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Decrypting the communication between microbes and the intestinal mucosa-A brief review on Pathogénie Microbienne Moléculaire's latest research

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5 **Decrypting the communication between microbes and the intestinal**
6 **mucosa – A brief review on PMM’s latest research**
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24 **ABSTRACT**
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28 Over the past 10 years, the “Pathogénie Microbienne Moléculaire”
29 (PMM) unit of Prof. Philippe Sansonetti has studied the molecular cross-talk
30 between the intestinal microbiota and the gut epithelium aiming to better
31 understand how this mutualistic symbiosis delineates homeostasis and, when
32 perturbed, prompts pathology. To do so, the unit has manipulated both
33 bacterial and epithelial cells, and used cutting-edge technology. More
34 recently, the lab has turned its focus also on studying the intestinal crypt and
35 more specifically the intestinal stem cell (ISC), for their role in epithelial
36 regeneration and long-term epithelium renewal. Here, we provide a brief
37 review summarizing recent results obtained from the lab, with particular
38 focus on the intestinal crypt.
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54 **INTRODUCTION**
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56 The microbiota is a complex ecosystem inhabiting a specific area of the
57 body (e.g. skin, oral cavity, intestine) and is comprised of a diverse
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3 community of microorganisms, predominately bacteria but also including
4 others such as archaea, fungi, protozoa and viruses. The best studied to-date
5 is the intestinal microbiota, in particular the one of the colon, where
6 Bacteroidetes and Firmicutes represent the dominant phyla.
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11 The microbiota has been referred to as the “forgotten organ” (O'Hara
12 and Shanahan, 2006) as it plays a central role in health and disease by
13 maintaining constant communication with the host epithelium either directly,
14 or via bacterial products and metabolites.
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20 Within the vast arsenal of microorganisms populating the gut, it is
21 important to define categories exceeding species and genera-based definitions
22 to encompass functionality and potential contribution to a healthy steady-
23 state. The so-called commensals (e.g. Lactobacilli and Bifidobacteria) have
24 established a symbiotic interaction with the host, while pathobionts, as is the
25 case for the segmented filamentous bacteria (SFB), have the ability to promote
26 immune maturation, but can also drive chronic inflammation, in the
27 susceptible host. Pathogens, like *Shigella flexneri* on the other hand, are
28 defined as microorganisms able to break the homeostatic barrier causing
29 acute inflammation and infection. Among them, opportunistic pathogens take
30 advantage of environmental changes, which may derive from a (transient)
31 weakening of the host immune defense, an altered microbiota or physical
32 damages, as in the example of *Streptococcus gallolyticus* (Hornef, 2015; Aymeric
33 and Dramsi, 2018; Schnupf *et al.*, 2018). The microbiota provides a natural
34 defense barrier against pathogens by mechanisms, coining the term
35 “colonization resistance”, which include the occupation of common ecological
36 niches and receptors, or the production of growth inhibitors, e.g. bacteriocins
37 (Corr *et al.*, 2007; Kommineni *et al.*, 2015). In addition, it has important
38 metabolic functions such as the fermentation of non-digestible dietary
39 residues, vitamin production, and the detoxification of dietary carcinogens
40 (Sansonetti, 2008). The microbiota influences specific physical properties of
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3 the intestinal barrier, such as the composition and thickness of the mucus
4 layer, epithelial tight junctions (Ulluwishewa *et al.*, 2011; Jakobsson *et al.*,
5 2015) and it also interacts with the mucosal immune system by stimulating its
6 development and maturation (Chow *et al.*, 2010). Notably, recent studies
7 report the multifaceted actions of the microbiome to extend the gut, by
8 showing its impact on host behavior and global metabolism. Overall, the
9 microbiota is a crucial component of a healthy intestinal environment
10 (Tremaroli and Bäckhed, 2012; Sommer and Bäckhed, 2013). Changes in its
11 composition complexity and/or distribution, leading to the loss of beneficial
12 bacteria in advantage of pathobionts, or global microbiota impoverishment (i.e.
13 **dysbiosis**) are correlated with a multitude of diseases, including diabetes,
14 obesity, atherosclerosis, and also colon cancer (Karlsson *et al.*, 2013; Vonaesch
15 *et al.*, 2018).

