity of cell Two general mechanisms can be envisaged to underlie<br>the maintenance of organized patterns despite cell inter-<br>mingling. One mechanism involves a plasticity of cell<br>specification, and local signals that cause any cells that the maintenance of organized patterns despite cell inter-<br>mingling. One mechanism involves a plasticity of cell<br>specification, and local signals that cause any cells that<br>cross into an adjacent territory to switch to the s mingling. One mechanism involves a plasticity of cell<br>specification, and local signals that cause any cells that<br>cross into an adjacent territory to switch to the same identity as their new neighbours. The other involves a

specific restriction of cell movement between adjacent cell<br>nopulations. There is good evidence for each of these specific restriction of cell movement between adjacent cell<br>populations. There is good evidence for each of these<br>mechanisms, which may act alone, or in parallel, to specific restriction of cell movement between adjacent cell<br>populations. There is good evidence for each of these<br>mechanisms, which may act alone, or in parallel, to<br>stabilize patterns and maintain sharp interfaces between populations. There is good evidence for each of these<br>mechanisms, which may act alone, or in parallel, to<br>stabilize patterns and maintain sharp interfaces between<br>distinct cell populations mechanisms, which may<br>stabilize patterns and mai<br>distinct cell populations.<br>There is much evidence bilize patterns and maintain sharp interfaces between<br>stinct cell populations.<br>There is much evidence for a key role of cell adhesion<br>plecules in stabilizing tissues by the establishment of

distinct cell populations.<br>There is much evidence for a key role of cell adhesion<br>molecules in stabilizing tissues by the establishment of There is much evidence for a key role of cell adhesion<br>molecules in stabilizing tissues by the establishment of<br>differences in cell–cell affinity. Classical experiments have<br>shown that when tissues are dissociated mixed an molecules in stabilizing tissues by the establishment of<br>differences in cell–cell affinity. Classical experiments have<br>shown that when tissues are dissociated, mixed and<br>reaggregated in vitro cells from different tissues s differences in cell–cell affinity. Classical experiments have<br>shown that when tissues are dissociated, mixed and<br>reaggregated *in vitro*, cells from different tissues sort out to<br>form segregated cell populations (Townes & shown that when tissues are dissociated, mixed and<br>reaggregated *in vitro*, cells from different tissues sort out to<br>form segregated cell populations (Townes & Holfreter<br>1955) This cell sorting can be explained by a model reaggregated *in vitro*, cells from different tissues sort out to<br>form segregated cell populations (Townes & Holfreter<br>1955). This cell sorting can be explained by a model in<br>which during intermingling cells of the same ty form segregated cell populations (Townes & Holfreter<br>1955). This cell sorting can be explained by a model in<br>which during intermingling, cells of the same type<br>preferentially associate because they have a stronger 1955). This cell sorting can be explained by a model in which during intermingling, cells of the same type which during intermingling, cells of the same type<br>preferentially associate because they have a stronger<br>affinity for each other than they do for a different cell type<br>(Steinberg 1970) Similar in vitm cell sorting occurs preferentially associate because they have a stronger<br>affinity for each other than they do for a different cell type<br>(Steinberg 1970). Similar *in vitro* cell sorting occurs between<br>cells expressing distinct cell adhesion affinity for each other than they do for a different cell type<br>(Steinberg 1970). Similar *in vitro* cell sorting occurs between<br>cells expressing distinct cell adhesion molecules, or<br>different levels of the same cell adhes (Steinberg 1970). Similar *in vitro* cell sorting occurs between cells expressing distinct cell adhesion molecules, or different levels of the same cell adhesion molecule (Nose  $et$ *allessing distinct cell adhesion molecules, or different levels of the same cell adhesion molecule (Nose <i>et al.* 1988; Friedlander *et al.* 1989). Taken together with the effect of null mutations and of blocking or ecton different levels of the same cell adhesion molecule (Nose *et al.* 1988; Friedlander *et al.* 1989). Taken together with the effect of null mutations, and of blocking or ectopic expression of cell adhesion molecules *in v* al. 1988; Friedlander *et al.* 1989). Taken together with the effect of null mutations, and of blocking or ectopic expression of cell adhesion molecules *in vivo* (e.g. Bradley *et al.* 1998; Godt & Tepass 1998; Gonzalez-R sion of cell adhesion molecules *in vivo* (e.g. Bradley *et al.* 1998; Godt & Tepass 1998; Gonzalez-Reyes & St Johnston 1998), these findings reveal a crucial role of tissue-<br>restricted cell adhesion molecules in stabiliz 1998; Godt & Tepass 1998; Gonzalez-Reyes & St Johnston<br>1998), these findings reveal a crucial role of tissue-<br>restricted cell adhesion molecules in stabilizing patterns of<br>cellular organization (reviewed by Takeichi 1991-1998), these findings reveal a crucial role of tissue-<br>restricted cell adhesion molecules in stabilizing patterns of<br>cellular organization (reviewed by Takeichi 1991;<br>Gumbiner 1996) Recent work has shown that another class restricted cell adhesion molecules in stabilizing patterns of<br>cellular organization (reviewed by Takeichi 1991;<br>Gumbiner 1996). Recent work has shown that another class<br>of molecules—Eph recentors and their ephrin ligands cellular organization (reviewed by Takeichi 1991;<br>Gumbiner 1996). Recent work has shown that another class<br>of molecules—Eph receptors and their ephrin ligands—<br>also contribute to the stabilization of tissue patterns. This Gumbiner 1996). Recent work has shown that another class<br>of molecules—Eph receptors and their ephrin ligands—<br>also contribute to the stabilization of tissue patterns. This<br>review will focus on the roles of Eph receptors an of molecules—Eph receptors and their ephrin ligands—<br>also contribute to the stabilization of tissue patterns. This<br>review will focus on the roles of Eph receptors and ephrins also contribute to the stabilization of tissue patterns. This<br>review will focus on the roles of Eph receptors and ephrins<br>in segmental patterning, and highlight the conclusions and<br>questions raised by these and other studi review will focus on the roles of Eph receptors and ephrins<br>in segmental patterning, and highlight the conclusions and<br>questions raised by these and other studies of their func-<br>tions in morphogenesis in segmental patterning, a<br>questions raised by these<br>tions in morphogenesis.

#### **2. EPH RECEPTORS AND EPHRINS**

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In vertebrates, Eph receptors comprise a family of 14 EFTI RECEPTORS AND EFTIRING<br>In vertebrates, Eph receptors comprise a family of 14<br>receptor tyrosine kinases that interact with a family of<br>eight membrane-bound ephrin ligands (Eph Nomen-In vertebrates, Eph receptors comprise a family of 14<br>receptor tyrosine kinases that interact with a family of<br>eight membrane-bound ephrin ligands (Eph Nomen-<br>clature Committee 1997) Recently an Eph receptor gene receptor tyrosine kinases that interact with a family of<br>eight membrane-bound ephrin ligands (Eph Nomen-<br>clature Committee 1997). Recently, an Eph receptor gene<br>has been found in *Caenorhabditis elegans* (George *et al* eight membrane-bound ephrin ligands (Eph Nomen-<br>clature Committee 1997). Recently, an Eph receptor gene<br>has been found in *Caenorhabditis elegans* (George *et al.*<br>1998), *Drosophila* (Scully *et al.* 1999) and sponges (Su has been found in *Caenorhabditis elegans* (George *et al.* different *Eph* receptor or *ephrin* is expressed in a specific 1998), *Drosophila* (Scully *et al.* 1999) and sponges (Suga *et* tissue (Wang & Anderson 1997; Fe multicellular animals. The most distinctive feature of Eph al. 1999), suggesting that they have an ancient role in multicellular animals. The most distinctive feature of Eph receptors is the primary structure of the extracellular region which includes two fibronectin type III moti multicellular animals. The most distinctive feature of Eph<br>receptors is the primary structure of the extracellular<br>region, which includes two fibronectin type III motifs<br>(Pasquale 1991) 20 conserved cysteines, many of whic receptors is the primary structure of the extracellular<br>region, which includes two fibronectin type III motifs<br>(Pasquale 1991), 20 conserved cysteines, many of which<br>are clustered in a cysteine-rich region and an N-termina region, which includes two fibronectin type III motifs<br>(Pasquale 1991), 20 conserved cysteines, many of which<br>are clustered in a cysteine-rich region, and an N-terminal<br>ligand-binding domain (Labrador *et al.* 1997: Lackma (Pasquale 1991), 20 conserved cysteines, many of which<br>are clustered in a cysteine-rich region, and an N-terminal<br>ligand-binding domain (Labrador *et al.* 1997; Lackmann<br>*et al.* 1998). Based on amino-acid sequence similar are clustered in a cysteine-rich region, and an N-terminal<br>ligand-binding domain (Labrador *et al.* 1997; Lackmann<br>*et al.* 1998). Based on amino-acid sequence similarities<br>(see Gale *et al.* 1996*a*) vertebrate Eph recent External ligand-binding domain (Labrador *et al.* 1997; Lackmann *et al.* 1998). Based on amino-acid sequence similarities  $\bigcup$  (see Gale *et al.* 1996*a*), vertebrate Eph receptors can be *et al.* 1998). Based on amino-acid sequence similarities (see Gale *et al.* 1996*a*), vertebrate Eph receptors can be divided into two subclasses, EphA (EphA1 to EphA8) and EphB (EphB1 to EphB6) Ephrins fall into two stru (see Gale *et al.* 1996*a*), vertebrate Eph receptors can be divided into two subclasses, EphA (EphA1 to EphA8) and EphB (EphB1 to EphB6). Ephrins fall into two structural classes with the enhrin-A proteins (ephrin-A1 to divided into two subclasses, EphA (EphA1 to EphA8)<br>and EphB (EphBl to EphB6). Ephrins fall into two structural classes, with the ephrin-A proteins (ephrin-A1 to<br>ephrin-A<sup>5</sup>) anchored in the plasma membrane through a and EphB (EphBI to EphB6). Ephrins fall into two structural classes, with the ephrin-A proteins (ephrin-AI to ephrin-A5) anchored in the plasma membrane through a tural classes, with the ephrin-A proteins (ephrin-Al to<br>ephrin-A5) anchored in the plasma membrane through a<br>glycosylphosphatidyl inositol linkage, whereas ephrin-B<br>proteins (ephrin-Bl to ephrin-B3) have a transmembrane ephrin-A5) anchored in the plasma membrane through a<br>glycosylphosphatidyl inositol linkage, whereas ephrin-B<br>proteins (ephrin-B1 to ephrin-B3) have a transmembrane<br>region and short cytoplasmic region. At the C-terminal glycosylphosphatidyl inositol linkage, whereas ephrin-B<br>proteins (ephrin-Bl to ephrin-B3) have a transmembrane<br>region and short cytoplasmic region. At the C-terminal<br>end of this cytoplasmic region are 33 highly conserved proteins (ephrin-B1 to ephrin-B3) have a transmembrane<br>region and short cytoplasmic region. At the C-terminal<br>end of this cytoplasmic region are 33 highly conserved<br>amino acids including five tyrosine residues. Interaction region and short cytoplasmic region. At the C-terminal<br>end of this cytoplasmic region are 33 highly conserved<br>amino acids including five tyrosine residues. Interactions<br>between Eph receptors and ephrins largely fall into t end of this cytoplasmic region are 33 highly conserved<br>amino acids including five tyrosine residues. Interactions<br>between Eph receptors and ephrins largely fall into two<br>binding-specificity, classes. EphA, receptors, bind, amino acids including five tyrosine residues. Interactions<br>between Eph receptors and ephrins largely fall into two<br>binding-specificity classes. EphA receptors bind the between Eph receptors and ephrins largely fall into two<br>binding-specificity classes. EphA receptors bind the<br>ephrin-A ligands, whereas EphB receptors bind the<br>ephrin-B proteins: an exception is the EphA4 receptor binding-specificity classes. EphA receptors bind the<br>ephrin-A ligands, whereas EphB receptors bind the<br>ephrin-B proteins; an exception is the EphA4 receptor<br>that binds ephrin-B2 and ephrin-B3 as well as ephrin-A ephrin-A ligands, whereas EphB receptors bind the<br>ephrin-B proteins; an exception is the EphA4 receptor<br>that binds ephrin-B2 and ephrin-B3 as well as ephrin-A<br>ligands (Gale *et al.* 1996*e*) ephrin-B proteins; an exception is the EphA4 receptor that binds ephrin-B2 and ephrin-B3 as well as ephrin-A ligands (Gale *et al.* 1996*a*). that binds ephrin-B2 and ephrin-B3 as well as ephrin-A<br>ligands (Gale *et al.* 1996*a*).<br>Membrane-bound ephrins trigger Eph receptor<br>phosphorylation, whereas soluble forms bind to Eph

receptor but do not trigger receptor activation (Davis *et* phosphorylation, whereas soluble forms bind to Eph<br>receptor but do not trigger receptor activation (Davis *et*<br>*al.* 1994). However, soluble ephrins activate the receptor<br>when they are artificially aggregated (Davis *et al* receptor but do not trigger receptor activation (Davis *et al.* 1994). However, soluble ephrins activate the receptor when they are artificially aggregated (Davis *et al.* 1994), and there is evidence that higher-order clu al. 1994). However, soluble ephrins activate the receptor when they are artificially aggregated (Davis *et al.* 1994), and there is evidence that higher-order clusters may stimulate distinct responses from dimers (Gale  $\$ when they are artificially aggregated (Davis *et al.* 1994), towards their targets (Henkemeyer *et al.* 1996; Orioli *et al.* and there is evidence that higher-order clusters may 1996; Wang & Anderson 1997; Dottori *et al* and there is evidence that higher-order clusters may<br>stimulate distinct responses from dimers (Gale & o<br>Yancopoulos 1997; Stein *et al.* 1998). These findings show ( stimulate distinct responses from dimers (Gale & Yancopoulos 1997; Stein *et al.* 1998). These findings show that Eph receptors and ephrins mediate contact-<br>dependent cell interactions and suggest that membrane that Eph receptors and ephrins mediate contact-<br>dependent cell interactions, and suggest that membrane that Eph receptors and ephrins mediate contact-<br>dependent cell interactions, and suggest that membrane<br>anchoring of ephrins may enable their clustering before<br>or upon binding to Eph receptor choring of ephrins may enable their clustering before<br>upon binding to Eph receptor.<br>The strong amino-acid sequence conservation in the<br>reacellular domain of ephrin-B family members raised

or upon binding to Eph receptor.<br>The strong amino-acid sequence conservation in the The strong amino-acid sequence conservation in the<br>intracellular domain of ephrin-B family members raised<br>the possibility that these proteins may themselves trans-<br>duce signals and this received indirect support from intracellular domain of ephrin-B family members raised<br>the possibility that these proteins may themselves trans-<br>duce signals, and this received indirect support from<br>analysis of mutants of the  $FbhR2$  grape (Henkemeyer et the possibility that these proteins may themselves trans-<br>duce signals, and this received indirect support from<br>analysis of mutants of the *EphB2* gene (Henkemeyer *et al.*<br>1996). Biochemical evidence was obtained in exper analysis of mutants of the  $EphB2$  gene (Henkemeyer *et al.* 1996). Biochemical evidence was obtained in experiments showing that tyrosine phosphorylation of ephrin-B1-B2 protein occurs upon interaction with clustered solu 1996). Biochemical evidence was obtained in experiments membrane-bound EphB2, presumably by recruitment of protein occurs upon interaction with clustered soluble or<br>membrane-bound EphB2, presumably by recruitment of<br>a cytoplasmic kinase to the ephrin-B cytoplasmic domain<br>(Holland *et al.* 1996: Bruckner *et al.* 1997) Thus inte membrane-bound EphB2, presumably by recruitment of<br>a cytoplasmic kinase to the ephrin-B cytoplasmic domain<br>(Holland *et al.* 1996; Bruckner *et al.* 1997). Thus, inter-<br>action between cells expressing Eph receptor with cel a cytoplasmic kinase to the ephrin-B cytoplasmic domain (Holland *et al.* 1996; Bruckner *et al.* 1997). Thus, interaction between cells expressing Eph receptor with cells expressing ephrin-B may lead to bidirectional sima (Holland *et al.* 1996; Bruckner *et al.* 1997). Thus, interaction between cells expressing Eph receptor with cells expressing ephrin-B may lead to bidirectional signal transduction with each component acting as both action between cells expressing Eph receptor with cells<br>expressing ephrin-B may lead to bidirectional signal<br>transduction, with each component acting as both<br>'receptor' and 'ligand' expressing ephrin-B m<br>transduction, with ea<br>'receptor' and 'ligand'.<br>Gene-expression stud transduction, with each component acting as both<br>
'receptor' and 'ligand'.<br>
Gene-expression studies have shown that, collectively,

