# Fringe: defining borders by regulating the Notch pathway

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The Notch pathway mediates cell–cell interaction in many developmental processes. Multiple proteins regulate the Notch pathway, among these are the products of the *fringe* genes. The first *fringe* gene was identified in *Drosophila*, where it is involved in the formation of the dorsal/ventral border of the wing disc. It has now been found to be crucial for determining the dorsal/ventral border of the *Drosophila* eye. In vertebrates, *fringe* genes play roles in the formation of the apical ectodermal ridge, the dorsal/ventral border in the limb bud, and in the development of somitic borders. The roles of *fringe* in the neural tube or the eyes of vertebrate embryos are not clear, although it is unlikely that these roles are evolutionarily related to those in the same tissues in *Drosophila*. Genetic evidences suggest that Fringe protein functions by modulating the Notch signaling pathway, perhaps through differential regulation of Notch activation by different ligands; however, the mechanism underlying Fringe function remains to be investigated.

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**Abbreviations**

| | |
|---|---|
| **AER** | apical ectodermal ridge |
| **A/P** | anterior/posterior |
| **Dl** | Delta |
| **D/V** | dorsal/ventral |
| **EGF** | epidermal growth factor |
| **En** | Engrailed |
| **ER** | endoplasmic reticulum |
| **FGF** | fibroblast growth factor |
| **Fng** | Fringe |
| **lFng** | lunatic Fringe |
| **mFng** | manic Fringe |
| **NICD** | Notch intracellular domain |
| **rFng** | radical Fringe |
| **Ser** | Serrate |
| **Wg** | Wingless |

## Introduction

In the past decade or so, many neural signaling pathways have been characterized, and it has been found that the same signaling pathways can be used in many apparently diverse processes. Mechanistically, some of these pathways are used similarly in different tissues, whereas others are used differently in different contexts. It is now clear that there are molecules that can impinge upon a signaling pathway and regulate it in such a way that a single pathway can play temporally and spatially distinct roles in multiple processes. During development, such regulation allows the same signaling pathway to function in a tissue- or developmental stage-specific manner.

The signaling pathway involving the transmembrane receptor Notch provides an excellent system to study how a single pathway functions in multiple developmental processes across species and how other molecules regulate the same pathway. When a pathway is used in similar structures of different species, it often leads to the conclusion that an evolutionarily conserved mechanism is responsible for forming these structures in different species. Studies of the Notch pathway show that, while evolutionary relationships can be inferred in some structures, it is hard to conclude an evolutionary relationship in other structures simply by examining the expression and function of a signaling pathway in different species.

We will begin here with a basic outline of Notch signaling, and then review the functional roles of the *fringe* (*fng*) genes, a family of Notch modulators in *Drosophila* and vertebrates. It appears that studies of these modulators not only reveal the function of a specific family of molecules, but also further our understanding of border formation, in particular, and regulation of cell–cell interaction, in general.

## The Notch signaling pathway

The first *Notch* mutation in *Drosophila* was discovered by Dexter in 1914 and the second one by Bridges in 1916. The *Notch*$^{8}$ allele, which was used often in earlier studies, was a deficiency identified by Mohr in 1919, found to be lethal in the embryo by Li in 1927, and mapped cytologically by Mackensen in 1935. The function of *Notch* in embryonic neural development was well characterized in the 1930s and 1940s by Poulson, who proposed that *Notch* promotes epidermal fate and inhibits neural fate. There are several excellent recent reviews on the Notch pathway [1•–4•], so we will only briefly summarize what is known as a basis for further discussions of its regulation.

The ligands for the Notch receptors are known as DSL proteins because the prototypical ligands are Delta (Dl) and Serrate (Ser) in *Drosophila* and Lag-2 in *C. elegans*. Apx-1 is another DSL ligand in *C. elegans*. DSL ligands in vertebrates include multiple Dl, Ser (also known as Jagged in mammals), and Dl-like (Dll) proteins. Typically, a DSL ligand contains a signal peptide, an amino-terminal domain, a DSL domain, a variable number of epidermal growth factor (EGF)-like repeats, a fragment of variable sequence followed by a single transmembrane domain, and a short intracellular domain. The DSL domain and EGF repeats are the more conserved sequences. The amino-terminal and the DSL domains are thought to be responsible for binding to the EGF repeats in the Notch receptors.