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30 The gut microbiota is present all along the intestinal tract but differs
31 notably depending on its location in the small intestine (duodenum, jejunum
32 and ileum) or the large intestine (caecum, colon and rectum). Generally
33 speaking, the microbiota increases in terms of complexity and concentration
34 from the duodenum to the colon. Moreover, in each region there is a different
35 local distribution of the microbiota due to morphological differences between
36 small and large intestine. The small intestine is characterized by the presence
37 of long epithelial protrusions extending into the intestinal lumen, the villi,
38 which increase the total surface area of the epithelium and therefore enable
39 maximal nutrient absorption, which is the primary function of this region.
40 Both the small and large intestine contain epithelial invaginations along the
41 mucosa, called intestinal crypts, which harbor proliferating progenitors and
42 intestinal stem cells (ISC) at the bottom. ISCs are essential for the regeneration
43 and repair of the intestinal tissue. Importantly, the ISC region of the small
44 intestinal crypts also contains Paneth cells, which support ISC regenerative
45 properties by secreting growth factors and metabolites, but also control the
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3 microbial environment of ISCs by the production of antimicrobial peptides
4 (AMPs) (Sato *et al.*, 2011; Clevers and Bevins, 2013).
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7 The interaction between the microbiota and intestinal cells (immune
8 and epithelial) is primarily maintained by host innate immune receptors
9 (pattern recognition receptors - PRRs) that recognize conserved bacterial and
10 viral motifs defined as “microbe-associated molecular patterns” (MAMPs)
11 (Kawai and Akira, 2011). The PRR family is comprised of membrane bound
12 receptors, notably Toll-like receptors (TLRs), and intracellular receptors such
13 as nucleotide oligomerization domain receptors (NODs), cGAS or RIG-I-like
14 receptors (RLR), which are involved in the sensing of bacteria outside and
15 inside the cells. MAMP recognition by PRRs canonically initiates an
16 inflammatory signaling cascade via activation of the transcription factor NF-
17 κ B (nuclear factor- kappa B) and subsequent production of pro-inflammatory
18 cytokines, including IL-1 β , IL-6, or IFN- γ . However, in the context of gut
19 health, PRRs have been associated with an additional function by promoting
20 a continuous bacterial-host communication crucial for the maintenance of
21 intestinal homeostasis (Abreu *et al.*, 2005; Kawai and Akira, 2011).
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38 Several studies have shown that bacteria or bacterial motifs play an
39 essential role in host intestinal development, which includes the epithelial
40 lining but also the immune system. While the recognition of the commensal
41 microflora by epithelial TLRs is required for intestinal homeostasis (Rakoff-
42 Nahoum *et al.*, 2004), the interaction between fragments of peptidoglycan and
43 NOD1 receptors is essential and exclusive in inducing the genesis of isolated
44 lymphoid follicles (Bouskra *et al.*, 2008).
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51 As previously mentioned, bacterial metabolites are important players in the
52 microbiota-epithelium dialogue, which includes a multitude of small
53 molecules such as bacteriocins, short chain fatty acids (SCFA), amino acids, or
54 vitamins – some of which having an additional function for the host
55 metabolism. For instance, in addition to serving as main energy substrate for
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3 colonocytes (Roediger, 1982), the SCFA butyrate, a potent histone deacetylase
4 inhibitor, has important anti-inflammatory properties by inhibiting the
5 expression of pro-inflammatory cytokines in macrophages (Chang *et al.*, 2014)
6 and by promoting the maturation of regulatory T cells (Treg) (Furusawa *et*
7 *al.*, 2013). Butyrate was also shown to induce the expression of the
8 antimicrobial molecule cathelicidin LL-37 in colonocytes (Schauber *et al.*,
9 2003).

