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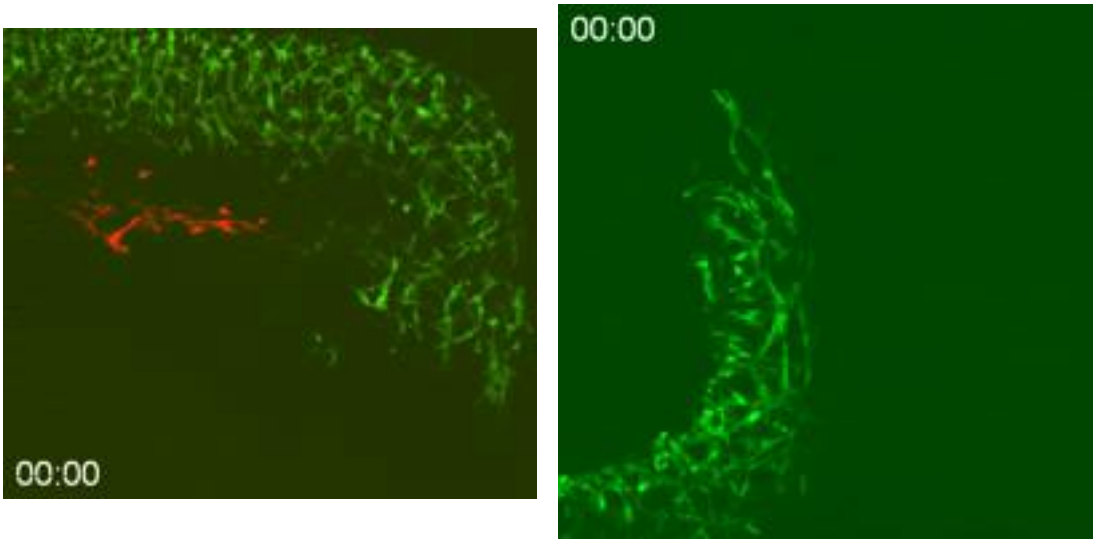
A fast track to gut innervation

August 30, 2012 – The gut is richly innervated with enteric neurons that autonomically control such behaviors as peristaltic movement, secretion and blood flow, earning this long stretch of the nervous system the distinction of being called "the second brain." During development, the enteric nervous system begins as a migratory population of neural crest cells, which wend their way down to populate the entire length of the intestines, which can be several times as long as the entire body. This long journey has always been thought to closely follow the winding course of the intestinal wall – when these neurons fail to complete the trip, it can lead to a complete lack of lower gut innervation as seen in the congenital condition called Hirschprung's disease. In some patients, however, there are gaps referred to as skip areas in sections of small intestine higher up in the gut as well, a phenomenon that the conventional model of enteric innervation cannot explain.

New work by Chihiro Nishiyama and others in the Laboratory for Neuronal Differentiation and Regeneration (Hideki Enomoto, Team Leader) shows how a subset of enteric neural crest cells in mouse takes a short cut across the mesentery on their way to innervate the large intestine. Published in *Nature Neuroscience*, these findings open up new and unexpected insights into the longest migration of neurons in the embryo.



Cells (KikumeGR, red) aligned with mesentery in E11.5 gut proliferate and migrate, giving rise to the neural network of the hindgut. A subpopulation of cells traverses the mesentery to reach the hindgut (right).

In the mouse, the population of the gut with enteric neurons takes place over the period of embryonic day 9.5 to 14, during which the guts itself undergoes extensive growth and morphological changes. At E10, the gut is still a nearly straight tube, but by the following day, it develops a hairpin loop that gives rise to the mid- and hindgut sections (which form the embryonic bases for the small and large intestines). By E11.5, these gut segments begin to pull away from each other. Nishiyama et al. used live imaging to observe the migration of cells in tissue sections from the gut over time, using embryos engineered to express one of two fluorescent proteins – GFP and KikumeGR. Both can be used to label enteric neurons, but KikumeGR has the added advantage of changing color from green to red on exposure to ultraviolet light, making it possible for the team to track the movements of select populations of cells through living gut tissue. Using this technique, they found that in the E12.5-13.5 embryo, precursor cells form bundles that spread in a network throughout the hindgut, with the wavefront region at the distal edge contributing more than 80% of the hindgut neural network.

But how did these precursors arrive at their destination? Looking more closely at the fluorescent-labeled E11.5 gut, Nishiyama found that the population arose around the time that the mid- and hindgut regions are brought into close proximity by the folding action. What the team saw next surprised them; they found that these wavefront cells are derived from a cell population that traveled straight across the mesentery from the mid to the hind region, not from cells taking the long way

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around the bend. This corner cutting by migrating cells plays a crucial role; when the team blocked this transmesenteric movement, they found a dramatic delay in hindgut colonization by precursors.

Next, using a mouse model of Hirschprung's disease developed by the lab in 2008, they looked for connections between this novel form of migration and disease. They found that in Hirschsprung's mice, the transmesenteric migration was markedly reduced, suggesting that a failure in this mechanism leads to the defects in gut innervation as well as the skip areas observed in some patients. Enteric neurons in Hirschsprung's disease are known to show reduced expression of GDNF, an important factor in neuronal development and differentiation, suggesting the intriguing possibility that the defects in transmesenteric migration may be tied to aberrant GDNF signaling.

"In mouse, the enteric neurons only have a window of a single day in which the mid- and hindgut are close enough to enable neurons to take this path across the mesentery, so it is really wonderful to see how tightly the timing of cell migration and morphological changes in the tissue are coordinated," says Enomoto. The lab plans to study differences in cell populations that take the transmesenteric shortcut and those that take the long way round, as well as to investigate possible applications of these findings in the development of cell therapies for Hirschsprung's disease.

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