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Review

Regulation and functions of cell division in the intestinal tissue

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ABSTRACT

In multicellular organisms, epithelial cells are key elements of tissue organization. In developing epithelial tissues, cellular proliferation and differentiation are under the tight regulation of morphogenetic programs to ensure correct organ formation and functioning. In these processes, proliferation rates and division orientation regulate the speed, timing and direction of tissue expansion but also its proper patterning. Moreover, tissue homeostasis relies on spatio-temporal modulations of daughter cell behavior and arrangement. These aspects are particularly crucial in the intestine, which is one of the most proliferative tissues in adults, making it a very attractive adult organ system to study the role of cell division on epithelial morphogenesis and organ function. Although epithelial cell division has been the subject of intense research for many years in multiple models, it still remains in its infancy in the context of the intestinal tissue. In this review, we focus on the current knowledge on cell division and regulatory mechanisms at play in the intestinal epithelial tissue, as well as their importance in developmental biology and physiopathology.

1. Introduction

The primary functions of the intestinal tract are digestion and absorption of nutrients while forming a barrier against luminal pathogens. It can be anatomically divided into the small intestine and the colon, where the small intestine can itself be segmented into the duodenum, jejunum and ileum. The intestinal lumen is lined with a cohesive, polarized simple columnar epithelium that covers upwards finger-like protrusions into the lumen called villi, downwards invaginations called crypts. Villi are differentiated compartments that serve to increase surface area to maximize absorption function. Crypts constitute the proliferative compartments. In the small intestine, intestinal stem cells (ISCs) reside in the niche at the crypt bottom, where their self-renewal capacity is promoted by niche Paneth cells and stroma cells [1–7]. Most of their progeny migrate upward the crypt to become transit amplifying (TA) cells that are simultaneously committing and proliferating. As they reach the villi, TA cells will differentiate into specialized absorptive (i.e. enterocyte) or secretory (i.e. enteroendocrine cells, goblet cells or tuft cells) lineages and further mature as they migrate towards the tip of the villi where they are eventually shed into the gut lumen. Altogether, it takes only 4–5 days for a cell to exit the stem cell niche and die at the top of the villus, making the intestinal epithelium one of the most actively self-renewing tissue in adult mammals [8]. Thus, cell division taking place in the crypt is crucial to: 1) constantly replenish the intestinal epithelium and 2) sustain proper expansion and functional integrity of the crypt. In this review, we will discuss the mechanisms that regulate cell division rates and orientation in the small intestine, as well as their impact on intestinal homeostasis.

2. Cell division compartmentalization in the intestinal tissue

2.1. Spatio-temporal distribution of dividing cells during intestinal development

Acute morphogenetic events drive intestinal morphogenetic development along with a compartmentalization of proliferative cells. In addition to expanding the villus structure to increase the exchanging surface between enterocytes and the gut lumen, crypt compartments are

Abbreviations: Apc, adenomatous polyposis coli; ASC, active stem cell; CDK, cyclin-dependent kinase; CKI, CDK inhibitor; EE, enteroendocrine cells; Hh, Hedgehog; Hopx, homeodomain-only protein homeobox; INM, interkinetic nuclear migration; ISC, intestinal stem cell; JNK, Jun-N-terminal kinase; Lgr5, leucine-rich repeat-containing G protein-coupled receptor 5; Lrgi1, MT, microtubule, leucine-rich glioma inactivated 1mitochondrial pyruvate carrier; QSC, quiescent or reserve stem cell; TA, transit-amplifying; Tert, telomerase reverse transcriptase; TIGAR, TP53-indeuced glycolysis and apoptosis regulator.

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defined, giving rise to the canonical functional unit of the intestinal tissue named crypt-villus axis (Fig. 1). Intestinal morphogenesis starts between E10.5 and E14.5. During this developmental window, the growing intestine can be described as a tube where the epithelial layer displays a pseudostratified aspect [9]. Proliferative cells are homogenously distributed as a push of proliferation occurs in almost all epithelial cells, and the mean cell cycle time is 16 h [10]. Villus structures start emerging at day E14.5 under the control of Hedgehog (Hh) and Bmp signaling pathways and the proliferation of subepithelial mesenchymal cell clusters [11–13]. The later locally modify shape of the above epithelial monolayer and activate cell division there [12,14]. Gumucio's lab further proposed that cell division directly participates to the initial stage of villus formation [14]. Although dividing cells exhibit an elongated cell shape in the pseudostratified epithelium, rounding of mitotic cells occurs during the villification time window. Subsequent pulling forces and invagination of the apical surface consecutive to mitotic rounding may constitute the first steps of monolayer deformation and epithelium folding that will trigger villus formation [14]. Concomitantly to villus formation, proliferating cells become restricted to the intervillus region. Remaining Bmp secreting cell clusters at the tip of the villus or specific activation of Wnt at the intervillus region may explain why dividing cells specifically pattern in the intervillus region [11,15].

Crypt formation occurs late during intestinal development. It takes place during gestation in humans (weeks 11–12 [16]) but during the first days after birth in mice [17,18]. Driving mechanisms that spawn crypt growth are subjected to conflicting views. This morphogenetic process may be initiated by remodeling of the intervillus region likely through the action of subepithelial myofibroblasts [19,20], or tissue invagination of the intervillus area as recently described by Lechler and colleagues [18]. In their study, the authors showed that crypt initiation depends on myosin-II activity and that crypt expansion on Rac1 activity [18]. Moreover, crypt curvature has been proposed to be self-generated. In this scenario, Wnt signaling may induce a positive curvature, and the positive curvature would in return reinforce Wnt activity [21]. More recently, Liberali and colleagues reported that in in vitro intestinal organoids, initiation of crypt formation results from a symmetry-breaking step which is concomitant to the appearance of Paneth cells, under the control of Wnt signaling. This symmetry-breaking phenomenon would be triggered by the local transient activation of YAP1, down-regulation of Notch signaling and subsequent Paneth cell generation [22]. However, it is important to note that Paneth cells appear only late during crypt maturation (between P7 and P14 [23,24]) in the mouse intestine. Thus, in contrast to the organoid model, if crypt formation requires Wnt signaling, this should involve additional cellular mechanisms other than Paneth cells in vivo.

