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Limb development: an international model for vertebrate pattern formation

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ABSTRACT Limb development is an excellent model for studying how patterns of differentiated cells and tissues are generated in vertebrate embryos. The cell interactions that mediate patterning have been discovered and, more recently, some of the molecules involved in these interactions have been identified. This has provided a direct link to genetics and thus to genes that cause human congenital limb defects.

KEY WORDS: *chick embryo, growth factors, retinoic acid, Hox genes*

Introduction

The study of vertebrate limb development has been truly international. Important embryological work on limb development was carried out in the United States particularly in the late 1940's and continuing into the 1950's and 60's. In Britain, much of the special contribution to the field is based on models of limb development which were formulated in the 1970's and which provided a framework for further experimental work. These models have assumed a new significance now that molecules which are important for limb development have been identified. The explosion of knowledge about the molecular biology of limb development has taken place in the space of less than ten years and has come not only from work in Europe but also from work in the United States and in Japan. As in other areas of active research, ideas about limb development have often caused controversy. This not withstanding, the field has been marked by cooperation between scientists in different countries and with different expertise and this has often been the key to advances in understanding.

Embryology of the vertebrate limb

There is a wealth of information about embryology of vertebrate limbs (reviewed Saunders, 1977; Tickle and Eichele, 1994). The study of limb development in chick embryos has been particularly important because it is relatively easy to manipulate the developing limb through a window in the egg shell. Work by Saunders and colleagues in the USA was mainly responsible for uncovering the three main interactions that operate in the limb bud and are necessary for limb development; an interaction between the thickened ectodermal rim of the limb bud, the apical (ectodermal) ridge, and underlying mesenchyme which mediates limb bud outgrowth: an ectodermal-mesenchymal interaction that controls dorso-ventral (extensor/flexor) patterning and a mesenchymal-mesenchymal interaction which controls antero-posterior patterning (e.g. in human hand, thumb to little finger).

Positional information and limb development

The ideas of positional information, put forward by Wolpert (1969; see also Wolpert, 1996) working at The Middlesex Hospital Medical School in London, have been particularly influential in making models of limb development. According to these ideas, pattern formation is a two step process; in the first step, cells are informed of their position and acquire a positional value; in the second step, cells then interpret this information in terms of appropriate differentiation. It was proposed that cell position in the limb bud would be specified in relation to the three main axes, proximo-distal, antero-posterior and dorso-ventral, as in a Cartesian co-ordinate system (Fig. 1). An experiment by Saunders and colleagues (1959) can be used to illustrate the basic concepts. They grafted presumptive thigh mesoderm of a chick leg bud (mesoderm from the proximal region of the bud nearest the body) to the distal tip of a chick wing bud and found that the grafted leg cells form toes. Thus, as a result of transplantation, the leg cells acquire distal positional values but because they originate from the leg, they interpret these values to form toes rather than fingers.

Abbreviations used in this paper: BMP, Bone morphogenetic protein; Dpp, Decapentaplegic; FGF, Fibroblast growth factor; Shh, Sonic hedgehog.

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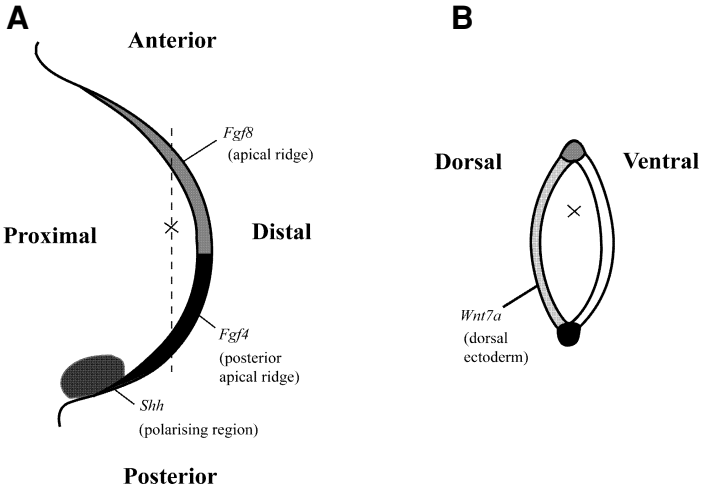