The *Ephreeser and 'ligand'*.<br> **Example:** Gene-expression studies have shown that, collectively,<br>
the *Eph* receptor and *ephrin* gene families are expressed in<br>
complex patterns in many perhans all tissues throughout Gene-expression studies have shown that, collectively,<br>the  $Eph$  receptor and *ephrin* gene families are expressed in<br>complex patterns in many, perhaps all tissues throughout<br>development and in the adult (for references se the *Eph* receptor and *ephrin* gene families are expressed in<br>complex patterns in many, perhaps all tissues throughout<br>development and in the adult (for references, see *Phil. Trans. R. Soc. Lond.* B (2000) *Phil. Trans. R. Soc. Lond.* B (2000)

Flanagan & Vanderhaeghen 1998; Wilkinson 2000). Individual members of the same *Eph* receptor or *ephrin* class Flanagan & Vanderhaeghen 1998; Wilkinson 2000). Individual members of the same *Eph* receptor or *ephrin* class<br>can have the same as well as distinct sites of expression,<br>raising the possibility that family members could h vidual members of the same  $Eph$  receptor or *ephrin* class<br>can have the same as well as distinct sites of expression,<br>raising the possibility that family members could have<br>overlapping or synergistic roles in some tissues can have the same as well as distinct sites of expression,<br>raising the possibility that family members could have<br>overlapping or synergistic roles in some tissues. Several<br>examples have been found in which in different spe raising the possibility that family members could have<br>overlapping or synergistic roles in some tissues. Several<br>examples have been found in which, in different species, a<br>different *Ebb* recentor or *ebhrin* is expressed overlapping or synergistic roles in some tissues. Several examples have been found in which, in different species, a different *Eph* receptor or *ephrin* is expressed in a specific tissue (Wang & Anderson 1997; Feldheim et al. 1998), tissue (Wang & Anderson 1997; Feldheim *et al.* 1998), suggesting that some members of the same class may be functionally interchangeable and have similar or identical biochemical properties. Importantly expression studie suggesting that some members of the same class may be<br>functionally interchangeable and have similar or identical<br>biochemical properties. Importantly, expression studies<br>have shown that interacting Eph receptors and ephrins biochemical properties. Importantly, expression studies have shown that interacting Eph receptors and ephrins biochemical properties. Importantly, expression studies<br>have shown that interacting Eph receptors and ephrins<br>are in some regions expressed in complementary<br>domains whereas in other regions there are overlaps (e.g. have shown that interacting Eph receptors and ephrins<br>are in some regions expressed in complementary<br>domains, whereas in other regions there are overlaps (e.g.<br>Elenniken et al. 1996: Gale et al. 1996a: Connor et al. 1998 are in some regions expressed in complementary<br>domains, whereas in other regions there are overlaps (e.g.<br>Flenniken *et al.* 1996; Gale *et al.* 1996*a*; Connor *et al.* 1998;<br>Adams *et al.* 1999: Sobieszczuk & Wilkinson 1 domains, whereas in other regions there are overlaps (e.g. Flenniken *et al.* 1996; Gale *et al.* 1996; Connor *et al.* 1998; Adams *et al.* 1999; Sobieszczuk & Wilkinson 1999). There have been major advances in understanding develop-Adams *et al.* 1999; Sobieszczuk & Wilkinson 1999). There<br>have been major advances in understanding develop-<br>mental roles of complementary Eph receptor and ephrin<br>expression, and recept work has started to elucidate the have been major advances in understanding developmental roles of complementary Eph receptor and ephrin expression, and recent work has started to elucidate the significance of overlapping expression mental roles of complementary Eph recexpression, and recent work has started<br>significance of overlapping expression. **3. ROLES IN AXONAL PATHFINDING** 

phosphorylation, mapping of projections (Drescher *et al.* 1995; Nakamoto *et*<br>Membrane-bound ephrins trigger Eph receptor *al.* 1996; Monschau *et al.* 1997; Zhou 1997; Feldheim *et al.*<br>1998). Eph receptors and ephrins c or upon binding to Eph receptor.<br>
The strong amino-acid sequence conservation in the receptors and ephrins may have a general role in<br>
intracellular domain of ephrin-B family members raised<br>
the possibility that these prot There is now much evidence that Eph receptors and **EXECUTE IN AXONAL PATHFINDING**<br>There is now much evidence that Eph receptors and<br>ephrins have key roles in guiding neuronal growth cones<br>(reviewed by Drescher *et al.* 1997: Orioli & Klein 1997: There is now much evidence that Eph receptors and<br>ephrins have key roles in guiding neuronal growth cones<br>(reviewed by Drescher *et al.* 1997; Orioli & Klein 1997;<br>Elanagan & Vanderbaeghen 1998; O'Leary & Wilkinson ephrins have key roles in guiding neuronal growth cones<br>(reviewed by Drescher *et al.* 1997; Orioli & Klein 1997;<br>Flanagan & Vanderhaeghen 1998; O'Leary & Wilkinson<br>1999) In the retinotectal system and other topographic (reviewed by Drescher *et al.* 1997; Orioli & Klein 1997; Flanagan & Vanderhaeghen 1998; O'Leary & Wilkinson 1999). In the retinotectal system and other topographic mans gradients of an EphA receptor in neurons and of Flanagan & Vanderhaeghen 1998; O'Leary & Wilkinson<br>1999). In the retinotectal system and other topographic<br>maps, gradients of an EphA receptor in neurons and of<br>enhrin-A ligands in the target tissue underlie a graded maps, gradients of an EphA receptor in neurons and of ephrin-A ligands in the target tissue underlie a graded maps, gradients of an EphA receptor in neurons and of<br>ephrin-A ligands in the target tissue underlie a graded<br>repulsion of growth cones that establishes a spatial<br>mapping of projections (Drescher *et al* 1995: Nakamoto *et* ephrin-A ligands in the target tissue underlie a graded<br>repulsion of growth cones that establishes a spatial<br>mapping of projections (Drescher *et al.* 1995; Nakamoto *et*<br>al. 1996: Monschau et al. 1997: Zhou 1997: Feldheim *al*. 1996; Monschau *et al.* 1997; *Alalahora 1996; Monschau <i>et al.* 1997; Zhou 1997; Feldheim *et al.* 1998; Frisen *et al.* 1998). Eph receptors and ephrins can also act as repellents at boundaries to prevent axons fro also act as repellents at boundaries to prevent axons from 1998; Frisen *et al.* 1998). Eph receptors and ephrins can<br>also act as repellents at boundaries to prevent axons from<br>entering specific territories, and thus channel them<br>towards their targets (Henkemever *et al.* 1996; O also act as repellents at boundaries to prevent axons from<br>entering specific territories, and thus channel them<br>towards their targets (Henkemeyer *et al.* 1996; Orioli *et al.*<br>1996; Wang & Anderson 1997; Dottori *et al.* towards their targets (Henkemeyer *et al.* 1996; Orioli *et al.* 1996; Wang & Anderson 1997; Dottori *et al.* 1998). Studies of growth cone collapse responses to ephrin repellents (Meima *et al.* 1997*a b*) and of the bio 1996; Wang & Anderson 1997; Dottori *et al.* 1998). Studies of growth cone collapse responses to ephrin repellents (Meima *et al.* 1997*a*,*b*), and of the biochemical pathways triggered by Eph receptor activation (review of growth cone collapse responses to ephrin repellents (Meima *et al.* 1997*a*,*b*), and of the biochemical pathways triggered by Eph receptor activation (reviewed by (Meima *et al.* 1997*a,b*), and of the biochemical pathways<br>triggered by Eph receptor activation (reviewed by<br>Bruckner & Klein 1998), suggest that the actin cyto-<br>skeleton is a major target of signalling It is therefore triggered by Eph receptor activation (reviewed by<br>Bruckner & Klein 1998), suggest that the actin cyto-<br>skeleton is a major target of signalling. It is therefore<br>believed that the complementary expression of Eph Bruckner & Klein 1998), suggest that the actin cyto-<br>skeleton is a major target of signalling. It is therefore<br>believed that the complementary expression of Eph<br>receptors and enhring may have a general role in skeleton is a major target of signalling. It is therefore<br>believed that the complementary expression of Eph<br>receptors and ephrins may have a general role in<br>preventing neuronal growth cones from entering inanbelieved that the complementary expression of Eph<br>receptors and ephrins may have a general role in<br>preventing neuronal growth cones from entering inap-<br>propriate territories. As will be discussed below there is a receptors and ephrins may have a general role in<br>preventing neuronal growth cones from entering inap-<br>propriate territories. As will be discussed below, there is a<br>strong parallel between roles in axonal pathfinding and at preventing neuronal growth cones from entering inappropriate territories. As will be discussed below, there is a strong parallel between roles in axonal pathfinding and at earlier stages of patterning.

### **4. RESTRICTION OF CELL INTERMINGLING DURING TION OF CELL INTERMINGLING I<br>HINDBRAIN SEGMENTATION HINDBRAIN SEGMENTATION**<br>The hindbrain is subdivided into repeated morpho-

logical units, termed rhombomeres, that underlie a The hindbrain is subdivided into repeated morphological units, termed rhombomeres, that underlie a segmental organization of nerves and of neural crest cells that migrate in streams into the branchial arches. These logical units, termed rhombomeres, that underlie a<br>segmental organization of nerves and of neural crest cells<br>that migrate in streams into the branchial arches. These<br>cellular patterns are established by the segmental expr segmental organization of nerves and of neural crest cells<br>that migrate in streams into the branchial arches. These<br>cellular patterns are established by the segmental expres-<br>sion of genes such as  $Kmv-20$  required for the that migrate in streams into the branchial arches. These cellular patterns are established by the segmental expression of genes such as *Krox-20* required for the formation of segments, and by *Hox* genes that confer anteroposterior sion of genes such as  $Krox-20$  required for the formation of<br>segments, and by Hox genes that confer anteroposterior<br> $(A-P)$  identity (reviewed by McGinnis & Krumlauf<br>1999: Wilkinson 1993: Lumsden & Krumlauf 1996) The segments, and by  $Hox$  genes that confer anteroposterior  $(A-P)$  identity (reviewed by McGinnis & Krumlauf 1992; Wilkinson 1993; Lumsden & Krumlauf 1996). The expression domains of these segmentation and segment (A–P) identity (reviewed by McGinnis & Krumlauf<br>1992; Wilkinson 1993; Lumsden & Krumlauf 1996). The<br>expression domains of these segmentation and segment



detected in the hindbrain.<br>identity genes have sharp boundaries, which are likely to Figure 1. Expression patterns of Eph receptors and ephrins in the developing hindbrain. The diagram illustrates the Figure 1. Expression patterns of Eph receptors and ephrins in<br>the developing hindbrain. The diagram illustrates the<br>expression domains in the hindbrain of ephrin-B proteins and<br>Enh receptors that they interact with. There the developing hindbrain. The diagram illustrates the<br>expression domains in the hindbrain of ephrin-B protein<br>Eph receptors that they interact with. There is both<br>complementarity and overlap between the expression de Eph receptors that they interact with. There is both<br>complementarity and overlap between the expression domains Eph receptors that they interact with. There is both<br>complementarity and overlap between the expression domains<br>of these ephrins and Eph receptors. The EphA2 and EphA7<br>receptors are also expressed in the hindbrain (not sho complementarity and overlap between the expression domains<br>of these ephrins and Eph receptors. The EphA2 and EphA7<br>receptors are also expressed in the hindbrain (not shown) but<br>ephrin-A ligands that interact with these hav receptors are also expressed in the hindbrain (not shown) but ephrin-A ligands that interact with these have not been

underlie a homogeneous specification of segments that identity genes have sharp boundaries, which are likely to<br>underlie a homogeneous specification of segments that<br>establishes precise patterns of neuronal organization.<br>Hindbrain patterning thus provides an example of an underlie a homogeneous specification of segments that<br>establishes precise patterns of neuronal organization.<br>Hindbrain patterning thus provides an example of an<br>important general question: What are the mechanisms establishes precise patterns of neuronal organization.<br>Hindbrain patterning thus provides an example of an<br>important general question: What are the mechanisms<br>that establish and maintain precise patterns of gene Hindbrain patterning thus provides an example of an<br>important general question: What are the mechanisms<br>that establish and maintain precise patterns of gene<br>expression and tissue organization? important general question: What a<br>that establish and maintain precise<br>expression and tissue organization?<br>Studies of cell lineage have shown t that establish and maintain precise patterns of gene<br>expression and tissue organization?<br>Studies of cell lineage have shown that whereas there is<br>substantial cell intermingling between presumptive rhom-