2.2. Organization of dividing cell populations in adult crypts

In physiological conditions, cell division is restricted to crypt compartments in adults, and epithelial proliferation is sustained by two subsets of cells: ISCs and progenitors TA cells [8,25] (Fig. 1). However, the current model proposes that two stem cell populations may coexist there. First, mitotically active ISCs are responsible for the bulk of cell cycling and homeostatic maintenance at the bottom crypt [8,26]. H. Clevers and colleagues pioneered this field of research and demonstrated that active ISCs express the receptor leucine rich-repeat-containing G-protein-coupled receptor 5 (Lgr5) or Grp49, a Wnt target gene [3]. Since then, Lgr5 has been proposed as a reliable marker for epithelial stem cells in other tissues such as the stomach [27], the colon [28], the skin [29], the uterine glands [30] and satellite cells [31].

Lgr5^{+}-ISCs are massively found during the vilification at E15.5, and their number decreases at E18.5 when they become confined to the intervillus region [32]. It is thought that Lgr5 would restrain Wnt activation during intestinal development [32]. After birth and during adult life, Lgr5^{+}-ISCs are restricted to the crypt bottoms [17], where they divide every 21.5 h on average [33] and their number constantly

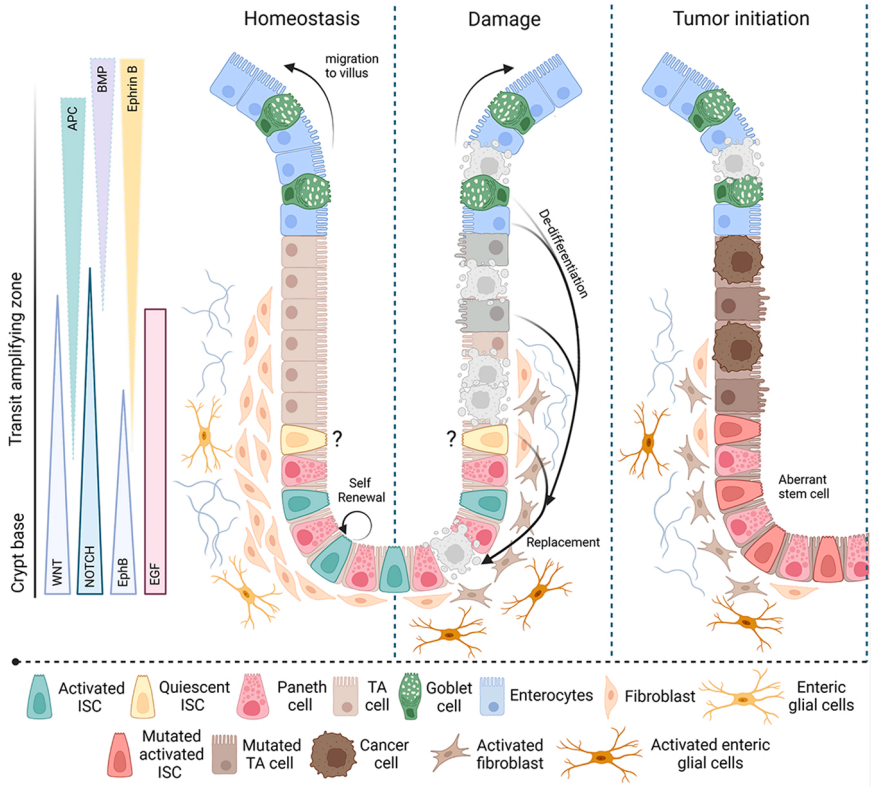

Fig. 1. Scheme depicting the homeostatic and pathological cell composition of the crypt compartment.

decreases with aging of the individual [17]. In contrast, in vitro experiments showed that during organoid early growth, ISCs stop expressing Lgr5, which become upregulated later on concomitantly with Paneth cell differentiation and crypt formation [22].

Lgr5^{+}-ISCs are small columnar cells interspersed with differentiated Paneth cells which are niche cells located at the base of the crypt [1,4,34,35] (Fig. 1). Paneth cells have been described to appear in the first 14 days after birth in mice, when crypts mature and multiply through fission event [23], and to remain stable up to 2 months [36]. Importantly, Paneth cells provide a short-range stemness signal, as they secrete growth factors required for ISC physiology such as Notch, EGF or Wnt ligands [4,37]. Thus, the way ISCs divide and re-intercalate between Paneth cells in the crypt base would be instrumental for their self-renewal capacity. In this context, McKinley and colleagues recently proposed that Paneth cells intercalate between ISC daughter cells at the end of cytokinesis in mouse organoids [38].

The second subset of ISCs, namely "reserve" or "quiescent stem cells" (QSCs), have been proposed at the + 4 position above the crypt base and would express Bmi1 [39], mTert [40], Hopx [41] and Lrig1 [42] (Fig. 1). + 4-QSCs would divide slower than Lgr5^{+}-ISCs and would be required for intestinal tissue replenishment and repair after damage or injury [40,42–44] (Fig. 1). However, the specificity of marker expression such as Bmi1 in + 4-QSCs is now controversial [45,46]. In addition, this existence of these + 4 reserve cells is an ongoing debate. TA cells and + 4-QSC cells may constitute overlapping cell populations, as lineage-committed TA cells are also able to regenerate the epithelium [47,48]. Moreover, transcriptional analyses of embryonic progenitors have revealed that distinct subpopulations give rise to Lgr5^{+}-ISCs, which may explain the heterogeneity of ISCs in adults [49]. Additional studies have also supported cellular plasticity and re-activation of a fetal-like program to sustain tissue repair in colitis model [50] or after parasite infection, for instance [51]. Along this line, A.Gregorieff and colleagues have uncovered at the crypt bottom the existence of rare stem cell subtype so called "revival stem cell" that exhibit high level of Clusterin expression and are quiescent in homeostatic conditions. Under intestinal damage, they are transiently overproduced in the crypt in a YAP-dependent manner to reconstitute the Lgr5-ISC pool and repair the epithelium [52]. In a similar manner, pericryptal fibroblasts in the mesenchyme induce the expansion of a Sca-1^{+}/Clusterin^{+} - revival-like stem cell population for the activation of tissue regeneration program or tumor initiation [53].