Fig. 1. Diagram to illustrate how the ideas of positional information have been applied in relation to a three-dimensional co-ordinate system to chick limb development and some of the signals that have been identified. (A) *Dorsal view of limb bud showing position of cell (X) with respect to proximo-distal and antero-posterior axes.* **(B)** *Section of limb bud taken along dotted line in (A) showing position of cell with respect to dorso-ventral axis. Sources of several known signals are stippled.*

Another important model around this time was the polar co-ordinate model which emphasised short range interactions rather than long range signalling between adjacent cells (French *et al.*, 1976). This model was also applied to limb development and stimulated much discussion (Iten and Murphy, 1980). The formation of the periodic pattern of skeletal elements in the vertebrate limb has also been modelled in terms of reaction-diffusion systems. These self-organising systems could generate a prepattern with concentration peaks of activators prefiguring the position in which the skeletal elements develop (see for example, Wilby and Ede, 1975; Wolpert and Stein, 1984).

The progress zone model and proximo-distal patterning

Signalling by the apical ectodermal ridge is required for bud outgrowth. Limb bud outgrowth is accompanied by the successive laying down of structures along the proximo-distal axis of the limb, starting with proximal structures such as humerus and ending with digits. But how do cells know which structure along this axis to form? One possibility is that the apical ridge signal changes with time. However, when apical ridges from limb buds at different stages were recombined with mesenchyme, in all cases the limbs developed normally (Rubin and Saunders, 1972). Another proposal was that a timing mechanism, which operates in the mesenchyme, controls proximo-distal pattern. According to this idea, the length of time that cells spend in the zone of undifferentiated mesenchyme, which is maintained by the apical ridge at the tip of the elongating bud, determines whether they will form proximal or distal structures. This region at the tip of the limb bud was called the progress zone (Summerbell *et al.*, 1973).

The idea that a mesenchymal timing mechanism might operate at the tip of the limb bud came from experiments in which tips of undifferentiated mesenchyme were exchanged between old and young limb buds (Summerbell *et al.*, 1973). The grafted tips behaved autonomously. This ruled out the possibility that structures already formed might dictate which structures formed next. In addition, if a graded signal from the apical ridge specified cell position along the proximo-distal axis, then one might have expected a normal limb pattern to be re-established.

Recent work shows that Fibroblast Growth Factors (FGFs) mediate apical ridge signalling. When the apical ridge is removed, truncated limbs are produced (Saunders, 1948; Summerbell, 1974). FGFs are expressed in the apical ridge and application of FGFs (FGF8, FGF4, FGF2) can rescue development of limb buds from which the apical ridge has been removed (Niswander *et al.*, 1993; Fallon *et al.*, 1994; Crossley *et al.*, 1996). The mechanisms of FGF signalling by the ridge and their relationship with progress zone function have not been characterised in any detail. It seems likely that FGF acts as a local signal and thus could not directly control the size of the progress zone. This suggests that secondary signals induced by FGFs secreted by the apical ridge are involved in maintaining the progress zone. It is also possible that mesenchymal cells measure time either directly or indirectly by the total amount of FGF to which they have been exposed.

