The Turing mechanism in vertebrate limb patterning

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In the Review 'Making digit patterns in the vertebrate limb'¹, Cheryll Tickle discusses evidence that the long-standing concept of 'positional information' — which was formulated to account for differences in the appearance of distinct skeletal elements — is inadequate to explain the number and arrangement of such elements. As an alternative means for laying out the overall template for the limb skeleton, she briefly mentions an entirely different mechanism of pattern formation: the reaction–diffusion model, which was originally put forward by the mathematician A.M. Turing². As Tickle states, "The series of many unpatterned digits, which develop in the absence of GLI3 function in mouse mutants, is reminiscent of the digit pre-pattern that has been proposed to function in combination with the morphogen gradient³. According to this proposal, a series of digit condensations, a pre-pattern, is specified by a wave-like distribution of a morphogen that is generated by a reaction–diffusion mechanism, with the peaks corresponding to the condensations. A gradient of another morphogen ... then provides each peak with a positional value and a digit identity. The number of peaks that are generated by the reaction–diffusion mechanism depends on the width of the limb."

The proposal that the limb skeleton is generated by a reaction–diffusion mechanism, with other morphogen gradients having a fine-tuning role, was first made not in the 1989 review³ that she cites, but a decade previously, in conjunction with a specific model for the production of patterns of pre-cartilage condensation through the interaction of mesenchymal cells with morphogens and with the extracellular matrix (ECM)⁴. More importantly, Tickle failed to describe research in the ensuing three decades that has seriously considered, tested and elaborated on the role of reaction–diffusion patterning in limb development. This includes a series of papers by Takashi Miura and his colleagues that provided evidence for a reaction–diffusion mechanism

over a mechanochemical alternative in an *in vitro* model for pre-chondrogenic pattern formation⁵, and for the functionality of transforming growth factor- β 2 (TGF β 2) as the activator in a reaction–diffusion scheme⁶. Later, it was demonstrated that lateral inhibition of limb pre-cartilage condensation (a necessary component of the reaction–diffusion framework) was induced by ectodermally produced fibroblast growth factors (FGFs)⁷. This was followed by a reaction–diffusion-based mathematical analysis⁸ that accounted for the coordinate effects of FGFs in laterally inhibiting and accelerating the rate of appearance of foci of pre-cartilage condensation. Miura and co-workers also showed that the peculiar 'thick–thin' morphology of limb skeletal elements that is observed in doublefoot-mutant mice could be understood by assuming that a reaction–diffusion mechanism underlies skeletal patterning⁹.

In the original reaction–diffusion-based limb model, the hypothesis that the non-diffusible ECM protein fibronectin mediates pre-cartilage condensation as part of a regulatory circuit that involves diffusible morphogens⁴ has been confirmed in several experimental studies (reviewed in REF. 10). Recently, the functionality of fibronectin has been incorporated into a 'reactor–diffusion' model¹¹ in which authentic cell behaviours are taken into account. The reactor–diffusion approach has enabled the introduction of increasing levels of biological detail, mathematical rigour and computational sophistication into models for chondrogenic pattern formation *in vivo* and *in vitro*^{12–15}.

Far from being a sideline in studies of vertebrate limb development, experimentally motivated reaction–diffusion and reactor–diffusion models, along with mathematically related models based on biochemical–genetic oscillations, have gained prominence in many areas of developmental biology^{10,16}, including patterning of the hair follicles¹⁷, feather germs¹⁸ and teeth¹⁹ and, in the oscillatory mode, somites²⁰. Indeed, with regard

to the generation of patterns with repeating elements, this class of dynamic mechanisms has eclipsed models that are based primarily on positional information.

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