**Sonic hedgehog** is not required for polarising activity in the Doublefoot mutant mouse limb bud

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**SUMMARY**

The mouse mutant Doublefoot (Dbf) shows preaxial polydactyly of all four limbs. We have analysed limb development in this mutant with respect to morphogenesis, gene expression patterns and ectopic polarising activity. The results reveal a gain-of-function mutation at a locus that mediates pattern formation in the developing limb. Shh expression is identical with that of wild-type embryos, i.e. there is no ectopic expression. However, mesenchyme from the anterior aspects of Dbf/+ mutant limb buds, when transplanted to the anterior side of chick wing buds, induces duplication of the distal skeletal elements. Mid-distal mesenchymal transplants from early, but not later, Dbf/+ limb buds are also able to induce duplication. This demonstration of polarising activity in the absence of Shh expression identifies the gene at the Dbf locus as a new genetic component of the Shh signalling pathway, which (at least in its mutated form) is able to activate signal transduction independently of Shh. The mutant gene product is sufficient to fulfil the signalling properties of Shh including upregulation of the direct Shh target genes Ptc and Gli, and induction of the downstream target genes Bmp2, Fgf4 and Hoxd13. The expression domains of all these genes extend from their normal posterior domains into the anterior part of the limb bud without being focused on a discrete ectopic site. These observations dissociate polarising activity from Shh gene expression in the Dbf/+ limb bud. We suggest that the product of the normal Dbf gene is a key active constituent of the polarising region, possibly acting in the extracellular compartment.

Key words: Sonic hedgehog (Shh), Zone of polarising activity (ZPA), Apical ectodermal ridge (AER), Fgf4, Doublefoot (Dbf), Limb bud, Mouse

**INTRODUCTION**

Within the vertebrate limb bud, three interacting tissues are responsible for the organisation of skeletal pattern. These are (1) the apical ectodermal ridge (AER), a thickened rim of distal ectoderm, (2) the progress zone (PZ), the mesenchyme underlying the AER, and (3) the zone of polarising activity (ZPA), a specialised area of posterior distal mesenchyme. By maintaining the PZ cells in a proliferative state, signals from the AER maintain proximodistal outgrowth of the limb bud, while cells leaving the PZ differentiate to form the limb skeletal pattern in a proximodistal sequence. Signals from the ZPA and AER maintain each other, and the ZPA governs the anteroposterior pattern of the limb, as shown most clearly by the three distinctly different digits of the chick wing. In most mammalian limbs, including mouse and human, the anteroposterior digital pattern is characterised by an anterior digit with two phalanges (numbered I) and four similar triphalangeal digits (numbered II-V).

The mouse mutant Doublefoot (Dbf), which shows polydactyly with 6-8 digits per limb, is a particularly informative resource for investigating the molecular mechanisms governing limb pattern. The phenotype is inherited in a single, dominant Mendelian manner and is fully penetrant; the mutation has been mapped to chromosome 1 (Lyon et al., 1996). There is no digit I, all digits invariably being triphalangeal. Some limbs have one or two digits that are not in contact with the carpus/tarsus (Fig. 1B); digital bifurcation is also sometimes present (Fig. 1C). The mutant also shows tibial defects and craniofacial abnormalities, including a broadened, bulbous skull, and facial clefts in homozygotes. Dbf limbs are morphologically distinguishable by E10.5, being enlarged along the anteroposterior axis (Fig. 2).

The protein Sonic hedgehog (Shh) has been implicated as the active signalling molecule of the ZPA (Riddle et al., 1993). The ZPA is defined as a region of posterior limb mesenchyme with the ability to induce mirror-image duplications when transplanted to the anterior margin of a chick wing bud (Saunders and Gasseling, 1968). Ectopic Shh gene expression mimics the effect of ZPA grafts and can induce the ectopic expression of downstream genes such as bone morphogenetic protein 2 (Bmp2), 5′HoxD genes and fibroblast growth factor 4 (Fgf4) (Lauf et al., 1994). These downstream targets only possess a subset of the properties of Shh itself (Francis et al.,...
an additional 34 cycles of steps 2-4. The cycler profile of (1) 94°C, 2 minutes; (2) 60°C, 45 seconds; (3) 94°C, 30 seconds; (4) 72°C, 5 minutes; (5) 72°C, 45 seconds; (6) 72°C, 5 minutes; (7) 72°C, 45 seconds; (8) 94°C, 2 minutes. The primers: 5'-GTGC-3' was amplified using the following PCR reaction:

GAGGTAGGGTGG-3'

An 810 bp fragment from the full-length clone of the murine patched gene (M. Scott) designated E0. E10.5-11.5 embryos were dissected into Tyrode's saline and their forelimbs were removed and processed for in situ hybridization (Wilkinson, 1992) with digoxigenin-labelled riboprobes for the genes Shh, Fgf4 (A. Mc Mahon), Hoxd13 (D. Duboule), Bmp2 (M. Jones), Gli (C. Hui) and Ptc (see above).

