

RESEARCH HIGHLIGHT

Thick and thin fingers point out Turing waves

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Abstract

Mouse genetics and computer simulations demonstrate that digit number and width are controlled by a Turing-type mechanism in which distal *Hox* genes modulate periodicity.

It is a simple yet profound question: How do genes make your hand? The vertebrate limb has long been one of the most iconic systems in the study of development, so it is perhaps surprising that the genetic patterning processes giving rise to the digits are poorly understood. Indeed, while the digits might appear to constitute a periodic pattern, namely one where an element is repeated at regular intervals, it has been unclear whether this is truly the case. Some theories of anteroposterior patterning of the limb have been based on a positional information model in which a morphogen gradient from the posterior to anterior of the limb sets up a coordinate system that identifies the position of each digit [1]. Each digit could - theoretically - be uniquely specified by a different set of coordinates, not by periodic repetitions of the same process. However, in a recent *Science* article, Sheth *et al.* provide convincing evidence that the digits are a truly periodic pattern, generated by a so-called reaction-diffusion (RD) or Turing mechanism, and provide clues about how it works [2].

Turing proposed a simple mathematical model to show that periodic patterns could be generated by reaction between two or more morphogens (defined as diffusing chemicals that influence biological pattern) from an initially near-homogeneous state [3]. These patterns include oscillations, traveling waves and, importantly, stationary waves with uniformly spaced peaks - in other words, stripes or spots. Turing's ideas have been influential in understanding pattern formation, most notably as developed by Meinhardt and Gierer, who demonstrated that periodic patterning requires a short-range positive feedback and a long-range negative feedback [4].

An activator morphogen thus not only activates its own accumulation, but also produces an inhibitor that spreads in its vicinity. Multiple activator centers can arise spontaneously, but not adjacently, leading to a regular spacing.

A reaction-diffusion mechanism in the limb

A model of digit patterning based on positional information was favored by the discovery some years ago of a gradient of Sonic Hedgehog protein (SHH) from the posterior limb (the zone of polarizing activity) to the anterior. Adding extra or ectopic SHH triggers polydactyly (that is, extra digits). However, 10 years ago, a major problem for a simple positional information gradient model of digit patterning arose when it was found that digit formation persists in the absence of the proposed morphogen. Specifically, *Gli3* (a *Shh*-antagonized repressor of *Shh* target genes) and *Shh;Gli3* null mutants, in which there is no gradient, also display polydactyly [1]. Therefore, while the SHH gradient clearly contributes to anteroposterior patterning of the limb, it is not required for the generation of the digits.

Turing mechanisms had previously been proposed to explain the pattern of digits seen in the limb [5], but there was a problem with this too: Turing mechanisms have a fixed periodicity, whereas the digits spread out fan-wise by increasing their width and spacing wavelength as they extend distally (Figure 1). By using a combination of mouse genetics and a minimal RD model, Sheth *et al.* have overcome this problem by demonstrating the existence of a wavelength-modulating mechanism. They have thus simultaneously validated the relevance of a Turing model and revealed some of the genes that make it work in the limb.

The study set out to examine a set of genes known to affect the patterning of digits, the distal *Hox* genes. A double deletion of *Gli3* and an allelic deletion series of the distal *Hox* gene *Hoxa13* were generated, with the perhaps surprising finding that a reduced *Hox* contribution results in an increased number of digits. Crucially, the increase in digit number occurred by decreasing the period of the digit pattern, not by expanding the hand. Even more emphatically, they went on to generate a triple deletion series for *Gli3*, *Hoxa13* and *Hoxd11-13* showing the same trend, with the triple knockout showing a spectacular array of up to 14 narrow, tightly spaced digits.

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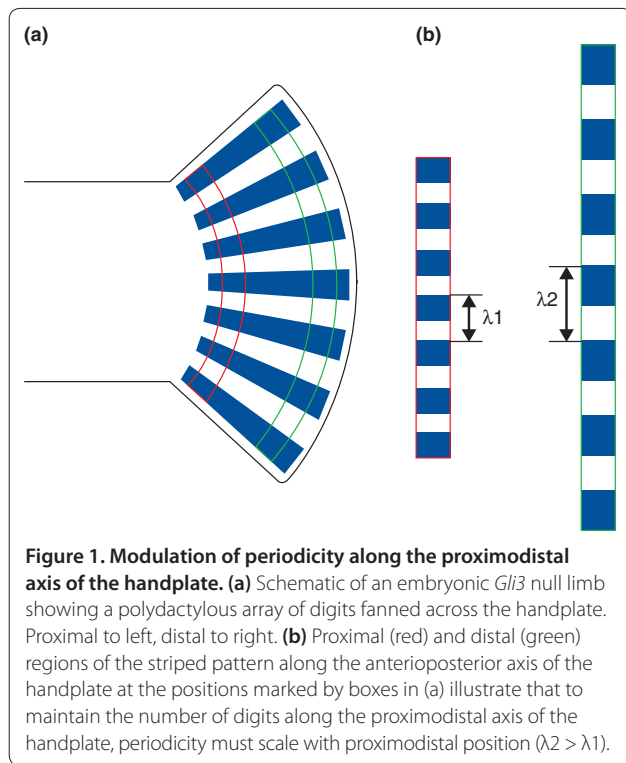


