Published in final edited form as:

Insect Biochem Mol Biol. 2015 December ; 67: 15–20. doi:10.1016/j.ibmb.2015.05.004.

The Multi-Tasking Gut Epithelium of Insects

Jia-Hsin Huang^a, Xiangfeng Jing^a, and Angela E Douglas^{a,b}

^aDepartment of Entomology, Cornell University, Ithaca, NY14853, USA

^bDepartment of Molecular Biology and Genetics, Cornell University, Ithaca, NY14853, USA

Abstract

The insect gut epithelium plays a vital role in multiple processes, including nutrition, immunity and osmoregulation. Recent research is revealing the molecular and biochemical basis of these functions. For example, the pattern of nutrient acquisition by the gut epithelium is integrated into the overall regulation of nutrient allocation, as illustrated by evidence for systemic controls over expression of key genes coding digestive enzymes and transporters in carbohydrate acquisition; and the abundance and diversity of microorganisms in the gut lumen is regulated by multiple molecular properties of the gut epithelial cells, including the synthesis of enzymes that produce reactive oxygen species and anti-microbial peptides. These traits are underpinned by the function of the gut epithelium as a selective barrier which mediates the controlled movement of water, ions, metabolites and macromolecules between the gut lumen and insect tissues. Breakdown of the gut epithelial barrier has been implicated in muscle paralysis of insects at low temperatures (chill coma) and in aging. The key challenge for future research is to understand how the multiple functions of the insect gut epithelium are integrated by signaling interactions among epithelial cells, the gut microbiota and other insect organs.

Keywords

epithelium; gut; immunity; microbiota; nutrient assimilation; stem cell

1. Introduction

A layer of epithelial cells defines every interface between the inner world of the insect body and the outer world of the external environment. Two generic functions of the epithelium underpin the appropriate response of insects to their environment: the capacity to communicate external cues to the internal world, and to protect the internal world against unfavorable abiotic conditions (e.g. desiccation) and biotic antagonists (pathogens, competitors, predators etc).

Corresponding author: AE Douglas. aes326@cornell.edu. jh735@cornell.edu, xj43@cornell.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

The gut epithelium has long been known to be exceptional among the epithelia that contact the external environment because it is the usual, and often sole, route by which insects acquire nutrients. Many of the cells in the gut epithelium are specialized for the production of digestive enzymes and assimilation of small organic nutrients (simple sugars, organic acids, amino acids etc), as well as the exchange of inorganic ions and water between the gut lumen and body fluids (Billingsley and Lehane, 1996; Douglas, 2013). A second fundamental role of the gut epithelium is also becoming increasingly apparent: the management of the gut microbiota. Until recently, the study of the microbiology of the insect gut focused on pathogenic microorganisms (Kaya and Vega, 2012) and specialized beneficial associations in a minority of insects that feed on nutrient-poor or unbalanced diets, e.g. the cellulolytic microorganisms in the enlarged proximal hindgut (“paunch”) of wood-feeding termites (Brune and Dietrich, 2015), and B-vitamin producing bacteria *Wigglesworthia* in specialized cells (“bacteriocytes”) of the tsetse fly *Glossina* midgut (Michalkova et al., 2014). It is now appreciated that, as with other animals, the guts of most insects are routinely colonized by microorganisms that play important and diverse roles including modulation of nutrient acquisition and immune function, essential for sustained health (Douglas, 2015).

Given its crucial location at interface between the internal and external worlds and its multiple roles in protection, nutrient acquisition and interactions with microorganisms, the gut epithelium might be expected to be structurally complex. In many respects, however, the insect gut epithelium is simple. It comprises a single cell layer, usually underlain by muscle, and includes just two differentiated cell types in most insects: the enterocytes and the endocrine cells (Billingsley and Lehane, 1996; Douglas, 2013; Lemaitre and Miguel-Aliaga, 2013). Most gut epithelial cells are enterocytes, which often have highly polyploid nuclei and a much-folded cell membrane. Midgut enterocytes, for example, have a brush border of microvilli on the apical membrane (facing the gut lumen) and irregularly convoluted infoldings of the basolateral membrane, consistent with their role in production of digestive enzymes and assimilation of nutrients. Transcript analyses indicate that enterocyte function varies widely with location in the gut (Buchon et al., 2013b; Marianes et al., 2013), but this functional specialization is rarely evident at the morphological level. For example, diagnostic ultrastructural differences between midgut epithelial cells responsible for secretion of digestive enzymes and assimilation of digested nutrients are generally lacking. The endocrine cells (also known as entero-endocrine cells) are distributed among the enterocytes. Although variable in shape and size, the endocrine cells have the common features of lacking microvilli and other infoldings, and they produce various peptide hormones (Beehler-Evans and Micchelli, 2015; Reiher et al., 2011; Zoephel et al., 2012). Some insects have other cell types with specialized function in ion transport, e.g. goblet cells of Lepidoptera and cuprophilic cells of some dipterans with H⁺-pumps that generate the highly alkaline and acidic conditions, respectively, in the gut lumen (Gomes et al., 2013; Shanbhag and Tripathi, 2009). The enterocytes and other more specialized gut epithelial cell types are the products of stem cells (also known as regenerative cells) which occur singly or in small groups (“nidi”), typically in contact with the basal lamina (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006).