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18 More recently, butyrate was found to promote cell cycle arrest and
19 differentiation of colonic progenitor cells (Kaiko *et al.*, 2016), suggesting that it
20 may also directly influence epithelial homeostasis. The concentration and
21 diversity of bacterial metabolites is heavily dependent on the diet, promoting
22 the notion that a dietary induced over-representation of specific bacterial
23 metabolites might be involved in certain pathogeneses.

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So-far, the extensive body of evidence confirming the microbial-
intestinal cross-talk has primarily been focusing on differentiated epithelial
cells, i.e. enterocytes. Therefore it is important to also consider the impact on
immature intestinal epithelial cells, including the stem cells residing in the
intestinal crypts. It is interesting to hypothesize that stem cells may also
respond to resident bacteria, either by direct recognition or via bacterial
metabolites. If so, this is suggestive of a conserved host-bacterial interplay,
which may have emerged from the coevolution of mammals with their gut
microbiota. The involved regulatory systems must be tightly controlled and
highlight a delicate balance by, on the one hand, protecting the vulnerable
crypt stem cells from microbial insults while, on the other hand, enabling the
sensing and integration of “positive” microbial signals to boost epithelial
regeneration.

Here we review the recent work from our laboratory, “Pathogénie
Microbienne Moléculaire” (PMM) unit headed by Philippe Sansonetti,
focusing on the molecular cross-talk that characterize the mutualistic signals

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3 exchanged between commensals and the host. We describe novel tools,
4 established by the laboratory, in order to functionally characterize commensal
5 organisms such as *Lactobacillus casei* and SFB. We also report results on how
6 specific symbionts affect the regulation of epithelial cell homeostasis and
7 show vice-versa, how changes in host homeostasis alter the composition of
8 the flora, possibly accelerating pathologic development. Finally, we delineate
9 the identification and characterization of a specific murine intestinal crypt
10 microbiota and present evidence indicating that an intimate dialogue between
11 bacteria and the crypt might impact ISCs fate and survival (Figure 1).
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27 **1. A bacterial close-up – investigations on symbiotic microbes**