Current understanding of the molecular nature of Shh-mediated patterning of the limb is incomplete. It is now known that the active amino peptide of Shh (Shh-N), even when cleaved from the C-terminal domain, is anchored to the cell surface by cholesterol (Porter et al., 1995, 1996). This lack of mobility, which is also indicated by co-localisation of Shh peptide and mRNA (Martí et al., 1995), spatially limits the range of direct influence of Shh at the molecular level in contrast to its functional influence, which spreads across the full width of the limb bud. Shh is the ligand for the cell surface receptor patched (Ptc) (Marigo et al., 1996a; Stone et al., 1996), which inhibits activation of another predicted cell surface receptor-like molecule, smoothened (Smo) (van den Heuvel and Ingham, 1996). Ptc also serves to reinforce the limited spatial distribution of hedgehog by sequestration of the ligand (Chen and Struhl, 1996). Binding of Shh to Ptc lifts the repression of Smo, leading to activation of the transcription factor Gli, and the transcription of direct target genes of Shh signalling. These include Ptc and Gli themselves (reviewed by Ruiz i Altuab, 1997).

Analysis of Dbf mutant limbs demonstrates that the as yet unidentified product of the Dbf locus ectopically activates the Shh signal transduction pathway in the absence of Shh gene expression. The resultant cascade of molecular genetic events is sufficient to confer polarising activity on anterior and distal limb bud mesenchyme cells. Our results identify a new element of the Shh pathway whose molecular function is integrated with those of Ptc and Smo, but which (at least in its mutated form) does not require Shh in order to activate signal transduction.

**MATERIALS AND METHODS**

**Embryos**
Embryos were obtained from matings between a Dbf/+ male and a C3H strain mouse. The day of the vaginal plug was designated E0. E10.5-11.5 Dbf/+ embryos were distinguished phenotypically from their wild-type littermates by their broader limb buds at E11.0/11.5 and by the characteristic diencephalic bulge on E10.5 (C. H., M. F. L. and G. M. M.-K., unpublished data).

**Cartilage and bone staining**
Mice were fixed overnight in 95% alcohol and eviscerated; the skin was then removed under a dissecting microscope with watchmakers forceps and the mice were stained as previously described (Watson, 1977).

**Ptc probe preparation**
An 810 bp fragment from the full-length clone of the murine Patched gene was amplified using the following PCR primers:

5'-TGACTGTTCGAGAGTGTC-3' and 5'-CGAATAGACAGAGGTTGAGG-3' with a thermal cycler profile of (1) 94°C, 2 minutes; (2) 94°C, 30 seconds; (3) 60°C, 45 seconds; (4) 72°C, 45 seconds; (5) 72°C, 5 minutes; (6) an additional 34 cycles of steps 2-4. The resulting blunt-end-polished fragment was inserted into the SphI site of pCR-script (Stratagene, La Jolla, CA), flanked by T3 and T7 promoters for the generation of antisense and sense riboprobes.

**In situ hybridization**
E10.5-11.5 embryos were dissected into Tyrode's saline and their forelimbs were removed and processed for in situ hybridization (Wilkinson, 1992) with digoxigenin-labelled riboprobes for the genes Shh, Fgf4 (A. Mc Mahon), Hoxd13 (D. Duboule), Bmp2 (M. Jones), Gli (C. Hui) and Ptc (see above).

**Grafts**
Mesenchymal cubes were cut from hindlimbs of freshly dissected E10.5-11.0 embryos (see above) following removal of the ectoderm; they were used as donor tissue for grafting under the anterior ectoderm of host Hamburger and Hamilton (1951) stage 20 chick wing buds (Izpisua-Belmonte et al., 1991b).