In this article, recent developments in our understanding of the processes by which the gut epithelium mediates multiple functions are reviewed. We focus on four topics of current and emerging research interest: how nutrient acquisition by the gut epithelium is regulated by the nutritional demands of the insect; how the epithelium interacts with gut microbiota; the functional significance of the gut epithelial barrier, especially in relation to water and thermal relations of the insect; and the changes in gut epithelial function in the aging insect. This article does not attempt a comprehensive review of the classical physiological study or structural organization and function of the gut epithelium, and consequently some topics that have received little recent attention, notably digestive enzyme secretion mechanisms and the molecular physiology of hindgut function, are not addressed in detail. For further information on topics not explored in this article, the reader is referred to the reviews of Buchon and Osman (submitted), Karasov and Douglas (2013), Lehane and Billingsley (1996) and Lemaitre and Miguel-Aliaga (2013).

2. The gut epithelium as a regulatory hub in insect nutrition

Insect nutrition can conveniently be divided into the complementary functions of nutrient acquisition and nutrient allocation (Fig. 1). A core function of the gut epithelium relates to nutrient acquisition: to produce the digestive enzymes and transporters that mediate the acquisition of nutrients from ingested food. It is becoming increasingly apparent that this function is integrated with system-wide controls over feeding behavior and nutrient allocation, such that insect nutrition is optimized within the constraints imposed by the amount and composition of available food and the physiological priorities of the insect (which may include growth, reproduction, preparation for metamorphosis or diapause etc.) (Simpson et al., 2015).

The importance of the regulated processing of ingested food in the gut to insect nutrition is illustrated by the impact of diet composition on the activity of midgut amylase, a digestive enzyme that mediates the hydrolysis of starch to oligosaccharides. When *Drosophila* is reared on diets containing high concentrations of free glucose or other nutritious sugars, amylase activity is much reduced (Benkel and Hickey, 1987). This effect can be attributed to the reduced transcription of amylase genes (Benkel and Hickey, 1987; Chng et al., 2014), and is known as glucose repression. Glucose repression is likely adaptive in that it prevents the costly production of amylase protein under conditions where substrate is unavailable or the energy requirements of the insect can be met entirely from free sugar. Consistent with this interpretation, transcriptome analyses of the *Drosophila* midgut have revealed that the amylase genes (*Amy-p*, *Amy-d* and *Amyrel*) are not the only α -glycosidase genes that are subject to glucose repression in the midgut: the expression of multiple genes with predicted activity against oligosaccharides (e.g. maltases, α -mannosidases) are also significantly reduced in *Drosophila* reared on glucose diets (Chng et al., 2014). Furthermore, glucose repression of two genes investigated in detail (*Amy-p* and *Mal-A1*) is dependent on the function in midgut enterocytes of the transcription factor SMAD2, an essential component of the TGF β signaling pathway; and the ligand which activates TGF β signaling in the enterocytes (the protein Dawdle) is synthesized in the fat body and presumably delivered to the gut via the hemolymph (Chng et al., 2014). The important implication is that glucose repression is not determined entirely by local inputs on nutrient availability in the gut but

also by the fat body, which is the master-regulator of nutrient allocation in the insect body. Although much of the detail remains to be determined, the gut epithelium is functioning as the effector of a system-wide nutritional regulatory network mediating glucose repression.

An intriguing aspect of the transcriptome analysis of glucose repression in the *Drosophila* midgut conducted by Chng et al. (2014) is the suppressed expression of genes with predicted function as glucose transporters in the midgut of insects feeding on high glucose diets. One possible explanation is that the transport function of these genes inferred from annotation *in silico* is incorrect; mis-annotation of insect sugar transporters has been reported in other contexts (Price and Gatehouse, 2014). Alternatively, the annotation may be correct and glucose transporter function is reduced under conditions of high glucose availability as an adaptive response to reduce the burden of excess energy inputs relative to other nutrients, especially protein. This interpretation is unexpected in two important ways. First, the animal gut is generally considered to function as a nutrient-maximizing organ, i.e. the enterocytes of the gut epithelium produce digestive enzymes that degrade dietary constituents and transporters that assimilate the digested products in proportion to the amount of the relevant food ingested (Karasov and Diamond, 1988). Second, matching of nutritional inputs to nutritional demand of the insect is achieved by neuro-endocrinal controls over the amount and composition of food eaten (Pool and Scott, 2014; Schwartz and Zeltser, 2013; Simpson et al., 2004). There is scant evidence for negative regulation of digestion or assimilation of nutritional classes that are in dietary excess. Exceptionally, one study of the locust *Locusta migratoria* reared on diets of systematically-varied carbohydrate and protein contents demonstrated reduced gut amylase activity in insects reared on the high carbohydrate:low protein (high C:low P) diet relative to high C:high P diet (Clissold et al., 2010). The role of the gut epithelium in the post-ingestive regulation of nutrient acquisition is worthy of further study.

