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Dendritic Tiling: New Insights from Genetics

Two papers in the current issues of *Neuron* (Gallegos and Bargmann) and *Cell* (Emoto et al.) identify a conserved kinase, SAX-1/Trc, and a large protein required for Trc activity, SAX-2/Fry, as essential elements in the control of dendritic branching and tiling in *Drosophila* and *C. elegans*. The tiling and ectopic branching phenotypes of *trc* mutants appear to be independently generated. Thus, this kinase is the first signaling protein to be associated specifically with tiling.

Dendritic tiling is a phenomenon in which the dendrites of a group of neurons with the same response characteristics completely innervate a tissue in a nonredundant manner. In the mammalian retina, the dendritic arbors of retinal ganglion cells (RGCs) of a particular subtype display little overlap. RGCs of different subtypes, however, have extensively overlapping arbors. The retina is separately tiled by several different classes of RGCs (Lin et al., 2004, and references therein).

For somatosensory neurons with sensory endings in the epidermis, tiling between neurons ensures that every location on the skin is innervated by a single cell of each sensory modality. Tiling within a neuron's arbor ensures that the receptive field of that neuron is innervated with an even density of sensory endings. Two papers in the current issues of *Cell* and *Neuron* define a conserved mechanism for the control of tiling and dendritic branching by sensory neurons in *Drosophila melanogaster* and *Caenorhabditis elegans* (Emoto et al., 2004; Gallegos and Bargmann, 2004). In both flies and worms, loss of a serine/threonine kinase, SAX-1/Tricornered (Trc), or of a large protein required for Trc kinase activity, SAX-2/Furry (Fry), causes sensory neurons to produce excessive numbers of dendritic branches. The absence of the Trc/Fry signaling system also produces a failure of tiling: in mutant *Drosophila* larvae, dendritic branches cross over other branches of the same neuron and invade territories occupied by branches of other neurons of the same class.

Class-specific tiling has been demonstrated for the two most complex subtypes of peripheral dendritic arborization (da) neurons in the *Drosophila* larval epidermis. The dendritic arbors of three class IV da neurons and five class III neurons separately cover the epidermal sheet within each half-segment. Class IV dendritic branches in fly larvae are very dynamic. They must grow rapidly between the first and third instar stages in order to innervate the entire epidermis as it expands. During arbor growth, dendritic branches of an individual neuron closely approach one another but avoid contact or crossing. This isoneuronal tiling ensures that the neuron's dendritic field is evenly covered by branches. Each neuron's dendritic branches also avoid the territories occupied by the branches of another class IV neuron. Heteroneuronal tiling produces an innervation pattern in which each spot on the epidermis is covered by branches from only one class IV neuron.

Both isoneuronal and heteroneuronal tiling of class IV neurons can be explained by a like-versus-like repulsion mechanism in which dendritic branches from neurons of the same class avoid contact with each other and turn away when they approach too closely. Class IV dendrites are not repelled by dendrites of class III neurons, however, and class III and class IV arbors exhibit extensive overlap (Figure 1). Ablation and duplication experiments have provided evidence for this repulsion model. Removal of a class IV neuron can cause expansion of the territory covered by an adjacent class IV cell's dendrites, and the arbors of duplicated class IV neurons occupy distinct and smaller territories (Grueber et al., 2002, 2003; Sugimura et al., 2003). In the mouse retina, however, genetic ablation of RGCs does not cause expansion of the dendritic fields of the remaining neurons of the same subtype, so a different mechanism might be responsible for tiling in this system (Lin et al., 2004).

trc and *fry* mutants have phenotypes in which single bristles and wing hairs split into branched structures, and genetic analysis demonstrates that Trc and Fry are likely to function within the same signaling pathway. Trc is a conserved kinase of the ACG family, and Fry is a large protein with HEAT/Armadillo repeats (Geng et al., 2000; Cong et al., 2001; Gallegos and Bargmann, 2004). The Emoto et al. paper now shows that Trc and Fry function cell-autonomously in da neurons. *trc* and *fry* mutations affect splitting of dendritic branches, and in mutant larvae, the number of class IV branches is increased by a factor of two. More interestingly, however, these dendritic branches fail to exhibit isoneuronal or heteroneuronal tiling. Within a neuron, dendritic branches freely cross one another, resulting in a tangled dendritic tree. They are also not repelled by branches of other class IV neurons, so the arbors of the different class IV neurons extensively overlap in these mutants (Figure 1).