**RESULTS**

**Dbf limbs show ectopic expression of Fgf4 but not Shh**
Shh and Fgf4 have been implicated as the main mediators of pattern along the anteroposterior and proximodistal axes of the developing limb (Lauffer et al., 1994). We therefore investigated the expression domains of these two genes in Dbf embryonic limb buds. In order to make the most efficient use of the limited mutant tissue available, in situ probes for both genes were hybridized on the same specimen. Shh was detected only in its normal posterior mesenchymal domain (Riddle et al., 1993), in both wild-type (C3H) and Dbf/+ limbs (Fig. 2A,B) (n=20). The double detection method Shh signal was comparatively weak; therefore, in order to rule out the possibility that the anterior mesenchyme possessed low levels of Shh transcripts, or that ectopic expression was delayed as in other polydactylous mouse mutants (Masuya et al., 1995), mutant limbs were hybridized with a probe for Shh alone at a range of stages from E10.5 hindlimbs to E11.5 forelimbs showing early digital condensation (n=20). Again, Shh expression was detected only in the posterior mesenchyme of Dbf/+ limb buds (Fig. 2C and data not shown). Fgf4 was expressed uniformly throughout the apical ectodermal ridge (AER) of Dbf/+ limbs (n=20), in contrast to its normal posterior domain (Niswander and Martin, 1992) as detected in wild-type AERs (Fig. 2A,B). Expression of Fgf4 has been shown to be induced and polarised by Shh (Lauffer et al., 1994).

The presence in the mutant limbs of an Fgf4 expression domain with both Shh-dependent and Shh-independent components indicates that the Dbf mutant gene product is sufficient to

**Fig. 1.** Forelimb skeletons (bone, red; cartilage, blue) of day 17 wild-type (A) and Dbf/+ (B,C) fetuses. Anterior is to the top. The Dbf/+ limbs show absence of digit I; (B) six triphalangeal digits and two interdigital digits and (C) digital bifurcations are evident. Scale bar = 1 cm.
Recent studies have shown ectopic Shh expression in several well-characterized mouse mutants, which phenotypically resemble the Dbf mutant, suggesting that the polydactyloous phenotype is due to ectopic polarising activity in the anterior limb bud mesenchyme. These mutants show ectopic Fgf4 in the overlying anterior AER and, in some cases, ectopic (anterior) HoxD gene expression, all concomitant with ectopic Shh expression (Chan et al., 1995; Masuya et al., 1995, 1997). The Dbf mutant differs significantly from these previously described mutants in having supernumerary digital elements suggestive of ectopic polarising activity in spite of the absence of Shh expression.

In order to establish unequivocally whether the anterior mesenchyme of the Dbf mutant limbs possesses polarising activity, we grafted portions of the anterior mesenchyme from E10.5 and E11 Dbf/+ and wild-type limbs into the anterior margin of stage 20 chick wing buds (Izpisua-Belmonte et al., 1991b); posterior and mid-distal mesenchyme was also grafted (Fig. 3A). The results are summarised in Table 1 and illustrated in Fig. 3B-F. By definition, the presence of duplications arising as a result of the grafts indicates polarising activity (Honig and Summerbell, 1985; Hinchliffe and Samson, 1985). As expected (Tickle et al., 1976), surviving limb grafts from wild-type anterior limb bud mesenchyme (Fig 3B) had no effect, whereas grafts of wild-type posterior limb bud mesenchyme induced mirror-image duplication (Fig. 3C, 4/5). Grafts from all three sites in Dbf/+ limbs induced duplications (Fig. 3D-F; 5/7 posterior, 14/17 anterior, 3/5 mid-distal), although the polarising activity of mid-distal mesenchyme was present in early (E10.5, 3/3) but not later stage (E11, 0/2) embryos.

A significant observation concerning the pattern of the induced digits is that, although the size and number of the induced digits was variable, grafts from all three mesenchymal sites from Dbf/+ embryos induced a greater number of supernumerary elements than grafts of posterior wild-type mesenchyme. Some successful mutant grafts, in addition to inducing extra elements in the region of the graft, had effects on the ‘host’ digits (i.e. those formed posterior to the graft site), e.g. thickening and bifurcation (Fig. 3E).

Fig. 2. Right E10.5 (A-E) or E11.5 (F,G) wild-type and Dbf/+ forelimb buds (anterior uppermost): whole-mount in situ hybridization to show expression domains of the genes indicated; Shh and Fgf4 are combined on A and B, Shh is shown separately on C. Dbf/+ limbs show ectopic anterior expression of Fgf4, Bmp2 and Hoxd13 but not Shh (distal/anterior boundaries of Fgf4 and Bmp2 domains are indicated by arrows). Scale bar = 100 μm.