There is a single *Notch* gene in *Drosophila* and two *Notch* orthologs in *C. elegans*: *lin-12* and *glp-1*. There are multiple *Notch* genes in vertebrates. Notch proteins are single transmembrane receptors. The extracellular part of a typical Notch receptor contains multiple EGF repeats and three LNG (Lin-12, Notch, Glp-1) repeats, whereas the intracellular part contains a RAM23 domain, six ankyrin repeats, and a variable sequence followed by a PEST sequence, which is indicative of protein instability. Among the 36 EGF repeats in *Drosophila* Notch, repeats 11 and 12 mediate the binding to Dl and Ser, whereas the precise roles of the other repeats are not well understood, although mutations in the other EGF or the LNG repeats result in abnormal Notch function.

There are two models of how Notch transduces its signal after being activated by a ligand [1•–4•]. One model proposes a crucial role for transcription factors of the CSL family (named after CBF1/RJBk in vertebrates, Suppressor of Hairless in *Drosophila*, and Lag-1 in *C. elegans*). In this model, a CSL protein binds to the Notch intracellular domain (NICD); upon ligand activation, CSL is released from NICD and translocates into the nucleus to regulate transcription of downstream genes. The other model proposes an interesting role for NICD itself in transcription regulation. In this model, ligand activation of the receptor causes proteolytic cleavage of Notch at a specific site, which results in the release of NICD from the membrane. NICD then moves into the nucleus and, together with CSL, regulates the transcription of downstream genes. Recent evidence strongly supports the latter model [5•–7•].

In *Drosophila*, there are several genes whose mutations cause a phenotype similar to those of *Notch* and *Dl*. These so-called neurogenic genes include *Enhancer of split*, *mastermind* (*mam*) and *neuralized* (*neu*), which encode putative transcription factors, and *big brain* (*bib*), which encodes a multiple transmembrane protein. The precise roles of the products of *mam*, *neu* and *bib* are still unknown. By contrast, genes whose functions do not overlap with that of *Notch* in embryonic neurogenesis have now been found to regulate Notch signaling in specific tissues.

## *Fringe* in *Drosophila* wing development

The wing of an adult *Drosophila* develops from epithelial cells in the wing imaginal disc of the larva. A wing disc is divided into anterior, posterior, dorsal and ventral compartments. Anterior/posterior (A/P) patterning requires the nuclear protein Engrailed (En) and secreted signals, including Hedgehog (Hh) and Decapentaplegic (Dpp). Dorsal/ventral (D/V) patterning requires the nuclear protein Apterous (Ap) and signaling molecules such as Wingless (Wg).

The D/V border of the wing disc forms the wing margin in the adult. During development, the D/V border plays an important role in promoting wing outgrowth. An elegant interplay of signaling molecules is used to form the D/V border in the wing disc [8–14]. The Notch pathway plays a central role in D/V border formation [8–14]. *Ser* is expressed in the dorsal compartment, and Ser protein is the signal sent by dorsal cells to activate Notch on the ventral side of the D/V border [8–10,13,14], whereas Dl is the signal expressed by the ventral cells to activate Notch on the dorsal side of the D/V border [11,12]. Thus, although Notch is expressed uniformly in the wing disc, it is only activated at the D/V border. Activation of Notch at the D/V border leads to expression of *wg*, *vestigial* (*vg*) and other genes specific for the D/V border, and promotes the outgrowth of the wing [10–14]. Since both Ser and Dl can activate Notch, there has to be a regulator that ensures Notch activation is limited to the D/V border. Fringe plays this crucial role.