The second subset of mitotically active cells in the intestinal epithelium are TA cells which derive from Lgr5^{+}-ISC divisions [54] (Fig. 1). TA cells are generated to continuously replenish the homeostatic epithelium. Several rounds of division (4−5) of 12 h on average occur in this tissue zone, and TA cells would generate up to 300 cells per crypt per day. They remain in the crypt for 48 h on average and then go towards terminal differentiation when crossing the crypt-villus boundary [55,56].

In addition to the classical mouse model, alternative animal models have emerged for the study of dividing cells in the intestinal tissue. In zebrafish (*Danio renio*) and teleost fish medaka (*Oryzias latipes*), the intestine does not display a tissue architecture as regular as the mammalian one but it exhibits tissue folds that may resemble the fetal villus in mammals. In this animal model, ISCs are restricted to fold bases or "intervillus pockets" during the first weeks of the postembryonic period, in a similar manner as for Lgr5^{+}-ISC patterning in newborn mice. Migration of cell progenies along folds, apoptosis at the villus tip and multiplication of secretory cells at the folded base occur starting from the third postembryonic week [57–60]. This arrangement is maintained through adult.

life in zebrafish, where "flat clusters" function as ISC niches [61]. In the adult *Drosophila* midgut, although the intestinal tissue remains flat and does not display any particular architecture, tissue compartmentalization also takes place. However, like mammalian ISCs [62], [63], fly ISCs are found in diverse midgut regions, where their number and gene expression signature vary, suggesting that their heterogeneity may sustain specific functionalization of tissue domains [64]. In addition, equivalent of TA cells or Paneth niche cells has not been yet reported in *Drosophila* [65,66].

3. Cell division mechanism and specificities in the intestinal tissue

Epithelia exhibit extensive cell divisions, needed for constant tissue renewal. Since epithelia provide critical barrier functions required for the compartmentalization of multicellular organisms, cell division must occur in a manner that preserves epithelial integrity. Mitotic cells must thus orient their division plane along the appropriate axis of the tissue while maintaining tight cell-cell junctions with their neighbors. When unchecked, spindle mis-positioning may impair the functional structure of a tissue and lead to various pathologies, including cancer. Such deregulations in division positioning may expose epithelial cells to non-conformal environments and cause dysplasia, hyperplasia, epithelial to mesenchymal transition, metastasis, or the emergence of cancer stem cell populations in stem cell niches [67–72]. In addition, in multiple tissues the orientation of cell divisions can influence fate lineage as well as the positioning of daughter cells along the tissue, ensuring a proper spatial distribution and balance of different cell types and giving rise to the functional structure of a tissue [73].

During cell division, the DNA material needs to be properly segregated between the two daughter cells to avoid the emergence of aneuploidy. This process is controlled by the mitotic spindle, a bipolar structure made of microtubules (MTs) and motors, with centrosomes located at spindle poles that nucleate and organize MTs. Spindles feature different populations of MTs, some that attach chromosomes through kinetochores, and astral MTs that radiate from spindle poles towards the cell cortex, and exert forces and torques that position and orient the spindle [67,74,75] (Fig. 2). In many cells, the distribution of MT forces depends on the spatial regulation of MT (-)-end directed dynein motors, which pull MTs. This spatial activity may be dictated by multiple cues including intrinsic polarity and environmental signals [67,69,76–78]. Therefore, how a mitotic spindle will be oriented in tissues may be influenced by diverse factors, including cell geometry [79], cell adhesion patterns [80], internal polarity cues [81], as well as external forces from the surrounding tissue [82,83].

Genetic studies in model organisms such as *Drosophila* and *C. elegans* first identified cell polarity regulators as key components in the molecular regulation of spindle orientation [84]. Pins (Partner of Inscuteable), the homolog of mammalian LGN (Leucine-Glycine-Asparagine), and Gαi subunits were for instance characterized in the control of spindle orientation in *Drosophila* embryonic neuroblasts [85–87]. In

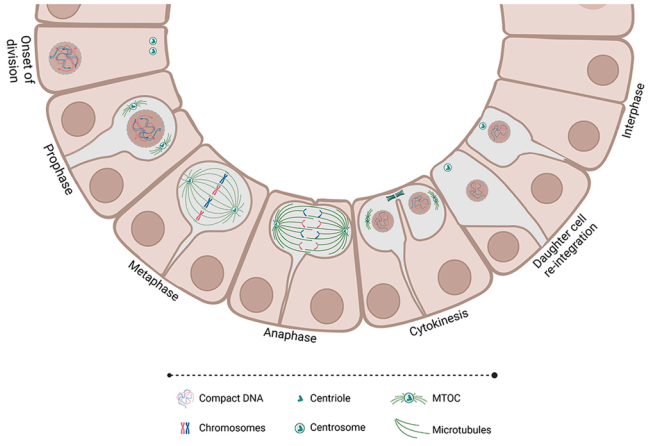

Fig. 2. Scheme depicting the process of cell division at the bottom crypt in the intestinal tissue.

most animal cells, this complex is localized at the cell cortex and interacts with NuMA (Nuclear and Mitotic Apparatus), which is released from the nucleus upon nuclear envelope breakdown at the onset of mitosis. NuMA recruits dynein to the cortex which binds and pulls astral MTs to orient the mitotic spindle and cell division [88,89]. Consequently, the localization of the LGN complex at a specific subcortical domain often dictates the site of force concentration that will act on astral MTs to orient the mitotic spindle and determine the division axis [90]. For instance, in many monolayered epithelia LGN and NuMA are recruited along the tissue plane at the level of apical cell junctions ensuring that the spindle lies in the plane of the tissue to safeguard tissue architecture [67].