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31 Setting out on elucidating the molecular mechanisms underlying the
32 delicate cross-talk between symbiotic microbes and intestinal mucosa, the
33 PMM unit started off by investigating interactions from the microbial point of
34 view. For instance, the lab established tools to functionally characterize
35 specific symbionts. Using *Lactobacillus casei* as a symbiotic model
36 microorganism, Licandro-Seraut *et al.* performed a genome-wide analysis
37 seeking to identify the functional phenotype required to establish symbiotic
38 colonization of the gut (Licandro-Seraut *et al.*, 2014). Utilizing a transposon-
39 based mutagenesis approach, the authors constructed a novel *L. casei*
40 collection encompassing 1100 mutant strains, which were then screened for
41 their ability to efficiently colonize the ligated rabbit ileal loop. Interestingly,
42 among the 47 genes identified as essential for *L. casei* colonization, the authors
43 found a high representation of genes involved in carbohydrate metabolism,
44 indicating that colonization and host cell homeostasis shared a common gene
45 pool. This library could serve a double purpose by on the one hand,
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3 providing a unique tool to investigate the molecular basis involved in the
4 stable establishment of *L. casei* in the gut, while on the other hand permitting
5 the identification bacterial effector mediated modulation of the host
6 physiology, for example by investigating host transcriptional response
7 towards different mutants.
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13 Another example for the class of commensals is the anaerobic
14 *Candidatus arthromitus*, also known as segmented filamentous bacterium
15 (SFB). Within the thousands of bacterial species present in the intestine, SFB
16 stands out due to its unique ability to stimulate post-natal maturation of the
17 B- and T-cell compartments and in particular, the induction of the Th17
18 response in the small intestine. Given SFB's unique impact on the immune
19 system, researchers have tried for decades to obtain *in vitro* cultures in order
20 to investigate its physiologic properties and the underlying mechanisms on
21 the host. In 2015, Schnupf *et al.* published for the first time, the successful
22 culture of mouse SFB *ex vivo* utilizing a SFB-host cell co-culturing system in
23 order to mimic its ecological niche (Schnupf *et al.*, 2015). The authors were
24 able to reproduce each of the SFB cell cycle steps, from the filaments to the
25 differentiated, intracellular offspring. Moreover, they confirmed the potent
26 stimulation of host innate defense genes by SFB, proving the system's
27 suitability for further mechanistic studies on host-bacterial interactions. Of note,
28 although SFB DNA has been isolated from multiple vertebrates, its presence in the
29 human intestine was only recently confirmed by sequencing, SEM and FISH
30 technology, thus indicating a potential role in the modulation of human immunity
31 (Chen *et al.*, 2018).
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51 The ability to cultivate and functionally characterize symbionts is a
52 major step in understanding the colonization of particular niches by
53 individual species and their differential contribution to host homeostasis.
54 However, many symbiotic bacteria of the gut remain uncultivable and
55 therefore their potential role in health and disease is still unexplored.
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8 **2. Players in health and disease - the microbial-host interface**
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11 Recent publications by our lab and others have emphasized on the
12 close interconnectivity of bacterial metabolites and the host epithelium and
13 thus highlighting the importance of this interdependency in the establishment
14 of specific bacterial communities. For instance, Matsuki and collaborators
15 showed that co-culture of intestinal cell lines with either *Lactobacillus casei* or
16 *Bifidobacterium breve* altered the expression of key cell cycle regulators
17 (Matsuki *et al.*, 2013). The two SCFAs acetate and lactate, produced upon
18 Lactobacilli and Bifidobacteria fermentation of complex sugars, were
19 sufficient to mediate Cyclins D1 and E1 down-regulation and to induce cell-
20 cycle arrest. These results show that SCFAs have an additional role to the
21 provision of fuel for colonocytes, by also influencing cell behavior. Another
22 example on the regulation of host cell homeostasis by bacterial symbionts was
23 reported in the recent publication by Tazi *et al.*, demonstrating the *L. paracasei*
24 and *E. coli*-mediated regulation of the host epithelial fatty acid metabolism,
25 both *in vivo* and *in vitro* (Tazi *et al.*, 2018). Mice colonized with either *E. coli* or
26 *L. paracasei* showed a decrease in the expression of important lipogenic
27 regulators within intestinal cells, including PPAR transcriptional targets,
28 SREBP1 and CHREBP. This phenotype was correlated with gene expression
29 changes in the liver and, in the case of *E. coli*, with reduced circulating
30 chylomicrons. Interestingly, *in vitro*, both strains led to the decrease of
31 enterocyte lipid secretion through two distinct mechanisms. While *L. paracasei*
32 increased fat storage, *E. coli* was shown to upregulate lipid catabolism. The
33 divergence in this mechanistic phenotype suggests species- or phylum-
34 specificity, indicating that the relative abundance of the individual phyla and
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3 their spatial distribution in the crypt-lumen axis bears importance to the
4 bacterial impact on host homeostasis. Importantly, colonization of mice with
5 *L. paracasei* was protective from high-fat diet (HFD) induced
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9 hypercholesterolemia and attenuated intestinal response to HFD showing
10 that this symbiont may be beneficial upon certain metabolic changes in the
11 host (Amar *et al.*, 2011; Öner *et al.*, 2014).
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15 HFD can induce obesity and associated metabolic complications and
16 has been linked to changes in the microbiota composition (Ley *et al.*, 2005). To
17 gain a better insight into the mechanisms involved, Tomas and co-authors
18 showed that a period of 30 days of HFD intake proved sufficient to
19 significantly shift the commensal bacterial composition marked by an increase
20 in the relative abundance of Proteobacteria or Verrucomicrobia, a decrease in
21 Bacteroidetes, as well as the complete absence of SFB in the ileum (Tomas *et*
22 *al.*, 2016). In addition, severe changes to the intestinal defenses were observed
23 upon short-time exposure to HFD, including the down-regulation of
24 antimicrobial peptide (AMP) expression, narrowing of the mucosal barrier
25 and subsequent colonization of the intervillous regions by bacteria.
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27 Interestingly, both phenotypes were mechanistically uncoupled as changes to
28 the defense system were largely dependent on the sensing of fat by
29 PPARgamma, while those to the microbial composition were linked to diet-
30 induced modifications to the metabolic substrates available in the lumen.
31 Taken together, these results confirm the importance of diet at the interface
32 between the intestine and the microbiota. However, since the microbial
33 composition appears modified prior to the onset of diseases (Tomas *et al.*,
34 2016), it also raises the question of a possible causative role for dysbiosis in
35 pathogenesis-associated with HFD.
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55 Dysbiosis could be directly or indirectly responsible for disease onset
56 by providing a suitable environment for the growth of pathobionts or
57 opportunistic pathogens. Aymeric *et al.* reported an example for such
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3 interactions in the context of intestinal tumorigenesis conferring growth
4 advantage to the opportunistic pathogen *Streptococcus gallolyticus* (Aymeric *et*
5 *al.*, 2018). Using mice genetically prone to colorectal cancer (CRC), the authors
6 showed that gut colonization by *S. gallolyticus* was higher in tumor-bearing mice than
7 in healthy controls. This advantage was linked to the upregulation of a bacteriocin
8 (“galloicin”) found constitutively expressed by *S. gallolyticus*. The authors showed
9 that in the presence of tumors, there is an enrichment of bile acid, which is required
10 for the activation of galloicin from its inactive state, therefore increasing the
11 competitive capacity of Streptococcus over other bacteria (i.e. Enterococci).
12 Confirming previous observations, these results indicate that the adhesion of *S.*
13 *gallolyticus* to tumoral cells promotes their proliferation rate, supporting the definition
14 of *S. gallolyticus* as opportunistic pathogen.