The *fng* gene was first identified by Irvine and Wieschaus [15] from an enhancer trap line that drove the expression of an inserted *lacZ* gene in the dorsal part of the *Drosophila* wing. The *fng* gene is normally expressed in the dorsal compartment of the wing disc [15]. Most interestingly, by either making mutant clones or ectopically expressing *fng*, it was found that juxtaposition of *fng*-expressing (*fng*$^+$) cells to *fng*-nonexpressing (*fng*$^-$) cells creates new D/V borders [15,16]. Thus, although *fng* is expressed in the entire dorsal compartment, it does not determine the dorsal fate; rather, it functions only to define the D/V border.

Further genetic studies revealed that *fng* functions by modulating the Notch pathway [17••,18,19•,20•]. In the wing disc, Panin *et al.* [17••] found that Fng inhibits Notch activation by Ser, but enhances Notch activation by Dl. Fleming *et al.* [18] also found that Fng inhibits Ser activation of Notch. However, since these effects were observed in genetic studies, it is unclear which effect is direct. Klein and Martinez Arias [19•] found that Fng inhibits the reception of the Ser signal (in cells expressing the Notch receptor), but also enhances the sending of the Ser signal [19•]. They did not find any stimulatory effect of Fng on Dl signaling. When tested in the embryonic central nervous system (CNS) or the adult peripheral nervous system (PNS), ectopically expressed Fng can inhibit Ser signaling [18,20•]. To make the situation more complicated, ectopically expressed Fng also inhibits Dl signaling in the PNS [20•].

Results from Panin *et al.* [17••] show that the effects of Fng on both Ser and Dl are cell-autonomous, suggesting that Fng functions in cells expressing the Notch receptor, rather than in those expressing the ligands. Klein and Martinez Arias [19•] suggest that although Fng inhibition of the reception of Ser signaling is through a direct effect of Fng on Notch, Fng may also inhibit the sending of the Ser signal indirectly. In the PNS, an inhibitory effect of Fng on both Ser and Dl was observed when Fng was co-expressed with either Ser or Dl [20•], suggesting that Fng is not functioning within the signal-receiving cells.

Thus, *fng* interacts genetically with genes in the Notch pathway to define the D/V border in the *Drosophila* wing. However, it is not yet known whether Fng directly regulates Ser, Dl, Notch or other components in the Notch pathway.

## *Fringe* in vertebrate limb development

The vertebrate limb develops from the limb bud in the embryo. The D/V border of a vertebrate limb bud is a specialized region called the apical ectodermal ridge (AER), which is essential in patterning the limb bud and in limb outgrowth. Juxtaposition of dorsal and ventral compartments in a wild-type embryo is important for AER formation [21], suggesting a possible role for signaling between the dorsal and ventral compartments in AER formation. Several genes of the Notch pathway are expressed in the limb bud. For example, *Ser2*, a vertebrate homolog of *Drosophila Ser*, is expressed in the dorsal compartment and the AER of the limb bud, and *Notch-1* is expressed in the AER [22,23]. *Ser2/Jagged2* mutations in the mouse cause abnormal AER formation [22–24,25•].

The vertebrate *radical Fng* (*rFng*) gene may play an important role in AER formation. *rFng* is expressed early in the dorsal compartment of the limb bud and later in the AER [22,23]. The expression of *rFng* is regulated positively by *Wnt-7a*, which promotes the dorsal fate [22], and negatively by *En-1*, which promotes the ventral fate [22,23]. These results indicate that *rFng* is downstream of the D/V patterning genes in the limb bud. In chick *limbless* mutant embryos, *rFng* is expressed throughout the limb bud ectoderm and there is no AER [23]. In chick *eudiplopodia* mutant embryos, there are ectopic AERs. Although other dorsal markers are uniformly expressed around the AERs in *eudiplopodia* mutants, *rFng* is expressed earlier in the presumptive dorsal parts of the original and ectopic AERs and later within the original and ectopic AERs [23]. These findings indicate that *rFng* is more predictive of the position of the AER than the juxtaposition of dorsal and ventral compartments. It also suggests the possibility that *eudiplopodia* mutants have disrupted a step downstream of D/V patterning but upstream of *rFng* expression. Taken together, the normal expression pattern of *rFng* and the expression of *rFng* in chick *eudiplopodia* mutant embryos are suggestive of a role for *rFng* in AER formation [22,23].