Dbf/+ limbs show ectopic expression of Shh downstream target genes

For further information on the position of the Dbf mutation in the polarising region signalling pathway, we investigated the expression of Bmp2 and Hoxd13, two downstream targets of Shh. In Dbf/+ limbs, Bmp2 showed ectopic expression extending throughout the anterodistal mesenchyme, in addition to its normal domain in the posterior and posterodistal mesenchyme (Hogan, 1996) (Fig. 2D,E). This pattern is reminiscent of that of the polydactyloous chick mutant, talpid, in which Bmp2 is expressed throughout the distal mesenchyme (Francis-West et al., 1995). Hoxd13, which is responsive to the polarising region (Izpisua-Belmonte et al., 1991a), was expressed uniformly throughout the distal mesenchyme of mutant limbs at E11, rather than being restricted to the posterodistal mesenchyme as in wild-type limbs (Fig. 2F,G). This information places the Dbf mutation genetically upstream of Bmp2 and Hoxd13.

Dbf limbs show ectopic polarising activity in the absence of Shh

Recent studies have shown ectopic Shh expression in several well-characterized mouse mutants, which
The position of Dbf in the Shh signalling pathway

To further elucidate the functional position of the Dbf gene and to rule out the possibility that the duplications and polarising activity were the result of a mutation in a parallel signalling pathway, we investigated the expression of elements of the Shh signal transduction pathway. The genes selected were (1) Ptc, which has been implicated as receiving the Shh signal by direct binding of Shh (Stone et al., 1996; Marigo et al., 1996a,b), and (2) the transcription factor Gli, a transactivator of Shh targets in cells responding to Shh (Alexandre et al., 1996; Hepker et al., 1997; Lee et al., 1997; von Ohlen et al., 1997), both of which are direct transcriptional targets of Shh, and thus immediate indicators of Shh pathway activation.

Ptc is normally expressed in the posterior limb mesenchyme, in a domain overlying but more extensive than that of Shh (Goodrich et al., 1996; Marigo et al., 1996b; Fig. 4A). In the Dbf/+ limbs, Ptc was expressed throughout the distal mesenchyme in a continuous posterior-to-anterior domain (Fig 4B). Gli also showed an exclusively posterior expression domain in wild-type embryos (Fig. 4C), but was expressed throughout Dbf/+ limb bud mesenchyme (Fig. 4D). In both Dbf/+ and wild-type limbs a specific downregulation of these two genes was observed in a small area of posterodistal mesenchyme corresponding to the Shh expression domain, as reported by others (Fig. 4 arrows; Marigo et al., 1996b,c; Goodrich et al., 1996). There was no equivalent region of lower transcript abundance in the ectopic anterior domain of either Ptc or Gli (n=40). This supports the Shh gene expression evidence indicating that there is no Shh present outside the posterodistal mesenchyme of Dbf limbs.

Analysis of the timing of Ptc expression revealed that, in younger Dbf/+ limbs, before the limb has significantly widened along the AP axis, Ptc is expressed in a broad anteroposterior band that then extends further anteriorly (compare E10.5 hindlimbs, Fig. 5A,B with E10.5 forelimbs, Fig 4A,B). In E11.0 forelimbs (not shown), the transcript level was lower mid-distally, indicating early downregulation in this site. In even older limb buds (E11.5 forelimbs, Fig. 5D), when Ptc expression has been down-regulated to barely detectable levels in wild-type limb buds, mutant limb buds showed downregulation in all regions except for the anterior domain, where relatively high transcript levels remained.

The results of these experiments indicate that polarising activity is located in a continuous band around the distal margin of the Dbf mutant limbs.

Table 1. Summary of the results of the grafting experiments

<table>
<thead>
<tr>
<th>Origin of graft</th>
<th>Normal</th>
<th>Nodule*</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>Radius/Ulna†</th>
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<td>0</td>
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<td>17</td>
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<td>0</td>
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<td>7</td>
</tr>
</tbody>
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For the digits, the posterior-most skeletal element induced is indicated.

*An induced ectopic cartilaginous element smaller than digit II, which could not be assigned a digital identity with any certainty.

†Indicates the duplication of the zeugopodal elements in the absence of digital duplications.

Fig. 4. Expression of Ptc in E10.5 wild-type (A) and Dbf/+ (B) forelimbs and Gli in E10.5 wild-type (C) and Dbf/+ (D) forelimbs, showing ectopic expression of both genes in the mutant limbs. Arrows indicate the posterior region of Ptc downregulation corresponding to the Shh expression domain. Scale bar = 100 μm.