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16 Research from our lab and others demonstrates the ability of some
17 bacteria to profoundly impact the intestinal metabolism and physiology.
18 Correspondingly, it is tempting to speculate that intestinal crypts, having a
19 central role in intestinal homeostasis, may also communicate with symbiotic
20 bacteria and may therefore play an important part in the epithelial response
21 towards the microbiota. Seeking to explore this hypothesis further,
22 subsequent research activities by the laboratory aimed to investigate the
23 existence and identity of bacteria in the vicinity of the crypt compartment.

24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 **3. Bacteria in the Crypt – microbiota and intestinal regeneration**

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46 The intestinal crypt represents a sensitive zone in the gut as it is home
47 to the ISCs. Given its crucial importance for epithelial health, the lab decided
48 to focus on this region and investigate a possible direct impact of the
49 microbiota on intestinal regeneration.

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55 In order to address the presence of bacteria within the intestinal crypts
56 of mice, Pedron *et al.* applied a novel technical approach combining Whartin-
57 Starr (silver/nitrate) staining and fluorescent in situ hybridization (FISH)
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3 with 16SrRNA probes (Pédron *et al.*, 2012). For the first time, the authors
4 identified the presence of a small cluster of bacteria in the crypts and
5 demonstrated their viability and metabolic activity. Interestingly, local
6 differences were noted as bacteria were found at the crypt bottom in the
7 caecum and proximal part of the colon, but absent in the duodenum-jejunum
8 and distal colon. To identify the crypt bacteria, a dedicated pipeline was
9 developed combining laser capture microdissection (LCM) and 16S rRNA
10 metagenomics. The analysis was carried-out on mice from different genetic
11 backgrounds and obtained from several providers to account for interspecies
12 and environmental variations. Comparing samples obtained from luminal
13 and crypt regions revealed the presence of fourteen bacterial phyla with
14 predominance of five phyla: Firmicutes (73%), Beta- and
15 Gammaproteobacteria (16%), Actinobacteria (3.5%), and Bacteroidetes (1.7%).
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30 Interestingly, marked differences were observed when comparing
31 samples from the luminal and the crypt region of the same mouse as
32 Firmicutes comprised the majority of sequences (95.5%) in the former, while
33 *Proteobacteria* represented the most abundant sequences (47.6%, versus 2.7%
34 for the lumen) in the latter region. Bacteroidetes were relatively poorly
35 represented within both crypt and luminal samples. In the crypt, the most
36 prominent bacterial family of *Proteobacteria* was the *Moraxellaceae* (23.7%); of
37 these, 23% identified as *Acinetobacter* spp. sequences in crypts versus 1.6% in
38 the lumen. Shared *Acinetobacter* OTUs were found among all crypts
39 suggesting a common bacterial phylogroup although quantitative variations
40 between mice of different background were noted. In all cases, the levels of
41 *Acinetobacter* spp. were significantly higher in crypt than in luminal samples.
42 Interestingly, *Acinetobacter* spp. belong to the family of strictly aerobic, non-
43 fermenting gamma-proteobacteria suggesting an oxygen enriched
44 environment in the proximity of the crypt.
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In order to investigate the potential benefits of these specific crypt