Rodriguez-Esteban *et al.* [22] and Laufer *et al.* [23] tested directly for *rFng* function in AER formation by ectopically expressing *rFng* in the limb bud. They used retroviral vectors to introduce *rFng* and found multiple classes of phenotypes in the limb bud, including disrupted AERs, ectopic AERs, missing AERs, and no AER. When the expression of *rFng* was probed either by *in situ* hybridization or by immunocytochemistry, it was found that an AER could be found only at the border of *rFng*-expressing and non-expressing cells [22,23]. These results demonstrate a striking similarity between *fng* function in vertebrate limb development and that in *Drosophila* wing disc development. In this context, it is surprising that removal of the *rFng* gene from the mouse genome did not lead to defects in AER formation or limb development [26,27].

The apparently similar roles of *fng* in the *Drosophila* wing disc and the vertebrate limb bud have been taken to suggest an evolutionarily conserved mechanism of D/V patterning in invertebrate and vertebrate appendages [22,23]. Similar conclusions of evolutionary relationships have also been made on the basis of molecular and developmental studies in other systems. In the appendages, there are genes whose functional roles are not conserved. For example, *en* is involved in the formation of the posterior compartment in the *Drosophila* wing disc, whereas *En-1* is involved in the formation of the ventral compartment in the vertebrate limb bud. The functions of *Drosophila wg* and vertebrate *Wnt-7a* genes are also different. Although fibroblast growth factors (FGFs) are crucial in vertebrate limb development, FGF has not been implicated in *Drosophila* appendage development. At this point, it cannot be ruled out that the repeated usage of some signaling molecules in invertebrates and vertebrates reflects a limited number of signaling molecules available during development. For genes such as *fng*, the unique function of defining a border by juxtaposition of $fng^+$ and $fng^-$ cells may make it convenient to be used in different animals in similar or different developmental processes. While none of these arguments alone can exclude an evolutionary relationship, they show that it is not safe to establish an evolutionary relationship by focusing on similarities and discounting differences. Indeed, Christen and Slack [28•] have examined the expression of *rFng*, *Wnt-7a*, *En-1*, and *Notch-1* in *Xenopus* limb buds, and they found that only *En-1* is expressed in the ventral compartment of *Xenopus* limb buds, in a pattern similar to those in mouse and chick embryos, whereas other genes, including *rFng*, are expressed uniformly. These observations are not consistent with an evolutionary conservation in D/V border formation between the *Drosophila* wing disc and the vertebrate limb bud.

## *Fringe* in vertebrate somitogenesis

In a different process of vertebrate development, *fng* also appears to be involved in defining borders. A molecular clock has been implicated in somite segmentation during somitogenesis [29]. The Notch pathway is required for normal segmentation of somites [30,31,32••]. *lunatic Fng* (*lFng*) has been found to be rhythmically expressed in the presomitic mesoderm in a pattern consistent with its being downstream of the molecular clock and upstream of the Notch pathway [33•,34•]. If lFng regulates Notch signaling during somitogenesis, it may then be the link between the molecular clock of segmentation and the Notch pathway [33•,34•]. *lFng* mutant mice have two noticeable defects: one in the formation of a nascent somite from the rostral presomitic mesoderm and the other in A/P patterning of the somite [35•,36•]. These results suggest that *lFng* is required for defining the borders between somites and between the anterior and posterior parts of each somite

[35•,36•]. It is not yet clear which role is the primary one and whether the other role is indirect [35•,36•]. So far, *Drosophila fng* has not been implicated in patterning the embryonic mesoderm. Thus, it is possible that *fng* is used in vertebrate somitogenesis for its border defining function, rather than a result of evolutionary conservation in mesoderm segmentation. It has been observed that the Notch signaling pathway is involved in segmentation in the *Drosophila* leg [37••,38•] and that *fng* also regulates this pathway [37••].