Dorso-ventral patterning

Signalling by the ectoderm covering the sides of the limb controls patterning along the dorso-ventral axis (Patou and Kieny, 1973; MacCabe *et al.*, 1974). This was shown by experiments in which limb buds were separated into mesenchyme and ectoderm components and these different tissues from right and left wing buds were recombined so that, for example, dorsal ectoderm was placed over ventral mesoderm. The distal part of the limb that developed after such an operation had reversed dorso-ventral pattern, in accordance with the change in ectoderm polarity. This dorso-ventral pattern was judged mainly by reference to muscle and tendon pattern which differs dorsally and ventrally (in flexor and extensor regions respectively) and by reference to epidermal differentiation which is controlled by the underlying dermis. When an apical ridge was grafted either to the dorsal surface or to the ventral surface of a wing bud, new limb outgrowths were induced with either a double-dorsal or a double-ventral pattern respectively. The simplest model to account for the effects of ectoderm is that both dorsal and ventral ectoderm produce graded signals that pattern each half of the limb (reviewed Tickle, 1995). Cartilage differentiation may be confined to the core of the limb bud by general inhibitory signals produced by the ectoderm (Solursh, 1984).

Signalling by dorsal ectoderm is mediated, at least in part, by *Wnt7a* (Parr and McMahon, 1995). The signalling molecule encoded by this gene is a member of a family of vertebrate signalling molecules related to the product of the *Drosophila* gene, *wingless*. This family also comprises one of the *int* genes, genes at integration sites of the mouse mammary tumour virus which lead to tumour formation. *Wnt7a* transcripts are confined to dorsal ectoderm in vertebrate limb buds and when *Wnt7a* was functionally inactivated in mice, the paws of these animals were found to have a double-ventral pattern. Ventral ectoderm expresses *En-1*, a gene encoding a transcription factor related to the product of the *Drosophila* gene, *engrailed*. When *En-1* is functionally inactivated in mice, the paws of the mice now have a double-dorsal pattern (Loomis *et al.*, 1996).

It is not clear whether the product of *Wnt7a* itself can act as a positional signal to specify dorsal pattern or whether indeed that this type of long range signalling is needed. Cells that will form muscles and tendons originally lie close to the ectoderm and then take up more central positions later in development (Hurlé *et al.*, 1990; Murray and Wilson, 1997). This displacement of muscles and tendons could be related to the formation of the dermis.

The phenotypes of the knockouts, just outlined above, showed that when genes expressed either dorsally or ventrally were functionally inactivated, dorso-ventral patterning was symmetrical. This resembles the pattern in outgrowths of chick limbs covered on both sides with either dorsal ectoderm or with ventral ectoderm. Indeed in the mice in which *En-1* was functionally inactivated, *Wnt7a* was found to be expressed ventrally as well as dorsally. However, in contrast, *En-1* is still expressed only ventrally in *Wnt7a* mutant mice. Thus, it seems that a signal independent of *En-1* expression governs ventral patterning. Such a signal could be produced by all the ectoderm both dorsal and ventral in normal limb development and be over-riden by *Wnt7a* dorsally (Parr and McMahon, 1995). Another possibility is that a ventralising signal is produced at the dorso-ventral interface and generates a symmetrical gradient both dorsally and ventrally (Akita, 1996).

Positional signalling by the polarising region

Patterning across the antero-posterior axis provides the best example of positional signalling in the limb bud. Grafting experiments by Saunders first revealed the signalling activity of mesenchyme cells at the posterior margin of the chick limb (Saunders and Gasseling, 1968). When grafts of these cells were placed at the anterior margin of a second limb bud, mirror-image symmetrical patterns of digits resulted (Fig. 2A,B).

The rules that govern additional digit formation were explored in an extensive series of grafting experiments. These showed that the character of a digit depends on distance from the polarising region and a model in which the polarising region produces a diffusible morphogen was proposed (Tickle *et al.*, 1975). According to the model, a morphogen gradient would be established across the limb and cells at different distances from the polarising region would then be exposed to different concentrations of morphogen (Fig. 2C). Local morphogen concentration would then provide a measure of distance across the limb bud. The main features of this model are that the polarising region morphogen acts long range and in a dose dependent fashion. Low concentrations of morphogen would specify an anterior digit; high concentrations a posterior digit. Such dose-dependent effects on digit specification were seen when different numbers of polarising cells were grafted (Tickle, 1981).