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3 microbes on the host, Saffarian *et al.* characterized and compared the genome
4 of two different strains of Acinetobacter (*A. modestus* CM11G and *A.*
5 *radioresistens* CM38.2) that were isolated from murine crypts (Saffarian *et al.*,
6 2017). Interestingly, both strains contained genes encoding for the presence of
7 a type IV pili system as well as the presence of genes associated with Type I
8 and Type II secretion systems. In contrast to these findings, pathogenic
9 Acinetobacter bacteria, including *A. baumannii*, possess a T6SS associated to
10 bacterial virulence (Repizo *et al.*, 2015). Most importantly, the authors
11 highlighted the potential of crypt *Acinetobacter* to metabolize xenobiotics due
12 to the expression of the benABCDE operon (involved in benzoate
13 metabolism), antABC operon (encoding the anthranilate dioxygenase) and, in
14 the case of *A. radioresistens*, the phenol hydroxylase gene cluster. Comparative
15 genomic analysis revealed that different *Acinetobacter* strains isolated from the
16 same biological niche, even though sharing the large majority of genes,
17 possess unique features that may play a specific role in the protection of the
18 intestinal crypt.

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36 Altogether, these results suggest that among the complex assemblage
37 of luminal and mucosal commensal species composing the microbiota, a
38 limited set of individual species may enter into a mutualistic interaction with
39 their host, potentially reflecting a longstanding co-evolution. This may have
40 led to the established symbiotic steady-state in which the epithelial
41 regenerative apparatus may benefit from microbiota-mediated protection and
42 vice-versa. These findings illustrate the importance of further investigations
43 in order to establish a cartography of commensals permitting a better
44 understanding on symbiosis.

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54 In order to decipher the significance of the CSCM-crypt symbiosis, we
55 explored the functional characterization of this community, i.e. the existence
56 of a true cross/talk between the microbiota and the cells of the crypt. For this,
57 Naito *et al.* have selected four members of each of the three main genera
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3 identified in the crypts: Acinetobacter, Stenotrophomonas, and Delftia and
4 investigated their impact on the colonic epithelium *in vivo* and *in vitro* (Naito
5 *et al.*, 2017). Given the implication of bacterial products (especially the
6 endotoxin) and the TLRs on intestinal homeostasis, the authors utilized
7 germfree mice and monocolonized them with *A. modestus*, *A. radioresistens*, *D.*
8 *tsuruhatensis*, and *S. maltophilia* followed by FISH analysis. Bacteria were
9 detected in the luminal space of the intestine, but also deep in the colonic
10 crypts, thus confirming the crypt tropism of these four species. In alignment
11 with previous results, crypts of the small intestine (duodenum, jejunum, and
12 ileum) and distal colon remained devoid of bacteria following mono-
13 colonization. Analyzing the impact of crypt colonization on colon epithelial
14 turnover, Naito *et al.* observed that CSCM members led to a decrease in the
15 number of proliferating cells and an increase in the number of dead cells.
16 Interestingly, these observations were found to be TLR4-dependent, *in vivo*.
17 Subsequently, LPS from the selected CSCM strains were purified and
18 evaluated for their effect on colonic organoid culture. In agreement with the
19 colonization model, treating organoids with the purified LPS, composed of
20 highly acylated lipid A, had a cytotoxic effect on ISCs and, most strikingly, on
21 progenitor cells through a mechanism identified as necroptosis. In addition to
22 the decrease of proliferative cells, cell differentiation was observed to be
23 increased and most profoundly towards the goblet cell lineage. These
24 observations imply that the intimate relationship between crypts and a subset
25 of colonic microbes could be important to regulate the turnover rate of ISCs at
26 homeostasis but may also affect cell differentiation. How the CSCM impacts
27 on ISCs self-renewal in a context of active epithelial regeneration remains an
28 important question to address.

