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Slit molecules prevent entrance of trunk neural crest cells in developing gut

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ABSTRACT

Neural crest cells emerge from the dorsal neural tube early in development and give rise to sensory and sympathetic ganglia, adrenal cells, teeth, melanocytes and especially enteric nervous system. Several inhibitory molecules have been shown to play important roles in neural crest migration, among them are the chemorepulsive Slit1–3. It was known that Slits chemorepellants are expressed at the entry to the gut, and thus could play a role in the differential ability of vagal but not trunk neural crest cells to invade the gut and form enteric ganglia. Especially since trunk neural crest cells express Robo receptor while vagal do not. Thus, although we know that Robo mediates migration along the dorsal pathway in neural crest cells, we do not know if it is responsible in preventing their entry into the gut. The goal of this study was to further corroborate a role for Slit molecules in keeping trunk neural crest cells away from the gut. We observed that when we silenced Robo receptor in trunk neural crest, the sympathoadrenal (somites 18–24) were capable of invading gut mesenchyme in larger proportion than more rostral counterparts. The more rostral trunk neural crest tended not to migrate beyond the ventral aorta, suggesting that there are other repulsive molecules keeping them away from the gut. Interestingly, we also found that when we silenced Robo in sacral neural crest they did not wait for the arrival of vagal crest but entered the gut and migrated rostrally, suggesting that Slit molecules are the ones responsible for keeping them waiting at the hindgut mesenchyme. These combined results confirm that Slit molecules are responsible for keeping the timeliness of colonization of the gut by neural crest cells.

1. Introduction

The neural crest is a group of cells that emerge early in development from the dorsal neural tube and migrate along pathways that are characteristic of their axial level of origin (Bronner-Fraser et al., 1991; Le Douarin et al., 1992). Neural crest cells give rise to a good portion of the peripheral nervous system (PNS) formation, cranio-facial structures and even endocrine organs. Probably the most intrinsic and characteristic feature of these cells is their precise migratory pathways along their rostro-caudal axis (Gammill and Roffers-Agarwal, 2010; Theveneau and Mayor, 2012). One of its classic pathways is that of vagal neural crest cells. These vagal neural crest cells emerge from the caudal hindbrain (between somites 1 and 7) and migrate into the developing gut where they extensively divide and eventually differentiate into the enteric nervous system (ENS) cells (Burns et al., 2002; Kuo and Erickson, 2010, 2011). In contrast, trunk neural crest cells (those arising from 8 to 25 somite levels) never enter the gut (Erickson and Goins, 2000b; Le Douarin and Teillet, 1974b).

Many inhibitory molecules have been shown to play critical roles in determining neural crest migration patterns, especially, ephrinB family members (Krull et al., 1997) and Semaphorins (Eickholt et al., 1999; Gammill et al., 2006). However, none of these molecules were able to explain the differences between the ability of vagal and trunk neural crest populations to enter the gut. A clue came from finding that only migrating trunk neural crest cells expressed Slit Robo receptors (De Bellard et al., 2003). Slit proteins are not only well known chemorepellants for axons and neuronal migration (Brose et al., 1999; Kidd et al., 1999; Kinrade et al., 2001; Zhu et al., 1999) but in addition they are powerful repellents of trunk neural crest cells (De Bellard et al., 2003).

Seminal work from the labs of Le Douarin and Erickson showed that trunk crest cells transplanted to the vagal position could enter

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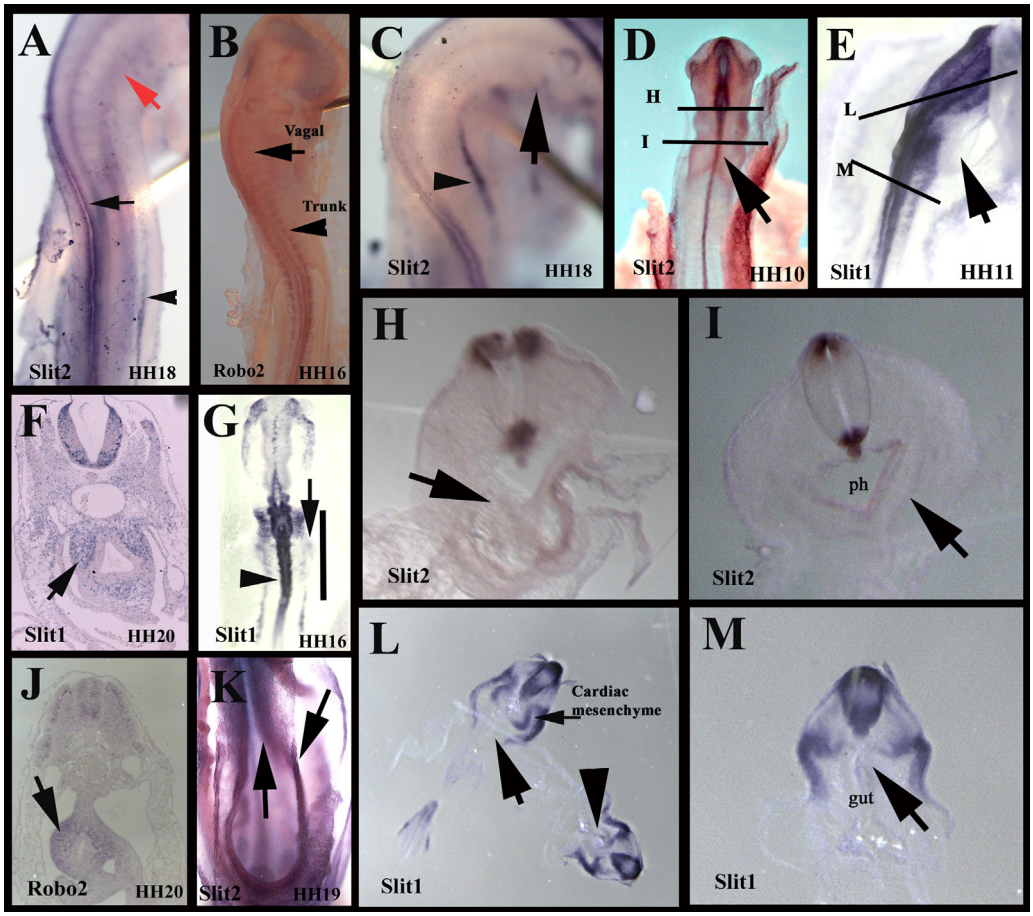