## *Fringe* in *Drosophila* and vertebrate eye development

Notch signaling is best characterized in *Drosophila* neural development, particularly in the formation of embryonic neuroblasts and in eye development. Recent studies have shown that *fng* plays an important role in *Drosophila* eye development. Specifically, *fng* is required for defining the D/V border in the *Drosophila* eye [39••–41••].

The *Drosophila* compound eye consists of about 750 units, the ommatidia, each of which is made up of multiple photoreceptors, pigment cells and cone cells. The distribution of photoreceptors within each ommatidium is asymmetric, conferring a certain orientation to each ommatidium. The orientations of ommatidia in the dorsal half of the eye are the same, which is opposite to the orientations of ommatidia in the ventral half of the eye. The physiological significance of the D/V differences is not well known, but the D/V border, or the equator, in the developing eye disc has been implicated as a signaling center for coordinating eye patterning and growth.

The *fng* gene has now been found to be expressed during early stages of eye disc development; its expression is localized to the ventral half of the eye disc [39••–41••]. Expression of *fng* in the ventral half is regulated by upstream genes involved in D/V patterning; transcription factors encoded by the *mirror* and *caupolican* genes, which are normally expressed in the dorsal half, are capable of repressing *fng* expression in the eye [40••,41••]. The juxtaposition of $fng^+$ and $fng^-$ cells is crucial for the formation of the equator [39••–41••]. Uniform expression of *fng* eliminates the equator, whereas creation of new $fng^+$ and $fng^-$ borders, either by making $fng^-$ clones in the ventral compartment or by ectopic expression of *fng* in the dorsal compartment near the D/V midline, causes the formation of ectopic equators [39••–41••]. The orientations of ommatidia at a distance of several ommatidia away from the ectopic equator are also altered [40••], suggesting either that *fng* can function at a distance or, more likely, that the equator has long-range organizing activities.

Similar to its function in the wing, *fng* also seems to function by regulating the Notch pathway to define the D/V border of the *Drosophila* eye [39••–41••]. Initially, *Dl* is expressed in the dorsal half of the eye disc, whereas *Ser* is expressed in the ventral half of the eye disc. This D/V difference in *Dl* and *Ser* expression patterns is maintained, albeit narrowed down to the region near the equator, until early third instar. *Notch* is expressed in the equator. Activation of Notch signaling is an essential step in equator formation. Fng seems to enable Dl to activate Notch, whereas it inhibits Ser activation of Notch [39••]. Activation of Notch is downstream of Fng action [39••–41••]. Once Notch is activated, the equator is defined. In addition to D/V asymmetry, this equator is also necessary for eye growth. Absence of the equator results in reduction or absence of the eye. Because of the requirement of a border of $fng^+$ and $fng^-$ cells in equator formation, both uniform expression of *fng* and loss-of-function mutations of *fng* lead to a small-eye phenotype [39••–41••]. Interestingly, it has also been observed that juxtaposition of $fng^+/fng^+$ to $fng^+/fng^-$ cells also creates an ectopic equator [41••], suggesting that concentration differences, rather than the presence and the absence, of Fng is sufficient to define a border [41••].

In vertebrates, *fng* may also be involved in eye development. However, it is unlikely that *fng* is involved in defining a D/V border of the vertebrate eye. *lFng* is expressed in the vertebrate eye [42], but not in a D/V differential pattern; it is expressed throughout the eye [42]. Notch signaling is however important in vertebrate eye development, where it is required for cell fate determination in the retina [43,44]. So far, there is no indication that Notch signaling is involved in D/V border formation in the vertebrate eye. The lack of D/V differences in *lFng* expression is consistent with the possibility that neither lFng nor the Notch pathway is involved in defining D/V border or in patterning the D/V axis of the vertebrate eye. Thus, there is no evidence for a conserved role of *fng* in *Drosophila* and vertebrate eye development.

