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Dendritic tiling through TOR signalling

Weizhe Hong and Liqun Luo*

Howard Hughes Medical Institute and Department of Biology, Stanford University, Stanford, CA, USA
*Correspondence to: lluo@stanford.edu

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In many sensory systems, dendrites of sensory neurons cover an entire sensory field in a complete and non-redundant manner, a phenomenon called dendritic tiling. Previous studies have identified the Tricornered (Trc) kinase as an important regulator of this process. In this issue of *The EMBO Journal*, Koike-Kumagai *et al* (2009) found that the target of rapamycin (TOR) complex 2 (TORC2) is essential for dendritic tiling by way of regulating Trc kinase activity in *Drosophila* class IV dendritic arborization (da) neurons. This study provides the first *in vivo* evidence for a function of TORC2 activity in nervous system development.

The function of the brain depends on the characteristic morphologies of individual neurons and specific connections between them. In many sensory systems, dendrites of individual neurons belonging to the same neuronal type cover the sensory field as completely as possible but with minimal overlap, to ensure a complete and non-redundant representation of sensory input. This phenomenon is referred to as 'tiling'. One classic example of dendritic tiling occurs in the mammalian retina, in which dendrites of the same type of retinal ganglion cells typically cover the retina with little overlap, whereas dendrites of different cell types overlap extensively (Wässle and Boycott, 1991). This neuronal type-specific tiling is an evolutionarily conserved mechanism that organizes dendritic fields in a variety of species from *Caenorhabditis elegans* and *Drosophila* to mammals (Parrish *et al*, 2007).

Drosophila da neurons tile their dendrites to cover the two-dimensional larval body wall in a cell type-specific manner, thus providing an excellent system for studying the cellular and molecular mechanisms underlying dendritic tiling (Figure 1A and E). Artificial doubling of class IV da neurons results in partitions of the dendritic fields with little overlap, whereas ablation of these neurons causes the dendrites of neighbouring class IV da neurons to invade the dendritic territory of the ablated neuron. These observations suggested that homotypic repulsion among dendrites is required for this complete and non-redundant dendritic coverage (Parrish *et al*, 2007). What are the molecular mechanisms underlying this homotypic repulsion? The NDR family kinase Trc and its activator Furry were identified as essential signalling components for regulating homotypic repulsion in class IV da neurons (Emoto *et al*, 2004). In this issue of *The EMBO Journal*, Koike-Kumagai *et al.* found a role for TORC2 in regulating dendritic tiling by activating the Trc kinase.

TOR is an evolutionarily conserved Ser/Thr protein kinase and is part of two distinct multi-protein complexes: TOR complexes 1 and 2 (TORC1 and TORC2) (Wullschleger *et al*, 2006). TORC1 is sensitive to rapamycin and is essential for regulation of cell growth by way of activating anabolic processes, including ribosome biogenesis and protein synthesis, and inhibiting catabolic processes such as autophagy and ubiquitin-dependent proteolysis. The biological roles

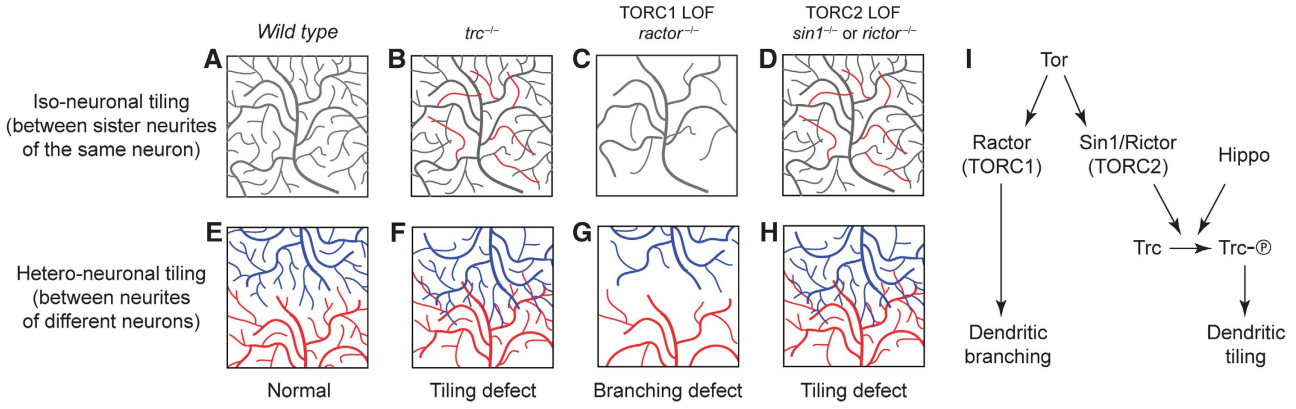

Figure 1 TORC2 is essential for dendritic tiling by way of regulating Trc kinase activity. (**A**–**H**) Schematics of dendritic tiling phenotypes in wild-type (**A**, **E**), $trc^{-/-}$ (**B**, **F**), $ractor^{-/-}$ (**C**, **G**), $sin1^{-/-}$ or $rictor^{-/-}$ (**D**, **H**). In wild-type, tiling occurs between sister neurites of the same neuron (iso-neuronal tiling, **A**) and between neurites of different neurons (hetero-neuronal tiling, **E**). Loss-of-function of *trc* or TORC2-specific components *sin1* and *rictor* leads to significant overlaps between dendritic branches, representing defects in both iso- and hetero-neuronal tiling (**B**, **D**, **F**, **H**); however, TORC1-specific *ractor* mutant results in a significant reduction of dendritic branches with no tiling defects (**C**, **G**). (**I**) Model for the distinct functions of TORC1 and TORC2 in dendritic branching and tiling, respectively.