Identification of limb morphogens

The first molecule that was discovered which could provide a positional signal to the developing limb was retinoic acid, a vitamin A derivative. Retinoic acid was applied to the chick limb because of its reported inhibitory effects on cell-cell communication (Pitts *et al.*, 1986). Completely unexpectedly it was found that when retinoic acid was applied to the anterior margin of a chick wing bud, this mimicked signalling of the polarising region (Tickle *et al.*, 1982). Retinoic acid fulfils two main criteria for a positional signal; it acts

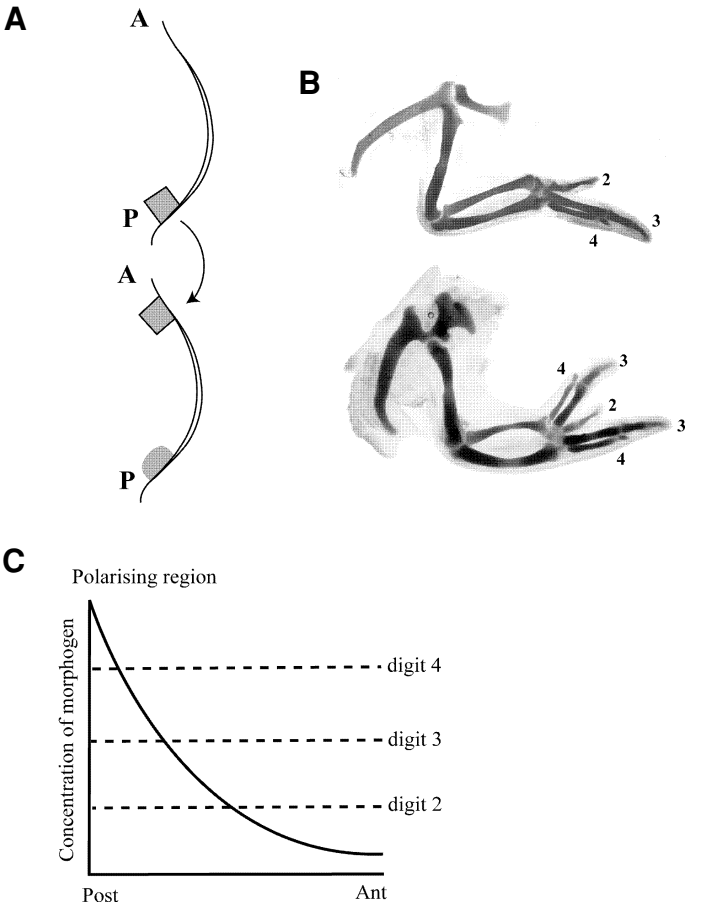

Fig. 2. Diagram to illustrate how the signalling properties of the polarising region were identified and a model to explain polarising region signalling. (A). *A cube of mesenchyme was cut out of the posterior margin of one chick wing bud and grafted to anterior margin of a second wing bud. A, anterior; P, posterior.* **(B)** *Normal wing skeleton (digit pattern 2 3 4) and, below, wing skeleton following operation in (A; digit pattern 4 3 2 3 4). Numbers refer to the digits.* **(C)** *Model for positional signalling by the polarising region. The proposal is that the polarising region secretes a diffusible morphogen that establishes a concentration gradient across the limb. Cells at different distances from the polarising region will be exposed to different morphogen concentrations. High morphogen concentrations will specify a digit 4 and lower concentrations a digit 2 .*

in a dose-dependent fashion and is readily diffusible in the limb (Tickle *et al.*, 1985). In addition, retinoic acid can be extracted from chick limb buds and has been shown to be enriched posteriorly where the polarising region is located (Thaller and Eichele, 1987). However, there is no evidence that cells at a distance from a source of retinoic acid respond directly to the local retinoic acid concentration. Moreover, when mesenchyme cells next to a retinoic acid source were implanted into a second wing bud, they were found to have acquired polarising activity and induced formation of additional digits (Noji *et al.,* 1991; Wanek *et al.,* 1991).