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56 In addition to LPS, other MAMPs produced by the CSCM may have a
57 direct impact on ISCs. To investigate this question, Nigro *et al.* subjected
58 murine small intestinal organoids to several bacterial products and assessed
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3 their effects on organoid survival (Nigro *et al.*, 2014). In contrast to the cell
4 toxicity observed by LPS, treatment with muramyl-dipeptide (MDP)
5 component of peptidoglycan resulted in higher yields of organoids, indicating
6 a protective role for MDP on ISCs. The authors showed that this mechanism
7 was NOD2-dependent and further confirmed the NOD2 gene to be highest
8 expressed in ISCs when compared to Paneth cells. Using co-culture of sorted
9 ISCs and Paneth cells from wt and/or NOD2KO mice, Nigro *et al.* showed that
10 direct sensing of MDP by NOD2 expressing ISCs conferred the protective
11 phenotype. Notably, this MDP-mediated protection was shown to be
12 important upon stress, i.e. following the isolation protocol, but not in steady-
13 state condition, as indicated by the normal appearance and viability of
14 NOD2KO mice. To validate this NOD2-dependent ISCs cytoprotection
15 pathway and to assess its activation by microbiota-produced MDP *in vivo*, the
16 authors utilized a NOD2KO mouse model and applied the chemotherapeutic
17 agent doxorubicin, to induce oxidative stress in proliferative cells.
18 Interestingly, in comparison to wt mice, NOD2KO mice lacked the ability to
19 regenerate the gut upon treatment with doxorubicin. Moreover, the wt mice
20 presented higher numbers of crypt survival compared to NOD2KO mice,
21 indicating a protective effect of NOD2. Crypts extracted from doxorubicin-
22 treated mice were much more responsive to MDP-dependent organoid
23 proliferation when compared to non-treatment further confirming the notion
24 of stress-dependence of the NOD2-cytoprotective pathway. These direct
25 interactions between bacterial products and ISCs highlight the potential of the
26 microbiota to impact ISCs development and therefore to affect mucosal health
27 (Nigro *et al.*, 2014). The molecular mechanisms underlying this cytoprotective
28 pathway in ISCs are subject of current investigations in the lab (Levy *et al.*,
29 under review). A closer contact of microbiota with the intestinal crypt has
30 been observed in several conditions including HFD or adenomas formation
31 (Ley *et al.*, 2005). Therefore, at the onset of pathogenesis the interactions
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3 between certain bacterial MAMPs and the ISCs may be altered when
4 compared to homeostasis, with potential to greatly impact the physiological
5 outcome. While ISCs express a number of PRRs, the expression level of
6 specific receptors vary depending on their location in the anteroposterior axis
7 of the intestine. In addition, our results suggest that PRR activation in ISCs
8 might trigger alternative pathway activation as compared to other cell types
9 in the intestine. Therefore, there is a need for future research to further define
10 the scope of these interactions and to elucidate the molecular implications on
11 the activation processes within ISCs.
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25 CONCLUSIONS