Fig. 5. Expression of Ptc in E10.5 wild-type (A) and Dbf/+ (B) hindlimbs and in E11.5 wild-type (C) and Dbf/+ (D) forelimbs. Ptc expression in early Dbf/+ limb buds (b) is in a single extended domain, confirming that the domain seen in E10.5 Dbf/+ forelimbs (Fig. 4B) is not due to the coalescence of a posterior and an ectopic anterior domain. When digital condensations begin to form, Ptc is down-regulated throughout its domain in wild-type limbs (C) but in Dbf/+ limbs (D) downregulation begins in the mid-distal region and expression is retained longest anteriorly. Scale bar, 100 μm.
DISCUSSION

Analysis of polarising activity and gene expression in the polydactylous mutant *Doublefoot* has shown that ectopic activity is present throughout the distal (progress zone) mesenchyme of the *Dbf* limb bud; it is not confined to the normal posterior ZPA region, nor is the ectopic activity localised to a discrete anterior region. This finding is reflected in the gene expression patterns. The results suggest that the *Dbf* mutation affects a gene encoding a key component of the Shh signalling pathway. The mutation represents a gain of function, showing both ectopic and enhanced activity of a normal function.

Detailed genetic mapping of the chromosomal assignment of the *Dbf* locus (Lyon et al., 1996) excludes the possibility that *Dbf* represents a mutation in a previously identified component of the vertebrate Shh signalling pathway (see Ruiz i Altaba, 1997 for a recent summary). Similarly, there is no known component of the *Drosophila* hedgehog pathway that fulfils a role equivalent to that of the *Dbf* gene (see Perrimon, 1995). Our analysis of the *Dbf* mouse indicates that the mutation is in a gene encoding a novel element of the Shh signalling pathway. The mutant gene product is able to fulfil the roles of Shh (polarising activity and induction of target genes) in the limb, in the absence of Shh. In our grafting experiments, wild-type ZPA grafts induced mirror-image duplications comparable to those induced by mouse-chick ZPA grafts (Tickle et al., 1976); in contrast, mesenchyme from all regions of the limb, including posterior mesenchyme, induced irregular patterns of extra digits that are very similar to those induced by the ectopic retroviral expression of Shh in the anterior of chick wing buds (Fig. 9 of Riddle et al., 1993). The failure of both Shh of retroviral origin and mesenchyme from *Dbf* limb buds to induce the mirror-image duplication pattern so consistently obtained with ZPA grafts, suggests that other factors, in addition to *Dbf* and Shh, are required to elicit the complete developmental sequence of events controlled by the endogenous ZPA.

We suggest that the activity of the normal *Dbf* gene product is under the control of Shh, confining activity within the limb to the region of the ZPA. In contrast, the mutant Dbf protein does not require Shh for its activity, which is therefore not restricted to the region of Shh ‘activation’. The fact that the ectopic polarising activity is widespread suggests that the *Dbf* gene product itself is widespread and that its normal function is closely related to the propagation and/or inhibition of signalling through the Shh signalling pathway.

The *Dbf* phenotype strongly resembles that of transgenic mice overexpressing Shh, which have polydactylyous limbs with supernumerary interdigital elements as well as bifurcations of the distal phalanges (Oro et al., 1997). The ability of a gain-of-function mutation at the *Dbf* locus to phenocopy a transgenic mouse ubiquitously expressing Shh supports the interpretation that the Shh and *Dbf* genes participate in a common pathway and that *Dbf* gene expression is widespread in the mutant limb bud mesenchyme. This is significant in that, if the normal *Dbf* peptide is also widespread in the developing limb, it cannot be under the transcriptional control of the Shh signalling pathway, unlike *Ptc* and *Gli*. This would account for the *Dbf* limbs having a single enlarged ZPA throughout the distal mesenchyme, in contrast to other polydactylyous mouse mutants, which possess a second discrete anterior polarising region (Chan et al., 1995; Masuya et al., 1995, 1997). The widespread polarising activity correlates with the phenotype of the *Dbf* limbs as a fan-shaped array of identical triphalangeal digits (Fig. 1; C. H., M. F. L. and G. M. M.-K. unpublished data).