Fig. 1. Slit and Robo expression in chicken embryos.
Wholemount and sections of in situ hybridization for Slit and Robo in HH16–20 in chicken embryos. (A) Slit2 is expressed in dorsal neural tube (arrow), mesonephros (arrowhead) and not in vagal region (red arrow) in HH18 embryo. (B) Robo2 receptor is expressed in migrating trunk neural crest (arrowhead) but not in vagal (arrow). (C) Slit2 is not expressed in the ventral region at vagal level (arrow), although it is expressed in gut region posterior to the heart (arrowhead) at HH18. (D) Slit2 is expressed in dorsal neural tube (arrow) in HH10 with lines for sections shown in (H) and (I). (E) Slit1 embryo at HH11 with lines for sections shown in (L) and (M) arrow points to pharynx. (F) sections in hindbrain region showing Slit1 expression in gut (arrow) of a HH20 embryo. (G) wholemount for Slit1 showing expression in dorsal neural tube (arrowhead) and absence where the gut is beginning to develop (arrow and line point to primordial gut mesenchyme). (H) and (I) sections of Slit2 embryo in (D) showing lack of Slit2 expression in pharynx region. (J) section at sacral level showing Robo2 expression of cells in gut (arrow) at HH20. (K) ventral side of HH19 embryo showing expression of Slit2 along gut mesenchyme (arrows) (L) and (M) sections of Slit1 embryo in (E) showing lack of Slit1 expression in pharynx region (arrows) Arrowhead corresponds to section on (M) at higher magnification.

the gut, albeit never to the extent of vagal crest (Burns et al., 2002; Le Douarin and Teillet, 1974a ; Le Douarin and Teillet, 1973). These findings showed that trunk crest cells will not enter the developing gut, not that they are incapable of doing so. The key to their poor colonization came from studies by Newgreen and Burns labs which showed that the trunk cells failed to match vagal crest cells in proliferation once in the intestinal tissue, (Newgreen et al., 1980; Zhang et al., 2010) due to lack of ret receptor (Delalande et al., 2008). In addition, Burns and co-workers showed that the enteric neural crest shows cell autonomous differences in their migratory properties, with the vagal neural crest being more invasive of the gut than the caudal, sacral crest population. The reasons for this difference in invasive capacity between vagal and sacral crest has yet to be determined (Burns, 2005).

Here we further examined and settled the potential role of Slit molecules in keeping trunk neural crest cells from entering the gut. Slits are expressed near the entrance to the gut during trunk migration but not during vagal migration, while trunk and sacral neural crest express Robo receptors. We tested the hypothesis of a functional role for Slits in keeping trunk neural crest from migrating into and populating the gut by loss-of-function experiments via electroporation of a dominant negative Robo receptor. The results show for the first time that trunk and sacral neural crest cells can enter the gut if Robo receptors are silenced, thus giving account for the differential ability of vagal but not trunk neural crest cells to invade and innervate the gut. Interestingly, we also found axial differences in the invading capabilities into the gut between more rostral trunk versus sympathoadrenal trunk (somites 18–24), suggesting the presence of another repellant for trunk neural crest cells in the gut.

2. Materials and methods

2.1. Animals

Chicken embryos were obtained by incubating fertilized chicken eggs at 38 °C as described by Hamburger and Hamilton (1951) until desired stage of development.

Mouse strains: all animals were generated in a mixed CD-1/129Sv/C57Bl6 background. Robo mutants were obtained by crossing mice with Robo1 and Robo2 mutant alleles already linked (Ma and Tessier-Lavigne, 2007). Slit mutants were obtained by crossing Slit1$^{-/-}$; Slit2$^{+/-}$; Slit3$^{+/-}$ animals (Plump et al., 2002; Yuan et al., 2003). PCR based genotyping was done following previously