The tiling and branching phenotypes produced by the *trc* and *fry* mutations appear to be independent of one another. Tiling phenotypes are observed in hypomorphic *fry* mutants that do not have increased branching, and dominant-negative Rac suppresses branching defects without affecting tiling.

In *C. elegans*, neurons in *sax-1* and *sax-2* mutants were previously shown to have ectopic dendritic branches (Zallen et al., 2000). The Gallegos and Bargmann paper now shows that these mutants also display phenotypes in which the dendrite of the PLM posterior mechanosensory neuron overlaps with that of its anterior counterpart ALM. This overlap is reminiscent of the tiling defects described above in *Drosophila* and may have similar consequences for the animal's behavior, but it is likely to arise by quite different mechanisms. In wild-type worms, there is a transient overlap between the ALM and PLM dendrites at an early stage in development. This overlap is resolved during a later phase in which the PLM dendrite grows more slowly than the body as a whole. The

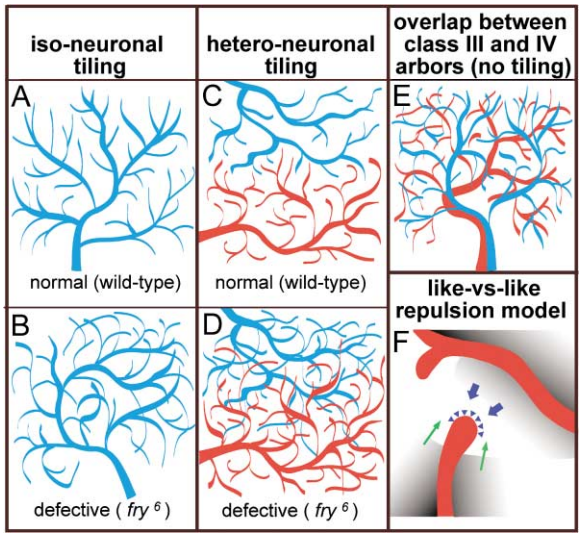

Figure 1. Dendritic tiling in *Drosophila*

(A and B) Isoneuronal tiling. (A) A simplified diagram of a portion of the dendritic arbor of a class IV neuron in a wild-type third instar larva. Note that the dendritic branches cover the entire field but do not cross each other. (B) A class IV neuron in a hypomorphic *fry* mutant, *fry*^{6}, where tiling but not branching is altered. Note the crossovers between dendritic branches, resulting in a tangled arbor. (C and D) Heteroneuronal tiling. (C) Dendritic arbors of two adjacent class IV neurons (red and blue) in wild-type. Note that the red and blue branches do not cross each other. (D) Two class IV neurons in a hypomorphic *fry* mutant. Note the crossovers between the red and blue branches. The neurons do not respect each other's territories. (E) Overlap between the dendritic arbors of a class III and a class IV neuron (red and blue) innervating the same area of the epidermis. (A)–(D) are based on drawings in Figures 1 and 4 of Emoto et al. (2004). (E) is based on a drawing in Figure 6 of Grueber et al. (2002). (F) A model for like-versus-like repulsion in which dendritic branches (red) have a receptor for a repulsive factor (black triangles) and also secrete that factor (gradient), which diffuses away from the branches. In this model, the factor is released only from the region behind the growing tip, so that the receptors on the tip transduce a repulsive signal (green thin arrows) that causes the tip to move forward, away from the factor released by its own branch. The tip receptors also receive a repulsive signal from the gradient of factor emanating from another branch (blue thick arrows), and this will cause the tip to turn away from the other branch. In the example shown here, the branch would presumably turn left, away from the factor gradient emanating from the branch in front of it. Figure drawn by Violana Nesterova.

pause in PLM dendritic growth is not caused primarily by like-versus-like repulsion, however, because it is not eliminated by ablation of ALM.