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27 Recent work by our unit, has set the intestinal crypt in the center of the
28 microbiota-epithelial dialogue. In addition to having an essential role in
29 epithelial regeneration, crypt stem cells were shown to possess the ability to
30 sense bacteria and bacterial signaling, potentially affecting epithelial
31 physiology and importantly, long-term memory. To investigate these
32 associations further, it is now crucial to develop novel, more integrated
33 analysis tools allowing for the *ex vivo* study of this system. For instance, one
34 approach could lie in the adaptation of the “gut on a chip” technology, which
35 is currently being developed by our laboratory. Another point of focus should
36 be the identification and functional analysis of the most important symbionts
37 required for host homeostasis, with particular emphasis on the CSCM.
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39 Finally, a better understanding of the mechanisms underlying signaling
40 distribution throughout the three players (microbes, immune and epithelial
41 cells) and how they integrate at the level of the crypt will be essential to
42 explain the onset and impact of dysbiosis. Joint efforts and a multidisciplinary
43 approach will be needed to then extend these concepts to other mammalian
44 species, including humans.
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17 **Conflict of Interest**

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19 The authors declare no conflict of interest.
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FIGURE LEGEND

Figure 1. Schematic summary of the recent publications by the PMM lab depicted in the relevant location along the intestine.

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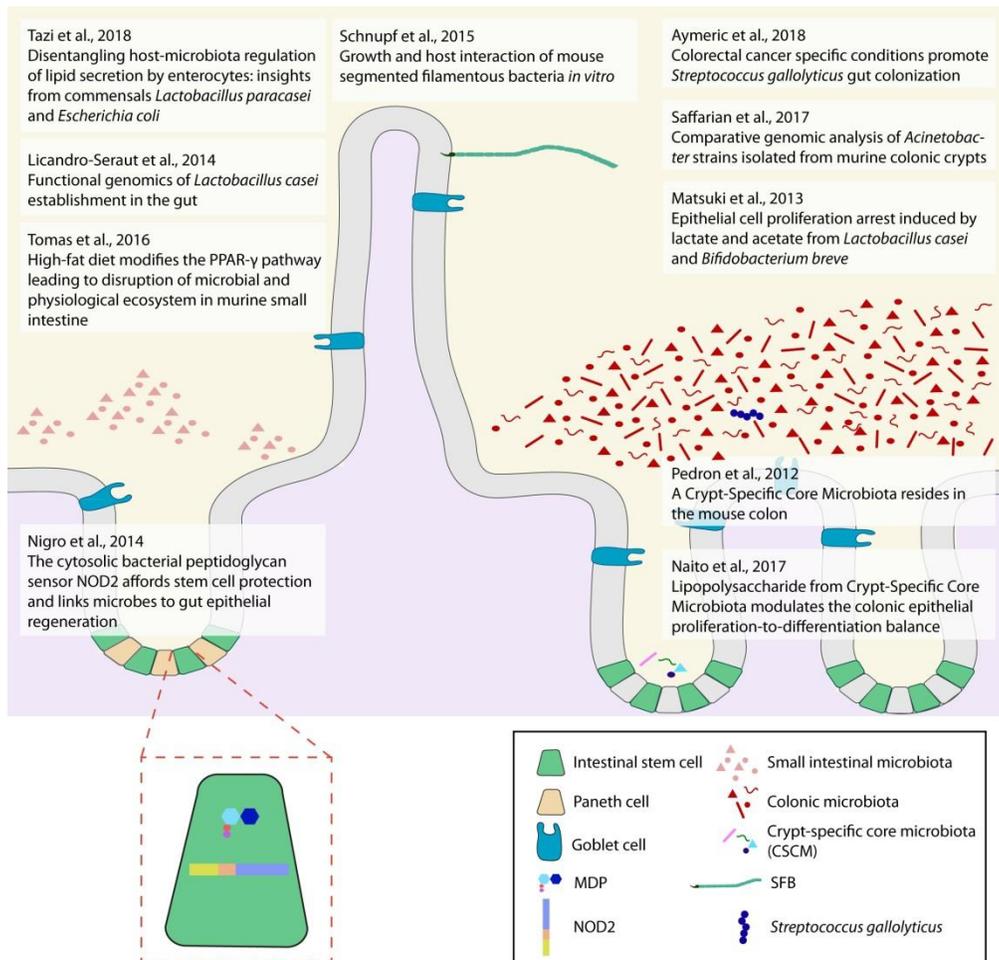
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Schematic summary of the recent publications by the PMM lab depicted in the relevant location along the intestine.

210x207mm (300 x 300 DPI)

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