More recently, peptide signalling molecules have been found to be expressed in the polarising region. Of these, Sonic Hedgehog (Shh) a member of the vertebrate family of molecules related to the product of the *Drosophila*, *hedgehog* gene, is able to polarise the limb (Riddle *et al.*, 1993). When *sonic hedgehog* was expressed anteriorly, mirror image duplicated patterns of digits

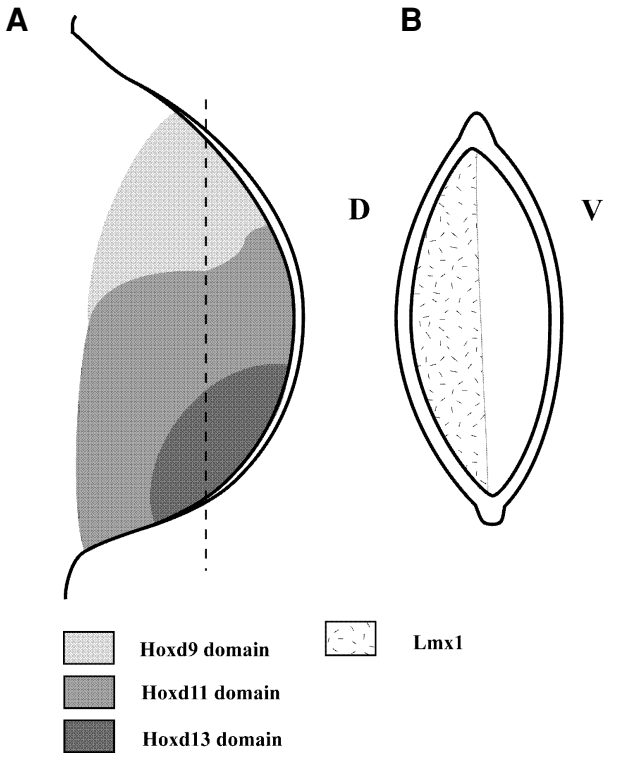

Fig. 3. Diagram to illustrate patterns of expression of genes encoding transcription factors; these patterns are established as a result of signalling in the chick limb bud. (A) *Overlapping patterns of Hoxd gene expression in early chick limb bud. Only three domains shown for clarity. Cells in postero-distal region of the bud express Hoxd9, Hoxd10, Hoxd11, Hoxd12 and Hoxd13 while cells in antero-distal region of bud express Hoxd9 only.* **(B)** *Section through chick limb bud showing expression of Lmx1 in dorsal mesenchyme. D, dorsal; V, ventral.*

resulted. Recent experiments suggest that Shh acts in a dose-dependent fashion and can exert long-range effects (Yang *et al.*, 1997). Different numbers of *sonic hedgehog* expressing cells or beads soaked in different concentrations of the amino-terminal peptide of Shh produced dose-dependent changes in digit pattern. Furthermore, DiI labelling experiments showed that cells at some distance from a bead soaked in Shh contributed to the additional digits. Although widespread diffusion of the amino-terminal peptide from the bead can be detected, it is not clear that Shh produced by polarising region cells and which has undergone cholesterol modification is freely diffusible. Thus, Shh itself may not directly signal digit formation at a distance.

Cells in the polarising region express genes that encode members of the Bone Morphogenetic Protein (BMP) family, for example, the *Bmp2* gene (Francis *et al.*, 1994). The *Bmp2* gene is a homologue of the *Drosophila* gene, *dpp*, and, in *Drosophila*, hedgehog signalling is mediated by dpp. Furthermore, when Shh was applied to the anterior margin of wing buds, *Bmp2* expression was activated in anterior mesenchyme (Laufer *et al.*, 1994; Yang *et al.*, 1997). Therefore, the idea that Bmps could mediate long range signalling by Shh in the vertebrate limb is attractive. However, when Bmps were applied to the anterior margin of a wing bud either by soaking beads in Bmp2 or by grafting cells expressing *Bmp2*, mirror-image duplicated patterns of digits were not obtained. At best, an additional digit *2* or a branched digit *3* has been obtained (Duprez *et al.*, 1996). In addition, when mesenchyme cells from next to a Shh bead are grafted to the anterior margin of a host limb bud, no additional digits were produced (Yang *et al.*, 1997). These results are not those that would be expected if Bmp signalling specifies additional digits.