In *sax-1* and *sax-2* mutants, the pause in PLM dendritic growth does not occur, and as a consequence, the PLM dendrite extends past its normal termination point (Gallegos and Bargmann, 2004). This continued dendritic growth may be more closely related to the excess branching phenotypes seen in both worms and flies than to the distinct tiling defects observed for fly da neurons. In *Drosophila*, tiling and branching phenotypes can be separated by examining their suppression by dominant-negative Rac (Emoto et al., 2004). Perhaps Rac could also be used as a tool to examine the relationships between the dendritic overlap and ectopic branching phenotypes in *C. elegans*.

Mutations in the *Neurospora cot-1* gene, which encodes an ortholog of Trc, cause excessive hyphal branching (Yarden et al., 1992). This suggests that the role of Trc in inhibiting hair and bristle splitting and dendritic branch formation may reflect an ancestral function for this kinase in formation of branched cellular structures. Trc activity might affect the actin and/or microtubule cytoskeleton so as to change the probability of branching (He and Adler, 2001). In *Neurospora*, Cot-1 appears to negatively regulate protein kinase A (PKA) activity, because inhibition of PKA suppresses the *cot-1* hyperbranching phenotype (Gorovits and Yarden, 2003). In *Drosophila*, Fry is necessary for Trc kinase activity, and Trc associates with Rac and negatively regulates accumulation of activated Rac-GTP (Emoto et al., 2004). These results suggest that cytoskeletal alterations that facilitate dendritic branching are favored when Trc activity is low and Rac-GTP levels are high.

The signaling pathways involved in control of tiling by Trc activity are unknown. The phenomenon of like-versus-like repulsion might be explained by a model in which every tiling class IV neuron expresses both a soluble repulsive factor and the receptor for that same factor. Trc and Fry would be required for signaling downstream of this receptor.

The model could be generalized to situations where there are several classes of neurons that independently tile a receptive sheet. In such cases, each class of tiling neuron would express a different factor/receptor pair, so that their dendrites would repel others of the same class but overlap with those of other classes. Trc and Fry could be downstream of each of these receptors and might thus be required for tiling by all the classes of neurons.

In this model, the putative factor would be released from dendritic branches, spread by diffusion, and sensed by receptors on other branches of the same neuron or branches of another class IV neuron. Growing branches would be traveling up a gradient of the factor as they approached branches from which it is being released and would turn away when they received a high enough level of the repulsive signal. The model suffers from the difficulty that the concentration of repulsive factor should be highest in the immediate vicinity of the branch that is releasing it, so that this branch's outgrowth should be inhibited more than that of other branches in its vicinity. However, if the receptor is located at growing branch tips and the repulsive factor is released from the regions behind the tips, each branch tip would grow away from its own branch's source of factor, but would be stopped by factor emanating from other branches (Figure 1). The actual mechanisms by which tiling is controlled in this system are likely to emerge from identification of other mutations that produce tiling phenotypes or by biochemical definition of the signaling pathways upstream of Trc and Fry.

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Cortical Development deNUDEd

The development of the cerebral cortex is a highly orchestrated process of cell division and migration. In this issue of *Neuron*, Feng and Walsh and Shu et al. examine the roles of two related proteins, Nde1 (mNudE) and Ndel1 (NUDEL), in cortical development. These proteins play a crucial role in centrosome positioning, with Nde1 functioning mainly during progenitor cell divisions and Ndel1 functioning in neuronal migration.

