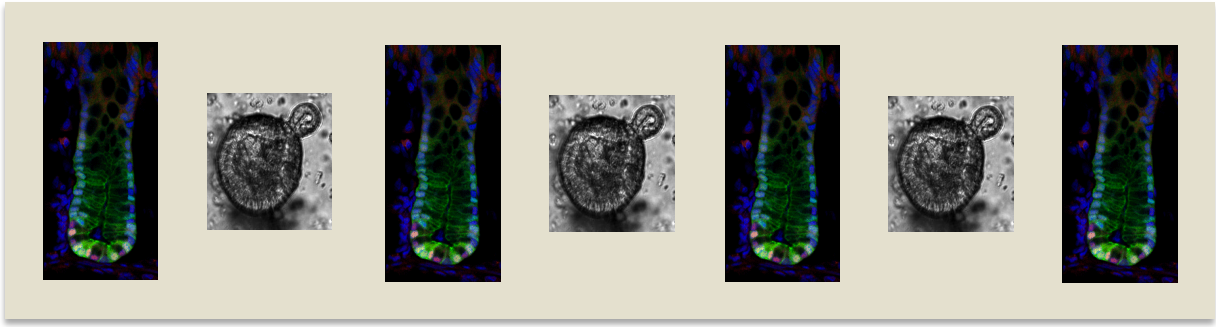

Intestinal Stem Cells

New concepts and methods

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Divisions asymmetric with regard to cell fate and mNumb contribute to lineage specification in mouse colon crypts

In the small intestine (SI) and colon, crypt base columnar (CBC) cells at positions 1-6 are the "working horses" stem cells (SCs) that fuel the extraordinary daily tissue renewal of intestinal epithelia[1]. In the SI and colon, they interdigitate with Paneth cells (PCs) or Goblet Like cells (GLCs), respectively, which act as niche-supporting cells providing many of the short-range signals for SC competence[2-3]. It is unclear at which level in the SC/progeny hierarchy lineage specification is initiated and to what extent and how cell fate decisions are timed in relation to the pattern of cell divisions. The Simons & Clevers model[4-5] proposes that (a) CBC cells undergo neutral competition for contact with niche-supporting cells whereby differentiation can only occur when one of them loses contact with the signals provided by the SC niche; (b) resulting CBC cell loss is compensated by symmetric self-renewal of a neighboring CBC cell; (c) subsequent lineage specification occurs upon stochastic lateral inhibitory Notch/Delta signaling among a general pool of interacting cells that are not necessarily sister pairs of cytokinesis (reviewed in[6]). This model therefore refutes any contribution of divisions asymmetric with regard to cell fate. The competing, but less well known Bjerknes & Chen model[7-8] proposes that (a) symmetric divisions of CBC cells are followed by divisions asymmetric with regard to cell fate when their immediate offspring (Mix progenitors) have reached cell positions located just above the CBC/PC zone; (b) these Mix progenitors commit to differentiation by setting up Delta-Notch lateral inhibition between the nascent sister cells during cytokinesis[9-11]; (c) this symmetry breaking leads to Notch and Delta expressing daughters which give rise to Hes1 or Atoh1-expressing cells which become the columnar or secretory cell lineage progenitors, respectively.

In a recent study[12] addressing the above questions, we reported in the SC/GLC niche of crypts of the descending colon the occurrence of sister cell doublets of cells in which Atoh1/Math1 was expressed in only one of the sister nuclei and proposed that these arose from Atoh1 (-) cells where one sister started expressing Atoh1 some time after completion of cell division. This is highly suggestive of cell divisions asymmetric with respect to cell fate and thus supports the model proposed by Bjerknes and Cheng. We next assessed whether some dividing cells showed characteristics of asymmetric division as indicated by vertical spindle reorientations, changes in cell shape or the asymmetric distribution of molecules. In the tubular crypt parts (positions >3) we uncovered a novel expression of planar cell polarity (PCP) designated longitudinally oriented basal asymmetry **(**LOBA) characterized by all interphase cells being bent at their base, uniformly oriented toward the crypt bottom. During the mitotic cycle dividing cells remain connected to the underlying lamina by a basal process (BP), as in the SI[13] and in addition it preserved LOBA. All the metaphase to telophase spindles planarly align with the apical cell surface and 80% of them longitudinally align with the crypt axis, thus fulfilling the criteria of oriented cell division (OCD)[14]. Near the crypt bottom too, all the spindles from metaphase on were aligned horizontally. These data therefore rule out for colon crypts a mechanism of division associated with reorienting vertically the spindle of CBC cells, reported in crypts of the SI[15]. Of note, we also found that the cells displaying spindle orientation parallel with the crypt axis set up the cleavage furrow in front of the BP, asymmetrically anchoring the daughter cell closest to the crypt bottom. During anaphase B spindle elongation, the apical pole elongated in the same direction so that the non-attached daughter moved up one position. OCD and LOBA therefore form a novel functional unit that orchestrates daughter cells placement and anisotropic movements. We believe the daughter cell breaking away from its sister staying in contact with the GLC cell can be considered as another expression of breakage of symmetry and propose a model in which in the colon, this activity promotes escape of CBC cells from the SC/GLC niche for them to become MIX progenitors. In the SI, it could also be used there at the border between the CBC/PC niche and the transit amplifying zone.

Earlier studies in the descending colon indicated that the CBC cells become committed in the lower part of the CBC/GLC niche [3, 16]. In order to assess whether the behavior of dividing cells described in our study could be linked to some form of asymmetric division, we investigated the distribution of proteins displaying asymmetric segregation as a cortical crescent adjacent to one spindle pole in several SC types[17]: Celsr1, Vangl2, NuMA and mNumb. Of these, only mNumb, an evolutionary conserved cell fate determinant acting in the Notch pathway[9, 18] was found in 60% of the telophases located predominantly in the CBC/GLC niche associated with cytoplasmic vesicular structures which segregate asymmetrically into only one daughter, where they became densely packed between the reassembled nucleus and the cleavage furrow of telophases. Strengthening the view that these observed asymmetric segregations are of functional significance, in 75% of the cases (12/16) in which mNumb-containing vesicles had asymmetrically distributed in one daughter, the latter had also inherited the BP asymmetrically. However, no asymmetric cortical segregation of mNumb, Vangl1, Celsr1 and NuMA was found, indicating an absence of a mechanism that coordinates spindle orientation and cell fate determinants as used in invertebrates [17] or vertebrate muscle[19] and skin cells[10]. Although these results provide first evidence that divisions asymmetric with regard to cell fate

and mNumb contribute to lineage specification in mouse colon crypts, it will be necessary to further assess cellular and mechanistic details of such SC divisions and to define the exact cells displaying this mode of lineage specification.

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