It seems likely that polarising region signalling involves a cascade of interacting signals. When *Shh* is functionally inactivated, the mice lack distal limb structures but some proximal development occurs (Chiang *et al.*, 1996). Work with inhibitors of retinoid synthesis and with retinoid antagonists has shown that retinoic acid signalling is required for early limb bud initiation (Helms *et al.*, 1996; Stratford *et al.*, 1996). Thus it seems likely that retinoic acid patterns the proximal part of the limb and Shh, probably with Bmps, the distal parts. Another possibility is that there are parallel pathways involving retinoic acid and Shh.

Molecular responses to signalling in the limb

Genes have been identified that are expressed in a position-dependent fashion in developing limbs and respond to patterning signals (Fig. 3; Izpisúa-Belmonte *et al.*, 1991; Nohno *et al.*, 1991). Genes in the 5' region of both the *HoxA* and *HoxD* gene clusters are expressed in overlapping domains in early limb buds of vertebrate embryos (Dollé *et al.*, 1989; Yokouchi *et al.*, 1991). Thus, cells at different positions express different combinations of *Hox* genes. Dorsal mesenchyme expresses *Lmx-1*, a gene encoding a transcription factor that also contains a homeodomain (Riddle *et al.*, 1995; Vogel *et al.*, 1995). Experimental manipulations in chick limb buds have shown that the pattern of *Hox* gene expression can be regulated by cooperative signalling between the polarising region (retinoic acid, or Shh, or Bmp-2) and the apical ridge (FGF). Dorsal ectoderm signalling (*Wnt7a*) can regulate *Lmx1* expression.

According to the ideas of positional information, these position-dependent patterns of gene expression might encode positional values and the results of ectopically expressing these transcription factors are to some extent consistent with this idea. When *Lmx1* was ectopically expressed ventrally, this led to the local formation of dorsal structures (Riddle *et al.,* 1995; Vogel *et al.*, 1995) and similarly, when posterior *Hoxd* genes were ectopically expressed anteriorly (Morgan *et al.*, 1992), this appeared to lead to the formation of posterior structures. However, it is clear from the limb phenotypes of mice in which individual *Hox* genes have been functionally inactivated, that there are complex interactions between different *Hox* genes both within the same cluster and between clusters (reviewed Rijli and Chambon, 1997).

Signalling interactions in the limb are mutually regulated. Thus FGF signalling by the posterior apical ridge, not only plays a role in inducing *Hoxd* expression but also is necessary for maintenance of *Shh* expression in posterior mesenchyme (Vogel and Tickle, 1993; Laufer *et al.*, 1994; Niswander *et al.*, 1994). In turn, Shh signalling by posterior mesenchyme maintains *Fgf4* expression in the overlying part of the apical ridge. Dorsal ectoderm via *Wnt7a* signalling also helps to maintain *Shh* expression (Yang and Niswander, 1995). Thus signalling along all three axes is coordinated and each "segment" of the limb pattern as it is laid down is thus correctly patterned along both antero-posterior and dorso-ventral axes.

Chick limb mutants

Chicken limb mutants have arisen and been studied in the UK, USA and Japan. The potential interest of these mutants was recognised by experimental embryologists, who examined cell interactions in mutant limb buds to try and identify the defective tissue(s) (reviewed Wolpert, 1976). More recently, gene expression has also been analysed.