The gut epithelium participates in a second set of interactions (additional to those involved in processing of ingested food) as a direct consequence of its location, and that is interactions with microorganisms in the gut lumen. We consider these epithelial-microbial interactions in the next section.

3. Managing microorganisms in the gut

The dynamics of microorganisms in the insect gut are inherently complex and variable (Fig. 2). Most microbial taxa are acquired orally, usually in the food. The fate of ingested microbial cells varies with the identity of both the microorganism and insect species. Some microbial cells may transit through the gut with the bulk flow of food; some may be killed by abiotic conditions (e.g. extreme pH or redox potential) and enzymes (e.g. lysozyme); others may persist for varying lengths of time in the gut lumen, sometimes as proliferating populations; and a minority may pass the gut epithelium to the hemolymph and internal tissues of the insect. Reflecting this diversity of interactions, the feces of insects are generally contaminated with both dead and live microbial cells with a taxonomic composition that, in some insects, is closely similar to the microbial community in the gut but, in other insects, is much-depleted in the dominant microbial taxa in the gut (Brune and Dietrich, 2015; Dillon and Dillon, 2004; Fink et al., 2013).

The molecular basis of the interactions between the insect gut epithelium and microorganisms has been investigated extensively in *Drosophila*. A key anti-microbial capability is the Duox enzyme, localized to the apical membrane of enterocytes. Duox generates reactive oxygen species (ROS), especially hydrogen peroxide and hypochlorite via its NADPH oxidase and myeloperoxidase activity, respectively (Ha et al., 2005). Multiple processes regulate Duox, including the transcription factor ATF2, the p38 MAPK pathway and an unidentified G-protein coupled receptor with bacterial-derived uracil as ligand (Ha et al., 2009; Lee et al., 2013). Precise controls over Duox function are important for sustained gut function because the Duox-generated ROS can also damage the epithelium (Buchon et al., 2009b; Ha et al., 2005). Interestingly, the *Drosophila* enterocytes bear a second NADPH oxidase, Nox, which is activated by the non-pathogenic *Lactobacillus* to produce ROS that are interpreted to function as a local signaling molecule without any apparent deleterious effects on the bacteria or gut epithelium (Jones et al., 2013).

Gut enterocytes also release humoral immune factors, including anti-microbial peptides, when the immune-related signaling pathways are triggered by microbial-associated molecular patterns, such as bacterial peptidoglycan and fungal β -glucans. Most research has been conducted on the Immune Deficiency (IMD) pathway, which is functional in the *Drosophila* midgut (Lemaitre and Miguel-Aliaga, 2013). Genetic ablation of any one of multiple transcription factors expressed in the gut leads to perturbation of IMD-dependent expression of anti-microbial peptide genes, resulting in changes in abundance and composition of the gut microbiota (Buchon et al., 2013b; Ryu et al., 2008). For example, the bacterium *Gluconobacter morbifer* is a minority member of the gut microbiota in wild-type *Drosophila* but increases dramatically in flies with RNAi-mediated knockdown of expression of the transcription factor *caudal*, resulting in the premature death of the insect (Ryu et al., 2008). These data indicate that the gut immune system in the healthy insect functions not only to eliminate pathogenic forms but also to maintain a benign microbiota. We are, however, critically lacking information on whether immune effectors have been selected for their low activity against benign gut microbes and whether these benign gut microbes have corresponding adaptations for resistance, suppression or evasion of the host immune response.

A complementary route by which the gut epithelium manages gut microorganisms is containment. In many insects, gut microorganisms do not make direct contact with the epithelial cells. They may be planktonic in the gut lumen or adherent to the cuticle bounding the foregut and hindgut and peritrophic envelope of the midgut (Brune and Dietrich, 2015; Dillon and Dillon, 2004; Lemaitre and Miguel-Aliaga, 2013). In particular, microbial cells cannot penetrate through the peritrophic envelope, which generally has a pore diameter <30 nm, unless facilitated by enzymes that degrade the chitin or proteins of the envelope (Shahabuddin et al., 1993; Toprak et al., 2012). The extracellular structures can also protect the gut epithelial cells against mechanical damage and toxins secreted by microbial pathogens. For example, the chitin-binding protein Drosocrystallin (Dcy) associated with the peritrophic envelope of *Drosophila* provides barrier function against the proteinaceous toxin monalysin produced by pathogenic bacterium *Pseudomonas entomophila*; the peritrophic envelope of mutant flies with much-reduced *dcy* expression in the midgut display enhanced

permeability to molecules >250 kDa, associated with dramatically-increased mortality when challenged with *P. entomophila* (Kuraishi et al., 2011).

