Stimulating cROSstalk between commensal bacteria and intestinal stem cells

Parthive H Patel, Julieta A Maldera and Bruce A Edgar*

DKFZ-ZMBH Alliance, Heidelberg, Germany.

*Correspondence to: b.edgar@dkfz.de

The EMBO Journal (2013) 32, 3009–3010. doi:10.1038/emboj.2013.244; Published online 5 November 2013

Commensal gut bacteria benefit their host in many ways, for instance by aiding digestion and producing vitamins. In a new study in *The EMBO Journal*, Jones *et al* (2013) report that commensal bacteria can also promote intestinal epithelial renewal in both flies and mice. Interestingly, among commensals this effect is most specific to *Lactobacilli*, the friendly bacteria we use to produce cheese and yogurt. *Lactobacilli* stimulate NADPH oxidase (dNox/Nox1)-dependent ROS production by intestinal enterocytes and thereby activate intestinal stem cells.

The human gut contains huge numbers of bacteria ($\sim 10^{14}$ /person) that play beneficial roles for our health, including digestion, building our immune system and competing with harmful microbes (Sommer and Backhed, 2013). Both commensal and pathogenic bacteria can elicit antimicrobial responses in the intestinal epithelium and also stimulate epithelial turnover (Buchon *et al*, 2013; Sommer and Backhed, 2013). In contrast to gut pathogens, relatively little is known about how commensal bacteria influence intestinal turnover. In a simple yet elegant study reported recently in

The EMBO Journal, Jones *et al* (2013) show that among several different commensal bacteria tested, only *Lactobacilli* promoted much intestinal stem cell (ISC) proliferation, and it did so by stimulating reactive oxygen species (ROS) production. Interestingly, the specific effect of *Lactobacilli* was similar in both *Drosophila* and mice. In addition to distinguishing functional differences between species of commensals, this work suggests how the ingestion of *Lactobacillus*-containing probiotic supplements or food (e.g., yogurt) might support epithelial turnover and health.

In both mammals and insects, ISCs give rise to intestinal enterocytes, which not only absorb nutrients from the diet but must also interact with the gut microbiota (Jiang and Edgar, 2012). The metazoan intestinal epithelium has developed conserved responses to enteric bacteria, for instance the expression of antimicrobial peptides (AMPs; Gallo and Hooper, 2012; Buchon *et al*, 2013), presumably to kill harmful bacteria while allowing symbiotic commensals to flourish. In addition to AMPs, intestinal epithelial cells use NADPH family oxidases to generate ROS that are used as microbicides (Lambeth and

Figure 1 Metazoan intestinal epithelial responses to commensal and pathogenic bacteria. (A) High reactive oxygen species (ROS) levels generated by dual oxidase (Duox) in response to uracil secretion by pathogenic bacteria. (B) Low ROS levels generated by NADPH oxidase (Nox) in response to commensal bacteria. In addition to acting as a microbicide, ROS in flies may stimulate JNK signaling and cytokine (Upd 1–3) expression in enterocytes, thereby stimulating ISC proliferation and epithelial turnover or regeneration. Whether this stimulation required damage to or loss of enterocytes has yet to be explored.

Neish, 2013). High ROS levels during enteric infections likely act non-discriminately against both commensals and pathogens, but controlled, low-level ROS can act as signalling molecules that regulate various cellular processes including proliferation (Lambeth and Neish, 2013). In flies, exposure to pathogenic Gram-negative bacteria has been reported to result in ROS (H_2O_2) production by an enzyme called dual oxidase (Duox; Ha *et al*, 2005). Duox activity in the fly intestine (and likely also the mammalian one) has recently been discovered to be stimulated by uracil secretion by pathogenic bacteria (Lee *et al*, 2013). In the mammalian intestine another enzyme, NADPH oxidase (Nox), has also been shown to produce ROS in the form of superoxide (O_2^-), in this case in response to formylated bacterial peptides (Lambeth and Neish, 2013). A conserved role for Nox in the *Drosophila* intestinal epithelium had not until now been explored.