of TORC2 are less well characterized—it is insensitive to rapamycin and is important for actin cytoskeleton organization (Wullschleger *et al*, 2006). TOR signalling is involved in a variety of processes in the nervous system, including neurite growth and dendritic branching. These processes, however, are sensitive to rapamycin, and are therefore likely mediated by TORC1-specific mechanisms (Swiech *et al*, 2008; Soulard *et al*, 2009). In contrast, neuronal functions for TORC2 are less well defined (Soulard *et al*, 2009).

Koike-Kumagai *et al.* found that TORC1 and TORC2 have distinct roles in dendrite morphogenesis in class IV da neurons—TORC1-specific component Ractor is required for dendritic branching, and TORC2-specific components Sin1 and Rictor are required for dendritic tiling (Figure 1C and D). MARCM (mosaic analysis with a repressible cell marker) analysis revealed a cell-autonomous requirement of TORC2-specific components in dendritic tiling. More interestingly, loss-of-function of TORC2-specific components impaired both iso-neuronal and hetero-neuronal tiling of class IV da neurons (Figure 1D and H). This phenotype is similar to that observed in Trc mutants, suggesting that TORC2 and Trc may act in the same pathway (Figure 1B and F; Koike-Kumagai *et al*, 2009). Does TORC2 therefore interact with Trc? The authors observed a strong tiling defect in trans-heterozygous mutants between Trc and TORC2 components, Sin1, Rictor or Tor, suggesting a genetic interaction between Trc and TORC2. Further biochemical assays demonstrated that Trc physically binds to TORC2-specific components Sin1 and Rictor, but does not bind to TORC1-specific component Raptor or the common protein Tor shared by TORC1 and TORC2 (Koike-Kumagai *et al*, 2009). The direct interaction between Trc and TORC2-specific components Sin1 and Rictor nicely explained how TORC1 and TORC2 have distinct roles in branching and tiling, respectively (Figure 1I).

How might TORC2 regulate Trc activity? Trc contains a conserved phosphorylation site at threonine 449 (Thr449) and the phosphorylation of threonine in the mammalian homologue of Trc is tightly correlated with its kinase activity (Hergovich *et al*, 2006). Does TORC2 have a role in Trc phosphorylation and activation? Indeed, TORC2 components Tor, Sin1 and Rictor were all required for the phosphorylation of Thr449, which leads to Trc activation. Conversely, Trc is not required for TORC2 activity (Koike-Kumagai *et al*, 2009), suggesting that TORC2 is upstream of the Trc signalling cascade (Figure 1I). How does TORC2 regulate Trc Thr449 phosphorylation? Previous studies showed that membrane targeting of human NDR1 leads to its constitutive activation (Hergovich *et al*, 2006). Membrane tagging of Trc also leads to a robust phosphorylation of Thr449 in S2 cells. Strikingly, overexpression of membrane-tagged Trc, but not wild-type Trc, rescues the loss-of-function phenotype of TORC2 components, suggesting that TORC2 may regulate the recruitment of Trc to the membrane in which Trc gets activated (Koike-Kumagai *et al*, 2009).

These findings suggest that TORC2 acts through the Trc signalling pathway to regulate dendritic tiling; this is the first report of neuronal function for TORC2 *in vivo*. These findings also raise several questions. The tumour suppressor kinase Hippo can directly regulate the phosphorylation of Trc (Emoto *et al*, 2006). Does TORC2 regulate membrane localization of Trc so that it can be phosphorylated by Hippo? How else might TORC2 and Hippo regulate the phosphorylation of Trc Thr449? Moreover, how does TORC2-dependent Trc activation regulate homotypic repulsion? Knock-down of Trc or TORC2 components showed severe cytoskeletal defects, suggesting that TORC2 regulates actin cytoskeleton, at least in part, by regulating Trc kinase activity (Koike-Kumagai *et al*, 2009). However, how TORC2-dependent Trc activation regulates actin cytoskeleton during tiling remains elusive. Does mammalian TORC2 also regulate dendritic morphogenesis, especially dendritic tiling?

Finally, how do cell surface molecules mediate the interactions between da neurons and initiate the intracellular signalling involving TORC2 and Trc? Although Dscam family proteins are involved in tiling of photoreceptor axons in *Drosophila* and retinal ganglion cells in mice, they are not required in da neuron tiling (Millard and Zipursky, 2008). Further identification of ligand(s) and receptor(s) for dendritic tiling in da neurons will help clarify how da neurons recognize each other, especially how they distinguish dendrites of the same neuronal class from those of a different class.

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Conflict of interest

The authors declare that they have no conflict of interest.

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