expression and tissue organization?<br>Studies of cell lineage have shown that whereas there is<br>substantial cell intermingling between presumptive rhom-<br>homeres after morphological segmentation there is a Studies of cell lineage have shown that whereas there is<br>substantial cell intermingling between presumptive rhom-<br>bomeres, after morphological segmentation there is a<br>major restriction to cell movement between adjacent substantial cell intermingling between presumptive rhom-<br>bomeres, after morphological segmentation there is a<br>major restriction to cell movement between adjacent<br>segments (Fraser et al. 1990) Taken together with studies bomeres, after morphological segmentation there is a major restriction to cell movement between adjacent segments (Fraser *et al.* 1990). Taken together with studies of segmental gene expression, these findings suggest tha major restriction to cell movement between adjacent segments (Fraser *et al.* 1990). Taken together with studies of segmental gene expression, these findings suggest that a local regulation of cell identity and the segmen segments (Fraser *et al.* 1990). Taken together with studies<br>of segmental gene expression, these findings suggest that<br>a local regulation of cell identity and the segmental<br>restriction of cell movement may both contribute of segmental gene expression, these findings suggest that<br>a local regulation of cell identity and the segmental<br>restriction of cell movement may both contribute to the<br>maintenance and sharpening of segmental domains a local regulation of cell identity and the segmental<br>restriction of cell movement may both contribute to the<br>maintenance and sharpening of segmental domains<br>(Irving *et al.* 1996). The restriction of cell movement restriction of cell movement may both contribute to the maintenance and sharpening of segmental domains (Irving *et al.* 1996). The restriction of cell movement maintenance and sharpening of segmental domains (Irving *et al.* 1996). The restriction of cell movement between adjacent segments is due to a cellular property that is present in alternating rhombomeres such that  $r$ <sup>9</sup>/ (Irving *et al.* 1996). The restriction of cell movement<br>between adjacent segments is due to a cellular property<br>that is present in alternating rhombomeres, such that r2/<br> $r^4/r6$  can intermingle with each other and so can between adjacent segments is due to a cellular property<br>that is present in alternating rhombomeres, such that  $r^2/r^4/r^6$  can intermingle with each other, and so can r3/r5,<br>but cells from even-numbered segments do not int that is present in alternating rhombomeres, such that  $r2/r5$ ,  $r4/r6$  can intermingle with each other, and so can  $r3/r5$ , but cells from even-numbered segments do not inter-<br>mingle with cells from odd-numbered segments (Gu r4/r6 can intermingle with each other, and so can r3/r5,<br>but cells from even-numbered segments do not inter-<br>mingle with cells from odd-numbered segments (Guthrie<br> $et \, al. 1993$ ) but cells from<br>mingle with<br>*et al.* 1993).<br>One poter mingle with cells from odd-numbered segments (Guthrie *et al.* 1993).<br>One potential mechanism for restricting intermingling

between rhombomeres is that a cell adhesion molecule(s) One potential mechanism for restricting intermingling<br>between rhombomeres is that a cell adhesion molecule(s)<br>underlies a differential adhesion of cells in odd- versus<br>even-numbered rhombomeres (Wizenmann & Lumsden between rhombomeres is that a cell adhesion molecule(s)<br>underlies a differential adhesion of cells in odd-versus<br>even-numbered rhombomeres (Wizenmann & Lumsden<br>1997) but an adhesion protein with alternating segmental underlies a differential adhesion of cells in odd- versus<br>even-numbered rhombomeres (Wizenmann & Lumsden<br>1997), but an adhesion protein with alternating segmental<br>expression, has not been discovered. The expression even-numbered rhombomeres (Wizenmann & Lumsden mosaic activation of Eph receptors is sufficient for cell<br>1997), but an adhesion protein with alternating segmental sorting. By analogy with the effects of differential cell<br>1 1997), but an adhesion protein with alternating segmental<br>expression has not been discovered. The expression<br>patterns of Eph receptors and ephrins are consistent with<br>the possibility that they restrict cell movements betwe expression has not been discovered. The expression<br>patterns of Eph receptors and ephrins are consistent with<br>the possibility that they restrict cell movements between<br>hindbrain segments  $FhhA4$   $FhhB2$  and  $FhhB3$  are patterns of Eph receptors and ephrins are consistent with<br>the possibility that they restrict cell movements between<br>hindbrain segments. *EphA4*, *EphB2* and *EphB3* are<br>expressed at high levels in rhombomeres r<sup>3</sup>/r<sup>5</sup> (Ni the possibility that they restrict cell movements between<br>hindbrain segments. *EphA4*, *EphB2* and *EphB3* are<br>expressed at high levels in rhombomeres r3/r5 (Nieto *et*<br>al. 1992: Becker *et al.* 1994: Henkemever *et al.* 1 *hindbrain* segments. *EphA4*, *EphB2* and *EphB3* are expressed at high levels in rhombomeres r3/r5 (Nieto *et al.* 1992; Becker *et al.* 1994; Henkemeyer *et al.* 1994), whereas *ephrin-B1 ephrin-B2* and *ephrin-B3* are expressed at high levels in rhombomeres r3/r5 (Nieto *et* Consistent with a repulsion or de-adhesion response, *al.* 1992; Becker *et al.* 1994; Henkemeyer *et al.* 1994), there are larger intercellular spaces at rhombomer al. 1992; Becker et al. 1994; Henkemeyer et al. 1994), whereas *ephrin-B1*, *ephrin-B2*, and *ephrin-B3* are expressed<br>at high levels in  $r2/r4/r6$  (Bergemann *et al.* 1995; Flen-<br>niken *et al.* 1996*;* Gale *et al.* 1996*b*) (figure 1). Due to this<br>complementary expression, int at high levels in  $r2/r4/r6$  (Bergemann *et al.* 1995; Flen-<br>niken *et al.* 1996; Gale *et al.* 1996*b*) (figure 1). Due to this<br>complementary expression, interactions of EphA4 and<br>EphB recentors with ephrin-B proteins will niken *et al.* 1996; Gale *et al.* 1996*b*) (figure 1). Due to this complementary expression, interactions of EphA4 and EphB receptors with ephrin-B proteins will occur at the EphB receptors with ephrin-B proteins will occur at the *Phil. Trans. R. Soc. Lond.* B (2000)

 $\frac{1}{1}$  is  $\infty$ <br>interface of adjacent rhombomeres. However, there are<br>also some overlaps in expression of Eph receptors and interface of adjacent rhombomeres. However, there are<br>also some overlaps in expression of Eph receptors and<br>ephrins at least in r2 and r3 (figure 1) also some overlaps in expression of Eph receptors and ephrins, at least in r2 and r3 (figure 1).

#### **5. CELLULAR RESPONSES REGULATED BY EPH RECEPTORS AND EPHRINS IN THE HINDBRAIN**

We obtained initial clues to roles of Eph receptors in We obtained initial clues to roles of Eph receptors in<br>the hindbrain in experiments in which truncated<br>Eph 44 lacking the kinase domain was expressed widely We obtained initial clues to roles of Eph receptors in<br>the hindbrain in experiments in which truncated<br>EphA4 lacking the kinase domain was expressed widely<br>in zebrafish embryos by RNA injection at the one- or EphA4 lacking the kinase domain was expressed widely<br>in zebrafish embryos by RNA injection at the one- or<br>two-cell stage (Xu *et al.* 1995). Due to the phenomenon in zebrafish embryos by RNA injection at the one- or in zebrafish embryos by RNA injection at the one- or two-cell stage (Xu *et al.* 1995). Due to the phenomenon of bidirectional activation, truncated EphA4 may act in a dominant negative manner to block endogenous. Eph two-cell stage (Xu *et al.* 1995). Due to the phenomenon<br>of bidirectional activation, truncated EphA4 may act in a<br>dominant negative manner to block endogenous Eph<br>receptors and as a ligand that ectonically activates of bidirectional activation, truncated EphA4 may act in a<br>dominant negative manner to block endogenous Eph<br>receptors, and as a ligand that ectopically activates<br>ephrin-B proteins. In contrast to control uniniected dominant negative manner to block endogenous Ephreceptors, and as a ligand that ectopically activates<br>ephrin-B proteins. In contrast to control uninjected<br>embryos (figure  $2a$ ) cells with  $r^3/r^5$  identity were often receptors, and as a ligand that ectopically activates<br>ephrin-B proteins. In contrast to control uninjected<br>embryos (figure 2*a*), cells with r3/r5 identity were often<br>present in r2/r4/r6 sometimes causing a fusion of r3 a ephrin-B proteins. In contrast to control uninjected<br>embryos (figure 2a), cells with r3/r5 identity were often<br>present in r2/r4/r6, sometimes causing a fusion of r3 and<br>r5 territories (figure 2c). Similar results were obt embryos (figure 2*a*), cells with  $r3/r5$  identity were often<br>present in  $r2/r4/r6$ , sometimes causing a fusion of r3 and<br> $r5$  territories (figure 2*c*). Similar results were obtained<br>when exogenous enhrin- $R2$  was widely ex present in  $r2/r4/r6$ , sometimes causing a fusion of r3 and<br>r5 territories (figure 2c). Similar results were obtained<br>when exogenous ephrin-B2 was widely expressed in<br>zebrafish embryos such that EphA4 and EphB recentors r5 territories (figure 2c). Similar results were obtained<br>when exogenous ephrin-B2 was widely expressed in<br>zebrafish embryos, such that EphA4 and EphB receptors<br>would be activated throughout  $r^{3}/r^{5}$ , rather than direczebrafish embryos, such that EphA4 and EphB receptors<br>would be activated throughout  $r3/r5$ , rather than directionally at rhombomere boundaries (figure  $2b$ ). These would be activated throughout  $r^3/r^5$ , rather than directionally at rhombomere boundaries (figure  $2b$ ). These phenotypes are consistent with several possible models.<br>Blocking or activation of Eph receptors or ephrins co tionally at rhombomere boundaries (figure 2*b*). These<br>phenotypes are consistent with several possible models.<br>Blocking or activation of Eph receptors or ephrins could<br>cause some cells with  $r^2/r^4/r^6$  identity to switch phenotypes are consistent with several possible models.<br>Blocking or activation of Eph receptors or ephrins could<br>cause some cells with  $r2/r4/r6$  identity to switch to  $r3/r5$ <br>identity or could block normal switches in ident Blocking or activation of Eph receptors or ephrins could cause some cells with  $r2/r4/r6$  identity to switch to  $r3/r5$  identity, or could block normal switches in identity that occur when cells intermingle between presumpti cause some cells with  $r2/r4/r6$  identity to switch to  $r3/r5$ identity, or could block normal switches in identity that<br>occur when cells intermingle between presumptive odd<br>and even segments. Alternatively, there could be a disrup-<br>tion of the normal restriction of intermingling betw tion of the normal restriction of intermingling between odd and even segments. and even segments. Altern<br>tion of the normal restri<br>odd and even segments.<br>To distinguish betwee In of the normal restriction of intermingling between<br>d and even segments.<br>To distinguish between these possibilities, we took<br>vantage of the extensive mixing of cells during early

odd and even segments.<br>To distinguish between these possibilities, we took<br>advantage of the extensive mixing of cells during early<br>zebrafish development such that when one cell is injected To distinguish between these possibilities, we took<br>advantage of the extensive mixing of cells during early<br>zebrafish development, such that when one cell is injected<br>with  $\sqrt{ac^2 RNA}$  at the eight-cell stage its descendan advantage of the extensive mixing of cells during early<br>zebrafish development, such that when one cell is injected<br>with  $lac\zeta$  RNA at the eight-cell stage, its descendants<br>have a scattered distribution at neurula stages ( zebrafish development, such that when one cell is injected<br>with  $lac\mathcal{Z}$  RNA at the eight-cell stage, its descendants<br>have a scattered distribution at neurula stages (figure 2*d*).<br>By co-injecting  $lac\mathcal{Z}$  and *ehhrin-B* with *lac* $\zeta$  RNA at the eight-cell stage, its descendants<br>have a scattered distribution at neurula stages (figure 2*d*).<br>By co-injecting *lac* $\zeta$  and *ephrin-B2* RNA, we could ask<br>whether mosaic activation of EphA4 a have a scattered distribution at neurula stages (figure 2d).<br>By co-injecting  $lac\mathcal{Z}$  and *ephrin-B2* RNA, we could ask<br>whether mosaic activation of EphA4 and EphB receptors<br>by this ephrin leads to changes in the identit By co-injecting *lac* $\chi$  and *ephrin-B2* RNA, we could ask whether mosaic activation of EphA4 and EphB receptors by this ephrin leads to changes in the identity or movement of cells within  $r3/r5$  (Xu *et al.* 1999). Cell whether mosaic activation of EphA4 and EphB receptors<br>by this ephrin leads to changes in the identity or<br>movement of cells within r3/r5 (Xu *et al.* 1999). Cells<br>expressing *ephrin-B2* were found to become restricted to by this ephrin-leads to changes in the identity or movement of cells within r3/r5 (Xu *et al.* 1999). Cells<br>expressing *ephrin-B2* were found to become restricted to<br>the boundaries of r3/r5, whereas in r2/r4/r6 expressing<br>cells are scattered throughout the segment (figure expressing *ephrin-B2* were found to become restricted to<br>the boundaries of  $r3/r5$ , whereas in  $r2/r4/r6$  expressing<br>cells are scattered throughout the segment (figure 2*e*).<br>The expression patterns of markers of  $r3/r5$  ide the boundaries of r3/r5, whereas in r2/r4/r6 expressing<br>cells are scattered throughout the segment (figure 2e).<br>The expression patterns of markers of r3/r5 identity are<br>not altered indicating that the mosaic expression of cells are scattered throughout the segment (figure  $2e$ ).<br>The expression patterns of markers of  $r3/r5$  identity are<br>not altered, indicating that the mosaic expression of<br> $e^{b h r i n} R2$  does not alter the identity of the exp The expression patterns of markers of r3/r5 identity are<br>not altered, indicating that the mosaic expression of<br>*ephrin-B2* does not alter the identity of the expressing or<br>adjacent cells. Similar cell sorting was observed not altered, indicating that the mosaic expression of *ephrin-B2* does not alter the identity of the expressing or adjacent cells. Similar cell sorting was observed after mosaic expression of truncated ephrin-B2 (lacking t  $ephrin-B2$  does not alter the identity of the expressing or adjacent cells. Similar cell sorting was observed after mosaic expression of truncated ephrin-B2 (lacking the adjacent cells. Similar cell sorting was observed after<br>mosaic expression of truncated ephrin-B2 (lacking the<br>intracellular domain) that can activate Eph receptors, but<br>cannot itself transduce a signal  $(X_1, et al. 1999)$ . Th mosaic expression of truncated ephrin-B2 (lacking the intracellular domain) that can activate Eph receptors, but cannot itself transduce a signal (Xu *et al.* 1999). Thus, mosaic activation of Eph receptors is sufficient f intracellular domain) that can activate Eph receptors, but<br>cannot itself transduce a signal ( $Xu$  *et al.* 1999). Thus,<br>mosaic activation of Eph receptors is sufficient for cell<br>sorting. By analogy with the effects of dif cannot itself transduce a signal (Xu  $et$  al. 1999). Thus, mosaic activation of Eph receptors is sufficient for cell sorting. By analogy with the effects of differential cell<br>adhesion (Steinberg 1970), sorting could be explained by<br>a cell repulsion response to Eph receptor activation that<br>leads to an affinity difference between  $r^{3}/r^{5$ adhesion (Steinberg 1970), sorting could be explained by<br>a cell repulsion response to Eph receptor activation that<br>leads to an affinity difference between  $r3/r5$  cells<br>expressing exogenous ephrin- $R2$  and those that are n a cell repulsion response to Eph receptor activation that<br>leads to an affinity difference between r3/r5 cells<br>expressing exogenous ephrin-B2 and those that are not.<br>Consistent with a repulsion or de-adhesion response leads to an affinity difference between r3/r5 cells<br>expressing exogenous ephrin-B2 and those that are not.<br>Consistent with a repulsion or de-adhesion response,<br>there are larger intercellular spaces at rhombomere expressing exogenous ephrin-B2 and those that are not.<br>Consistent with a repulsion or de-adhesion response,<br>there are larger intercellular spaces at rhombomere<br>boundaries (Lumsden & Keynes 1989: Heyman *et al* Consistent with a repulsion or de-adhesion response, occurring. 1993) where Eph receptor-ephrin-B interactions are<br>occurring.<br>In view of evidence that ephrin-B proteins may