An important chicken mutant with respect to antero-posterior patterning is the *talpid* mutant and the *talpid*^{3} mutant has been studied in the UK (the US mutant is *talpid*^{2}). In *talpid* mutants, the limbs are polydactylous and up to 10 digits develop with uniform morphology (Hinchliffe and Ede, 1967). This uniformity of digit morphology in *talpid*^{3} has been shown to be associated with uniformity of *Hoxd* gene expression at the tips of the limb buds (Izpisúa-Belmonte *et al*., 1992). Grafting experiments with both *talpid*^{2} and *talpid*^{3} (the American and British *talpids* respectively) showed that polarising activity is more widespread but it is now known that *Shh* gene expression is posteriorly restricted. However, *Bmps* are expressed uniformly throughout the mesenchyme and *Fgf4* throughout the ridge (Francis-West *et al*., 1995). Recently, it has been shown that the defect in *talpid*^{3} is based on failure to express high levels of *Ptc* in response to Shh signalling (Lewis *et al*., 1999). *Ptc* is the gene that encodes the Shh receptor. It seems likely that this change in response of *talpid* ^{3} cells to hedgehog signalling could account for all the other developmental defects in the mutant embryos in addition to the limb defects.

Interpretation of positional information

The ways in which the expression of different *Hox* genes governs cell behaviour and controls tissue and cell arrangements are not known. There is only a limited repertoire of cell behaviour. Cells proliferate, die, change shape, move, adhere to other cells and/or extracellular matrix, and differentiate. These activities will have to be locally co-ordinated by, for example, gap junctional communication to produce specific local patterns of cells and tissues.

A major conceptual difficulty is how different positional values (such as those that lead to the development of each of the three different digits in a chick wing) lead to differentiation of the same cell type (cartilage). To some extent, this issue has been addressed by the idea of non-equivalence (Lewis and Wolpert, 1976). This suggests, for example, that other cellular properties such as proliferation may depend on positional value. Non-equivalence can also explain how the same positional values may lead to different outcomes depending on the history of the cells. A good example is the development of the chick wing versus the leg. The signalling regions in both wing and leg buds were found to be interchangeable and the molecules produced in the apical ridge, polarising region and ectoderm appear to be the same. Moreover, dorsal mesenchyme in both wing and leg expresses *Lmx1* (Vogel *et al*., 1995) and patterns of expression of most *Hox* genes are also similar in both wing buds and leg buds at early stages (Nelson *et al.,* 1996). Nevertheless, cells, for example, at the anterior distal edge of the leg bud form a “big toe” while those in the equivalent position in the wing form a “thumb”. Thus, the origin of the cells from different axial levels –either wing or leg levels– affects how they interpret the same positional values in the limb bud. Molecules that are responsible for these properties of “wingness” and “legness” have now been identified (see below).

Initiation of limb development

The development of four limbs is a hallmark of the tetrapod body plan. Thus issues about control of position, number and type of limbs in vertebrate embryos are fundamental (reviewed Cohn and Tickle, 1996). Fibroblast Growth Factors can induce ectopic limb development in chick embryos (Cohn *et al*., 1995) and specific family members have been identified that could act very early as endogenous limb initiation signals (Crossley *et al.,* 1996; Ohuchi *et al.,* 1997). *Fgf10* , for example, is expressed very early in presumptive limb-forming regions of chick embryos (Ohuchi *et al*., 1997) and functional inactivation of *Fgf10* in mouse embryos leads to failure of limb development (Min *et al*., 1998; Sekine *et al.,* 1999).

The position in which factors operate to initiate forelimbs and hindlimbs must be part of the patterning process that governs the head to tail axis of the embryo. A number of different lines of evidence, both from transgenic mice and experimental manipulation in chicken embryos implicates *Hox* gene expression in the lateral plate mesoderm as encoding position, i.e., one combination of *Hox* gene expression specifies presumptive wing level, another combination, the interlimb level, and yet another, the leg level (Cohn *et al*., 1997). These differences will be set up very early long before the limb buds appear.