## Fringe and Notch in vertebrate neural tube development

It is clear that *fng* is not involved in the development of the ventral nerve cord in the *Drosophila* embryo [15], but it remains possible that *fng* plays a role in the development of the vertebrate neural tube. It is therefore not clear that *fng* or similar genes can play conserved roles in the embryonic CNS of *Drosophila* and vertebrates.

*lFng* and *mFng* are expressed in the neural tube [23,42,45,46]. In the spinal cord, they are expressed in three stripes, which correspond to the primary neurons. This pattern of *fng* expression is similar to that of *Dl* or *Dl-like* genes and is complementary to that of *Ser* (or *Jagged* in mammals) [23,42,45,46]. It should be noted that the co-localization of *fng* with *Dl*, rather than *Ser*, in the vertebrate neural tube is opposite to that of *fng* co-localization with *Ser* in *Drosophila* wing and eye discs. Nonetheless, because the Notch pathway is crucial for the formation of primary neurons in the neural tube [47], it is tempting to speculate that *fng* may also function in this process, although there is no direct evidence yet for *fng* function in neural tube development.

It is presently difficult to know exactly what functional role Fng may play in the vertebrate neural tube. Notch activation in vertebrates reduces the number of primary neurons, whereas inhibition of the Notch pathway increases the number of primary neurons [47]. This resembles, superficially, the phenotypes of gain- or loss-of-function mutations of *Notch* in *Drosophila* embryos, respectively. In *Drosophila* embryos, *Notch* clearly plays a role in singling out a neuroblast from a group of ectodermal cells with initially equal potentials in cell fate decisions: cells with high Notch activation become epidermoblasts, whereas those with low or no Notch activation become neuroblasts. In vertebrates, although Notch signaling is involved in neurogenesis, the choices of fates are not those of neuroblast and epidermoblasts. It is not clear whether there is an alternative fate that cells take when inhibited from forming the primary neurons, and if so, what is the fate alternative to that of the primary neurons. On the basis of the primary neuron phenotype of Notch activation in vertebrates, it is reasonable to speculate *fng* may regulate the number of primary neurons.

However, in all situations where functional roles of *fng* have been characterized, *fng* acts to define a developmental border by juxtaposing $fng^+$ and $fng^-$ cells. If one has to use this analogy to speculate a function for *fng* in the vertebrate neural tube, then one has to propose borders between $fng^+$ ($Dl^+/Ser^-$) and $fng^-$ ($Dl^-/Ser^+$) cells within the neural tube. Such borders are not known previously from anatomical, developmental, or functional differences. Although the proposal of such borders is interesting and provocative, there is presently no molecular markers expressed specifically at these hypothetical borders to prove the existence, or facilitate the study, of these borders.

Thus, comparisons across species is not as straightforward as usually practiced and can lead to dilemma in deciding what, if any, features are actually conserved evolutionarily. Depending on the molecular markers available at different time points of research, one could potentially be confused by relying on beliefs of evolutionary conservation.

## Mechanism of Fringe function

While the *fng* genes are important for development, little is known about the biochemical mechanism of the Fng proteins. All predicted Fng proteins contain a signal peptide at the amino terminus indicative of translocation into the endoplasmic reticulum (ER) and the secretory pathway [15]. Some of the Fng proteins contain a pro-region that can be cleaved from the carboxy-terminal mature region [42,45]. When overexpressed in cultured cell lines, the mature region can either be secreted extracellularly [42,45] or be associated with the cell membrane [17••]. These cellular and biochemical data, even when considered together with the genetic data, do not resolve the issue as to the site of Fng action. We still cannot distinguish whether Fng proteins act in the secretory pathway (i.e. the ER or the Golgi complex), on the cell membrane, or in the extracellular space.

