matoid arthritis. Should these drugs also prove effective in the treatment of cherubism it can be hoped that the interval from laboratory discovery to clinical use would be short.

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Hairy Math: Insights into Hair-Follicle Spacing and Orientation

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DOI 10.1016/j.cell.2006.12.020

Hair follicles in the skin have a characteristic spacing and orientation. Two recent papers (Sick et al., 2006; Wang et al., 2006) report the use of contrasting mathematical models and experimental manipulations to gain insight into the mechanisms underlying patterns of hair-follicle distribution and orientation.

Mathematical and computational models can play an important role in integrating the wealth of information generated by molecular biology and genetics. They are particularly useful for understanding how phenomena at the tissue or organismal level arise from networks of molecular interactions within and between cells. Ideally, such models should offer biological insight into complex phenomena and generate new testable hypotheses. The authors of two recent papers (Sick et al., 2006; Wang et al., 2006) appearing in *Science* and in the *Proceedings of the National Academy of Sciences* use contrasting approaches to model observed patterns of hair-follicle distribution and orientation. In one case the model is based on known and predicted properties of key signaling molecules, whereas the other model is more abstract and does not depend on specific biological parameters.

Hair follicles are found in regular arrays in which large primary follicles that develop first are interspersed with smaller, secondary follicles that develop at a later stage. Both primary and secondary follicles are uniformly oriented in an anterior-posterior direction (Figure 1). Turing first proposed a reaction-diffusion (R-D) model that explained how amplification of small initial fluctuations of an activator and inhibitor that interact could lead to development of a spatial pattern of morphogens (Turing, 1952). More recently, Jung et al. showed in skin explant cultures from chick embryos that the size, number, and distribution of developing feather buds could be altered by addition of specific morphogens (Jung et al., 1998). Insight into the molecular signals that direct initial patterning of the hair-follicle array has been provided by experiments showing that follicle induction is blocked by ectopic expression of Dickkopf1 (Dkk1), a secreted Wnt/β-catenin inhibitor (Andl et al., 2002), and is stimulated by constitutive activation of the Wnt/β-catenin pathway (Gat et al., 1998; Kuraguchi et al., 2006). These observations identify Wnt/β-catenin signaling as a key initiating