4. Crossing the border: regulated transport across the gut epithelium

The roles of the gut epithelium in insect nutrition and management of the gut microbiota, described in the previous sections of this article, are founded on the sustained function of the gut epithelium as a selective barrier that mediates the uptake of nutrients and controlled exchange of ions and water, while excluding toxic compounds and microorganisms. Transport across the gut epithelium (to or from the gut lumen) is mediated by two routes: across the epithelial cells by the transcellular route, and between the epithelial cells by the paracellular route (Fig. 3).

Two processes contribute to transcellular transport across the gut epithelium. Inorganic ions and small organic molecules are translocated by membrane-bound transporters on the apical and basolateral membranes, with diffusion across the cytoplasm of the cell; and large molecules, notably proteins and virus particles are translocated in membrane-bound vesicles, a process known as transcytosis. The translocation of orally-administered lectins, albumins and immunoglobulins to the hemolymph and internal tissues of multiple insects is generally inferred to be mediated by transcytosis, but this has rarely been demonstrated and some data are indicative of paracellular translocation for certain proteins (Bonning and Chougule, 2014; Jeffers and Michael Roe, 2008).

The selectivity achieved by transcellular transport by the gut epithelium is dependent on the parallel restriction of nonspecific leakage of water and solutes across the intercellular space between the epithelial cells. Paracellular transport across the gut epithelium and other epithelia with barrier function (e.g. glial cells at the blood-brain barrier and tracheolar cells) is limited by septate junctions that girdle the lateral margin of every cell of the epithelium. In this way, the septate junction of insects is functionally equivalent to the tight junction in mammals, although these two types of junction are ultrastructurally distinct and their constituent proteins are not orthologous (Limmer et al., 2014; Matter and Balda, 2003).

The gut epithelium plays a critical role in maintaining the water content of the body fluids. In biological systems, water is invariably transported down the osmotic gradient, i.e. from a dilute to a more concentrated compartment, and flux across biological membranes is increased up to 1,000-fold by channels known as aquaporins (Spring et al., 2009). (Some aquaporins are permeable to other molecules, e.g. polyols, ammonia, CO₂, additionally or alternatively to water.) It is becoming increasingly apparent that water transport across the gut epithelium can play a crucial role in the responses of insects to osmotic challenge and to temperature.

Osmotic challenge is particularly acute for insects feeding through the life cycle on plant sap. Relative to insect hemolymph with osmotic pressure *ca* 1 MPa, plant phloem sap is hyperosmotic (3-5 MPa) and plant xylem sap is hypo-osmotic (0.1 MPa), linked to their roles in the transport of photo-assimilated sugars and water, respectively (Malone et al., 2002; Wilkinson et al., 1997). The selective movement of water across the gut epithelium protects insects feeding on these diets from either loss of water from body fluids to the gut

and dehydration (phloem feeding) and uncontrolled gain of water from the gut to the body fluids (xylem feeding). The physiological basis of this process has been studied in the pea aphid *Acyrtosiphon pisum* feeding on phloem sap. A water-permeable aquaporin is expressed in the epithelium of the proximal stomach and distal region of the gut, which are closely apposed as a result of stereotypical looping of the gut in the insect body (Shakesby et al., 2009). The osmotic pressure in the distal lumen contents is reduced, as a result of sugar assimilation and transformation of free monosaccharides to oligosaccharides (Ashford et al., 2000), resulting in net movement of water from distal to proximal regions of the gut. RNAi-mediated knockdown of expression of the gut aquaporin leads to increased osmotic pressure of the insect hemolymph (Shakesby et al., 2009). The expression of the orthologous aquaporin in phloem-feeding whitefly is localized to abutting proximal and distal regions of the gut, indicative of a comparable role of the epithelium in osmoregulation (Mathew et al., 2011).

The importance of the gut epithelium in shaping the thermal response of insects relates to the phenomenon of chill coma: that insects subjected to low temperatures enter a reversible state of paralysis. The physiological basis of chill coma has been investigated in the cricket *Gryllus pennsylvanicus* (MacMillan and Sinclair, 2011; MacMillan et al., 2012). Energy-consuming ion transport across the gut epithelium plays an important role in maintaining the ionic and water balance of the hemolymph in physiologically-active insects, but these functions break down at low temperatures, resulting in the net transfer of Na^+ ions and water from the hemolymph to the gut. The resultant rise in $\text{K}^+:\text{Na}^+$ in the hemolymph causes depolarization of the K^+ -equilibrium potential of muscle, blocking muscle contraction/relaxation. The paralysis (coma) is reversed when the insect is re-warmed, but the time to recovery is not determined by the temperature of the insect tissues but by the time required for full osmotic recovery, i.e. re-distribution of Na^+ ions and water across the gut epithelium from the gut lumen back to the hemolymph (MacMillan et al., 2012). The cricket hindgut epithelium is likely the primary site of osmotic rebalancing during recovery from chill coma. The important implication of these results is that any explanation of inter- and intra-specific variation in insect response to temperature should include gut epithelial function, particularly the temperature-dependent flux of ions and water across the gut epithelium (Andersen et al., 2015).