On the basis of the sequence similarities of the predicted Fng proteins, Fng has been suggested to be a glycosyltransferase [48•]. However, since the similarities are not very high, it is not clear whether Fng would function as a glycosyltransferase or whether the region in Fng similar to the glycosyltransferases is simply a carbohydrate-binding motif. The target(s) of Fng, either as a substrate or a binding partner, is also unknown, although the most attractive candidates are ligands and receptors in the Notch pathway.

Genetic studies in *Drosophila* demonstrate that, if the amino-terminal and DSL domains in Dl are replaced by the same domains in Ser, then the function of the chimeric Dl protein is also inhibited by Fng [18], suggesting that Fng inhibition of Ser is specific for the amino-terminal and DSL domains of Ser. However, this effect could be explained either by a direct effect of Fng on the amino-terminal and DSL domains in Ser or by a direct effect of Fng on the region in Notch that is involved in binding to the Ser domains [18]. There is no evidence that Fng can bind, or transfer carbohydrate moieties, to ligands, receptors or other components in the Notch pathway.

If Fng is a glycosyltransferase, it may also help us postulate the functions of other genes. For example, it is quite tempting to speculate on a possible function for the product of *bib*, which remains mysterious despite its cloning almost a decade ago [49]. Because the Bib protein has significant sequence similarities to membrane transporters of small neutral molecules [49], one hypothesis is that Bib could be a transporter for carbohydrates (perhaps even galactose), which is used by enzymes such as Fng to modify Notch or its ligands. In the embryonic CNS and PNS, such glycosylation is proposed to be required for Notch signaling. For this hypothesis to be correct, there has to be another Fng-like enzyme in the embryo because *fng* is not functionally required there. In any case, it seems that studies of the functional mechanisms of Fng proteins may not only solve the puzzle of how Fng works, but also shed light on the function of other genes, which would be helpful for a better understanding of pattern formation and neural development.

## Conclusions

The *fng* genes encode a new family of signaling molecules that function in both invertebrates and vertebrates. The most interesting aspect of *fng* functioning is that the juxtaposition of $fng^+$ and $fng^-$ cells can determine developmental borders. All functions of Fng characterized so far can be attributed to its modulation of the Notch pathway, which could also conceivably be the mechanism for those functional roles of Fng remaining to be investigated. However, it is clear that Fng is not involved in all processes that require Notch signaling. While it is debatable whether the roles for *fng* in *Drosophila* and vertebrate appendage development indicate evolutionary conservation, it seems less likely that *fng* plays evolutionarily conserved roles in *Drosophila* and vertebrate neural development.

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These two papers [33•,34•] report that, similar to *hairy-1*, *lFng* is expressed in a rhythmic pattern in the presomitic mesoderm. However, unlike *hairy-1*, *lFng* expression is dependent on protein synthesis, and may thus be downstream of *hairy-1* or other earlier acting components in the molecular clock involved in somite segmentation.

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See annotation [36•].

36. Evrard YA, Lun Y, Aulehla A, Gan L, Johnson RL: ***lunatic fringe* is an**
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These two papers [35•,36•] report the phenotype of *lFng* knockout mice. This paper provides an interesting model on the functional mechanism of *Fng* action.

37. Bishop SA, Klein T, Martinez Arias A, Couso JP: **Composite**
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Fly legs develop from concentric segments along the proximal–distal axis. The authors found that *Ser*, *Dl* and *Notch* have roles in leg segmentation. *fng* inhibits both *Ser* and *Dl*. There is a twist to the relationship of *Ser* and *Dl*: *Ser* is expressed in concentric rings proximal to the presumptive joints, whereas *Dl* is expressed throughout the leg; however, a high level of *Dl* is expressed in the same region as *Ser*. *fng* seems to inhibit *Ser* and *Dl* both in cells expressing *Ser* and in those neighboring them.

38. Rauskolb C, Irvine KD: **Notch-mediated segmentation and growth**
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See annnotation [41••].

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•• **morphogenesis in the Drosophila eye.** *Nature* 1998, **396**:272-276.
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