Vagus Topographic Map: Wandering through a gRAdient

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Topographic map development requires precise coordination between navigating axons and their targets in a dynamic environment. In this issue of *Developmental Cell*, Isabella et al. describe a mechanism in which a changing gradient of the morphogen retinoic acid regulates the expression of guidance factors to shape topographic axon targeting.

Axons in the developing nervous system must precisely navigate a noisy and dynamic environment to reach their specific targets. How do different neuronal populations integrate a complex mixture of extrinsic signals over time and space to establish intricate innervation patterning? In this issue of *Developmental Cell*, Isabella et al. (2020) reveal a new mechanism they term "temporal matching." At its center is a gradient of the morphogen retinoic acid (RA), which changes over developmental time. The RA domain temporally matches the expression of the Met receptor in distinct subsets of pharyngeal motor neurons with the expression of its chemoattractive ligand Hgf at their corresponding synaptic target sites. This temporospatial matching of receptor and ligand expression shapes topographic mapping of vagus motor neurons to their respective pharyngeal target areas.

Topographic maps, in which spatial relations between projecting sets of neurons and their targets are preserved, are a common feature of nervous system organization. Since the 1960s, the prevalent model underlying topographic map formation has been Sperry's chemoaffinity theory, in which molecular tags on axons integrate spatially segregated environmental cues to reach specific targets (Sperry, 1963). Indeed, in the visual system, the retinotopic map forms as Eph receptors expressed by retinal axons direct them to a particular location in the optic tectum expressing complementary gradients of Ephrin ligands (Cang and Feldheim, 2013). In contrast, the olfactory system utilizes a predetermined timing order of olfactory sensory axon outgrowth to generate topographic organization (Eerdunfu et al., 2017). Additionally, axon-axon competition and neuronal activity can refine topographic maps (Luo and Flanagan, 2007). Thus, topographic maps across motor and sensory nervous systems are established by different developmental mechanisms, yet it is unclear if and how these and other mechanisms intersect to achieve precise topographic targeting.

Vagus motor neurons (mX) that comprise cranial nerve X innervate pharyngeal arch (PA)-derived muscles, such as those for swallowing and speech, in an evolutionarily conserved topographic map. In humans and zebrafish, mX neurons in the posterior hindbrain are arranged such that anterior mX neurons innervate anterior PAs while posterior mX neurons innervate posterior PA targets (Figure 1). In the zebrafish embryo, PAs 4–7 arise sequentially from a primordium and are subsequently innervated by mX axon branches that develop from anterior to posterior (A-P). Previously, the Moens lab reported that the A-P position of individual mX neurons determines target selection (Barsh et al., 2017), suggesting there are unknown instructive cues along the A-P axis.

In a tour de force to identify genes that establish the vagus motor topographic map, Moens and colleagues used photoconversion to selectively mark anterior or posterior mX neurons in developing embryos, then sorted mX neurons by flow cytometry and performed RNA-seq analysis. They discovered that genes associated with RA, a morphogen that plays crucial roles early in embryonic development including A-P embryonic patterning, are enriched in the posterior vagus nucleus (Isabella et al., 2020). Moreover, a direct reporter for RA is turned on in the posterior but not anterior mX nucleus. The authors hypothesized that an RA gradient is required for specific axon targeting of mX neurons. To test this idea, they decreased RA signal transduction in individual mX neurons, causing posterior neurons to project to anterior targets. Conversely, single-neuron expression of constitutively active RA receptors induced anterior mX neurons to project to more posterior targets. Thus, low levels of RA signaling in the anterior mX nucleus promote anterior targeting, whereas higher levels in the posterior mX nucleus promote posterior targeting.

How does RA influence mX axon targeting? RNA-seq on hindbrains treated with RA revealed the receptor tyrosine kinase *met* was downregulated upon RA treatment. *In situ* hybridization confirmed *met* expression in the anterior mX nucleus, while its chemoattractive ligand *hepatocyte growth factor* (*hgf*) is expressed in anterior PAs. These expression domains shrink upon RA treatment and expand posteriorly with RA inhibition.