5. Renewal and aging of the insect gut epithelium

In previous sections of this review, we have treated the gut epithelium as a tissue, i.e. a coherent layer of cells that is remarkably dynamic in its biochemistry and physiology. An important complementary perspective is the individual epithelial cell. The cell dynamics of the gut epithelium is best-understood in *Drosophila*. In the larva, the epithelium is non-dividing and grows by cell expansion accompanied by increased ploidy of the nucleus (Micchelli and Perrimon, 2006; Ohlstein and Spradling, 2006; Takeshima et al., 2011). By contrast, the adult intestinal stem cells are actively dividing, with one of the two daughter cells differentiating via the enteroblast progenitor cell to a mature epithelial cell, usually enterocytes but occasionally endocrinal cells interspersed among the enterocytes. These newly-formed epithelial cells replace mature cells that are lost continuously by delamination (Buchon et al., 2013a). Turnover of the *Drosophila* midgut epithelium takes approximately

one week, but the rate is influenced by many factors. For example, cellular damage and colonization by microbial cells promote epithelial turnover (Buchon et al., 2009b; Chatterjee and Ip, 2009).

As the fly ages, the process of cell proliferation and differentiation becomes perturbed. Specifically, the gut of old flies commonly displays dysplasia, i.e. undifferentiated cells accumulate because they are produced more rapidly than they differentiate into mature epithelial cells (Biteau et al., 2008; Buchon et al., 2009a). Dysplasia predisposes the epithelium to hyperplasia and tumor formation. Multiple correlates of dysplasia have been identified, including high ROS, chronic activation of the JNK signaling pathway and impaired barrier function of the epithelium (Biteau et al., 2008; Choi et al., 2008; Rera et al., 2012). The gut microbiota has also been implicated, as indicated by the elevated bacterial load in old conventional flies, i.e. flies with unmanipulated microbiota, (Broderick et al., 2014; Ren et al., 2007) and reduced incidence of dysplasia in old germ-free flies (Buchon et al., 2009a). Intriguingly, age-associated gut dysplasia is altered by modulating IMD activity in the gut of conventional flies but not germ-free flies (Chen et al., 2014; Guo et al., 2014). Further study is needed to elucidate how the interaction between gut microbiota and the gut IMD pathway regulates the dynamics of epithelial cell division and differentiation during aging.

What is the significance of the gut epithelial dysplasia in the aging process? Recognizing that gut epithelial function is critical for insect health, impairment to its function may trigger multiple cascading perturbations to other organ systems, resulting in whole-body dysfunction and early death. The demonstration that loss of barrier function (as quantified by passage of dye to the body tissues) is a strong predictor of early death (Rera et al., 2012) can be interpreted as evidence for a central role of gut epithelium dysfunction in the aging process. However, the data are also compatible with the possibility that gut epithelial dysplasia and other dysfunctional traits of the aging gut epithelium may be a downstream consequence of age-related malaise in other organs or at the whole-body level, e.g. mediated by perturbation of the EGF/Wnt/JAK-STAT signaling networks that regulate gut epithelial homeostasis (Buchon et al., 2013a); and further research is required to dissect the causal basis of the relationships among age-related malaise, gut epithelial function and the gut microbiota.

6. Concluding Comments

The linked disciplines of insect biochemistry and molecular biology have undergone radical changes in recent years. Descriptions of function in terms of processes mediated by isolated molecules and cells are being replaced by system-level explanations of organ and whole body function in terms of the underlying molecular interactions. As reviewed in this article, the gut epithelium is emerging as a key player in multiple functions. Nevertheless, major areas of uncertainty remain. For example, there is tantalizing evidence for local signaling interactions, both among the cells of the epithelium and between the epithelial cells and the microbiota, that collectively mediate the regulation of nutritional, immunological and barrier functions of enterocytes and the dynamics of epithelial cell growth and turnover. There are also indications that these local interactions may be integrated with long-distance

interactions between the gut and other organs, including the central nervous system, the fat body and, in adults, the reproductive organs, as dictated by neuro-endocrine factors. For this web of interactions, the identity of the signaling molecules and their mode of action at the molecular level are starting to be elucidated. The research challenges ahead can best be addressed by combining approaches from multiple disciplines, including the biochemistry of chemical and subcellular fractions, the transport and electrophysiological properties of isolated cells and tissues, and ‘omic analyses, together with genetic strategies to elucidate the function of individual genes, and whole insect physiological function. Many of the ground-breaking discoveries are being made with *Drosophila*, facilitated by the genetic tractability and genomic resources available for this species. But the guts of insects are diverse in structure and function, and sustained comparative research on multiple insect systems is essential to gain a comprehensive understanding of the multi-tasking gut epithelium.