transduce signals, we analysed the effect of activating these

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Figure 2. Roles of Eph receptors and ephrins in the control of cell movement. The panels summarize the results of different approaches to investigate responses to Eph receptor and ephrin signalling in zebrafish hindbrain patterning and in an *in vitro* Figure 2. Roles of Eph receptors and ephrins in the control of cell movement. The panels summarize the results of different approaches to investigate responses to Eph receptor and ephrin signalling in zebrafish hindbrain approaches to investigate responses to Eph receptor and ephrin signalling in zebrafish hindbrain patterning and in an *in vitro*<br>system.  $(a-c)$  Effects of widespread blocking or ectopic activation of Eph receptors. The ind RNA injection at the one- to two-cell stage *in vivo.* (*a*) Control uninjected embryo showing sharply restricted r3/r5 domains marked by Krox-20 gene expression (blue stain). (*b*) After widespread expression of ephrin-B2 RNA injection at the one- to two-cell stage *in vivo.* (*a*) Control uninjected embryo showing sharply restricted r3/r5 domains<br>marked by Krox-20 gene expression (blue stain). (*b*) After widespread expression of ephrin-B marked by Krox-20 gene expression (blue stain). (*b*) After widespread expression of ephrin-B2 to ectopically activate Eph<br>receptors there are ectopic r3/r5 cells and often a fusion of these segments. (*c*) A similar phen expression of truncated EphA4 that will block Eph receptor activation, and activate ephrin-B proteins. (*d–f*) Effects of mosaic activation of Eph receptors or ephrin-B proteins *in vivo*. The indicated proteins were coex expression of truncated EphA4 that will block Eph receptor activation, and activate ephrin-B proteins. (*d–f*) Effects of mosaic activation of Eph receptors or ephrin-B proteins *in vivo*. The indicated proteins were coe activation of Eph receptors or ephrin-B proteins *in vivo*. The indicated proteins were coexpressed with  $\beta$ -galactosidase in a fashion by RNA injection into one cell at the eight-cell stage. The distribution of  $\beta$ -gal marker of r3/r5 (red stain) was visualized. (*d*) Control injection of only lacZ RNA showing mosaic distribution due to<br>intermingling during early development. (*e*) If RNA encoding ephrin-B2 is co-injected, the expressing of r3/r5 (arrowheads). (*f*) If RNA encoding truncated EphA4 is co-injected, the expressing cells sort to the boundaries of r2/r4/ intermingling during early development. (*e*) If RNA encoding ephrin-B2 is co-injected, the expressing cells sort to the boundar<br>of r3/r5 (arrowheads). (*f*) If RNA encoding truncated EphA4 is co-injected, the expressing c of r3/r5 (arrowheads). (f) If RNA encoding truncated EphA4 is co-injected, the expressing cells sort to the boundaries of r<br>r6.  $(g-i)$  Fishball assays for cell intermingling *in vitro*. Zebrafish animal caps labelled with fluorescein dextran (green signal) were juxtaposed, cultured overnight and the distribution of cells visualized by confocal<br>microscopy. (*g*) In a control assay with no co-injected reagents, cell intermingling occurs. (*h* fluorescein dextran (green signal) were juxtaposed, cultured overnight and the distribution of cells visualized by confocal<br>microscopy. (g) In a control assay with no co-injected reagents, cell intermingling occurs. (*h*) microscopy. (g) In a control assay with no co-injected reagents, cell intermingling occurs. (h) Expression of EphB2 receptor in<br>one population and of ephrin-B2 in the other leads to bidirectional signalling that restricts **intermingling.** (*j*−*l*) Fishball assays for gap junctional communication *in vitro*. Zebrafish animal caps labelled with rhodamine in vitrorian caps labelled with rhodamine truncated EphB2 in one population and of ephrin-B2 in the other leads to unidirectional signalling, but this does not restrict cell<br>intermingling.  $(j-l)$  Fishball assays for gap junctional communication *in vitro*. Zebrafi intermingling. (*j*-*l*) Fishball assays for gap junctional communication *in vitro*. Zebrafish animal caps labelled with rhodamin dextran (red) or Lucifer yellow (green signal) are juxtaposed and cultured overnight. Trans dextran-labelled cells via gap junctions is seen as a yellow signal. (*j*) In a control assay with no co-injected reagents gap junctional communication.<br>Junctional communication occurs. (*k*) Bidirectional activation of Ep (*<sup>l</sup>*) Unidirectional activation of ephrin-B2 by truncated EphB2 restricts gap junctional communication despite cell intermingling. Data in (*c*) from Xu *et al*. (1995); data in (*<sup>d</sup>*^*<sup>f</sup>*) from Xu *et al*. (1999); data in (*<sup>g</sup>*^*<sup>l</sup>*) from Mellitzer *et al*. (1999).

proteins in a mosaic fashion in the hindbrain. We visualized the distribution of cells expressing truncated EphA4 proteins in a mosaic fashion in the hindbrain. We visual-<br>ized the distribution of cells expressing truncated EphA4<br>that can activate ephrin-B proteins, but cannot itself trans-<br>duce a signal  $(X_1 \notin al, 1999)$ . Cells expres ized the distribution of cells expressing truncated EphA4<br>that can activate ephrin-B proteins, but cannot itself trans-<br>duce a signal (Xu *et al.* 1999). Cells expressing truncated<br>EphA4 were found to sort adjacent to the that can activate ephrin-B proteins, but cannot itself trans-<br>duce a signal (Xu *et al.* 1999). Cells expressing truncated<br>EphA4 were found to sort adjacent to the boundaries of r2/<br>r4/r6 that express endogenous ephrin-B duce a signal (Xu *et al.* 1999). Cells expressing truncated EphA4 were found to sort adjacent to the boundaries of  $r2/r4/r6$  that express endogenous ephrin-B proteins, whereas labelled cells are frequently present in central regions of

 $r3/r5$  (figure 2*f*). One explanation is that ephrin-B activation can drive cell sorting via differences in cell–cell  $r3/r5$  (figure  $2f$ ). One explanation is that ephrin-B activation can drive cell sorting via differences in cell–cell<br>affinities due to a repulsion or de-adhesion response  $r3/r5$  (figure  $2f$ ). One explanation is that ephrin-B activation can drive cell sorting via differences in cell–cell affinities, due to a repulsion or de-adhesion response similar to that occurring after Enh receptor act activation can drive cell sorting via differences in cell–c<br>affinities, due to a repulsion or de-adhesion respon-<br>similar to that occurring after Eph receptor activation.<br>These findings indicate that mosaic activation of E inities, due to a repulsion or de-adhesion response<br>nilar to that occurring after Eph receptor activation.<br>These findings indicate that mosaic activation of Eph<br>ceptors or of ephrin-B proteins can each drive cell

similar to that occurring after Eph receptor activation.<br>These findings indicate that mosaic activation of Ephreceptors or of ephrin-B proteins can each drive cell<br>sorting but it is not clear why the cells expressing ligan These findings indicate that mosaic activation of Eph receptors or of ephrin-B proteins can each drive cell sorting, but it is not clear why the cells expressing ligand

 $($ truncated receptor or ephrin $)$  sort to rhombomere<br>houndaries rather than within the segment. One possibi-(truncated receptor or ephrin) sort to rhombomere<br>boundaries rather than within the segment. One possibi-<br>lity is that interactions of endogenous Enh receptors and boundaries rather than within the segment. One possibility is that interactions of endogenous Eph receptors and boundaries rather than within the segment. One possibility is that interactions of endogenous Eph receptors and<br>ephrins at rhombomere boundaries create a zone with<br>lower cell–cell affinities compared with non-boundary lity is that interactions of endogenous Eph receptors and<br>ephrins at rhombomere boundaries create a zone with<br>lower cell–cell affinities compared with non-boundary<br>regions. Due to repulsive interactions, cells expressing ephrins at rhombomere boundaries create a zone with<br>lower cell–cell affinities compared with non-boundary<br>regions. Due to repulsive interactions, cells expressing<br>ligand may have a similar lower affinity for their neighlower cell–cell affinities compared with non-boundary<br>regions. Due to repulsive interactions, cells expressing<br>ligand may have a similar lower affinity for their neigh-<br>hours and thus sort preferentially to the boundaries ligand may have a similar lower affinity for their neighbours and thus sort preferentially to the boundaries.

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### **6. REGULATION OF CELL INTERMINGLING 6. REGULATION OF CELL INTERMINGLING<br>AND COMMUNICATION BY EPH RECEPTORS** N OF CELL INTER<br>CATION BY EPH R<br>AND EPHRINS

**AND EPHRINS**<br>The finding that mosaic activation of Eph receptors or The finding that mosaic activation of Eph receptors or<br>of ephrin-B proteins can drive cell sorting suggests that<br>they may each trigger responses that affect cell affinities The finding that mosaic activation of Eph receptors or<br>of ephrin-B proteins can drive cell sorting suggests that<br>they may each trigger responses that affect cell affinities.<br>This raises the question as to whether hidirecti of ephrin-B proteins can drive cell sorting suggests that<br>they may each trigger responses that affect cell affinities.<br>This raises the question as to whether bidirectional<br>activation at interfaces of Enh recentor-enhrin ex they may each trigger responses that affect cell affinities.<br>This raises the question as to whether bidirectional<br>activation at interfaces of Eph receptor–ephrin expression<br>domains has an important role. To test this we es  $\bigcirc$  activation at interfaces of Eph receptor–ephrin expression<br>domains has an important role. To test this, we estabactivation at interfaces of Eph receptor–ephrin expression<br>domains has an important role. To test this, we estab-<br>lished and used an *in vitro* assay (Mellitzer *et al.* 1999).<br>One-cell stage zebrafish embryos are injected domains has an important role. To test this, we established and used an *in vitro* assay (Mellitzer *et al.* 1999).<br>One-cell stage zebrafish embryos are injected with fluor-<br>escent lineage tracer and then animal cans disse lished and used an *in vitro* assay (Mellitzer *et al.* 1999).<br>One-cell stage zebrafish embryos are injected with fluor-<br>escent lineage tracer and then animal caps dissected at<br>the 1000-cell stage. After inxtanosing two a One-cell stage zebrafish embryos are injected with fluorescent lineage tracer and then animal caps dissected at the 1000-cell stage. After juxtaposing two animal caps, escent lineage tracer and then animal caps dissected at<br>the 1000-cell stage. After juxtaposing two animal caps,<br>one labelled with rhodamine dextran and the other with<br>fluorescein dextran, they rapidly adhere to form a fish the 1000-cell stage. After juxtaposing two animal caps,<br>one labelled with rhodamine dextran and the other with<br>fluorescein dextran, they rapidly adhere to form a fishball<br>that is cultured overnight. Confocal microscopy rev one labelled with rhodamine dextran and the other with<br>fluorescein dextran, they rapidly adhere to form a fishball<br>that is cultured overnight. Confocal microscopy reveals<br>that intermingling occurs between control animal ca fluorescein dextran, they rapidly adhere to form a fishball<br>that is cultured overnight. Confocal microscopy reveals<br>that intermingling occurs between control animal caps<br>(figure  $2g$ ). In contrast when cells expressing en that is cultured overnight. Confocal microscopy reveals<br>that intermingling occurs between control animal caps<br>(figure 2*g*). In contrast when cells expressing ephrin-B2 that intermingling occurs between control animal caps<br>(figure 2g). In contrast when cells expressing ephrin-B2<br>are juxtaposed with cells expressing EphB2 and/or<br>EphA4 there is a major restriction of interminating (figure 2g). In contrast when cells expressing ephrin-B2<br>are juxtaposed with cells expressing EphB2 and/or<br>EphA4, there is a major restriction of intermingling<br>between the cell populations (figure 2h) This restriction are juxtaposed with cells expressing EphB2 and/or<br>EphA4, there is a major restriction of intermingling<br>between the cell populations (figure 2*h*). This restriction<br>does not occur if Eph receptor or ephrin is omitted from EphA4, there is a major restriction of intermingling accessive and between the cell populations (figure 2h). This restriction side does not occur if Eph receptor or ephrin is omitted from exame of the two cell populations between the cell populations (figure 2*h*). This restriction<br>does not occur if Eph receptor or ephrin is omitted from<br>one of the two cell populations, indicating that activation<br>of any endogenous EphB receptors or ephrin-B does not occur if Eph receptor or ephrin is omitted from<br>one of the two cell populations, indicating that activation<br>of any endogenous EphB receptors or ephrin-B proteins is not sufficient to restrict cell intermingling. To test whether of any endogenous EphB receptors or ephrin-B proteins is<br>not sufficient to restrict cell intermingling. To test whether<br>the restriction of cell intermingling requires bidirectional<br>activation, we carried out fishball assay not sufficient to restrict cell intermingling. To test whether<br>the restriction of cell intermingling requires bidirectional<br>activation, we carried out fishball assays in which there<br>was unidirectional activation of EphA4 o the restriction of cell intermingling requires bidirectional<br>activation, we carried out fishball assays in which there<br>was unidirectional activation of EphA4 or EphB2<br>receptor by truncated ephrin-B2 or of ephrin-B2 by activation, we carried out fishball assays in which there<br>was unidirectional activation of EphA4 or EphB2<br>receptor by truncated ephrin-B2, or of ephrin-B2 by<br>truncated EphB2. We found that after unidirectional was unidirectional activation of EphA4 or EphB2<br>receptor by truncated ephrin-B2, or of ephrin-B2 by<br>truncated EphB2. We found that after unidirectional<br>signalling there is extensive interminaling between the receptor by truncated ephrin-B2, or of ephrin-B2 by truncated EphB2. We found that after unidirectional signalling there is extensive intermingling between the truncated EphB2. We found that after unidirectional tis<br>signalling there is extensive intermingling between the<br>two cell populations (figure 2*i*) (Mellitzer *et al.* 1999). A<br>caveat is raised by the possibility that the i signalling there is extensive intermingling between the<br>two cell populations (figure 2i) (Mellitzer *et al.* 1999). A<br>caveat is raised by the possibility that the intracellular<br>domain of Eph receptor or of ephrin-B is req two cell populations (figure 2*i*) (Mellitzer *et al.* 1999). A caveat is raised by the possibility that the intracellular domain of Eph receptor or of ephrin-B is required for them to be fully active as ligands for examp caveat is raised by the possibility that the intracellular<br>domain of Eph receptor or of ephrin-B is required for<br>them to be fully active as ligands, for example by<br>mediating interactions with intracellular proteins that domain of Eph receptor or of ephrin-B is required for<br>them to be fully active as ligands, for example by<br>mediating interactions with intracellular proteins that<br>could cluster them (Hock *et al.* 1998; Torres *et al.* 1998 them to be fully active as ligands, for example by<br>mediating interactions with intracellular proteins that<br>could cluster them (Hock *et al.* 1998; Torres *et al.* 1998;<br>Bruckner *et al.* 1999; Buchert *et al.* 1999; Lin *e* mediating interactions with intracellular proteins that<br>could cluster them (Hock *et al.* 1998; Torres *et al.* 1998;<br>Bruckner *et al.* 1999; Buchert *et al.* 1999; Lin *et al.* 1999). To<br>test this we took advantage of the could cluster them (Hock *et al.* 1998; Torres *et al.* 1998; Bruckner *et al.* 1999; Buchert *et al.* 1999; Lin *et al.* 1999). To test this, we took advantage of the different binding specificities of Eph recentors and e Bruckner *et al.* 1999; Buchert *et al.* 1999; Lin *et al.* 1999). To test this, we took advantage of the different binding specificities of Eph receptors and ephrins to reconstruct bidirectional signalling from unidirect test this, we took advantage of the different binding<br>specificities of Eph receptors and ephrins to reconstruct<br>bidirectional signalling from unidirectional activation in<br>each direction using truncated Eph receptor and eph specificities of Eph receptors and ephrins to reconstruct<br>bidirectional signalling from unidirectional activation in<br>each direction using truncated Eph receptor and ephrin<br>as ligands Cell intermingling was restricted in th bidirectional signalling from unidirectional activation in each direction using truncated Eph receptor and ephrin as ligands. Cell intermingling was restricted in this situaeach direction using truncated Eph receptor and ephrin<br>as ligands. Cell intermingling was restricted in this situa-<br>tion (Mellitzer *et al.* 1999). Thus, bidirectional signalling<br>between two cell populations restricts thei as ligands. Cell intermingling was restricted in this situation (Mellitzer *et al.* 1999). Thus, bidirectional signalling between two cell populations restricts their intermingling, but unidirectional signalling does not tion (Mellitzer *et al.* 1999). Thus, bidirective two cell populations restricts the but unidirectional signalling does not.<br>A further mechanism that may stabil tween two cell populations restricts their intermingling,<br>t unidirectional signalling does not.<br>A further mechanism that may stabilize patterns in the<br>odbrain is suggested by the observation that there is a