Genes that are expressed specifically in wing lateral plate mesoderm and in leg lateral plate mesoderm were first discovered in mice. These are *Tbx* genes which encode transcription factors and are related to the large T mouse gene (Chapman *et al*., 1996). It was shown in chick embryos that *Tbx* gene expression is stable when wing cells are transplanted to leg and vice versa (Gibson-Brown *et al*., 1998; Isaac *et al*., 1998; Logan *et al*., 1998; Ohuchi *et al*., 1998). Furthermore, work in the US and in Japan showed that, when the *Tbx* gene normally expressed in the leg is now expressed in the wing, this leads to development of ectopic leg structures (Logan and Tabin, 1999; Rodriguez-Esteban *et al*., 1999; Takeuchi *et al*., 1999).

An important feature of limb bud initiation is the positioning of the signalling regions. The apical ridge in both wing and leg forming regions in chick embryos arises at a dorsal-ventral compartment boundary in the ectoderm (Altabef *et al*., 1997) and work by two groups in the USA has shown that *radical fringe* signalling is involved (Laufer *et al*., 1997; Rodriguez-Esteban *et al*., 1997). This dorsal-ventral ectodermal compartment has also been detected in the interlimb region and thus can account for the positioning of ectopic limb buds in register with the normal limb buds along the sides of the body.

There is good evidence from work in both mice and chicks that positioning of the polarising region in the forelimb /wing is associated with *Hoxb8* gene expression in lateral plate mesoderm (Charité *et al*., 1994; Lu *et al*., 1997; Stratford *et al*., 1997) . Rather surprisingly, the potential to form a polarising region is not reprogrammed by application of FGF to the interlimb region of a chick embryo. Thus, a polarising region expressing *Shh* was found to develop anteriorly in ectopic limb buds rather than posteriorly leading eventually to a reversed antero-posterior limb pattern (Cohn *et al*., 1995).

Chick limb development as a model for mammalian limb development

The patterning mechanisms involved in limb development are conserved between different vertebrates. Thus, for example, the posterior margin of a mouse limb bud has polarising activity and can induce additional digits in a chick wing (Tickle *et al.*, 1976) and the ectodermal jacket (including the apical ridge) from a rat limb bud can maintain development of chick wing mesenchyme (Jorquera and Pugin, 1971). Molecular analysis shows that *Shh* is expressed in the polarising region of both mouse and chick limb buds. Furthermore, the patterns of *Hox* gene expression at least in early buds are similar. This again shows the importance of interpretation of positional information, this time giving rise to the differences in morphology between vertebrates. Thus, grafts of mouse limb polarising region provide the same signal as grafts of the chick limb polarising region but when grafted to the chick wing lead to the formation of chick digits not mouse digits. A major challenge is to uncover the basis for the subtle differences in cell behaviour that lead to development of a chick "finger" rather than a mouse "finger".

There are over 100 different mutations known in mice that affect development of the limb. The ability to graft tissues from mouse limb buds into chicken wing buds and to monitor expression of genes that pattern the limb has given some interesting new insights into conditions, such as extra digits or loss of digits. Furthermore, the genes affected in such mouse mutants are rapidly being discovered. In many cases similarities to human conditions can be recognised (Winter, 1988). It has been shown, for example, that the mouse mutant, Hypodactyly, is due to a mutation in the *Hoxa13* gene. A mutation in the same gene has been discovered in a patient with hand-genital syndrome (reviewed Scott, 1997). Other direct links have been made between experimental embryology and human clinical genetics.

With modern molecular techniques, the genetic bases of many human congenital malformations are rapidly being uncovered. Many inherited conditions are very rare and therefore the world-wide availability of families to study is limited. This is an instance where work in different centres and even in different countries can be particularly valuable. In Britain, a notable example has been the discovery in two different centres of the involvement of FGF receptors in a series of craniosynostosis conditions, some associated with limb defects (Reardon *et al.*, 1994; Wilkie *et al.*, 1995). Another example is the identification of the involvement of a *Tbx* gene in Holt-Oram syndrome in a family in the UK and in a family in the USA (Basson *et al.*, 1997; Li *et al.*, 1997). These clinical discoveries provide a new dimension and stimulus to basic research on limb development.

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