3.1. Cellular remodeling during division

During intestinal development, between E10.5 and E14.5, interkinetic nuclear migration (INM) takes place, and nuclei are alternating in the epithelial layer. However, two distinct pools of dividing cells can be considered according to their shape in the pseudostratified epithelium: elongated cells that participate in the expansion of the epithelial layer, and rounded cells that participate in the invagination of the apical surface and initial stages of villus formation [14].

In the adult intestine, epithelial cells are tall columnar cells with a high aspect ratio and their nuclei sit basally during interphase. When cell division is initiated, in prophase, the DNA condenses, and the nucleus is pushed towards the apical side through an INM process [91–94] (Fig. 2). Concomitantly, the cell undergoes shape changes into a more rounded cell shape positioned towards the apical side of the monolayer. Throughout the rest of the division phases, the apical side of the mitotic cell stays intact, aligned with the rest of the monolayer but the basal membrane is remodeled and the cell stays in contact with the basement membrane through an extension of the membrane filled with actin cables that is referred to as the “basal foot” or “filopodial pathfinding” [10,38,95] (Fig. 2). The basal process would be essential for daughter cells to move back to the basal side of the monolayer, and reintegrate the tissue at the end of cytokinesis [38,95].

Although diverse cytoskeletal networks have been reported as key to the INM process, their exact function remains controversial. In fact, MTs and actomyosin networks may constitute the main force generators described so far [95,96]. By analyzing the ventricular zone of the developing mouse central nervous system, Shenk and colleagues pioneered this field of research [97]. They demonstrated that myosin-II activity was required for force generation during the INM process, as blebbistatin treatment abolished apical DNA migration in progenitors [97]. In addition, Meyer et al. showed that the basal process is enriched in actin which is required for apical migration of the DNA in *Drosophila* imaginal discs [96]. Yet so far, the basal process does not exhibit any contractile property, as activated myosin is only located at the mitotic cortex for cell rounding and DNA migration [96]. Similar observations were made in the intestinal crypt [95]. On the other side, MTs are not enriched in the mitotic basal process of *Drosophila* imaginal discs [96]. However, Näthke and colleagues proposed a significant contribution of the MT cytoskeleton during intestinal INM, based on immunofluorescence analyses of fixed samples [95].

3.2. Orientation of cell division in the crypt

Once INM is complete, spindles form and mitosis proceeds. Centrosomes are located apically in interphase cells and become aligned laterally with condensed chromosomes during prometaphase (Fig. 2). The angle of the mitotic spindle relative to the apical surface of the monolayer can greatly vary before stabilizing at metaphase [98]. As in most of the epithelial tissues, the spindles in the mammalian intestine have been shown to display a planar orientation [93,98] but, to date, how polarity complexes, MTs and force generators may be distributed in dividing intestinal cells to control planar spindle orientation remains poorly addressed. In a recent preprint, 3D imaging of dividing cells in the crypt suggests that polarity effectors including NuMA, LGN and Afadin, may be segregated to the basal poles of mitotic cells away and orthogonal to mitotic spindles [99]. This unconventional localization sharply contrasts with the stereotypical recruitment of these effectors at the level of apical junctionsin other model epithelia. Using mathematical models and high-resolution expansion microscopy, the authors suggest that mitotic astral MTs may be limited by Kif18b, a depolymerizing kinesin which allow spindles to probe local apical cellular geometries to orient the spindle in the tissue plane.

However, the orientation of ISC division per se remain controversial. Pioneering studies by Fleming et al. demonstrated that ISCs divide symmetrically [98]. Similar data were further reported in the subsequent literature [34,93]. Particularly, 3D imaging of the entire crypt compartment allowed to demonstrate that 100% of dividing cells orient horizontally in the tissue plane [93]. In this context, planar cell polarity might be a good candidate for spindle orientation, as loss of planar cell polarity components in other systems disrupt spindle orientation [100–102]. In sharp contrast, Quyn et al. showed that a spatial regulation of cell division orientation occurs in the crypts: dividing cells orient orthogonal to the epithelium plane in the stem cell compartment but in a planar manner in the TA area [70]. More recently, Sei and colleagues reported that ISCs would preferentially follow an orthogonal cell division by computational modeling as well as following ISC mitosis in vivo [103]. Changes in spindle orientation along the normal crypt axis might contribute to the maintenance of a constant number of ISCs in the niche [70,103]. The distribution of symmetric or asymmetric spindle orientation may rely on the opposite Wnt and Apc gradients [104,105]. Moreover, a delay in the transition between symmetric and asymmetric divisions is observed following a mutation in *Apc* in the crypt [105–107].

It is important to mention at this stage that a great deal of confusion has arisen in this field of research, and, commonly, the terms symmetry/asymmetry are used to designate either the orientation of cell division or the cell fate progeny. In fly, asymmetry in the division plane is directly linked to cell fate asymmetry. Indeed, 70–90% of ISCs generate asymmetric division and will generate asymmetric progeny, i.e. one new ISC and one enteroblast which will ultimately differentiate into enterocyte or enteroendocrine cell [65,66]. Symmetric ISC division occurs during ISC duplication in this model [108,109]. Whether the orientation of ISC division is also directly related to cell fate decisions in mammals remains to be investigated and deserves careful examination with optimal spatio-temporal resolution.

3.3. Daughter cell re-integration

The correct positioning of daughter cells after division within the epithelial layer is important to determine the architecture of the tissue [110–112]. At the end of mitosis, the cleavage furrow extends toward the apical surface and progressively separates the nascent daughter cells [98] (Fig. 2). In intestinal crypts, cytokinesis is tightly controlled. For instance, Cdc42 regulates Rab8a vesicle trafficking during cytokinesis [113], and Cdc42 depletion in mice provokes defective cytokinesis, decreased clonal expansion of $Lgr5^{+}$-ISCs, crypt enlargement and ultimately hyperplasia [113–115]. Moreover, Zhang et al. revealed that a conditional Cdc42-KO leads to a reduction of ISCs and an increase of TA cells. They concluded that Cdc42 may be at the center of Hippo/YAP signaling for the control of cell fate balance between ISCs and TA cells [115].

