The Roundabout Way of Finding the Right Target

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Of the organ systems, the nervous system is the first to initiate and last to complete. The neural system of a child undergoes tremendous development, with more than 2,500 synapses per neuron at birth that increase to 15,000 synapses per neuron by age 3 years. Proper functioning of the neural circuit requires not only the production of nerve cells, which are the building blocks of the nervous system, but also detailed guidance to locations that result in their making appropriate connections.

The neurons have a cell body with extensions called axons and dendrites. Whereas dendrites are smaller and branched, axons are longer and single. The neural circuit functions only when the axon of one neuron makes a long-lasting and faithful connection (synapse) with the dendrite of the other. The selection of the axon and dendrite partners for the synaptic connection is not straightforward or random; it is the result of an educated sequence of trial and error attempts of the axons and dendrites to connect to each other correctly.

In bilaterally symmetric animals, the information processed from the left and right halves of the central nervous system (CNS) must be integrated for proper cognition and motor function. This requires some of the nerves from one half of the CNS to cross the midline and connect to the other half. The precision of the neural connections depends largely on the leading edge of the axon, referred to as the “growth cone,” which bestows the axons with the impetus to navigate over long distances in response to guidance molecules released in the local environment.

The accuracy of axon crossing at the midline is dependent on the guidance of secreted molecules that attract (chemoattractant) and repel (chemorepellent) the axons. These chemoattractant and chemorepellent proteins are produced by the combination of a ligand and an axonal cone receptor of the ligand (in biochemistry, a ligand is a substance that forms a complex with a biomolecule to serve a biological purpose). One of the predominant guidance signaling complexes at the midline is the SLIT-ROBO pathway, with SLIT being the ligand and the axon’s roundabout guidance (ROBO) re-
The ROBO gene was first discovered in the fruit fly *Drosophila melanogaster* in a large-scale mutation screening. Mutation in this gene led to improper crossing and re-crossing of axons in the midline of the CNS, with the resultant pathways resembling a circular traffic junction (roundabout), hence the name “roundabout gene.” The equivalent of the ROBO gene in humans (ROBO homolog) was first isolated from a human fetal brain cDNA library using the sequence obtained from the *Drosophila* gene.

Currently, there are four ROBO receptors identified in vertebrates: ROBO1, ROBO2, ROBO3, and ROBO4. Whereas the genes of ROBO1 and ROBO2 are located on the short arm of chromosome 3 (3p12.3), ROBO3 and ROBO4 are located on the long arm of chromosome 11 (11q24.2).

The ROBO proteins that form the receptors belong to the immunoglobulin transmembrane receptor superfamily. The proteins consist of five immunoglobulin-like domains, three fibronectin type III repeats, a transmembrane domain, and a cytoplasmic domain.

In most invertebrates and vertebrates, the ROBO1 and ROBO2 receptors mediate axon repulsion when binding to SLIT ligands, thus preventing midline crossing (Figure 1). In agreement with this notion, several studies on mutant ROBO1 and ROBO2 receptors revealed that the axons with ineffective ROBO1 or ROBO2 receptors invade and then cross CNS midlines that they normally would avoid or repel. However, in ROBO3 knock-out mice, increased midline repulsion was observed that decreased midline crossing. This observation was unexpected given that SLIT-ROBO signals usually repel the axons away from the midline, so knocking them out should promote crossing. This led to the hypothesis that ROBO3 had a different role than other ROBO receptors and served as a negative regulator of the SLIT-ROBO repulsion in pre-crossing axons. Recent studies have confirmed the hypothesis that ROBO3 functions by contributing to midline attraction and crossing by mediating attractive responses while suppressing other SLIT-ROBO repulsion.

Human ROBO3 was identified as the candidate gene for horizontal gaze palsy with progressive scoliosis (HGPPS) in a genome-wide linkage analysis of unrelated consanguineous families of diverse ethnic origin with this disorder. HGPPS is a rare autosomal recessive disorder that affects eye movements and the spine. The condition is characterized by the absence of horizontal (side-to-side) eye movements beginning in childhood or adolescence, which necessitates turning one's head because those with this condition have no ability to horizontally track objects with the eyes. Alternatively, vertical eye movements are intact. Additionally, these individuals have a progressive scoliosis. Homozygous mutations in the ROBO3 gene were most common in consanguineous families afflicted with HGPPS, whereas compound heterozygous mutations were more frequent in non-consanguineous families. With respect to the nature of the mutations, non-sense, missense, frame-shift, and splice site mutations have been reported. So far, 22 distinct ROBO3 mutations have been identified in families of different ethnicities affected by HGPPS. Analysis of the ROBO genes in patients with congenital gaze palsy is indicated to make an early diagnosis of the scoliosis. The precise mechanism by which ROBO3 mutations lead to gaze palsy and scoliosis has not yet been elucidated.

SLIT-ROBO interactions have been shown to regulate angiogenesis, myogenesis, kidney morphogenesis, and leukocyte migration in addition to neurogenesis.

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