Acknowledgements

We thank Dr Alyssa Bost and Dr Nicolas Buchon for helpful comments on the manuscript. This work was supported by NIH grant 1R01GM095372 and NIFA grant NYW-2011-04650.

References

- Andersen JL, Manenti T, Sorensen JG, MacMillan HA, Loeschcke V, Overgaard J. How to assess *Drosophila* cold tolerance: chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Funct. Ecol.* 2015; 29:55–65.
- Ashford DA, Smith WA, Douglas AE. Living on a high sugar diet: the fate of sucrose ingested by a phloem-feeding insect, the pea aphid *Acyrtosiphon pisum*. *J. Insect Physiol.* 2000; 46:335–341. [PubMed: 12770238]
- Beehler-Evans R, Micchelli CA. Generation of enteroendocrine cell diversity in midgut stem cell lineages. *Development.* 2015; 142:654–664. [PubMed: 25670792]
- Benkel BF, Hickey DA. A *Drosophila* gene is subject to glucose repression. *Proc. Natl Acad. Sci. U S A.* 1987; 84:1337–1339. [PubMed: 16593815]
- Billingsley, PF.; Lehane, MJ. Structure and ultrastructure of the insect midgut.. In: Lehane, MJ.; Billingsley, PF., editors. *Biology of the Insect Midgut*. Chapman and Hall; London, UK: 1996. p. 3-30.
- Biteau B, Hochmuth CE, Jasper H. JNK activity in somatic stem cells causes loss of tissue homeostasis in the aging *Drosophila* gut. *Cell Stem Cell.* 2008; 3:442–455. [PubMed: 18940735]
- Bonning BC, Chougule NP. Delivery of intrahemocoelic peptides for insect pest management. *Trends Biotechnol.* 2014; 32:91–98. [PubMed: 24331760]
- Broderick NA, Buchon N, Lemaitre B. Microbiota-induced changes in *Drosophila melanogaster* host gene expression and gut morphology. *mBio.* 2014; 5:e01117–01114. [PubMed: 24865556]
- Brune A, Dietrich C. The gut microbiota of termites: digesting the diversity in the light of ecology and evolution. *Annu. Rev. Microbiol.* in press. 2015
- Buchon N, Broderick NA, Chakrabarti S, Lemaitre B. Invasive and indigenous microbiota impact intestinal stem cell activity through multiple pathways in *Drosophila*. *Genes Dev.* 2009a; 23:2333–2344. [PubMed: 19797770]
- Buchon N, Broderick NA, Poidevin M, Pradervand S, Lemaitre B. *Drosophila* intestinal response to bacterial infection: activation of host defense and stem cell proliferation. *Cell Host Microbe.* 2009b; 5:200–211. [PubMed: 19218090]
- Buchon N, Broderick NA, Lemaitre B. Gut homeostasis in a microbial world: insights from *Drosophila melanogaster*. *Nat. Revs Microbiol.* 2013a; 11:615–626. [PubMed: 23893105]