After cytokinesis, both daughter cells slowly migrate basally until their nuclei align with adjacent interphase cells, and assume a columnar shape (Fig. 2) [38]. Whereas apical migration has been suggested to be an active process, basal re-integration after cytokinesis may involve more passive processes [95].

During development, between E10.5 and E14.5 in the pseudostratified epithelium, two modes of daughter cell re-integration have

been reported [10]. In the first scenario, the daughter cell that possesses a basal process re-integrates twice faster than the other one by taking advantage of this pre-existing contact with the basal surface. The remaining daughter cell has to project between neighboring cells in a "pathfinding" manner. In the second scenario, both daughter cells use the pathfinding mode and thus delay their re-integration in the epithelial monolayer by intercalating between neighboring cells [10]. At this developmental stage, Wnt5a is instrumental for filopodial pathfinding mode during daughter cell re-integration. In the absence of Wnt5a expression in the mesenchyme, daughter cells fail to extend filopodial extrusion, remain at the apical surface and some die through apoptosis [10].

In adults, daughter cell repositioning displays a strong asymmetric behavior, as only one daughter cell exhibits a basal process. Interestingly, this phenomenon is accompanied by an anisotropic displacement of cells in the plane of the epithelial monolayer along the crypt axis [93]. Moreover, it has been observed that some sister cells remain neighbors, while other sisters separate and reintegrate into the tissue apart from each other. This latter process is called interspersion and would promote the ISC exit from the niche toward the TA zone and their subsequent differentiation [38,95]. Interestingly, it was suggested that sister ISC separation is more efficient when an ISC divides on top of stiff and strongly adherent substrates [116,117]. In general, the functional significance of the heterogeneity of daughter cell re-integration is still missing. It will be interesting to determine in the future whether the mode and geometry of daughter cell intercalation in the epithelial monolayer influence their fate and behavior.

3.4. Symmetry and asymmetry of cell fate

With respect to cell fate and to sustain tissue renewal in a balanced manner, ISCs can divide symmetrically to generate two new ISCs or two TA cells, or asymmetrically with the formation of one ISC and one TA [56]. The initial dogma arose from genetic models in vivo and mathematic modeling. The choice between the two scenarios helps preserve ISC and progeny pools, and is crucial for tissue homeostasis. However, lineage tracing studies proposed that cell division would follow a stochastic pattern described as "neutral drift" dynamics, in which Lrg5 + divisions are predominantly symmetric and compete for space within the niche [34,118]. Thus, ISC asymmetry in terms of fate would be established at the level of the entire pool of ISC as they lose contact with neighboring Paneth cells [34,118]. Recently, a counter-proposal has favored instead an asymmetric cell division-dominant neutral drift model, which was also validated in vivo [103]. Nevertheless, the mode of ISC divisions would be temporally regulated, as in newborns symmetric modes of division occur whereas asymmetric divisions would sustain crypt growth and differentiated lineage implementation in adult mice [119]. More recently, a study that combined tracking of single cells after time-lapse microscopy of mouse intestinal organoids and computational modeling, demonstrated that a vast majority of dividing cells divide symmetrically in terms of cell progeny. This mechanism would allow to maintain a constant number of cells under proliferation in the crypt compartment [120].

Despite accumulating knowledge, our understanding of the regulation of intestinal cell fate is very limited, particularly with regard to the mechanisms that take place during cell division and that determine the symmetry or asymmetry of ISC fate. In particular, the orientation of the mitotic spindle relative to intrinsic polarity cues, as observed in neuroblasts, remains a hypothesis of choice to coordinate the spatial segregation of cellular determinants with the physical division of the two daughter cells. The control of spindle orientation has been suggested to be one of the principal factors in the specification of symmetric versus asymmetric intestinal cell division [121]. Importantly, cell fate asymmetry is not necessarily linked to an asymmetry in the size or shape of daughter cells. Bellis et al., demonstrated that some planar (symmetric) ISC divisions display asymmetric mNumb segregation and subsequent daughter cell asymmetry with respect to cell fate [93]. Other asymmetric events during mitosis have also been proposed as determinants of asymmetric cell fate. As an example, the anisotropy of daughter cell movement would trigger asymmetry in terms of fate [93].

In other tissue models, additional mitotic events are associated with the spindle itself, such as the inheritance of the mother or daughter centrosome, and that of the "mid-body" in one of the two daughter cells [122–124]. Others concern the asymmetric segregation of adhesion factors or of cytoplasmic constituents associated with degradation processes, such as lysosomes, mitophagosomes or autophagosomes [125]. As an example, Katajisto et al., demonstrated that newly synthetized mitochondria preferentially segregate in ISC daughter cells that retain their stemness [126]. Finally, asymmetries may also occur at the level of genome or epigenome segregation. In some cases, preferential inheritance of the old DNA strand by the stem cell has been observed [127]. Similarly, asymmetric segregation of new and old histone proteins has been described and shown to be driven by mitotic histone modifications [128,129].

Even if an asymmetric division is likely the triggering event for a change in cell fate, very often other aspects of post-mitotic maturation allow the complete differentiation of one of the two daughter cells. Central to intestinal homeostasis, different cell types surrounding the niche maintain the activity and the fate of ISCs by producing signaling factors. In particular, canonical factors like Wnt, Notch and Noggin, secreted by the neighboring Paneth cells, maintain the self-renewal of $Lgr5^{+}$-ISCs within the niche. In addition, the ISC fate is also controlled by the overall architecture of the tissue and by cell-to-cell contacts, which can exert physical signals that control ISC division. Over the last ten years, accumulating experimental evidence has led to the conclusion that mechanical tissue properties have the power of directing a variety of cell functions including cell proliferation and differentiation [130]. Cells interact with their environment through cell-substrate and cell-cell adhesions, and sense the mechanical status of the matrix and neighboring cells. Through mechano-transduction processes, cells can thus adapt in response to the physical properties of the tissue and modulate their organization and homeostasis [131,132]. For example, matrix rigidity regulates ISC proliferation, organoid expansion and intestinal differentiation [133]. To date, only few studies concentrated on the ISC niche by itself. Nevertheless, it is known that the rigidity of the extracellular matrix and the activity of myofibroblasts are important for ISC homeostasis and that their alteration is found in certain pathologies such as fibrosis or cancer development [134–138].

