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Intestinal regeneration as an insect resistance mechanism to entomopathogenic bacteria

Anaïs Castagnola[1] and Juan Luis Jurat-Fuentes[2]

The intestinal epithelium of insects is exposed to xenobiotics and entomopathogens during the feeding developmental stages. In these conditions, an effective enterocyte turnover mechanism is highly desirable to maintain integrity of the gut epithelial wall. As in other insects, the gut of lepidopteran larvae have stem cells that are capable of proliferation, which occurs during molting and pathogenic episodes. While much is known on the regulation of gut stem cell division during molting, there is a current knowledge gap on the molecular regulation of gut healing processes after entomopathogen exposure. Relevant information on this subject is emerging from studies of the response to exposure to insecticidal proteins from the bacterium *Bacillus thuringiensis* (Bt) as model intoxicants. In this work we discuss currently available data on the molecular cues involved in gut stem cell proliferation, insect gut healing, and the implications of enhanced healing as a potential mechanism of resistance against Bt toxins.

Addresses
[1] Center for Insect Science, University of Arizona, Tucson, AZ 85721, USA
[2] Department of Entomology and Plant Pathology, University of Tennessee, Knoxville, TN 37996, USA

Corresponding author: Jurat-Fuentes, Juan Luis (jurat@utk.edu)

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Introduction to the larval intestine of Lepidoptera

The insect intestinal epithelium has two overarching functions; provide a barrier between ingested items (including microorganisms) and the main body cavity (hemocoel), and nutrient uptake [1]. The monolayer epithelium of Lepidoptera larvae includes four major cell types: intestinal stem cells (ISCs), goblet cells (GCs), columnar cells or enterocytes (ECs), and enteroendocrine cells (EEs) (Figure 1). Basal to the epithelial cell layer is an extracellular matrix (ECM) of circular and longitudinal muscle fibers interwoven with trachea that provide oxygen used during peristaltic muscle contractions that move the food bolus along the digestive tube [2].

Each cell type in the gut epithelium has a defined role and contributes to unique microenvironments in the tissue. For instance, the unique physicochemical conditions in the gut lumen of Lepidoptera larvae are mostly maintained by the action of vacuolar ATPase pumps and secretions from GCs [3], while the absorptive role of ECs is evidenced by their elongated apical microvilli. Endoreplication of ECs results in polyploidy, further contributing to increased cell size and digestive capabilities [4]. Homeostasis and epithelial renewal are ISC-mediated, since these stem cells are the only gut cell type capable of division and thus represent the only source of new cells during tissue repair and growth. The ability of ISC to proliferate is remarkable, as the gut surface area increases approximately 200-fold during larval development [5]. The role of EEs in insects is secretory in nature and regulates the immune response [6], metabolic/endocrine functions associated with growth [7], lipid metabolism [8], and paracrine/endocrine peptide secretion [9].

Intestinal regenerative mechanisms in Lepidoptera

Midgut growth at each larval instar is initiated by increasing rates of stem cell proliferation [10] and subsequent differentiation to increase the total cell number [5]. This process has been best characterized in *Drosophila* adult gut epithelium as a relevant genetic model. In *Drosophila* epithelium, asymmetric ISC divisions assure maintenance of a constant number of ISC cells. Alternatively, gut ISCs may also undergo symmetrical division which may be followed by differentiation to provide a net increase in the number of midgut cells in response to abundant nutrients [11]. However, once gut stem cells differentiate they are incapable of reverting to stem cells [12], in contrast to dedifferentiation processes documented in alternative insect tissues [13].

In Lepidoptera, much progress has been made using primary midgut cell cultures from larvae. These cultures are optimal models to study gut regeneration, as they preserve the proliferative and differentiation features observed during molting [14•] and during the regenerative response to gut injury [15]. Similar to observations in *Drosophila*, isolated Lepidoptera ISCs undergo asymmetric cell division during epithelial growth and repair (Figure 1), and ISC symmetric differentiation has also