Figure 1. Modulation of periodicity along the proximodistal axis of the handplate. (a) Schematic of an embryonic *Gli3* null limb showing a polydactylous array of digits fanned across the handplate. Proximal to left, distal to right. (b) Proximal (red) and distal (green) regions of the striped pattern along the anteroposterior axis of the handplate at the positions marked by boxes in (a) illustrate that to maintain the number of digits along the proximodistal axis of the handplate, periodicity must scale with proximodistal position ($\lambda_2 > \lambda_1$).

In wild-type and *Gli3* nulls, digit thickness and spacing increases smoothly with proximodistal (PD) position to maintain the fanning out arrangement of the pattern. The authors showed that reducing the *Hox* gene dosage, by contrast, led to a leveling off of the period along the PD axis: the wavelength ceased to increase such that the digit-stripes bifurcated (that is, split in two) rather than broadened. This point was clearly illustrated by their simulations: when the period was scaled along the PD axis, digits did not bifurcate, but when periodicity was uniform throughout, bifurcations were seen.

The authors argue that their findings have significance for limb evolution, pointing to the similarity between their highly polydactylous phenotypes and the many skeletal elements in the fins of sharks and basal ray-finned fish [6]. They speculate that the evolution of the pentadactyl limb of amniotes could be the result of the modulation of the *Hox* system.

Reaction-diffusion mechanisms in experimental systems

Sheth *et al.* tantalizingly leave the identity of the key molecular players unknown. Most importantly, they fail to identify the core activator and inhibitor of the RD system. This is in contrast to the periodic patterning of hair follicles, feather buds, feather barb ridges, the rugae (ridges) in the hard palate and zebrafish stripes, which are all examples of systems with identified key activator-inhibitor pairs [7-9].

Sheth *et al.* also admit that as the distal *Hox* genes do not show any graded PD expression, an unknown factor with a PD-graded activity must be an additional component of this patterning system. The authors suggest fibroblast growth factor (FGF) as a candidate for this role, and FGF does indeed have a suitable PD gradient [1]. Unfortunately, FGF is also necessary for limb growth, which makes it the ideal mediator of PD scaling, but also makes it harder to test as a digit spacing modulator.

The paper by Sheth *et al.* is part of a resurgence of interest in Turing RD-type patterning mechanisms. RD models fell into some disrepute in the 1970s and 1980s, partly because there was little molecular validation of them (despite their impressive ability to produce convincing simulations of real patterns) and partly because positional information theories seemed to contradict them: whereas RD couples the morphogen distribution to the eventual pattern, positional information explicitly uncouples them, allowing smooth morphogen gradients to generate any pattern you want. But we are now beginning to reconcile these ideas: in the limb, Sheth *et al.* show that RD establishes stripes, while longer-range morphogen gradients modulate the wavelength proximodistally and impose digit identity differences anteroposteriorly. In fact, RD and positional information mechanisms are probably generally interdependent in another way. Setting up a reliable morphogen gradient in the first place requires some type of long-range feedback inhibition, for which an RD system is well suited, while stripes and spots merely represent peaks in morphogen gradients such that each can generate its own local positional information. For example, Müller *et al.* [10] demonstrated that the developmental regulators Nodal and Lefty act as a differentially diffusing activator-inhibitor pair in the patterning of left-right asymmetry. Similar activator-inhibitor dynamics have been implicated in dorsoventral patterning [4]; the period in these cases is a single wavelength corresponding to the entire axis.

2012 was the centenary year of Turing's birth, and it is fitting that it was also the year that saw real integrations of RD theory into molecular genetics. The linking of genes to how the hand is made is still highly incomplete, but it is perhaps now within reach.

Abbreviations

FGF, fibroblast growth factor; PD, proximodistal; RD, reaction-diffusion; SHH, Sonic Hedgehog.

Competing interests

The authors declare that they have no competing interests.

Acknowledgements

This work was supported by a grant from the Medical Research Council.

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doi:10.1186/gb-2013-14-1-101

Cite this article as: Economou AD, Green JBA: **Thick and thin fingers point out Turing waves.** *Genome Biology* 2013, **14**:101.