

How inhibitory cues can both constrain and promote cell migration

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Collective cell migration is a common feature in both embryogenesis and metastasis. By coupling studies of neural crest migration *in vivo* and *in vitro* with mathematical modeling, Szabó et al. (2016, *J. Cell Biol.*, <http://dx.doi.org/10.1083/jcb.201602083>) demonstrate that the proteoglycan versican forms a physical boundary that constrains neural crest cells to discrete streams, in turn facilitating their migration.

During embryonic development, cells undergo extensive rearrangements and migrations that are critical for proper organ formation. The neural crest is one of the most migratory of embryonic cell types. Originating as neuroepithelial cells within the central nervous system, neural crest cells subsequently undergo an epithelial to mesenchymal transition to become migratory cells that move extensively within the periphery of the vertebrate embryo (Le Douarin, 1982). In fact, their invasive behavior has been likened to that of cancer cells undergoing metastasis, which is an apt analogy given that several highly invasive cancers, such as melanoma, neuroblastoma, and glioma, originate from neural crest–derived cells. After migrating to often distant locations, neural crest cells differentiate into diverse cell types, ranging from melanocytes of the skin to neurons and glia of the peripheral nervous system and the skeletal elements of the face.

Over the past several decades, numerous mechanisms have been proposed to explain the apparently directional migration by which neural crest cells home to diverse tissues. One possibility is that chemoattractants function at a distance to direct these cells to their proper destinations. Indeed, there is evidence in the literature to support the role of numerous attractants that influence neural crest cells, including factors such as stromal derived factor and VEGF, which are expressed in a graded fashion and are critical for the migration of subpopulations of neural crest cells in particular species. For example, stromal derived factor attracts cranial neural crest cells in frog (Theveneau et al., 2010) and trunk sympathetic precursors in chick (Kasemeier-Kulesa et al., 2010), whereas VEGF appears to attract chick cranial neural crest cells (McLennan et al., 2010, 2015). Repulsion also plays an important role in confining neural crest cells to particular pathways. Most notably, at spinal cord levels in amniotes, semaphorins (Gammill et al., 2006) and ephrins (Krull et al., 1997; Wang and Anderson, 1997) play a critical role in restricting neural crest cells to streams that migrate through only a portion of each somite. This is important

not only for causing neural crest cells to migrate in a segmental fashion but also for the subsequent segmental organization of the peripheral nervous system. Chemorepellant proteins, called Slits, also are important for preventing neural crest cells from entering certain migratory pathways (De Bellard et al., 2003).

A further complication to understanding neural crest cell migration is that these cells not only interact with their environment but also with each other. For example, neural crest cells exhibit contact inhibition of locomotion (Abercrombie, 1979), such that when two neural crest cells come into contact, their membranes freeze at the site of contact and then the cells reverse directions (Carmona-Fontaine et al., 2008). Coupled with this, neural crest cells also display mutual attraction using the complement component C3 and its receptor C3R (Carmona-Fontaine et al., 2011). This results in neural crest cells that can attract each other but that change their direction upon close contact, such that they migrate collectively but not as an epithelial sheet.

Despite numerous examples of attractants and repellants, either autocrine or paracrine, how these various signals are integrated to produce directional cell migration has remained a mystery. In this issue, Szabó et al. help to resolve this conundrum by examining cranial neural crest migration in frog embryos. In the head region of the embryo, neural crest cells emerge from the neural tube, the future brain, and migrate in defined streams of cells from their site of origin toward destinations in the branchial arches (Fig. 1 A), structures that give rise to elements of the facial skeleton. These discrete streams are separated by regions that lack neural crest cells. Szabó et al. (2016) report that a member of the versican family of proteoglycans is expressed in a pattern that is complementary to the paths followed by neural crest cells, exactly filling the neural crest–negative domains (Fig. 1 A). This raised the intriguing possibility that versican might function as an inhibitory cue that restricts neural crest cells to defined streams.