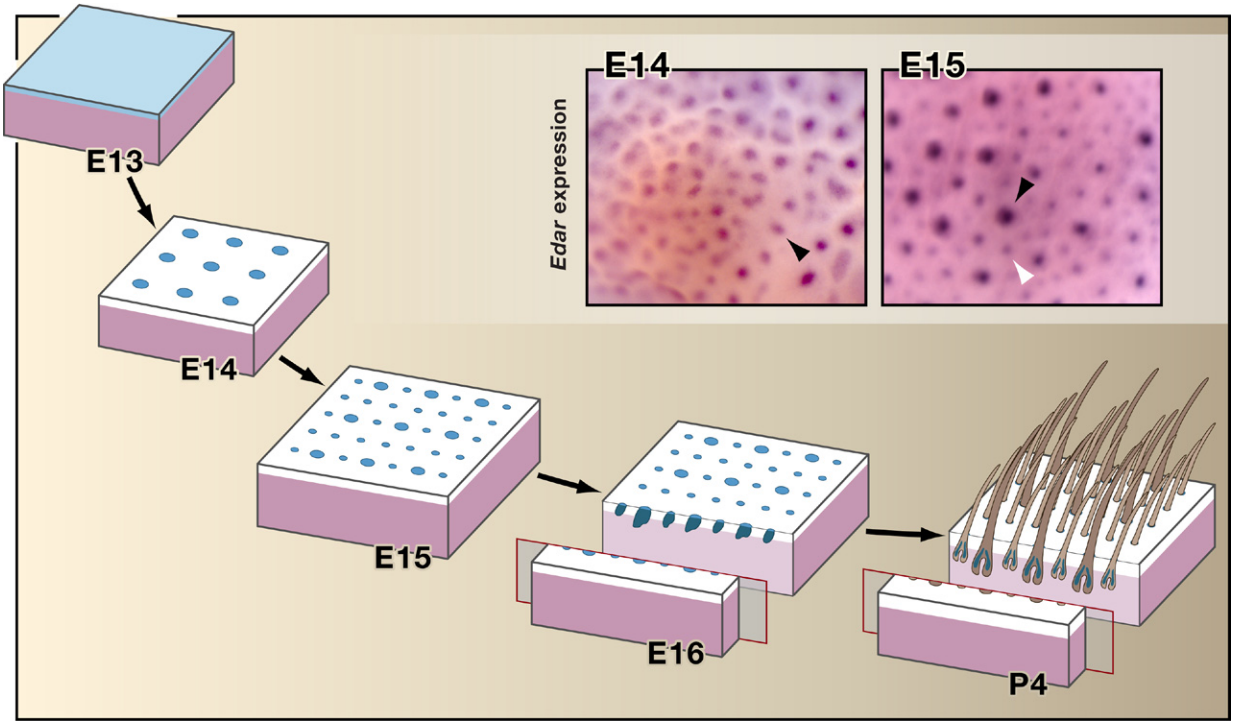

Figure 1. Stages of Hair-Follicle Development

Whole-mount in situ hybridization with digoxygenin-labeled probe for the hair-follicle placode marker *Edar* and schematic depiction of key stages of hair-follicle development. *Edar* expression reveals initiation of primary hair-follicle placode development (black arrowheads) at mouse embryonic day (E) 14 and formation of secondary hair-follicle placodes (white arrowhead) interspersed between the primary placodes at E15. In the schematic depiction, Wnt activation (blue) is initially uniform in the epithelium but is subsequently upregulated at sites adopting a hair-follicle fate and downregulated in interfollicular regions. Smaller spots of Wnt activation, corresponding to secondary hair-follicle placodes, appear at points furthest distant from the primary placode spots. Placode cells subsequently invade the underlying dermis (pink) and the developing hair bulb structures begin to become oriented with respect to the anterior-posterior axis of the embryo, eventually resulting in the growth of hair shafts that are oriented posteriorly. In this diagram, anterior is to the left and posterior to the right.

factor in hair-follicle development but do not explain how Wnt/β-catenin activity becomes localized at particular positions within the skin.

Sick et al. demonstrate that the Wnt/β-catenin antagonist *Dkk4* is expressed early in hair-follicle placode formation and suggest that an R-D mechanism involving WNT-DKK interactions patterns hair-follicle fate assignment (Sick et al., 2006). They show that *Dkk4* expression can be regulated by Wnt/β-catenin signaling. They further assume that Wnt controls its own expression, and that WNT proteins are both more stable and less diffusible than DKK, properties that have yet to be demonstrated definitively. Using these parameters the authors construct R-D equations to simulate the first wave of hair-follicle development. To model a second wave of follicle formation, they fix the spots obtained from the first simulation and assume that these produce both activator and inhibitor at constant rates. This model produces an array of large primary spots interspersed with smaller secondary spots, similar to the pattern of hair follicles observed in vivo. It predicts that moderate overexpression of activator will increase follicular density and that moderate overexpression of inhibitor during the initial inductive phase will increase follicle spacing. During the secondary wave of induction, excess inhibitor can impede formation of secondary follicles and also causes formation of rings of high activator levels around pre-existing follicles, leading, under some conditions, to development of clusters of secondary follicles around primary follicles.

The authors test these predictions using transgenic mice in which *Dkk* expression is controlled by a *Foxn1* promoter, which is activated following follicle initiation. Mice expressing high levels of transgenic *Dkk2* develop a limited number of primary hair follicles due to delayed activity of the *Foxn1* promoter relative to primary hair-follicle induction, but secondary hair follicles are suppressed. Consistent with the authors' mathematical model, rings of Wnt/β-catenin signaling activity are observed around developing follicles in *Foxn1::Dkk2* transgenic mice expressing moderate levels of *Dkk2*, and clusters of follicles, separated by skin lacking follicles, form in place of the normal pattern.