but unidirectional signalling does not.<br>A further mechanism that may stabilize patterns in the<br>hindbrain is suggested by the observation that there is a<br>disruption to cell communication via gan junctions across A further mechanism that may stabilize patterns in the<br>hindbrain is suggested by the observation that there is a<br>disruption to cell communication via gap junctions across<br>rhombomere boundaries (Martinez et al. 1999) Gap j hindbrain is suggested by the observation that there is a disruption to cell communication via gap junctions across rhombomere boundaries (Martinez *et al.* 1992). Gap junctions form by assembly of connexin proteins into c disruption to cell communication via gap junctions across<br>rhombomere boundaries (Martinez *et al.* 1992). Gap junc-<br>tions form by assembly of connexin proteins into channels<br>between cells that allow passage of  $\lt 1.2 \text{ k$ rhombomere boundaries (Martinez *et al.* 1992). Gap junctions form by assembly of connexin proteins into channels between cells that allow passage of  $\lt$  1.2 kDa molecules (Bruzzone *et al*. 1996; Kumar & Gilula 1996), and can be

through these channels. The developmental roles of gap junctional communication are currently unclear, but it is through these channels. The developmental roles of gap<br>junctional communication are currently unclear, but it is<br>likely that by allowing cells to share low molecular<br>weight secondary messengers they enable coordination of junctional communication are currently unclear, but it is<br>likely that by allowing cells to share low molecular<br>weight secondary messengers they enable coordination of<br>cell proliferation or differentiation. Thus, disruption likely that by allowing cells to share low molecular<br>weight secondary messengers they enable coordination of<br>cell proliferation or differentiation. Thus, disruption to<br>gap iunctional communication may be essential for weight secondary messengers they enable coordination of<br>cell proliferation or differentiation. Thus, disruption to<br>gap junctional communication may be essential for<br>adiacent cell populations to acquire differences in fate cell proliferation or differentiation. Thus, disruption to<br>gap junctional communication may be essential for<br>adjacent cell populations to acquire differences in fate or<br>proliferation. It seemed possible that the larger int gap junctional communication may be essential for<br>adjacent cell populations to acquire differences in fate or<br>proliferation. It seemed possible that the larger inter-<br>cellular spaces at rhombomere boundaries (Lumsden & adjacent cell populations to acquire differences in fate or<br>proliferation. It seemed possible that the larger inter-<br>cellular spaces at rhombomere boundaries (Lumsden &<br>Keynes 1989: Heyman *et al.* 1993) are due to cell re proliferation. It seemed possible that the larger inter-<br>cellular spaces at rhombomere boundaries (Lumsden &<br>Keynes 1989; Heyman *et al.* 1993) are due to cell repul-<br>sion mediated by Eph receptor-ephrin interactions and cellular spaces at rhombomere boundaries (Lumsden &<br>Keynes 1989; Heyman *et al.* 1993) are due to cell repul-<br>sion mediated by Eph receptor-ephrin interactions, and<br>that this prevents stable cell contacts required for gap Keynes 1989; Heyman *et al.* 1993) are due to cell repulsion mediated by Eph receptor-ephrin interactions, and that this prevents stable cell contacts required for gap iunction assembly. We tested this in fishball assays sion mediated by Eph receptor–ephrin interactions, and<br>that this prevents stable cell contacts required for gap<br>junction assembly. We tested this in fishball assays in<br>which one animal can labelled with Lucifer vellow (gre that this prevents stable cell contacts required for gap<br>junction assembly. We tested this in fishball assays in<br>which one animal cap labelled with Lucifer yellow (green<br>in the confocal image) is juxtanosed with another junction assembly. We tested this in fishball assays in<br>which one animal cap labelled with Lucifer yellow (green<br>in the confocal image), is juxtaposed with another<br>labelled with rhodamine dextran (red fluorescence) which one animal cap labelled with Lucifer yellow (green<br>in the confocal image), is juxtaposed with another<br>labelled with rhodamine dextran (red fluorescence)<br>(Mellitzer et al. 1999) In control fishballs Lucifer yellow in the confocal image), is juxtaposed with another<br>labelled with rhodamine dextran (red fluorescence)<br>(Mellitzer *et al.* 1999). In control fishballs, Lucifer yellow<br>transfers into rhodamine dextran-labelled cells (the labelled with rhodamine dextran (red fluorescence)<br>(Mellitzer *et al.* 1999). In control fishballs, Lucifer yellow<br>transfers into rhodamine dextran-labelled cells (the<br>overlan leading to a vellow signal) indicating that g (Mellitzer *et al.* 1999). In control fishballs, Lucifer yellow transfers into rhodamine dextran-labelled cells (the overlap leading to a yellow signal), indicating that gap transfers into rhodamine dextran-labelled cells (the overlap leading to a yellow signal), indicating that gap junctions have formed between the cell populations (figure 2i). However, when  $\text{Fph} A4$  or  $\text{Fph} B2$  were overlap leading to a yellow signal), indicating that gap<br>junctions have formed between the cell populations<br>(figure 2*j*). However, when EphA4 or EphB2 were<br>expressed in one animal cap and ephrin-B<sup>2</sup> in the other junctions have formed between the cell populations<br>(figure 2*j*). However, when EphA4 or EphB2 were<br>expressed in one animal cap and ephrin-B2 in the other,<br>Lucifer vellow did not diffuse between the cell popula-(figure 2*j*). However, when EphA4 or EphB2 were<br>expressed in one animal cap and ephrin-B2 in the other,<br>Lucifer yellow did not diffuse between the cell popula-<br>tions (figure 2*k*). Furthermore, gan iunction formation expressed in one animal cap and ephrin-B2 in the other,<br>Lucifer yellow did not diffuse between the cell popula-<br>tions (figure 2*k*). Furthermore, gap junction formation<br>was prevented by unidirectional activation of ephrin-Lucifer yellow did not diffuse between the cell populations (figure 2*k*). Furthermore, gap junction formation was prevented by unidirectional activation of ephrin-B2 or of EphR2 by truncated ligand (figure 2*h*) (Mellitz tions (figure 2*k*). Furthermore, gap junction formation<br>was prevented by unidirectional activation of ephrin-B2<br>or of EphB2 by truncated ligand (figure 2*l*) (Mellitzer *et*<br> $\frac{d}{dt}$  1999) was prevented by unidirectional activation of ephrin-B2<br>or of EphB2 by truncated ligand (figure 2*l*) (Mellitzer *et*<br>*al.* 1999).

detected by the ability of Lucifer yellow to diffuse

These results can be explained by a model in which the activation of Eph receptor or ephrin each triggers a repul-These results can be explained by a model in which the activation of Eph receptor or ephrin each triggers a repulsion or de-adhesion response. At the interface of cells expressing Eph receptor and cells expressing ephrin-B activation of Eph receptor or ephrin each triggers a repulsion or de-adhesion response. At the interface of cells expressing Ephrin-B, bidirectional activation leads to a mutual repulsion that sion or de-adhesion response. At the interface of cells<br>expressing Eph receptor and cells expressing ephrin-B,<br>bidirectional activation leads to a mutual repulsion that<br>prevents the movement of each cell population into th expressing Eph receptor and cells expressing ephrin-B,<br>bidirectional activation leads to a mutual repulsion that<br>prevents the movement of each cell population into the<br>other and restricts gap junction formation. In the hin bidirectional activation leads to a mutual repulsion that<br>prevents the movement of each cell population into the<br>other, and restricts gap junction formation. In the hind-<br>brain, this coordinated restriction of cell intermi prevents the movement of each cell population into the<br>other, and restricts gap junction formation. In the hind-<br>brain, this coordinated restriction of cell intermingling<br>and communication may be crucial for the stabilizat other, and restricts gap junction formation. In the hind-<br>brain, this coordinated restriction of cell intermingling<br>and communication may be crucial for the stabilization<br>of segmental patterns. In contrast, unidirectional brain, this coordinated restriction of cell intermingling<br>and communication may be crucial for the stabilization<br>of segmental patterns. In contrast, unidirectional signal-<br>ling will renel one population but the cells expre and communication may be crucial for the stabilization<br>of segmental patterns. In contrast, unidirectional signal-<br>ling will repel one population, but the cells expressing<br>truncated Enh receptor or enhrin are not repelled a of segmental patterns. In contrast, unidirectional signal-<br>ling will repel one population, but the cells expressing<br>truncated Eph receptor or ephrin are not repelled, and<br>can invade adiacent territory, leading to interming ling will repel one population, but the cells expressing<br>truncated Eph receptor or ephrin are not repelled, and<br>can invade adjacent territory, leading to intermingling. However, repulsion of only one of the two cell populacan invade adjacent territory, leading to intermingling.<br>However, repulsion of only one of the two cell popula-<br>tions is sufficient to prevent stable cell–cell contacts<br>required for gan junction assembly leading to an unco However, repulsion of only one of the two cell populations is sufficient to prevent stable cell–cell contacts required for gap junction assembly, leading to an uncou-<br>pling of restrictions to cell mixing and communication tions is sufficient to prevent stable cell–cell contacts<br>required for gap junction assembly, leading to an uncou-<br>pling of restrictions to cell mixing and communication.<br>Since truncated forms of Enh recentors exist due to required for gap junction assembly, leading to an uncoupling of restrictions to cell mixing and communication.<br>Since truncated forms of Eph receptors exist due to alter-<br>native splicing (reviewed by Pasquale 1997) it is po pling of restrictions to cell mixing and communication.<br>Since truncated forms of Eph receptors exist due to alternative splicing (reviewed by Pasquale 1997) it is possible<br>that unidirectional activation occurs in vive It w Since truncated forms of Eph receptors exist due to alternative splicing (reviewed by Pasquale 1997) it is possible that unidirectional activation occurs *in vivo*. It will be native splicing (reviewed by Pasquale 1997) it is possible<br>that unidirectional activation occurs *in vivo*. It will be<br>interesting to examine whether this could prevent gap<br>innetional communication between interminaled cel that unidirectional activation occurs *in vivo*. It will be interesting to examine whether this could prevent gap junctional communication between intermingled cell populations populations.