Figure 1

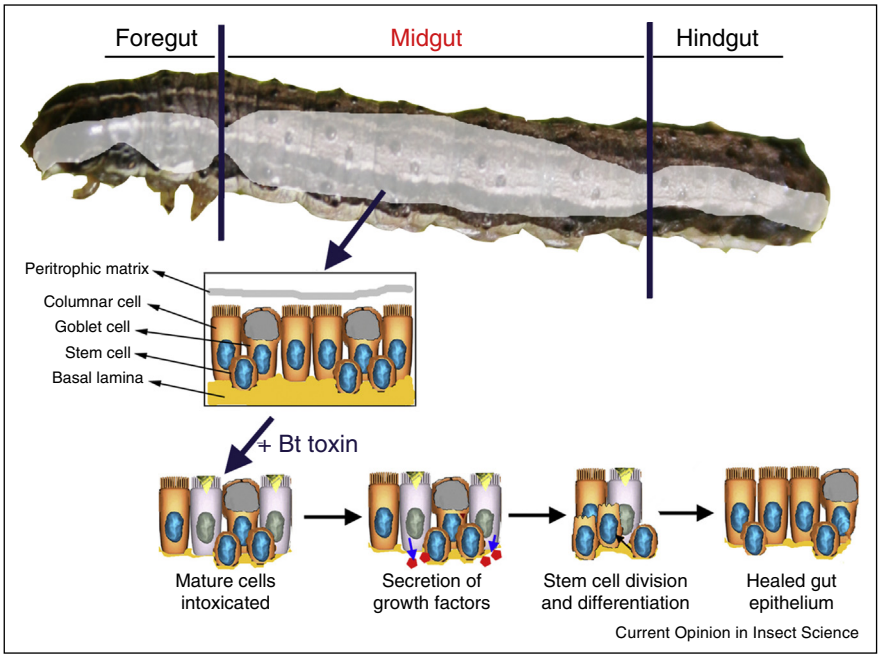

Diagram of the main cell types in the midgut of lepidopteran larvae and the steps in the process of epithelial healing in response to intoxication with toxins from *Bacillus thuringiensis* (Bt). Less abundant enteroendocrine cells are also present in the midgut are not represented in the figure.

been observed with some midgut differentiation factors (MDFs), as detailed below [16]. This dual fate of stem cells is also detected in cultured midgut stem cells from *Heliothis virescens* larvae; differentiation progressed in the presence of fetal bovine serum, while proliferation was observed in the presence of Albumax II [17]. Other mitogens for cultured stem cell systems were identified from conditioned media and hemolymph (reviewed in [18]). The first MDF identified from conditioned media was a 30 amino acid peptide with high identity to the C-terminus of fetuin [19], a protein that promotes cell attachment and growth in mammals [20]. Undigested fetuin did not have an effect on *H. virescens* midgut cell cultures and only after tryptic digestion one of the resulting peptides was identified as midgut growth factor MDF2 [19]. Additional peptides inducing midgut stem cell differentiation (MDF3 and MDF4) were isolated from chymotryptic digestion of *Lymantria dispar* hemolymph [21]. However, 100% differentiation of Lepidoptera stem cell cultures has never been observed with these MDFs, suggesting the existence of additional differentiation factors, including ecdysone [22], α-arylphorin [23] and insulin-related bombyxin [24]. In the case of α-arylphorin there is also evidence for mitogenic activity on gut cells *in vivo* [25], where 4th instar *Manduca sexta* larvae displayed weight gain after feeding on arylphorin.

Apart from ISCs, regeneration of midgut epithelia in Lepidoptera is also regulated by tracheal stem cells (TSCs) within the ECM and basal lamina. These cells are cued to undergo cell division during the larval molt to increase the amount of trachea supporting the muscle layer as the size of the epithelium increases with ISCs division and differentiation [26].

Similar to the gut growth process observed during molting, ISCs proliferate and differentiate to restore gut epithelial integrity after diverse biotic and abiotic injuries. However, at least in some cases gut healing may involve additional processes distinct from ISC proliferation. For example, gut healing in *Bombyx mori* larvae after physical perforation involved recruitment of hemocytes and production of a melanized scab, and stem cell proliferation detected as DNA duplication [27]. In contrast, the response to infection with the bacterium *Bacillus thuringiensis* (Bt) involved a regenerative mechanism [28,29], which *in vitro* it has been shown to depend on asymmetrical ISC division [15]. Interestingly, an increase in the number of midgut cells producing MDF1 peptide was detected after treatment with Bt toxins [15], suggesting a potential role for this peptide in response to intoxication.

Ingestion of plant xenobiotics can also have a drastic effect on these healing defensive responses to concurrent entomopathogen ingestion. For instance, the sloughing of virus-infected midgut cells occurred at a higher rate in insects that fed on cotton compared to artificial diet. Cellular sloughing contributed to the prevention of