These data provide the first molecular evidence that an R-D mechanism involving WNT and DKK underlies hair-follicle patterning. As with any important advance, these results raise a host of new questions. First, ectopic hair-follicle development has

been observed to date only following constitutive Wnt pathway activation downstream of the receptor (Gat et al., 1998; Kuraguchi et al., 2006). It will be important to test the authors' model further by determining whether expression of excess secreted WNT ligand can produce the predicted effects on hair-follicle formation. Second, DKK2 inhibits Wnt/β-catenin signaling activity in the presence of the DKK receptor Kremen2 but activates Wnt/β-catenin in the absence of Kremen2 (Mao and Niehrs, 2003). As Kremen2 is induced in developing appendages, it is conceivable that DKK2 could inhibit Wnt activity within hair follicles but activate signaling in the epidermis immediately surrounding the follicle, leading to the observed rings of Wnt activity in *Foxn1:Dkk2* transgenics. Despite these caveats, R-D remains a compelling model for explaining follicle patterning. Analysis of the effects on patterning of single and combined loss-of-function mutations in *Dkk* genes, in particular *Dkk4*, will provide important further clues as to whether WNT-DKK interactions lie at the heart of this process.

Once the hair-follicle fate pattern is established, hair-follicle precursor cells invade the underlying dermis in a controlled fashion and gradually differentiate to produce a hair shaft and its surrounding root sheaths. The follicles become oriented within the plane of the skin, such that hair shafts point posteriorly (Figure 1). Molecular genetic dissection of planar cell polarity (PCP) has identified a set of core proteins that become specifically localized to either the proximal or distal cell membrane (Barrow, 2006). In the *Drosophila* wing, the Wnt receptor Fz acts cell autonomously to recruit Dishvelled (Dsh) to the cell membrane and non-cell autonomously to influence recruitment of the transmembrane protein Van-gogh/Strabismus (Vang) and the cytoplasmic regulator Prickle (Pk) at the adjacent membrane of a neighboring cell. Vang and Pk autonomously prevent Fz recruitment of Dsh. Thus subtle directional biases in any of these core proteins, presumably occurring in response to global signaling cues, are sufficient to set up polarity that can be transferred from cell to cell (Barrow, 2006). The nature of the global signal is less clear and may be context dependent. PCP signaling is distinct from the Wnt/β-catenin pathway, although it shares some molecular components such as Fz and Dsh.

A first indication that PCP signaling governs the polarity of mammalian hair follicles came from observations by Jeremy Nathans and coworkers of aberrant hair patterns, including whorls and waves, in the coats of *Fz6* null mice. These are strikingly similar to the patterns seen in *Fz* mutant *Drosophila* wings. Wang et al. now show that wild-type hair follicles are initially oriented roughly with respect to the A-P axis, followed by a gradual narrowing in the distribution of follicle angles. In contrast, *Fz6* null follicles are initially oriented randomly but progressively form larger spatial domains of correlated orientation, resulting in the observed mutant patterns in which hundreds of adjacent follicles within a field are oriented nonrandomly with respect to each other, but adjacent follicle fields have different overall orientations (Wang et al., 2006). This suggests that hair-follicle polarity is established in two phases: an early, *Fz6*-dependent phase that roughly orients follicles with respect to the A-P axis in response to a presumed global signal, and a later-acting *Fz6*-independent phase in which local signaling between follicles orients them with respect to their neighbors. Analyses of wild-type:*Fz6* null chimeras and wounding experiments show that the local signals can act over distances of a few follicle diameters to re-orient neighboring follicles.

Prior mathematical models for PCP (e.g., Amonlirdviman et al., 2005) have relied on complex sets of equations that make multiple assumptions about the properties of individual molecular components of the PCP pathway. The authors of the current paper note that PCP displays similarity to the patterning of electron spins in a ferromagnet, in which local interactions favor the alignment of adjacent electron spins. A global external magnetic field can also bias the alignment of individual spins. Wang et al. adapt a generalized Ising model of ferromagnetism to understand hair-follicle orientation. Hair follicles are assumed to lie in a regular lattice, and the orientation of each follicle is repeatedly updated by adding a scaled average of the orientations of neighboring follicles. Patterns similar to those seen in *Fz6* mutants are observed when the initial orientation is random. Application of a relatively weak bias in initial orientation (as seen in wild-type skin) leads to uniform orientation. Finally, a region in which vectors are initially oriented uniformly can over time influence an adjacent region that has vectors initially oriented randomly, consistent with observations of hair-follicle orientations in *Fz6* null:wild-type chimeras.