In fly, ISC fate asymmetry would be accomplished by an integrin-dependent cell-basal membrane interaction that provokes the redistribution of apical polarity complex Par in the apically positioned daughter cell [139]. More recently, Bardin and colleagues showed that Numb distribution during cell division conditions the cell fate choice in EE cells in *Drosophila* [140]. In addition, a direct role of cell division orientation in fly ISC fate has been provided by Hu et al. [141]. The authors showed that in young flies, a balance between asymmetric and symmetric cell division with respect to fate takes place to modulate organ size. This switch in cell fate is ensured by a change in the cell division mode, from oblique to planar, respectively. At the molecular level, this change is triggered by the phosphorylation of Jun-N-terminal kinase (JNK) and its consecutive binding to Wrd62 at the spindle pole, the repression of Kif1a expression and loss of cortical Mud, the homologue of NuMA [141].

Note that all the aforementioned diverse hypotheses, which are not mutually exclusive, have been put forward and tested in different invertebrate models in majority, or in isolated mammalian stem cell culture systems without a niche. However, to date, they remain unstudied in the mammalian intestinal tissue.

4. Regulation of cell division rate in the intestinal tissue

Diverse physiological situations modulate the rate of cell division, in addition to canonical cell cycle regulators such as cyclin-dependent

kinase (CDKs), CDK inhibitors (CKIs) and transcription factors.

4.1. Development and aging

Proliferation rates are exacerbated during development. By using theoretical models and single-molecule FISH experiments, Itzkovitz et al. demonstrated that over-proliferation of ISCs occurs during crypt growth in the mouse intestine. On the other side, the ISC cycling time increases during adult life [119]. In addition, ISC division rate decreases with aging in human and mouse small intestine due to reduced Wnt signaling [142–146]. Such a decrease in the proliferative capacity of intestinal tissues has serious consequences for tissue repair following stress in elderly individuals. Interestingly, a recent study showed that the defective potential of aging crypts can be modulated by down-regulating Cdc42 activity and thereby recovering the regenerating potential of ISCs, opening new perspectives for the repair of the aging intestinal epithelium [144]. In flies, it has been shown that aging is accompanied by a disturbance in mitochondrial metabolism and an elevation in oxidative stress, causing ISC over-proliferation and an impairment of differentiation. This phenomenon often leads to intestinal dysplasia in old specimens [147–149].

4.2. Microenvironment

The microenvironment modulates the regenerative response by influencing the activity of various signaling pathways, including Wnt, Notch, EGFR/MAPK, Apc, EpbB, BMP, Hippo or Activin A among others [150–153]. Wnt signaling would not be required during the first stage of intestinal development, before villus formation [154,155]. Under the depletion of beta-catenin or Lrp5–6 (Frizzled co-receptors), no impact on the proliferation of embryonic progenitors was observed in the pseudostratified epithelium. Effects of Wnt signaling would only occur later during villification (E15.5) [156]. In adult physiological situations, titration of the Wnt signaling cascade constitutes a key pathway that regulates ISC proliferation and differentiation [6,35,157,158]. Apc and Wnt signaling components (e.g., survivin) are required for mitosis. This molecular mechanism establishes a zone in the lower crypt where conditions are optimal for maximal cell division rates and mitotic spindle orientation [105,159].

Intestinal proliferation is not only controlled by cellular components, but also in response to mechanical inputs such as stretch or compression as reviewed recently [160]. Moreover, under stress or damage provoked by treatments with genotoxins or pathogens, there is an increase of ISC division in flies [161,162].

More surprisingly, cell division would be subject to a regulation by the circadian rhythm. Habits such as bedtime/ getting up at regular times or light limitation at night would be critical to keeping the homeostasis of the circadian rhythm and therefore a proper intestinal function [163]. Interestingly, fluctuations in cell proliferation during the day have been reported in mice [164]. In addition, in the zebrafish intestine, M phase may be light-sensitive and under the control of the circadian clock, through molecular modulations of p21, PCNA and Cdck2 gene expression [165]. This mode of cell division regulation may probably be indirect and related to the consequences per se of the circadian rhythm such as hormone levels or variations in body temperature [163]. Modulation of cell division by the circadian rhythm would be crucial for gut physiology [166,167], as evidenced as an example by Bishehsari and colleagues [168]. Finally, disruption of the circadian rhythm worsens the inflammatory response and polyp development in predisposed mice mutated for *Apc* [168].

4.3. Radiotherapy

Ionizing radiation is commonly used in the treatment of various cancers. However, this therapy has side effects such as diarrhea or intestinal failure. This radiosensitivity may emerge from a strong decrease in the number of active proliferating ISCs after treatment [169,170]. This is largely due to the fact that ISCs cannot activate DNA repair pathways and therefore undergo p53-dependent apoptosis [171,172]. However, + 4 quiescent ISCs are not affected by ionizing radiation and are thus radioresistant [173,174]. + 4 quiescent ISCs become mitotically active upon exposure to radiations but fail to replenish the intestinal epithelium in the long run. However, the overexpression of TP53-induced glycolysis and apoptosis regulator (TIGAR) has been recently reported to boost + 4 quiescent ISC proliferation after irradiation treatment [175].