Grafts of posterior limb bud mesenchyme from both chick (Saunders and Gasseling, 1968) and mouse (Tickle et al., 1976) limb buds show a characteristic complete or partial mirror-image pattern. This pattern was only shown by the wild-type posterior grafts in our experiments; it was not induced by any of the *Dbf* mutant mesenchyme grafts. Some grafts from *Dbf/+* limb buds, including those from the posterior region, showed both extra digits (mainly the more posterior digits, i.e. frequently III and more rarely IV) and an effect on the host digital pattern. These results indicate that the *Dbf/+* mesenchyme, including that of the region expressing Shh, has a greater ability to repattern the chick limb bud mesenchyme than that of wild-type embryos and that the effect is independent of Shh. Nevertheless, the ectopic upregulation of *Ptc* and *Gli* indicates that *Dbf* is signalling through the pathway that is normally Shh-dependent.

In terms of the spatial extent of polarising activity, the *Dbf* mutant shows some similarity to the polydactylyous chick mutant *talpid*, although the ectopic polarising activity of *talpid* wing buds becomes progressively weaker in a posterior-to-anterior gradient (Francis-West et al., 1995), whereas *Dbf* limbs do not show this graded pattern of polarising activity. The number of *Dbf* mutant mesenchyme-induced skeletal elements is also greater than that induced by the ectopic polarising region of *Strong’s Luxoid* mice, which in similar grafting experiments showed weak activity (extra digit II or other skeletal abnormality) in only 4 out of 39 grafts (Chan et al., 1995). Taking together the evidence from the limb phenotype, the observation of polarising activity in both

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**Fig. 6.** Hypothesis for the role of the normal *Dbf* gene product in Shh signalling. (A) Current model of Shh signalling (see text). (B) New model, based on data presented here: following direct or indirect activation by Shh, *Dbf* activates *Smo* initiating signal transduction through *Smo*. According to this interpretation, Shh target genes can be activated in both an Shh-dependent and an Shh-independent manner, i.e. both within and outside the ZPA.
anterior and mid-distal grafts, and the continuous anteroposterior expression domains of downstream Shh target genes in the limb bud, our results indicate that, in the Dbf mutant, polarising activity is located in a continuous band around the distal limb bud margin.

Current understanding of the Shh signalling pathway is summarised in Fig. 6A. Outside the ZPA, Ptc is present at basal levels; the low level of Ptc here is presumably sufficient to repress the pathway in the absence of Shh. Within the ZPA, the presence of Shh lifts the repressive influence of Ptc on Smo, enabling the signal to be transduced, leading to Gli-mediated transcription of target genes, including Ptc and Gli, and ultimately more downstream targets such as Fgf4, Bmp2 and the 5’ HoxD genes. This model makes Shh, through its binding to Ptc, solely responsible for polarising activity and hence pattern formation in the developing limb. This model does not fit the new information derived from analysis of the Dbf mutant.

We have considered the possibility that the Dbf mutation could represent the inactivation of a negative regulator. Negative regulators are a feature of the Shh/Hh pathway, including Patched (Ingham et al., 1991), cAMP-dependent protein kinase A (PKA) (Jiang and Struhl, 1995; Hammerschmidt et al., 1996) and Costal2 (Robbins et al., 1997). Our grafting experiments demonstrate the ability of the Dbf gene product to act in a non-cell-autonomous fashion, i.e. to signal to surrounding cells and to divert their developmental fate. This property strongly suggests that the Dbf gene encodes a secreted signalling molecule that acts as a positive regulator of Shh-related signalling, although the possibility that it acts as a negative regulator cannot be categorically excluded.

We therefore propose the scheme summarised in Fig. 6B, in which the Dbf gene product interacts with Smo. In this model, in the ZPA Shh would activate Dbf, possibly via a Shh/Ptc/Smo/Dbf ligand-receptor complex, the activated Dbf protein then activating Smo in and around the ZPA; this would result in signal transduction and Shh-dependent gene expression in an extensive posterior domain reflected by the domain of Ptc gene expression. According to this interpretation, the normal expression domain of Ptc indicates the sphere of influence of activated Dbf protein. A possible route for the Dbf-Smo-activated pathway is through PKA. PKA is epistatic to smo, which itself has similarities to G-protein-coupled receptors, which signal through PKA (van den Heuvel and Ingham, 1996). This model requires Shh for the activation and spatial control of the pathway, but not for signal transduction in all cells, thereby taking into account the non-diffusibility of Shh. The Dbf mutant phenotype would then result from generalised activation of Dbf protein, leading to widespread Dbf-Smo-generated signal transduction. In this molecular context, the upper limit of the number of digits formed would be dependent on the amount of mesenchyme that can be generated during the developmental period in which the genes are active.

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Shh-independent polarising activity