Thus a simple model that does not require detailed biological information is able to predict how small biases in initial orientation can generate a field of uniformly oriented follicles. A drawback is that it cannot make specific predictions about the properties of the contributing molecular components that could aid in their identification. Outstanding questions that need to be answered at the biological level include the nature of the global signal, or signaling center, that is sensed by *Fz6* and generates the initial rough A-P bias in follicle orientation. It will also be important to test the hypothesis that core components of the planar cell polarity pathway in addition to *Fz6* are involved in generating hair-follicle polarity.

These two thought-provoking papers illustrate how relatively simple mathematical techniques can reveal underlying principles governing the complex signaling pathways and cellular behaviors that generate biological patterns. Importantly, such models provide useful information and predictions and can be used to generate testable hypotheses, even when the details of the biology (such as protein half-lives or diffusion constants) are unknown, or known only imprecisely.

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Polo Delivers a PICH to the Kinetochore

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DOI 10.1016/j.cell.2006.12.022

In this issue, Baumann et al. (2007) identify a helicase PICH that localizes to "threads" that remain connected between sister kinetochores after they have separated in anaphase. These threads are thought to be catenated centromeric DNA. PICH contributes to the mitotic checkpoint by recruiting Mad2 to kinetochores and is proposed to regulate checkpoint signaling by monitoring tension at centromeres.

Accurate chromosome segregation depends on the proper attachment of chromosomes to spindle microtubules. Kinetochores—proteinaceous structures that are associated with centromeric heterochromatin—are assembled on both sister chromatids to allow chromosomes to be captured by microtubules emanating from the two opposite spindle poles. The stochastic nature by which highly dynamic spindle microtubules attach to chromosomes requires that the centromere-kinetochore complex be able to capture microtubules and discern the quality of the kinetochore-microtubule interaction. Once kinetochores establish bipolar connections, the polymerization and depolymerization of the attached microtubules produces a series of oscillatory motions that result in their alignment at the spindle equator. Coordinating microtubule dynamics at sister kinetochores requires that each knows what the other is doing despite being physically separated by what is a vast stretch (1 μm) of centromeric chromatin. Presently, approximately 100 different proteins of the centromere-kinetochore complex have been identified, and many are now known to be directly involved in microtubule attachment and in quality control of these attachments (Chan et al. 2005).

Plk1 (Polo) is the founding member of an evolutionarily conserved family of mitotic kinases that participates in centrosome and spindle functions, regulation of chromatid cohesion, kinetochore functions, and mitotic exit (Barr et al. 2004). Plk1 contains a phospho binding motif, called the Polo-box domain (PBD), that recruits it to proteins that are phosphorylated at a specific consensus site (Elia et al. 2003). To identify proteins that associate with the PBD of Plk1 in mitosis, Baumann and colleagues (2007) used this domain to probe for proteins that associate with Plk1. This led to the identification of PICH (Plk1 interacting checkpoint helicase), which was subsequently found to localize to kinetochores. Immunofluorescence staining revealed that in prometaphase cells, PICH was mostly concentrated in between kinetochores. In metaphase cells, PICH was localized to numerous short threads that stretched between sister kinetochores of the aligned chromosomes. This pattern at first glance was reminiscent of the chromosome passenger complex (Vagnarelli and Earnshaw 2004), which also undergoes a dramatic relocalization from the inner centromere to the microtubules within the spindle midzone at the onset of anaphase. The surprising difference was that the PICH threads do not appear to be microtubules and seem to connect sister kinetochores well after the chromatids have separated. Although the number of threads progressively decreased as anaphase ensued, the length of the threads increased as the chromatids moved farther apart from each other.