# **7. RELATIONSHIPS BETWEEN CELL MIXING ELATIONSHIPS BETWEEN CELL MIXING<br>AND IDENTITY IN THE HINDBRAIN**

The work discussed above suggests that Eph receptors<br>dephrims are involved in restricting cell interminaling **ENTITY IN THE HINDBRAIN**<br>The work discussed above suggests that Eph receptors<br>and ephrins are involved in restricting cell intermingling<br>between hindbrain segments. In view of the possibility The work discussed above suggests that Eph receptors<br>and ephrins are involved in restricting cell intermingling<br>between hindbrain segments. In view of the possibility<br>that such restrictions act in parallel with a plasticit and ephrins are involved in restricting cell intermingling<br>between hindbrain segments. In view of the possibility<br>that such restrictions act in parallel with a plasticity and local regulation of segmental identity, it is important to that such restrictions act in parallel with a plasticity and<br>local regulation of segmental identity, it is important to<br>consider why disruptions to  $r3/r5$  organization are seen<br>after widespread expression of truncated  $\text$ local regulation of segmental identity, it is important to<br>consider why disruptions to  $r3/r5$  organization are seen<br>after widespread expression of truncated EphA4 (Xu<br>et al. 1995). Ectonic cells with  $r3/r5$  identity are n consider why disruptions to r3/r5 organization are seen<br>after widespread expression of truncated EphA4 (Xu<br>*et al.* 1995). Ectopic cells with r3/r5 identity are never

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coherent groups contiguous with r3/r5. After injection of RNA encoding truncated EphA4 into one cell at the coherent groups contiguous with r3/r5. After injection of RNA encoding truncated EphA4 into one cell at the eight-cell stage, r3/r5 were altered in shape in only 5% of the embryos compared with  $> 50\%$  after injection at RNA encoding truncated EphA4 into one cell at the eight-cell stage,  $r3/r5$  were altered in shape in only 5% of the embryos, compared with  $> 50\%$  after injection at the two-cell stage. These data are consistent with the eight-cell stage, r3/r5 were altered in shape in only 5% of<br>the embryos, compared with  $> 50\%$  after injection at<br>the two-cell stage. These data are consistent with the<br>blocking of EphA4 in an increasing proportion of  $r$ the embryos, compared with  $> 50\%$  after injection at<br>the two-cell stage. These data are consistent with the<br>blocking of EphA4 in an increasing proportion of r3/r5<br>cells causing a greater number to intermingle into r2/r4 the two-cell stage. These data are consistent with the blocking of EphA4 in an increasing proportion of  $r3/r5$  cells causing a greater number to intermingle into  $r2/r4/r6$  It can be envisaged that local community effects w blocking of EphA4 in an increasing proportion of  $r3/r5$  cells causing a greater number to intermingle into  $r2/r4$  r6. It can be envisaged that local community effects will cells causing a greater number to intermingle into  $r2/r4$ <br>r6. It can be envisaged that local community effects will<br>switch isolated ectopic  $r3/r5$  cells to an even-numbered<br>identity whereas larger groups of ectopic cells r6. It can be envisaged that local community effects will<br>switch isolated ectopic r3/r5 cells to an even-numbered<br>identity, whereas larger groups of ectopic cells can main-<br>tain their identity. According to this view. Enh identity, whereas larger groups of ectopic cells can main-<br>tain their identity. According to this view, Eph receptor-<br>There is ev identity, whereas larger groups of ectopic cells can maintain their identity. According to this view, Eph receptor-<br>ephrin interactions may be required *in vivo* to prevent the<br>intermingling of cells from being so excessiv tain their identity. According to this view, Eph receptor-<br>ephrin interactions may be required *in vivo* to prevent the<br>intermingling of cells from being so excessive that identity<br>switching mechanisms are not able to mai ephrin interactions may be required *in vivo* to prevent the intermingling of cells from being so excessive that identity switching mechanisms are not able to maintain sharp patterns. It will be important to test this mode intermingling of cells from being so excessive that identity  $\Box$  switching mechanisms are not able to maintain sharp  $\rightarrow$  patterns. It will be important to test this model by transswitching mechanisms are not able to maintain sharp<br>patterns. It will be important to test this model by trans-<br>planting groups of cells between rhombomeres, and<br>analysing the relationship between cell intermingling and patterns. It will be important to test this model by trans-<br>planting groups of cells between rhombomeres, and<br>analysing the relationship between cell intermingling and<br>identity for example using green fluorescent protein planting groups of cells between rhombomeres, and<br>analysing the relationship between cell intermingling and<br>identity, for example using green fluorescent protein<br>reporter genes to visualize cell identity in living embryos analysing the relationship between cell intermingling and<br>identity, for example using green fluorescent protein<br>reporter genes to visualize cell identity in living embryos.<br>Since the restriction of cell intermingling betwe

entity, for example using green fluorescent protein<br>porter genes to visualize cell identity in living embryos.<br>Since the restriction of cell intermingling between<br>pombomeres by Eph receptors and ephrins requires that reporter genes to visualize cell identity in living embryos.<br>Since the restriction of cell intermingling between<br>rhombomeres by Eph receptors and ephrins requires that<br>they are segmentally expressed it is important to unde Since the restriction of cell intermingling between<br>rhombomeres by Eph receptors and ephrins requires that<br>they are segmentally expressed, it is important to understand how this expression is regulated. Currently, nothing they are segmentally expressed, it is important to understand how this expression is regulated. Currently, nothing<br>is known regarding the regulation of ephrin-B gene<br>expression but  $\mathit{F}bhA4$  gene expression has been show stand how this expression is regulated. Currently, nothing<br>is known regarding the regulation of ephrin-B gene<br>expression, but  $EphA4$  gene expression has been shown to<br>be under the direct control of the Krox-20 zinc finger is known regarding the regulation of ephrin-B gene<br>expression, but  $EphA4$  gene expression has been shown to<br>be under the direct control of the Krox-20 zinc finger<br>transcription factor (Theil *et al.* 1998). In addition to expression, but *EphA4* gene expression has been shown to<br>be under the direct control of the Krox-20 zinc finger<br>transcription factor (Theil *et al.* 1998). In addition to be under the direct control of the Krox-20 zinc finger<br>transcription factor (Theil *et al.* 1998). In addition to<br>being required for the formation of definitive r3/r5<br>(Schneider-Maunoury *et al.* 1993: Swiatek & Gridley transcription factor (Theil *et al.* 1998). In addition to<br>being required for the formation of definitive r3/r5<br>(Schneider-Maunoury *et al.* 1993; Swiatek & Gridley<br>1993) Krox-20 requires the expression of the *Hora*<sup>2</sup> a being required for the formation of definitive r3/r5<br>(Schneider-Maunoury *et al.* 1993; Swiatek & Gridley<br>1993), Krox-20 regulates the expression of the *Hoxa2* and<br>*Hoxh2* genes (Sham *et al.* 1993: Nonchey *et al.* 1996) *(Schneider-Maunoury <i>et al.* 1993; Swiatek & Gridley 1993), Krox-20 regulates the expression of the *Hoxa2* and *Hoxb2* genes (Sham *et al.* 1993; Nonchev *et al.* 1996). There is thus a coupling between segmentation  $A-P$ 1993), Krox-20 regulates the expression of the *Hoxa2* and  $H \circ x b^2$  genes (Sham *et al.* 1993; Nonchev *et al.* 1996). There is thus a coupling between segmentation, A-P positional specification and the segmental restric Hoxb2 genes (Sham *et al.* 1993; Nonchev *et al.* 1996). There is thus a coupling between segmentation,  $A-P$  positional specification and the segmental restriction of cell moveis thus a coupling between segmentation, A–P positional<br>specification and the segmental restriction of cell move-<br>ment, and this may be important for the maintenance of<br>segmental domains with distinct identity. Furthermore specification and the segmental restriction of cell move-<br>ment, and this may be important for the maintenance of<br>segmental domains with distinct identity. Furthermore,<br>there is evidence that expression of  $FbbA47$  in  $r^3/r$ ment, and this may be important for the maintenance of<br>segmental domains with distinct identity. Furthermore,<br>there is evidence that expression of *EphA7* in r3/r5 is<br>downstream of *Hoxa*? (Taneia *et al.* 1996) and that o segmental domains with distinct identity. Furthermore,<br>there is evidence that expression of *EphA7* in r3/r5 is<br>downstream of *Hoxa2* (Taneja *et al.* 1996), and that of<br>*EphA2* in r4 is downstream of *Hoxal* and *Hoxh1* ( there is evidence that expression of *EphA7* in r3/r5 is<br>downstream of *Hoxa2* (Taneja *et al.* 1996), and that of<br>*EphA2* in r4 is downstream of *Hoxal* and *Hoxb1* (Studer *et*<br>*al.* 1998) indicating that there is also downstream of *Hoxa2* (Taneja *et al.* 1996), and that of *EphA2* in r4 is downstream of *Hoxa1* and *Hoxb1* (Studer *et al.* 1998), indicating that there is also coupling at a different step of the regulatory bierarchy Ho  $EphA2$  in r4 is downstream of *Hoxal* and *Hoxbl* (Studer *et al.* 1998), indicating that there is also coupling at a different step of the regulatory hierarchy. However, the role of these Eph receptors in the hindbrain is currently unknown.

#### **8. ROLES IN RESTRICTING NEURAL CREST CELL MIGRATION**

**EXAMPLE 1998**<br>in most if not all regions of the developing embryo (Gale<br> $et$  al. 1996a), raises the question as to whether they have The complex expression of Eph receptors and ephrins<br>in most if not all regions of the developing embryo (Gale<br>*et al.* 1996*a*) raises the question as to whether they have<br>general roles in stabilizing patterns of tissue or The complex expression of Eph receptors and ephrins in most if not all regions of the developing embryo (Gale *et al.* 1996*a*) raises the question as to whether they have general roles in stabilizing patterns of tissue organization.<br>Although little is currently known rega *et al.* 1996*a*) raises the question as to whether they have general roles in stabilizing patterns of tissue organization.<br>Although little is currently known regarding their roles in many tissues, there is evidence that E  $\overline{ }$ general roles in stabilizing patterns of tissue organization.<br>Although little is currently known regarding their roles in<br>many tissues, there is evidence that Eph receptors and<br>enhrins are involved in restricting the movem Although little is currently known regarding their roles in<br>many tissues, there is evidence that Eph receptors and<br>ephrins are involved in restricting the movement of cells<br>in the neural crest and during somite formation many tissues, there is evidence that Eph receptors and ephrins are involved in restricting the movement of cells in the neural crest and during somite formation.

Neural crest cells arise by the delamination of cells in the neural crest and during somite formation.<br>Neural crest cells arise by the delamination of cells<br>from the dorsolateral edge of the neural epithelium, and<br>migrate along a variety of pathways to specific destina-Neural crest cells arise by the delamination of cells<br>from the dorsolateral edge of the neural epithelium, and<br>migrate along a variety of pathways to specific destina-<br>tions (Le Douarin 1982: Bronner-Fraser 1993). In chick from the dorsolateral edge of the neural epithelium, and<br>migrate along a variety of pathways to specific destina-<br>tions (Le Douarin 1982; Bronner-Fraser 1993). In chick<br>and rodent embryos, trunk neural crest cells migrate migrate along a variety of pathways to specific destinations (Le Douarin 1982; Bronner-Fraser 1993). In chick and rodent embryos, trunk neural crest cells migrate tions (Le Douarin 1982; Bronner-Fraser 1993). In chick<br>and rodent embryos, trunk neural crest cells migrate<br>through the anterior but not the posterior half of each<br>somite (Rickmann et al. 1985; Bronner-Fraser 1986) and and rodent embryos, trunk neural crest cells migrate<br>through the anterior but not the posterior half of each<br>somite (Rickmann *et al.* 1985; Bronner-Fraser 1986), and<br>this segmental migration underlies formation of the through the anterior but not the posterior half of each somite (Rickmann *et al.* 1985; Bronner-Fraser 1986), and this segmental migration underlies formation of the this segmental migration underlies formation of the *Phil. Trans. R. Soc. Lond.* B (2000)

998 Q. Xu and others *Eph receptors and ephrins in segmental patterning*<br>found to be isolated within  $r2/r4/r6$ , but rather form repeated pattern of dorsal root and sympathetic ganglia<br>coherent groups contiguous with r3/r5. repeated pattern of dorsal root and sympathetic ganglia<br>(Kalcheim & Teillet 1989: Goldstein & Kalcheim 1991) repeated pattern of dorsal root and sympathetic ganglia<br>(Kalcheim & Teillet 1989; Goldstein & Kalcheim 1991).<br>If the orientation of somites is reversed along the A–P If the orientation of somites is reversed along the  $A-P$ (Kalcheim & Teillet 1989; Goldstein & Kalcheim 1991).<br>If the orientation of somites is reversed along the A–P<br>axis, there is a corresponding reversal of the pattern of<br>migration of neural crest cells (Bronner-Fraser & Ste If the orientation of somites is reversed along the A–P<br>axis, there is a corresponding reversal of the pattern of<br>migration of neural crest cells (Bronner-Fraser & Stern<br>1991) A similar restriction imposed by the somites axis, there is a corresponding reversal of the pattern of<br>migration of neural crest cells (Bronner-Fraser & Stern<br>1991). A similar restriction imposed by the somites also<br>occurs for trunk motor axons (Keynes & Stern 1984) migration of neural crest cells (Bronner-Fraser & Stern 1991). A similar restriction imposed by the somites also occurs for trunk motor axons (Keynes & Stern 1984). Somites therefore guide neural crest cells and motor 1991). A similar restriction imposed by the somites also occurs for trunk motor axons (Keynes & Stern 1984).<br>Somites therefore guide neural crest cells and motor axons perhans due to attractive cues within the anterior occurs for trunk motor axons (Keynes & Stern 1984).<br>Somites therefore guide neural crest cells and motor axons, perhaps due to attractive cues within the anterior<br>half of each somite and/or repulsive cues within the Somites therefore guide neural crest cells and motor axons, perhaps due to attractive cues within the anterior half of each somite and/or repulsive cues within the nosterior half axons, perhaps<br>half of each so<br>posterior half.<br>There is evid If of each somite and/or repulsive cues within the<br>sterior half.<br>There is evidence implicating a number of molecules<br>pressed in the posterior half of somites in the restriction