- Buchon N, Osman D, David FP, Fang HY, Boquete JP, Deplancke B, Lemaitre B. Morphological and molecular characterization of adult midgut compartmentalization in *Drosophila*. *Cell Rep.* 2013b; 3:1725–1738. [PubMed: 23643535]
- Buchon N, Osman D. All for one and one for all: regionalization of the *Drosophila* intestine. *Insect Biochem. Mol. Biol.* submitted.
- Chatterjee M, Ip YT. Pathogenic stimulation of intestinal stem cell response in *Drosophila*. *J. Cell. Physiol.* 2009; 220:664–671. [PubMed: 19452446]
- Chen H, Zheng X, Zheng Y. Age-associated loss of lamin-B leads to systemic inflammation and gut hyperplasia. *Cell.* 2014; 159:829–843. [PubMed: 25417159]
- Chng WB, Bou Sleiman MS, Schupfer F, Lemaitre B. Transforming growth factor beta/activin signaling functions as a sugar-sensing feedback loop to regulate digestive enzyme expression. *Cell Rep.* 2014; 9:336–348. [PubMed: 25284780]
- Choi NH, Kim JG, Yang DJ, Kim YS, Yoo MA. Age-related changes in *Drosophila* midgut are associated with PVF2, a PDGF/VEGF-like growth factor. *Aging Cell.* 2008; 7:318–334. [PubMed: 18284659]
- Clissold FJ, Tedder BJ, Conigrave AD, Simpson SJ. The gastrointestinal tract as a nutrient-balancing organ. *Proc. Roy. Soc. B.* 2010; 277:1751–1759.
- Dillon RJ, Dillon VM. The gut bacteria of insects: nonpathogenic interactions. *Annu. Rev. Entomol.* 2004; 49:71–92. [PubMed: 14651457]
- Douglas, AE. Alimentary canal, digestion and absorption.. In: Simpson, SJ.; Douglas, AE., editors. *The Insects: Structure and Function*. Cambridge University Press; New York: 2013. p. 46-80.
- Douglas AE. Multiorganismal insects: diversity and function of resident microorganisms. *Annu. Rev. Entomol.* 2015; 60:17–34. [PubMed: 25341109]
- Fink C, Staubach F, Kuenzel S, Baines JF, Roeder T. Noninvasive analysis of microbiome dynamics in the fruit fly *Drosophila melanogaster*. *Appl. Environ. Microbiol.* 2013; 79:6984–6988. [PubMed: 24014528]
- Gomes FM, Carvalho DB, Machado EA, Miranda K. Ultrastructural and functional analysis of secretory goblet cells in the midgut of the lepidopteran *Anticarsia gemmatilis*. *Cell Tiss. Res.* 2013; 352:313–326.
- Guo L, Karpac J, Tran SL, Jasper H. PGRP-SC2 promotes gut immune homeostasis to limit commensal dysbiosis and extend lifespan. *Cell.* 2014; 156:109–122. [PubMed: 24439372]
- Ha EM, Lee KA, Park SH, Kim SH, Nam HJ, Lee HY, Kang D, Lee WJ. Regulation of DUOX by the Galphag-phospholipase C β -Ca $^{2+}$ pathway in *Drosophila* gut immunity. *Dev. Cell.* 2009; 16:386–397. [PubMed: 19289084]
- Ha EM, Oh CT, Bae YS, Lee WJ. A direct role for dual oxidase in *Drosophila* gut immunity. *Science.* 2005; 310:847–850. [PubMed: 16272120]
- Jeffers LA, Michael Roe R. The movement of proteins across the insect and tick digestive system. *J. Insect Physiol.* 2008; 54:319–332. [PubMed: 18177888]
- 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. Symbiotic lactobacilli stimulate gut epithelial proliferation via Nox-mediated generation of reactive oxygen species. *EMBO J.* 2013; 32:3017–3028. [PubMed: 24141879]
- Karasov WH, Diamond J. Interplay between physiology and ecology in digestion. *BioScience.* 1988; 38:602–611.
- Karasov WH, Douglas AE. Comparative digestive physiology. *Comp. Physiol.* 2013; 3:741–783.
- Kaya, HK.; Vega, FE. Scope and basic principles of insect pathology.. In: Vega, FE.; Kaya, HK., editors. *Insect Pathology*. Elsevier; Amsterdam: 2012. p. 1-12.
- Kuraishi T, Binggeli O, Opota O, Buchon N, Lemaitre B. Genetic evidence for a protective role of the peritrophic matrix against intestinal bacterial infection in *Drosophila melanogaster*. *Proc. Natl Acad. Sci. U S A.* 2011; 108:15966–15971. [PubMed: 21896728]
- Lee KA, Kim SH, Kim EK, Ha EM, You H, Kim B, Kim MJ, Kwon Y, Ryu JH, Lee WJ. Bacterial-derived uracil as a modulator of mucosal immunity and gut-microbe homeostasis in *Drosophila*. *Cell.* 2013; 153:797–811. [PubMed: 23663779]