Jones *et al* (2013) checked seven different commensal bacterial to see which would stimulate ROS production by the fly's intestinal epithelium, and found that only one species, a Gram-positive *Lactobacillus*, could stimulate significant production of ROS in intestinal enterocytes. Five bacterial species were checked in mice or cultured intestinal cells, and again it was a *Lactobacillus* that generated the strongest ROS response. Although not all of the most prevalent enteric bacteria were assayed, those others that were—such as *E. coli*—induced only mild, barely detectable levels of ROS in enterocytes. Surprisingly, although bacteria pathogenic to *Drosophila*, like *Erwinia caratovora*, were expected to stimulate ROS production via Duox, Jones *et al* (2013) did not observe this using the ROS detecting dye hydrocyanine-Cy3, or a ROS-sensitive transgene reporter, Glutathione S-transferase-GFP, in flies. Further, Jones *et al* (2013) found that genetically suppressing Nox in either *Drosophila* or mice decreased ROS production after *Lactobacillus* ingestion. Consistent with the important role of Nox, Duox appeared not to be required for ROS production after *Lactobacillus* ingestion. In addition, Jones *et al* (2013) found that *Lactobacilli* also promoted DNA replication—a metric of cell proliferation and epithelial renewal—in the fly's intestine, and that this was also ROS- and Nox-dependent. Again, the same relationship was found in the mouse small intestine. Together, these results suggest a conserved mechanism by which *Lactobacilli* can stimulate Nox-dependent ROS production in intestinal enterocytes and thereby promote ISC proliferation and enhance gut epithelial renewal.

In the fly midgut, uracil produced by pathogenic bacteria can stimulate Duox-dependent ROS production, which is thought to act as a microbicide (Lee *et al*, 2013), and can also promote ISC proliferation (Buchon *et al*, 2009). However, Duox-produced ROS may also damage the intestinal epithelium itself and thereby promote epithelial regeneration indirectly through stress responses. In this disease scenario, ROS appears to be sensed by the stress-activated Jun N-terminal Kinase (JNK; Figure 1A), which can induce pro-proliferative cytokines of the Leptin/IL-6 family (Unpaireds, Upd1–3) (Buchon *et al*, 2009; Jiang *et al*, 2009). These cytokines activate JAK/STAT signalling in the ISCs, promoting their growth and proliferation, and accelerating regenerative repair of the gut epithelium (Buchon *et al*, 2009; Jiang *et al*, 2009). It is also possible, however, that low-level ROS, or specific types of ROS (e.g., H_2O_2) might induce ISC proliferation directly by acting as a signal between enterocytes and ISCs. Since commensal *Lactobacillus* stimulates ROS production via Nox rather than Duox, this might be a case in which a non-damaging ROS signal promotes intestinal epithelial renewal without stress signalling or a microbicidal effect (Figure 1B). However, Jones *et al* (2013) stopped short of ruling out a role for oxidative damage, cell death or stress signalling in the intestinal epithelium following colonization by *Lactobacilli*, and so these parameters must be checked in future studies. Perhaps even the friendliest symbiotes cause a bit of 'healthy' damage to the gut lining, stimulating it to refresh and renew. Whether damage-dependent or not, the stimulation of *Drosophila* ISC proliferation by commensals and pathogens alike appears to involve the same cytokine (Upd3; Buchon *et al*, 2009), and so some of the differences between truly pathogenic and 'friendly' gut microbes might be ascribed more to matters of degree than qualitative distinctions. Future studies exploring exactly how different types of ROS signals stimulate JNK activity, gut cytokine expression and epithelial renewal should be able to sort this out, and perhaps help us learn how to better manage the ecosystems in our own bellies. From the lovely examples reported by Jones *et al* (2013), an experimental back-and-forth between the *Drosophila* and mouse intestine seems an informative way to go.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Buchon N, Broderick NA, Chakrabarti S, Lemaitre B (2009) Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev* **23**: 2333–2344
- Buchon N, Broderick NA, Lemaitre B (2013) Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat Rev Microbiol* **11**: 615–626
- Gallo RL, Hooper LV (2012) Epithelial antimicrobial defence of the skin and intestine. *Nat Rev Immunol* **12**: 503–516
- Ha EM, Oh CT, Bae YS, Lee WJ (2005) A direct role for dual oxidase in *Drosophila* gut immunity. *Science* **310**: 847–850
- Jiang H, Edgar BA (2012) Intestinal stem cell function in *Drosophila* and mice. *Curr Opin Genet Dev* **22**: 354–360
- Jiang H, Patel PH, Kohlmaier A, Grenley MO, McEwen DG, Edgar BA (2009) Cytokine/Jak/Stat signaling mediates regeneration and homeostasis in the *Drosophila* midgut. *Cell* **137**: 1343–1355
- Jones RM, Luo L, Ardita CS, Richardson AN, Kwon YM, Mercante JW, Alam A, Gates CL, Wu H, Swanson PA, Lambeth JD, Denning PW, Neish AS (2013) Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J* **32**: 3017–3028
- Lambeth JD, Neish AS (2013) Nox enzymes and new thinking on reactive oxygen: a double-edged sword revisited. *Annu Rev Pathol* (advance online publication, 13 September 2013; doi:10.1146/annurev-pathol-012513-104651)
- Lee KA, Kim SH, Kim EK, Ha EM, You H, Kim B, Kim MJ, Kwon Y, Ryu JH, Lee WJ (2013) Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. *Cell* **153**: 797–811
- Sommer F, Backhed F (2013) The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol* **11**: 227–238