posterior half.<br>There is evidence implicating a number of molecules<br>expressed in the posterior half of somites in the restriction<br>of neural crest cells, and/or motor axons, including a There is evidence implicating a number of molecules<br>expressed in the posterior half of somites in the restriction<br>of neural crest cells and/or motor axons, including a<br>neanut lectin-hinding glyconrotein type IX collagen an expressed in the posterior half of somites in the restriction<br>of neural crest cells and/or motor axons, including a<br>peanut lectin-binding glycoprotein, type IX collagen and<br>F-spondin (Stern et al. 1986; Davies et al. 1990; of neural crest cells and/or motor axons, including a<br>peanut lectin-binding glycoprotein, type IX collagen and<br>F-spondin (Stern *et al.* 1986; Davies *et al.* 1990; Krull *et al.*<br>1995; Ring *et al.* 1996; Debby-Brafman *e* F-spondin (Stern *et al.* 1986; Davies *et al.* 1990; Krull *et al.* 1995; Ring *et al.* 1996; Debby-Brafman *et al.* 1999). In addition to these factors, ephrin-B proteins (ephrin-B1 in the chick ephrin-B2 in rodents) ar 1995; Ring *et al.* 1996; Debby-Brafman *et al.* 1999). In addition to these factors, ephrin-B proteins (ephrin-B1 in the chick, ephrin-B2 in rodents) are expressed in the posterior half of somites and *in vitro* strine a addition to these factors, ephrin-B proteins (ephrin-Bl in<br>the chick, ephrin-B2 in rodents) are expressed in the<br>posterior half of somites, and *in vitro* stripe assays show<br>that they renel trunk neural crest cells and mot the chick, ephrin-B2 in rodents) are expressed in the posterior half of somites, and *in vitro* stripe assays show that they repel trunk neural crest cells and motor axons that express EphB recentors (Krull *et al.* 1997: posterior half of somites, and *in vitro* stripe assays show<br>that they repel trunk neural crest cells and motor axons<br>that express EphB receptors (Krull *et al.* 1997; Wang &<br>Anderson 1997) As observed in stripe assays of that they repel trunk neural crest cells and motor axons<br>that express EphB receptors (Krull *et al.* 1997; Wang &<br>Anderson 1997). As observed in stripe assays of retinal that express EphB receptors (Krull *et al.* 1997; Wang & Anderson 1997). As observed in stripe assays of retinal axons (Walter *et al.* 1987), the rate of neural crest cell migration is not slower on a uniform ephrin subst Anderson 1997). As observed in stripe assays of retinal axons (Walter *et al.* 1987), the rate of neural crest cell migration is not slower on a uniform ephrin substrate, but rather they act as directional repellents when axons (Walter *et al.* 1987), the rate of neural crest cell<br>migration is not slower on a uniform ephrin substrate, but<br>rather they act as directional repellents when presented at<br>houndaries or in a gradient (Krull *et al.* migration is not slower on a uniform ephrin substrate, but<br>rather they act as directional repellents when presented at<br>boundaries or in a gradient (Krull *et al.* 1997; Wang &<br>Anderson 1997) Furthermore *in nino* blocking rather they act as directional repellents when presented at boundaries or in a gradient (Krull *et al.* 1997; Wang & Anderson 1997). Furthermore, *in vivo* blocking experiments in chick trunk explants show that EnhB-enhrin boundaries or in a gradient (Krull *et al.* 1997; Wang & Anderson 1997). Furthermore, *in vivo* blocking experiments in chick trunk explants show that EphB – ephrin-B interactions are required to prevent neural crest cells Anderson 1997). Furthermore, *in vivo* blocking experiments in chick trunk explants show that EphB-ephrin-B interactions are required to prevent neural crest cells from entering the posterior half of somites (Krull *et al* ments in chick trunk explants show that EphB-ephrin-B<br>interactions are required to prevent neural crest cells<br>from entering the posterior half of somites (Krull *et al.*<br>1997). However, a null mutation in *ephrin-B2* does from entering the posterior half of somites (Krull *et al.* 1997). However, a null mutation in *ephrin-B2* does not affect neural crest or motor axon pathfinding, and this 1997). However, a null mutation in *ephrin-B2* does not affect neural crest or motor axon pathfinding, and this may be due to the continued presence of other guidance cues in somites (Wang *et al.* 1998) affect neural crest or motor axon<br>may be due to the continued prese<br>cues in somites (Wang *et al.* 1998).<br>Segmental migration of neural c ay be due to the continued presence of other guidance<br>es in somites (Wang *et al.* 1998).<br>Segmental migration of neural crest also occurs in the<br>anchial region of vertebrate embryos, from rhombo-

cues in somites (Wang *et al.* 1998).<br>Segmental migration of neural crest also occurs in the<br>branchial region of vertebrate embryos, from rhombo-Segmental migration of neural crest also occurs in the<br>branchial region of vertebrate embryos, from rhombo-<br>meres to specific branchial arches where they differentiate<br>to form specific patterns of bones and cartilage (Lums branchial region of vertebrate embryos, from rhombomeres to specific branchial arches where they differentiate<br>to form specific patterns of bones and cartilage (Lumsden<br>et al. 1991: Sechrist et al. 1993: Birgbauer et al. 1 meres to specific branchial arches where they differentiate<br>to form specific patterns of bones and cartilage (Lumsden<br>*et al.* 1991; Sechrist *et al.* 1993; Birgbauer *et al.* 1995;<br>Kontges & Lumsden 1996; Saldivar *et al.* to form specific patterns of bones and cartilage (Lumsden *et al.* 1991; Sechrist *et al.* 1995; Kontges & Lumsden 1996; Saldivar *et al.* 1996). There is et al. 1991; Sechrist et al. 1993; Birgbauer et al. 1995; Kontges & Lumsden 1996; Saldivar et al. 1996). There is evidence from transplantation experiments and studies of Hax gene expression for both segmental specificatio Kontges & Lumsden 1996; Saldivar *et al.* 1996). There is<br>evidence from transplantation experiments and studies of<br>*Hox* gene expression for both segmental specification and<br>plasticity in the A-P identity of branchial neur evidence from transplantation experiments and studies of  $Hox$  gene expression for both segmental specification and plasticity in the A-P identity of branchial neural crest cells (Noden 1983: Hunt *et al.* 1991–1998: Saldi Hox gene expression for both segmental specification and plasticity in the A-P identity of branchial neural crest cells (Noden 1983; Hunt *et al.* 1991, 1998; Saldivar *et al.* 1996). In an analogous manner to that discuss cells (Noden 1983; Hunt *et al.* 1991, 1998; Saldivar *et al.* 1996). In an analogous manner to that discussed above  $(\S 7)$  for the hindbrain, the targeted migration of cells may act together with local signals regulatin 1996). In an analogous manner to that discussed above  $(\S 7)$  for the hindbrain, the targeted migration of cells may act together with local signals regulating identity to maintain  $A-P$  patterning of the branchial arch ne (§7) for the hindbrain, the targeted migration of cells<br>may act together with local signals regulating identity to<br>maintain A<sup>-</sup>P patterning of the branchial arch neural<br>crest crest. Notation A–P patterning of the branchial arch neural<br>
In *Xenopus* embryos, premigratory branchial neural<br>
est is segmented into three adjacent groups of cells that

In *Xenopus* embryos, premigratory branchial neural crest is segmented into three adjacent groups of cells that are destined to enter the first, second and third plus crest is segmented into three adjacent groups of cells that<br>are destined to enter the first, second and third plus<br>fourth arches, respectively (Sadaghiani & Thiebaud<br>1987) The complementary expression of ephrin-B2 in are destined to enter the first, second and third plus<br>fourth arches, respectively (Sadaghiani & Thiebaud<br>1987). The complementary expression of ephrin-B2 in<br>second-arch neural crest and mesoderm, and of EphA4 fourth arches, respectively (Sadaghiani & Thiebaud 1987). The complementary expression of ephrin-B2 in second-arch neural crest and mesoderm, and of EphA4 plus EphBI in third-arch neural crest and mesoderm has 1987). The complementary expression of ephrin-B2 in<br>second-arch neural crest and mesoderm, and of EphA4<br>plus EphBl in third-arch neural crest and mesoderm, has<br>been implicated in the targeted migration of cells (Smith second-arch neural crest and mesoderm, and of EphA4<br>plus EphBI in third-arch neural crest and mesoderm, has<br>been implicated in the targeted migration of cells (Smith<br>et al. 1997). After blocking or ectonic activation of th plus EphBl in third-arch neural crest and mesoderm, has<br>been implicated in the targeted migration of cells (Smith<br>*et al.* 1997). After blocking or ectopic activation of these<br>Eph receptors, there is an abnormal migration been implicated in the targeted migration of cells (Smith *et al.* 1997). After blocking or ectopic activation of these Eph receptors, there is an abnormal migration of third-<br>arch neural crest cells into adjacent territor *et al.* 1997). After blocking or ectopic activation of these<br>Eph receptors, there is an abnormal migration of third-<br>arch neural crest cells into adjacent territory, consistent<br>with ephrin-B2 acting to restrict these cell Eph receptors, there is an abnormal migration of third-<br>arch neural crest cells into adjacent territory, consistent<br>with ephrin-B2 acting to restrict these cells from inter-<br>mingling with second-arch neural crest arch neural crest cells into adjacent terr<br>with ephrin-B2 acting to restrict these<br>mingling with second-arch neural crest.

# **9. ROLES AT MULTIPLE STAGES OF PATTERNING**

**ROLES AT MULTIPLE STAGES OF PATTERNING**<br>Somite formation occurs progressively along the  $A-P$ <br>is by the aggregation of groups of mesenchymal cells to  $\bullet$  axis by the aggregation of groups of mesenchymal cells to<br>form entihelial halls. Each somite is subdivided into Somite formation occurs progressively along the  $A-P$ <br>axis by the aggregation of groups of mesenchymal cells to<br>form epithelial balls. Each somite is subdivided into<br>anterior and posterior halves that are demarcated by a form epithelial balls. Each somite is subdivided into anterior and posterior halves that are demarcated by a morphological boundary (Keynes & Stern 1984). As each form epithelial balls. Each somite is subdivided into<br>anterior and posterior halves that are demarcated by a<br>morphological boundary (Keynes & Stern 1984). As each<br>somite differentiates the sclerotomal component anterior and posterior halves that are demarcated by a<br>morphological boundary (Keynes & Stern 1984). As each<br>somite differentiates, the sclerotomal component<br>(presumptive cartilage) becomes mesenchymal yet its morphological boundary (Keynes & Stern 1984). As each<br>somite differentiates, the sclerotomal component<br>(presumptive cartilage) becomes mesenchymal, yet its<br>segmentation is maintained to later form the reneated somite differentiates, the sclerotomal component<br>(presumptive cartilage) becomes mesenchymal, yet its<br>segmentation is maintained to later form the repeated<br>vertebrae Restrictions to cell intermingling may therefore (presumptive cartilage) becomes mesenchymal, yet its<br>segmentation is maintained to later form the repeated<br>vertebrae. Restrictions to cell intermingling may therefore<br>stabilize the distinct identity of somite derivatives a segmentation is maintained to later form the repeated<br>vertebrae. Restrictions to cell intermingling may therefore<br>stabilize the distinct identity of somite derivatives along<br>the body axis, and of the anterior and posterior vertebrae. Restrictions to cell intermingling may therefore<br>stabilize the distinct identity of somite derivatives along<br>the body axis, and of the anterior and posterior half of<br>each somite that contribute to distinct parts stabilize the distinct identity of somite derivatives along<br>the body axis, and of the anterior and posterior half of<br>each somite that contribute to distinct parts of each<br>vertebra (Goldstein & Kalcheim 1992) Intriguingly the body axis, and of the anterior and posterior half of<br>each somite that contribute to distinct parts of each<br>vertebra (Goldstein & Kalcheim 1992). Intriguingly,<br>there is a complementary expression of ephrin-B2 in the each somite that contribute to distinct parts of each<br>vertebra (Goldstein & Kalcheim 1992). Intriguingly,<br>there is a complementary expression of ephrin-B2 in the<br>posterior half of somites (Bergemann *et al.* 1995; Krull *e* vertebra (Goldstein & Kalcheim 1992). Intriguingly,<br>there is a complementary expression of ephrin-B2 in the<br>posterior half of somites (Bergemann *et al.* 1995; Krull *et*<br> $a l$  1997: Wang & Anderson 1997) and of Eph A4 in t there is a complementary expression of ephrin-B2 in the posterior half of somites (Bergemann *et al.* 1995; Krull *et al.* 1997; Wang & Anderson 1997) and of EphA4 in the anterior half of forming somites (Nieto *et al.* 19 posterior half of somites (Bergemann *et al.* 1995; Krull *et al.* 1997; Wang & Anderson 1997) and of EphA4 in the anterior half of forming somites (Nieto *et al.* 1992; Irving *et al.* 1996) in the chick and mouse and a s *al.* 1997; Wang & Anderson 1997) and of EphA4 in the anterior half of forming somites (Nieto *et al.* 1992; Irving *et al.* 1996) in the chick and mouse, and a similar expression of these genes occurs in zehrafish embryos

sion of these genes occurs in zebra*fish embryos* (Durbin *et al.* 1996) in the chick and mouse, and a similar expresion of these genes occurs in zebrafish embryos (Durbin *et et al.* 1996) in the chick and mouse, and a similar expression of these genes occurs in zebrafish embryos (Durbin *et al.* 1998). Furthermore, overexpression in zebrafish embryos of truncated or full-length ephrins that w sion of these genes occurs in zebrafish embryos (Durbin *et al.* 1998). Furthermore, overexpression in zebrafish embryos of truncated or full-length ephrins that will ectonically activate Eph $A4$  leads to the disruption o al. 1998). Furthermore, overexpression in zebrafish<br>embryos of truncated or full-length ephrins that will<br>ectopically activate EphA4 leads to the disruption of<br>somite boundaries (Durbin et al. 1998). The reciprocal embryos of truncated or full-length ephrins that will<br>ectopically activate EphA4 leads to the disruption of<br>somite boundaries (Durbin *et al.* 1998). The reciprocal<br>expression of Eph receptors and ephrins may therefore ectopically activate EphA4 leads to the disruption of somite boundaries (Durbin *et al.* 1998). The reciprocal expression of Eph receptors and ephrins may therefore have a role analogous to that in the hindbrain in somite boundaries (Durbin *et al.* 1998). The reciprocal<br>expression of Eph receptors and ephrins may therefore<br>have a role, analogous to that in the hindbrain, in<br>restricting intermingling between the anterior and expression of Eph receptors and ephrins may therefore<br>have a role, analogous to that in the hindbrain, in<br>restricting intermingling between the anterior and<br>nosterior halves of somites have a role, analogous to<br>restricting intermingling l<br>posterior halves of somites.<br>Taken together with the tricting intermingling between the anterior and<br>sterior halves of somites.<br>Taken together with the studies of trunk neural crest<br>d motor axon migration, these findings show that

posterior halves of somites.<br>Taken together with the studies of trunk neural crest<br>and motor axon migration, these findings show that<br>expression domains of Eph recentors and ephrins act at Taken together with the studies of trunk neural crest<br>and motor axon migration, these findings show that<br>expression domains of Eph receptors and ephrins act at<br>multiple steps of patterning. At early stages, repulsion and motor axon migration, these findings show that<br>expression domains of Eph receptors and ephrins act at<br>multiple steps of patterning. At early stages, repulsion<br>mediated by these proteins may restrict interminaling expression domains of Eph receptors and ephrins act at<br>multiple steps of patterning. At early stages, repulsion<br>mediated by these proteins may restrict intermingling<br>hetween anterior and posterior half somites. In addition multiple steps of patterning. At early stages, repulsion removing or ectopically expressing ephrin-A5 on axonal<br>mediated by these proteins may restrict intermingling behaviour in stripe assays reveals that persistent Eph<br>b mediated by these proteins may restrict intermingling<br>between anterior and posterior half somites. In addition<br>to allowing correct patterning of somite derivatives, this<br>restriction stabilizes the enhrin expression domains between anterior and posterior half somites. In addition<br>to allowing correct patterning of somite derivatives, this<br>restriction stabilizes the ephrin expression domains later<br>used as pathfinding cues by migrating cells and to allowing correct patterning of somite derivatives, this<br>restriction stabilizes the ephrin expression domains later<br>used as pathfinding cues by migrating cells and axons. An<br>analogous proposal that ephrin domains may sta restriction stabilizes the ephrin expression domains later<br>used as pathfinding cues by migrating cells and axons. An<br>analogous proposal that ephrin domains may stabilize an<br>early pattern later used as a pathfinding cue can used as pathfinding cues by migrating cells and axons. An analogous proposal that ephrin domains may stabilize an early pattern later used as a pathfinding cue can be made for branchial arch mesoderm in *Xenotus* embryos ( analogous proposal that ephrin domains may stabilize an<br>early pattern later used as a pathfinding cue can be made<br>for branchial arch mesoderm in *Xenopus* embryos (Smith<br>et al. 1997), and for the countergradients of ephrin *early pattern later used as a pathfinding cue can be made for branchial arch mesoderm in <i>Xenopus* embryos (Smith *et al.* 1997), and for the countergradients of ephrins and Fnh recentors in the tectum (Connor *et al.* 19 for branchial arch mesoderm in *Xenopus* embryos (Smith *et al.* 1997), and for the countergradients of ephrins and Eph receptors in the tectum (Connor *et al.* 1998).