The cerebral cortex arises from a complex interplay of cell division, differentiation, and migration during embryogenesis. An error in one of these processes during development will impact brain function throughout the life of the organism. This is particularly evident in humans when cortical malformations cause mental retardation and epilepsy. An example of one such disorder is lissencephaly, where the normally convoluted cerebral cortex is smooth due to defects in neuronal cell migration (Gupta et al., 2002; Olson and Walsh, 2002). *LIS1*, which is mutated in an autosomal form of lissencephaly, is part of an evolutionarily conserved network of interacting proteins that regulates the function of the microtubule motor cytoplasmic dynein. In the filamentous fungus *A. nidulans*, the ortholog of *LIS1* is *nudF* (for *nu*clear *d*istribution*F*)—one of several mutants isolated for their inability to move their nuclei into the tube-like mycelium (Xiang et al., 1995). This process is dependent upon the proper regulation of microtubules and cytoplasmic dynein. Further genetic screens in *A. nidulans* found that *nudE* overexpression could suppress the nuclear positioning defect due to a mutation in *nudF* (Efimov, 2003). Two highly related mammalian orthologs of nudE, Nde1 and Ndel1 (formerly mNudE and NUDEL), were isolated by their ability to bind to LIS1, suggesting that there is a functional relationship between LIS1, Nde1, and Ndel1 in mammals (Feng et al., 2000; Niethammer et al., 2000; Sasaki et al., 2000). Somehow these proteins facilitate cytoplasmic dynein's motor activity or its ability to bind cargo. While it is gratifying to see conservation of the *nud* interactions across evolution, what role do these proteins play in brain development? Two papers in this issue of *Neuron* (Feng and Walsh [2004] and Shu et al. [2004]) address this question for both Nde1 and Ndel1, respectively.

Feng and Walsh describe the consequences of inactivating Nde1, which is expressed by the proliferative progenitors that produce the neocortical neurons. During corticogenesis, a single layer of proliferative precursors that line the ventricle produces the six-layered cerebral cortex. Because neurons born in this ventricular zone will be unable to divide again, the precursor population has to balance the production of proliferative and postmitotic progeny: if too many neurons are produced ahead of schedule, there will be a shortage of progenitors to produce neurons later in development. Retrospective studies that date the birth of cortical neurons have shown that, early in development, progenitors produce more mitotic progenitors. Then, in midcorticogenesis, there is a switch (one that piques the interests of any developmental biologist) in which asymmetric divisions generate both proliferative and postmitotic progeny (Caviness et al., 1995). In Nde1 mutant mice, Feng and Walsh found that this control system appears to break down, since depletion of Nde1 resulted in a cortex that is smaller than that in wild-type animals. Subsequent analyses revealed that the later-born neurons that normally populate the superficial layers are missing, whereas earlier-born deep layer neurons are unaffected. If anything, slightly more neurons are born at earlier times. These results led Feng and Walsh to investigate asymmetric divisions in the *Nde1*$^{-/-}$ embryos.

Asymmetric division in a number of invertebrate organisms is accomplished by orienting the mitotic spindle so that developmental determinants are differentially inherited upon cleavage of the two daughter cells (Doe and Bowerman, 2001). This seems to hold true for the mammalian cerebral cortex, where time-lapse imaging has shown that the angle of mitotic cleavage can predict an asymmetric division (Chenn and McConnell, 1995; Haydar et al., 2003; Noctor et al., 2004). Feng and Walsh found defects in the cell division behavior of cortical progenitors of *Nde1*$^{-/-}$ mice. First, there was an accumulation of progenitor cells in mitosis along the ventricular surface, indicating a delay in mitosis. Normally in rodents, progenitors divide their chromosomes in a plane perpendicular to the ventricular surface. However, in the *Nde1*$^{-/-}$ mice, a larger portion of mitotic cells had division planes that varied from the normal 90°. The authors speculate that the absence of Nde1 randomizes the division plane, causing more divisions to produce neurons rather than progenitors. A delay in the cell cycle may also affect the fate of the daughter cells: transplantation studies have shown that a cortical progenitor's fate is cell cycle dependent (McConnell and Kaz-