To test this possibility, they examined the role of versican in neural crest migration by performing gain- and loss-of-function experiments *in vitro* and *in vivo*. Szabó et al. (2016) find that loss of versican *in vivo* results in aberrant neural crest migration and demonstrate that this effect is a result of the presence of versican in the environment (Fig. 1 B). Consistent with their *in vivo* results, they demonstrate that versican inhibits neural crest migration *in vitro*; whereas neural crest cells migrate happily on fibronectin substrates, they avoid fibronectin

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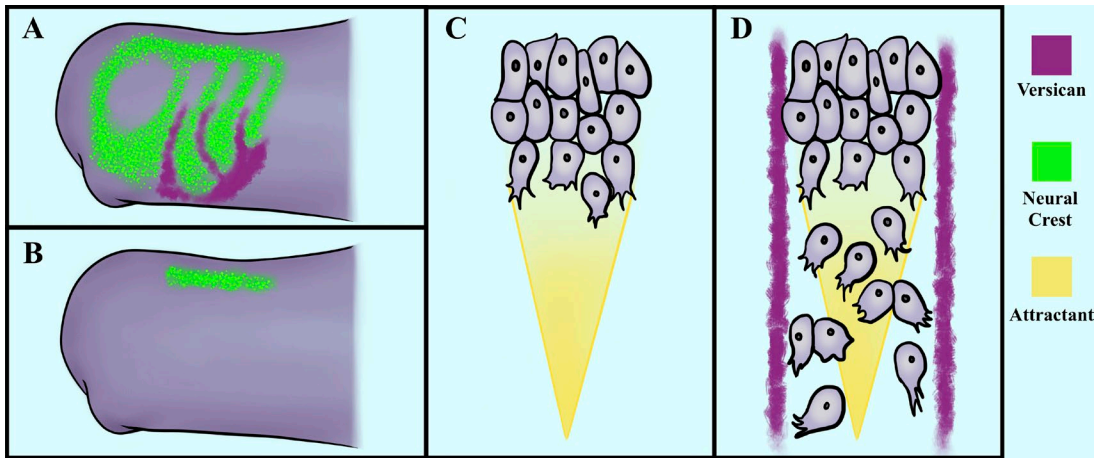


Figure 1. Versican borders neural crest streams and helps facilitate migration. (A) A schematic diagram of the head of a frog embryo viewed from the side, showing migrating cranial neural crest cells (green) that emerge from dorsal neural tube (top) and migrate in streams ventrally toward the branchial arches. Anterior is to the left and posterior to the right. The proteoglycan versican (purple) is expressed in a pattern that is complementary to the neural crest streams. (B) After knockdown of versican, the neural crest cells (green) fail to migrate efficiently. (C) When neural crest cells are grown on permissive substrates and exposed to a gradient of chemoattractant (yellow), they migrate downward toward the source of attractant. (D) However, when an inhibitory molecule like versican (purple) borders the neural crest migratory stream, the neural crest stream progresses downward faster and more efficiently. Thus, optimal migration involves a combination of chemoattraction, coattraction, and contact inhibition of locomotion together with confinement of the stream by inhibitory cues like versican.

plus versican substrates. Together, these results suggest that versican forms boundaries between streams of neural crest cells that confine the cells to individual streams within the versican-negative region. Essentially, the results suggest that versican forms a border that restricts available neural crest migratory paths to discrete regions, much as barriers on the freeway confine traffic to designated lanes.

To further test the idea that spatial confinement facilitates and enhances migration, Szabó et al. (2016) turned to a computational approach. Using a mathematical model that combines contact inhibition of locomotion, coattraction, and confinement, they demonstrate that imposing boundaries on cells that exhibit both contact inhibition of locomotion and coattraction leads to more rapid and efficient migration of the neural crest streams, resulting in optimal directional collective migration (Fig. 1, C and D). Importantly, this also helps to explain scaling of the migratory streams that occurs between species. Across vertebrates, there are marked differences in the numbers of migrating neural crest cells within a particular stream. The model proposed by Szabó et al. (2016) shows that persistence of migration varies with the width of the stream and that width is optimized based on the numbers of migrating neural crest cells. Thus, there is an optimal confinement width for a particular cell number.

Collectively, the data suggest that cell confinement plays an important role in promoting collective cell migration. Szabó et al. (2016) identify versican as one example of such an inhibitory molecule. Other examples, like ephrins and semaphorins, may function in a similar manner in other contexts. These experiments nicely resolve the apparent discrepancy in the literature regarding how a repulsive molecule like versican can act as a repellent and yet be important for promoting efficient forward migration. Moreover, the work highlights the importance of confining cells to particular streams to facilitate their effective movement; although neural crest cells are inherently highly migratory, they generally lack directionality of migration. This study demonstrates that confining these cells to streams promotes their forward and directional movement.

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