4.4. Nutrition and metabolism

Although the concept is appealing, molecular mechanisms by which nutrients may influence gut homeostasis are not yet fully demonstrated. So far, a dietary control of the intestinal niche through nutrient availability and modulation of metabolic pathways, has been proposed in the literature with direct repercussions on ISC proliferation rates, fates and modes of division [176,177]. Starvation leads to the expansion of the ISC population in zebrafish and flies [141,165]. Besides, the fasting of mice during 24 h increases ISC renewal and provokes crypt expansion, at the expense of cell differentiation [176,178]. Similarly, the intestinal tissue of patients under parenteral nutrition (and therefore, under food uptake restriction) displays villus atrophy and a decrease in the mitotic index but the number of crypts remains constant [179]. In fact, caloric restriction regulates mitochondrial metabolism, thus delaying the effects of aging and protecting against many diseases [148,180]. Fasting would thus be a way to boost ISC biology and tissue regeneration in aged or injured individuals [176,181]. The mTOR (mechanistic Target Of Rapamycin) pathway is at the core of a nutrient-sensing mechanism. Indeed, food uptake limitation and consequently calorie restriction leads to mTOR complex-1 (mTORC1) signaling downregulation in Paneth cells to favor $Lgr5^{+}$-ISC stemness [44,178].

On the other hand, over-nutrition which leads to obesity and prediabetes, engenders ISC hyperproliferation and accelerated differentiation in mice [182]. As an example, high fat diet causes global morphological changes in the crypt-villus axis. Indeed, fatty acids stimulate cell proliferation, threatening intestinal regeneration and increasing the risk of hyperplasia and tumor formation in mice [183,184]. From a mechanistic point of view, this would involve the transcription factor PPAR-delta (Peroxisome Proliferator-Activated Receptor) which reinforces the ISC stemness potential [183].

Interestingly, food intake in mice may modify the orientation of cell division by itself. When mice are fed *ad libidum*, a majority of divisions are planar but when fasting, divisions become mainly orthogonal to the tissue surface [185]. In addition, glucose supplementation in intestinal organoids switches the cell division orientation towards symmetric/planar [185]. Moreover, a direct link between starvation and increase of JNK signaling, loss of Mud cortical recruitment, abolishment of astral MT-based spindle orientation and planar cell division has been reported in flies [141].

5. Impact of cell division on the intestinal tissue

Cell division impacts tissue organization during developmental or pathological processes. Any fluctuation in the orientation or rate of cell division has large-scale repercussions on tissue homeostasis. We describe here few examples for the intestinal tissue.

5.1. Crypt dynamics

Crypt fission is a normal developmental event that is required for intestinal expansion during embryogenesis and childhood in humans, and postnatal maturation before weaning in mice [186–188]' [189]. Low rate of crypt fission takes place during adulthood under physiological conditions [189]. When the intestinal epithelium is damaged, for

instance in case of inflammatory bowel disease [190] or after irradiation [191], the rate of this morphogenetic event dramatically increases upon regeneration to generate new crypts. This phenomenon is driven by a slow and continuous process that is composed of three distinct phases and called the "crypt cycle" [192]. First, during the "development phase", the crypt grows in size up to a threshold equivalent to twice the initial ISC pool. At this point, a tissue bud develops at the crypt bottom crypt and triggers a "bifurcation phase". This bifurcation process will go up along the crypt until a total fission gives rise to two identical crypts. Any disruption during this crypt cycle spawns so-called "corrupted crypts" which participate in the development of adenomas and adenocarcinomas in the small intestine and colon [193–196]. Those aberrantly shaped crypts would be the result of the hyperproliferation of mutated cells, which causes a truncated crypt cycle, resulting in mostly asymmetric or incomplete fission. Among the mutations involved, *KRas*, *TP53* and *Apc* are associated with adenocarcinoma development in 45%, 54% and 85% of cases [197].

The link between abnormal cryptogenesis and tumor initiation is best described in the context of mutations in the tumor-suppressor gene *Apc*. Heterozygous mutations in humans (Familial adenomatous polyposis (FAP) patients) or $Apc^{+/-}$ mice, lead to a loss of proper crypt organization and a dramatic over-proliferation of niche cell populations that progress towards the top of the crypt [106]. In addition, the *Apc* mutation also impacts the orientation of mitotic spindles by regulating the (+)-end dynamics of MTs [122]. In the case of a heterozygous mutation of *Apc* (in humans and mice), the preferential mode of planar division orientation is lost and could be at the origin of changes in cell shape and consequently in the structure of the crypt [70,93]. Along this line, Boman and Fields [105] have proposed a Apc:Wnt counter-gradient which would explain the dual effect of *Apc* mutations. According to their study, the default in spindle orientation following the loss of Apc leads to the expansion of cells along the crypt, resulting in a net increase in cell proliferation. As tissue budding is no longer confined to the bottom of the crypt, asymmetrical crypt fission prevails [195,196] with the generation of corrupted crypts and micro-adenomas, i.e. precursors of adenomas, that can degenerate into adenocarcinomas [107,194,195].

5.2. Microenvironment

Over the last decade, accumulating experimental evidence has led to the conclusion that mechanical tissue properties have the power of directing a variety of cell functions including cell proliferation and differentiation [130]. Cells interact with their environment through cell-substrate and cell-cell adhesions, and sense the mechanical status of the matrix and neighboring cells. Using mechano-transduction pathways, cells can thus adapt in response to the physical properties of the tissue and modulate their organization and homeostasis [131,132]. As an example, Farge and colleagues elegantly demonstrated that colonic tumor physical properties directly impinge on healthy surrounding tissues. Their study stemmed from the initial observation of the existence of a mechanical pressure induced by hyper-proliferation in tumoral areas. By mimicking such mechanical pressure on healthy tissue by injecting magnetic nano-crystals and applying mechanical stresses, they caused a nuclear translocation of beta-catenin in yet non-tumoral areas, followed by the overexpression of beta-catenin target genes and tumor-related abnormalities in the colon tissue [198]. Moreover, matrix rigidity regulates stem cell proliferation, organoid expansion and intestinal differentiation [133] and extracellular matrix stiffness and physical constraints applied to the niche compartment influence the ISC behavior [136,137]. In addition, physical properties are able to directly modulate the orientation of cell division in diverse tissues [83,133,199,200] but this assumption remains to be demonstrated in the intestinal tissue.