In both *met* and *hgf* mutants, mX neurons fail to innervate the PAs, demonstrating the necessity for these genes in mX axon targeting. Met/Hgf were one of the first pairs of neurotropic factors identified for motor axons but have been challenging to study due to their important roles in mammalian muscle precursor development and motor neuron survival, which are unaffected in zebrafish (Caton et al., 2000; Ebens et al., 1996). Thus, the developing zebrafish may be an accessible *in vivo* system to study the downstream signaling pathway that mediates Met/Hgf-dependent chemoattraction. As RA can both activate and repress gene transcription, how does it repress expression of *met* and *hgf*? The authors speculate that

if this repression is indirect, the target may be found in the list of upregulated genes in their RNA-seq dataset.

The authors previously demonstrated that timing of axogenesis can drive targeting of mX neurons independent of birth order and A-P organization in the mX nucleus (Barsh et al., 2017), indicating that a classical Met/Hgf spatial gradient is insufficient to explain vagus axonal targeting. Moreover, the authors observe that the RA signaling domain recedes over time rather than forming a static gradient. This correlates with an expansion of *met* and *hgf* expression from anterior to posterior in both the PA region and in mX neurons, thereby approximating the timing of innervation of each PA along the A-P axis. Thus, RA signaling coordinates the Met and Hgf wavefronts in the mX nucleus and PA, respectively, thereby enabling successive A-P innervation of topographic targets. An important next question will be to identify the source of the RA and to understand how this gradient is regulated across time and space.

Together, these data provide a new view of axon targeting that is independent of cell birth timing, suggesting a model in which changing morphogen gradients induce local expression of chemoattractants to direct topographic map formation. Intriguingly, the authors demonstrate that Met/Hgf-dependent temporal matching is independent of the timing of axogenesis and *hox5a*, two previously described drivers of topographic innervation of PAs, indi-
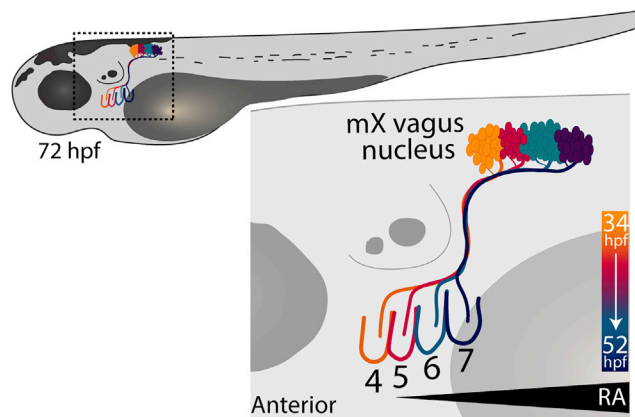

Figure 1. Topographic Mapping of mX Neurons to Pharyngeal Arches
Schematic of a 72 h post fertilization (hpf) zebrafish larvae. Inset outlined in box highlights innervation of pharyngeal arches (PAs) 4–7 by vagus motor neurons (mX) within the spinal cord. Colors indicate both topographic mapping between mX neurons in the vagus nucleus and their relative PA targets and the approximate developmental time at which the mX axons are navigating. Concurrently, an anterior to posterior retinoic acid (RA) gradient recedes over time.

cating that mX neurons employ multiple mechanisms to mediate axon targeting (Barsh et al., 2017; Isabella et al., 2020). The authors speculate that these mechanisms might synchronize targeting signals between navigating axons and their targets. This idea is reminiscent of axons pausing at a plexus to enable maturational changes in the target tissue prior to limb innervation (Wang and Scott, 2000). Future work will be required to elucidate the coordination of these different mechanisms for axon targeting and refinement.

Finally, as there are topographic maps all throughout the nervous system, it will be exciting to discover if and to what extent dynamic morphogenic gradients synergize with other mechanisms to mediate axon targeting during development as well as during regeneration.

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