#### **10. POTENTIAL ROLES IN CELL ADHESION**

There is accumulating evidence that in neuronal There is accumulating evidence that in neuronal<br>growth cones, Eph receptor activation restricts growth<br>cone movement by triggering a local depolymerization of There is accumulating evidence that in neuronal<br>growth cones, Eph receptor activation restricts growth<br>cone movement by triggering a local depolymerization of<br>the actin cytoskeleton leading to a collapse response. It growth cones, Eph receptor activation restricts growth<br>cone movement by triggering a local depolymerization of<br>the actin cytoskeleton leading to a collapse response. It<br>seems likely that collapse of filanodia of neural cre cone movement by triggering a local depolymerization of<br>the actin cytoskeleton leading to a collapse response. It<br>seems likely that collapse of filapodia of neural crest cells<br>(Jesuthasan, 1996), could also be triggered by the actin cytoskeleton leading to a collapse response. It<br>seems likely that collapse of filapodia of neural crest cells<br>(Jesuthasan 1996) could also be triggered by Eph<br>receptor activation However, it is not known whether seems likely that collapse of filapodia of neural crest cells (Jesuthasan 1996) could also be triggered by Eph receptor activation. However, it is not known whether (Jesuthasan 1996) could also be triggered by Eph<br>receptor activation. However, it is not known whether<br>such responses occur in epithelial tissues such as the hind-<br>brain. There is some evidence that Eph receptors and receptor activation. However, it is not known whether<br>such responses occur in epithelial tissues such as the hind-<br>brain. There is some evidence that Eph receptors and<br>enhring could cause de-adhesion by regulating the func such responses occur in epithelial tissues such as the hind-<br>brain. There is some evidence that Eph receptors and<br>ephrins could cause de-adhesion by regulating the func-<br>tion of cell adhesion molecules (Winning et al. 199 brain. There is some evidence that Eph receptors and example, do Eph receptors and ephrins act in parallel ephrins could cause de-adhesion by regulating the function, and/or regulate, cell adhesion molecules? What are tion ephrins could cause de-adhesion by regulating the func-<br>tion of cell adhesion molecules (Winning *et al.* 1996; Zisch<br>*et al.* 1997; Jones *et al.* 1998). Furthermore, although Eph<br>receptors and ephrin-B proteins, and what tion of cell adhesion molecules (Winning  $et$   $al$ . 1996; Zisch segments (Xu *et al*. 1999), *in vitro* sorting of cells from odd receptor activation can drive cell sorting in hindbrain<br>segments (Xu *et al.* 1999), *in vitro* sorting of cells from odd<br>and even rhombomeres requires cell adhesion molecules<br>(Wizenmann & Lumsden 1997) One possibility is segments (Xu *et al.* 1999), *in vitro* sorting of cells from odd<br>and even rhombomeres requires cell adhesion molecules<br>(Wizenmann & Lumsden 1997). One possibility is that an<br>adhesive system that is uniformly expressed is and even rhombomeres requires cell adhesion molecules<br>(Wizenmann & Lumsden 1997). One possibility is that an<br>adhesive system that is uniformly expressed is locally *Phil. Trans. R. Soc. Lond.* B (2000)

regulated by activation of Eph receptors or ephrins. Alternatively, differentially expressed cell adhesion molecules may act in parallel with Eph receptors and ephrins. tively, differentially expressed cell adhesion molecules<br>ay act in parallel with Eph receptors and ephrins.<br>In contrast to the repulsion or de-adhesion of cells<br>served in a number of systems. Eph receptor activation

may act in parallel with Eph receptors and ephrins.<br>In contrast to the repulsion or de-adhesion of cells<br>observed in a number of systems, Eph receptor activation<br>has been found to increase cell adhesion in some situaobserved in a number of systems, Eph receptor activation<br>has been found to increase cell adhesion in some situaobserved in a number of systems, Eph receptor activation<br>has been found to increase cell adhesion in some situa-<br>tions. Activation of Eph receptors with clustered soluble<br>enhrins leads to an assembly of endothelial cells i has been found to increase cell adhesion in some situations. Activation of Eph receptors with clustered soluble ephrins leads to an assembly of endothelial cells in culture into capillary-like networks (Stein *et al.* 199 tions. Activation of Eph receptors with clustered soluble<br>ephrins leads to an assembly of endothelial cells in culture<br>into capillary-like networks (Stein *et al.* 1998), and<br>promotes angiogenic sprouting (Adams *et al.* 1 ephrins leads to an assembly of endothelial cells in culture<br>into capillary-like networks (Stein *et al.* 1998), and<br>promotes angiogenic sprouting (Adams *et al.* 1999). Intri-<br>puingly the assembly of endothelial cells onl into capillary-like networks (Stein *et al.* 1998), and<br>promotes angiogenic sprouting (Adams *et al.* 1999). Intri-<br>guingly, the assembly of endothelial cells only occurred<br>after clustering of enhrins into complexes greate promotes angiogenic sprouting (Adams *et al.* 1999). Intri-<br>guingly, the assembly of endothelial cells only occurred<br>after clustering of ephrins into complexes greater than<br>dimers suggesting that higher-order clustering of guingly, the assembly of endothelial cells only occurred<br>after clustering of ephrins into complexes greater than<br>dimers, suggesting that higher-order clustering of Eph<br>receptors may trigger a cellular response distinct fro after clustering of ephrins into complexes greater than<br>dimers, suggesting that higher-order clustering of Eph<br>receptors may trigger a cellular response distinct from<br>dimerization (Stein *et al.* 1998). Recent work has sho dimers, suggesting that higher-order clustering of Eph<br>receptors may trigger a cellular response distinct from<br>dimerization (Stein *et al.* 1998). Recent work has shown<br>that Eph receptor activation can increase cell adhesi receptors may trigger a cellular response distinct from dimerization (Stein *et al.* 1998). Recent work has shown that Eph receptor activation can increase cell adhesion to dimerization (Stein *et al.* 1998). Recent work has shown<br>that Eph receptor activation can increase cell adhesion to<br>extracellular matrix via integrins (Huyn Do *et al.* 1999).<br>These findings raise the important question a that Eph receptor activation can increase cell adhesion to extracellular matrix via integrins (Huyn Do  $et$   $al$ . 1999).<br>These findings raise the important question as to what underlies repulsion versus adhesion responses t extracellular matrix via integrins (Huyn Do *et al.* 1999).<br>These findings raise the important question as to what<br>underlies repulsion versus adhesion responses to Eph<br>receptor activation. One explanation could be that thi These findings raise the important question as to what<br>underlies repulsion versus adhesion responses to Eph<br>receptor activation. One explanation could be that this is<br>due to a cell type-specific response. However as discus underlies repulsion versus adhesion responses to Eph<br>receptor activation. One explanation could be that this is<br>due to a cell type-specific response. However, as discussed<br>helow recent studies in the retinotectal system su receptor activation. One explanation could be that this is<br>due to a cell type-specific response. However, as discussed<br>below recent studies in the retinotectal system suggest<br>another possibility due to a cell type-specific response. However, as discussed<br>below recent studies in the retinotectal system suggest<br>another possibility.

Although many studies have emphasized the role of another possibility.<br>Although many studies have emphasized the role of<br>complementary expression of Eph receptors and ephrins,<br>it is now clear that overlaps in expression occur in a Although many studies have emphasized the role of<br>complementary expression of Eph receptors and ephrins,<br>it is now clear that overlaps in expression occur in a<br>number of tissues (Elenniken *et al.* 1996; Connor *et al.* complementary expression of Eph receptors and ephrins,<br>it is now clear that overlaps in expression occur in a<br>number of tissues (Flenniken *et al.* 1996; Connor *et al.*<br>1998; Sobieszczuk & Wilkinson 1999). One such site<br>o number of tissues (Flenniken *et al.* 1996; Connor *et al.* 1998; Sobieszczuk & Wilkinson 1999). One such site occurs in the retina, in which uniform expression of Enh A4 overlaps with enhrin-A5 in axons in the anterior 1998; Sobieszczuk & Wilkinson 1999). One such site<br>occurs in the retina, in which uniform expression of<br>EphA4 overlaps with ephrin-A5 in axons in the anterior<br>retinal leading to persistent receptor activation in these occurs in the retina, in which uniform expression of<br>EphA4 overlaps with ephrin-A5 in axons in the anterior<br>retina, leading to persistent receptor activation in these<br>axons (Connor *et al* 1998) Analysis of the effects of EphA4 overlaps with ephrin-A5 in axons in the anterior<br>retina, leading to persistent receptor activation in these<br>axons (Connor *et al.* 1998). Analysis of the effects of<br>removing or ectonically expressing ephrin-A5 on axo retina, leading to persistent receptor activation in these<br>axons (Connor *et al.* 1998). Analysis of the effects of<br>removing or ectopically expressing ephrin-A5 on axonal<br>behaviour in strine assays reveals that persistent axons (Connor et al. 1998). Analysis of the effects of receptor activation desensitizes growth cones to behaviour in stripe assays reveals that persistent Eph<br>receptor activation desensitizes growth cones to<br>exogenous ephrin, such that they navigate further up the<br>ephrin gradient in the tectum (Hornberger et al. 1999) A receptor activation desensitizes growth cones to exogenous ephrin, such that they navigate further up the ephrin gradient in the tectum (Hornberger *et al.* 1999). A similar conclusion can be drawn from experiments in exogenous ephrin, such that they navigate further up the<br>ephrin gradient in the tectum (Hornberger *et al.* 1999). A<br>similar conclusion can be drawn from experiments in<br>which retinal axons encounter artificial gradients of ephrin gradient in the tectum (Hornberger *et al.* 1999). A similar conclusion can be drawn from experiments in which retinal axons encounter artificial gradients of ephrins in strine assays (Rosentreter *et al.* 1998). B experiments in which retinal axons encounter artificial gradients of ephrins in stripe assays (Rosentreter *et al.* 1998). Based on these findings it will be interesting to determine whether which retinal axons encounter artificial gradients of ephrins in stripe assays (Rosentreter *et al.* 1998). Based on these findings, it will be interesting to determine whether ephrins in stripe assays (Rosentreter *et al.* 1998). Based on these findings, it will be interesting to determine whether the persistent activation of Eph receptor at other sites of overlan with enhrins desensitizes a re these findings, it will be interesting to determine whether<br>the persistent activation of Eph receptor at other sites of<br>overlap with ephrins desensitizes a repulsion response. An<br>intriguing possibility is that below the th the persistent activation of Eph receptor at other sites of<br>overlap with ephrins desensitizes a repulsion response. An<br>intriguing possibility is that below the threshold level for<br>repulsion, persistent Eph receptor activat overlap with ephrins desensitizes a repulsion response. An<br>intriguing possibility is that below the threshold level for<br>repulsion, persistent Eph receptor activation leads to an<br>adhesive response (Huyn Do *et al* 1999) intriguing possibility is that below the thin<br>repulsion, persistent Eph receptor activat<br>adhesive response (Huyn Do *et al.* 1999). adhesive response (Huyn Do *et al.* 1999).<br>**11. CONCLUDING PERSPECTIVES** 

**11. CONCLUDING PERSPECTIVES**<br>In conclusion, studies of Eph receptors and ephrins have<br>own that they have important roles in morphogenesis in IT. CONCLODING PERSPECTIVES<br>In conclusion, studies of Eph receptors and ephrins have<br>shown that they have important roles in morphogenesis, in<br>which they regulate both repulsion and adhesion responses In conclusion, studies of Eph receptors and ephrins have<br>shown that they have important roles in morphogenesis, in<br>which they regulate both repulsion and adhesion responses<br>that establish or stabilize patterns of cellular shown that they have important roles in morphogenesis, in<br>which they regulate both repulsion and adhesion responses<br>that establish or stabilize patterns of cellular organization.<br>These advances raise, many important, quest which they regulate both repulsion and adhesion responses<br>that establish or stabilize patterns of cellular organization.<br>These advances raise many important questions. For<br>example, do Eph receptors and ephrins act in paral that establish or stabilize patterns of cellular organization.<br>These advances raise many important questions. For<br>example, do Eph receptors and ephrins act in parallel<br>with and/or reculate cell adhesion molecules? What are These advances raise many important questions. For example, do Eph receptors and ephrins act in parallel<br>with, and/or regulate, cell adhesion molecules? What are<br>the intracellular transduction pathways activated by Eph<br>receptors and ephrin-B proteins and what underlies with, and/or regulate, cell adhesion molecules? What are<br>the intracellular transduction pathways activated by Eph<br>receptors and ephrin-B proteins, and what underlies<br>repulsion versus adhesion responses? Do ephrin-A the intracellular transduction pathways activated by Eph receptors and ephrin-B proteins, and what underlies<br>repulsion versus adhesion responses? Do ephrin-A<br>proteins transduce signals? Do different family members<br>trigger the same or different responses? It is likely that repulsion versus adhesion responses? Do ephrin-A<br>proteins transduce signals? Do different family members<br>trigger the same or different responses? It is likely that<br>important insights into their roles in morphogenesis will proteins transduce signals? Do different family members<br>trigger the same or different responses? It is likely that<br>important insights into their roles in morphogenesis will<br>come from further dissection of biochemical pathw trigger the same or different responses? It is likely that<br>important insights into their roles in morphogenesis will<br>come from further dissection of biochemical pathways,

**BIOLOGICAL**<br>SCIENCES CIENCES systematic genetic analysis in amenable systems such as<br>*Drosobhila* and *C* elegans as well as studies of cellular *Drosophila* and *C. elegans*, as well as studies of cellular systematic genetic<br>*Drosophila* and *C*.<br>responses *in vivo*.

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