Damages to the intestinal stem cell niche can result from mechanical stress, infections, chronic inflammation or cytotoxic therapies. Stem cells develop specific DNA damage responses [201], the underlying mechanisms of which are incompletely understood. Recent work by Romagnolo and colleagues showed that intestinal stem cells have an increased capacity to prevent the accumulation of genetic damage [202]. Unlike other proliferative intestinal cells, stem cells show very few DNA double-strand breaks following irradiation. However, this resistance to DNA damage is abolished when autophagy is inhibited [202]. In addition, mesenchymal cells and immune cells become more abundant and secrete signaling molecules that promote the regenerative process [48]. This regeneration process is influenced by the nutritional state [185], the microbiome [203], [204], GLI1-expressing mesenchymal cells and Wnt signaling [205–207], the extracellular matrix [50,208] and the enteric nervous system [7]. During injury, a single clone of ISC is sufficient to induce crypt regeneration [209]. However, if the injury has destroyed some crypts, the few surviving crypt stem cells can divide to increase their numbers, and subsequently restore sufficient numbers of crypts by crypt fission, to maintain epithelial homeostasis [209].

5.3. Tumor development

Rapid cell proliferation constitutes one characteristic feature of cancer development but dysregulation of cell division is also directly associated with oncogenic transformation and expansion of cancer stem cells [210,211] (Fig. 3). In *Drosophila*, centrosome amplification or kinetochore disruption causes chromosome mis-segregation, aneuploidy and ultimately the accumulation of ISCs, leading to an intestinal dysplasic phenotype [212,213]. In the same model, spindle orientation disruption by abolishment of integrin-dependent signaling or direct perturbations of spindle positioning leads to an over-proliferative phenotype [139]. Furthermore, regulators of cell division orientation are often proposed to participate in tumor progression. For example, the mutation of Pins (LGN) in *Drosophila* causes tumor-like phenotypes in neuroblasts [214].

Although a direct link between division mis-orientation and intestinal tumorigenesis has been well described in invertebrates, a precise demonstration of such effect in mammals is still lacking [215]. Mutations accumulate during stem cell division during aging, and this increases the risk of cancer [145,216]. Along this line, *Apc* mutations constitute a first hit of colorectal cancer initiation [217]. Loss of Apc in Lgr5 + ISCs leads to rapid adenoma formation within 3–4 weeks, whereas no microadenomas are observed when *Apc* deletion is induced in non-stem cells such as TA cells, with only the formation of non-progressing adenomatous crypts. Thus, Apc depletion in ISC drives intestinal neoplasia [218]. On the other hand, Näthke and colleagues showed that the Apc^{Min} mutation in mouse or human adenoma leads to randomly oriented cell division of both ISCs and TA cells and defective basal foot formation, provoking changes in crypt architecture and crypt base enlargement [70,95]. By contrast, Bellis et al., showed that both daughter cells grow a basal process in $Apc^{Min/+}$ mice. As a result, directed movement of cells within the crypt is abolished in the mutant tissue [93]. However, it remains difficult to distinguish between the role of Apc in modulating signaling pathways and cell proliferation on the one hand, from its role in the orientation of divisions for tumor initiation on the other hand.

6. Conclusion

The intestinal tissue is one of the most proliferative in the body and is, therefore, one of the most exposed to the development of tumorigenesis and other diseases. To date, however, the current literature that documents how the division process is regulated in intestinal crypt tissues, remains sparse, with many fundamental aspects that remain unanswered or controversial. Contrasting findings may be related to experimental details, e.g., nutrient availability, harsh environment, micro-injuries or aging both in mice and flies [77], [48] or from the frequent confusion between cell fate symmetry and geometries of cell divisions.

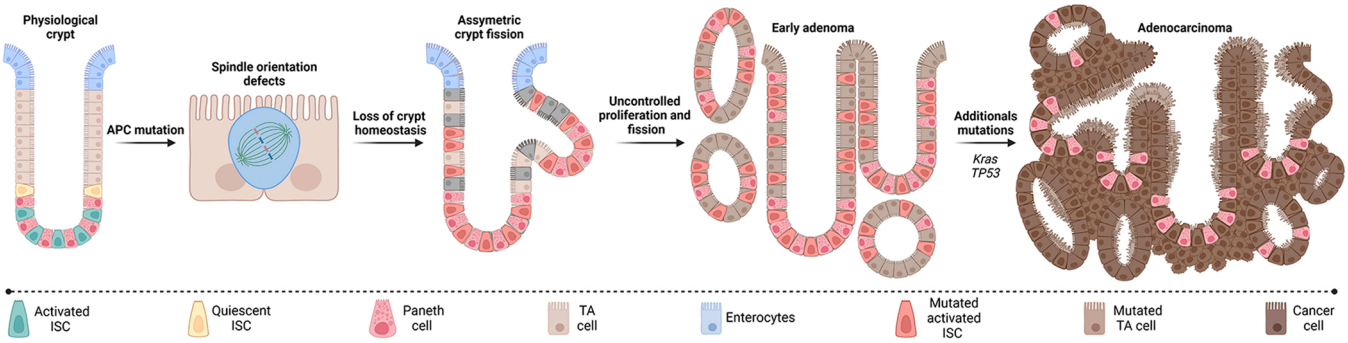

Fig. 3. Scheme showing the successive steps leading to tumor development in the intestine.

The popularization of intestinal organoid cultures and biomimetic micro-engineered devices will now make it possible to overcome variabilities in environmental factors as well as to gain in spatio-temporal resolution within crypt domains [25,160,219–222]. This approach will certainly foster fundamental knowledge of how cell division processes are regulated and how they promote the organization and function of the intestinal tissue [25,38,73,223].

Conflict of interest

All authors declare no conflict of interest.

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