- Lehane, MJ.; Billingsley, PF., editors. *Biology of the Insect Midgut*. Chapman and Hall; London, UK: 1996.
- Lemaitre B, Miguel-Aliaga I. The digestive tract of *Drosophila melanogaster*. *Annu. Rev. Gen.* 2013; 47:377–404.
- Limmer S, Weiler A, Volkenhoff A, Babatz F, Klambt C. The *Drosophila* blood-brain barrier: development and function of a glial endothelium. *Frontiers Neurosci.* 2014; 8:365.
- MacMillan HA, Sinclair BJ. The role of the gut in insect chilling injury: cold-induced disruption of osmoregulation in the fall field cricket, *Gryllus pennsylvanicus*. *J. Exp. Biol.* 2011; 214:726–734. [PubMed: 21307058]
- MacMillan HA, Williams CM, Staples JF, Sinclair BJ. Reestablishment of ion homeostasis during chill-coma recovery in the cricket *Gryllus pennsylvanicus*. *Proc. Natl Acad. Sci. U S A.* 2012; 109:20750–20755. [PubMed: 23184963]
- Malone M, Herron M, Morales MA. Continuous measurement of macronutrient ions in the transpiration stream of intact plants using the meadow spittlebug coupled with ion chromatography. *Plant Physiol.* 2002; 130:1436–1442. [PubMed: 12428008]
- Marianes A, Spradling AC, Brand A. Physiological and stem cell compartmentalization within the *Drosophila* midgut. *Elife.* 2013; 2:e00886. [PubMed: 23991285]
- Mathew LG, Campbell EM, Yool AJ, Fabrick JA. Identification and characterization of functional aquaporin water channel protein from alimentary tract of whitefly, *Bemisia tabaci*. *Insect Biochem. Mol. Biol.* 2011; 41:178–190. [PubMed: 21146609]
- Matter K, Balda MS. Signalling to and from tight junctions. *Nat. Rev. Mol. Cell Biol.* 2003; 4:225–236. [PubMed: 12612641]
- Micchelli CA, Perrimon N. Evidence that stem cells reside in the adult *Drosophila* midgut epithelium. *Nature.* 2006; 439:475–479. [PubMed: 16340959]
- Michalkova V, Benoit JB, Weiss BL, Attardo GM, Aksoy S. Vitamin B6 generated by obligate symbionts is critical for maintaining proline homeostasis and fecundity in tsetse flies. *Appl. Environ. Microbiol.* 2014; 80:5844–5853. [PubMed: 25038091]
- Ohlstein B, Spradling A. The adult *Drosophila* posterior midgut is maintained by pluripotent stem cells. *Nature.* 2006; 439:470–474. [PubMed: 16340960]
- Pool AH, Scott K. Feeding regulation in *Drosophila*. *Curr. Op. Neurobiol.* 2014; 29:57–63. [PubMed: 24937262]
- Price DR, Gatehouse JA. Genome-wide annotation and functional identification of aphid GLUT-like sugar transporters. *BMC Genomics.* 2014; 15:647. [PubMed: 25091229]
- Reiher W, Shirras C, Kahnt J, Baumeister S, Isaac RE, Wegener C. Peptidomics and peptide hormone processing in the *Drosophila* midgut. *J. Proteome Res.* 2011; 10:1881–1892. [PubMed: 21214272]
- Ren C, Webster P, Finkel SE, Tower J. Increased internal and external bacterial load during *Drosophila* aging without life-span trade-off. *Cell Metab.* 2007; 6:144–152. [PubMed: 17681150]
- Rera M, Clark RI, Walker DW. Intestinal barrier dysfunction links metabolic and inflammatory markers of aging to death in *Drosophila*. *Proc. Natl Acad. Sci. U S A.* 2012; 109:21528–21533. [PubMed: 23236133]
- Ryu JH, Kim SH, Lee HY, Bai JY, Nam YD, Bae JW, Lee DG, Shin SC, Ha EM, Lee WJ. Innate immune homeostasis by the homeobox gene caudal and commensal-gut mutualism in *Drosophila*. *Science.* 2008; 319:777–782. [PubMed: 18218863]
- Schwartz GJ, Zeltser LM. Functional organization of neuronal and humoral signals regulating feeding behavior. *Annu. Rev. Nutr.* 2013; 33:1–21. [PubMed: 23642202]
- Shahabuddin M, Toyoshima T, Aikawa M, Kaslow DC. Transmission-blocking activity of a chitinase inhibitor and activation of malarial parasite chitinase by mosquito protease. *Proc. Natl Acad. Sci. U S A.* 1993; 90:4266–4270. [PubMed: 8483942]
- Shakesby AJ, Wallace IS, Isaacs HV, Pritchard J, Roberts DM, Douglas AE. A water-specific aquaporin involved in aphid osmoregulation. *Insect Biochem. Mol. Biol.* 2009; 39:1–10. [PubMed: 18983920]
- Shanbhag S, Tripathi S. Epithelial ultrastructure and cellular mechanisms of acid and base transport in the *Drosophila* midgut. *J. Exp. Biol.* 2009; 212:1731–1744. [PubMed: 19448082]

- Simpson SJ, Clissold FJ, Lihoreau M, Ponton F, Wilder SM, Raubenheimer D. Recent advances in the integrative nutrition of arthropods. *Annu. Rev. Entomol.* 2015; 60:293–311. [PubMed: 25341097]
- Simpson SJ, Sibly RM, Lee KP, Behmer ST, Raubenheimer D. Optimal foraging when regulating intake of multiple nutrients. *Animal Behav.* 2004; 68:1299–1311.
- Spring JH, Robichaux SR, Hamlin JA. The role of aquaporins in excretion in insects. *J. Exp. Biol.* 2009; 212:358–362. [PubMed: 19151210]
- Takeshima S, Younossi-Hartenstein A, Ortiz PA, Hartenstein V. A novel tissue in an established model system: the *Drosophila* pupal midgut. *Dev. Genes Evol.* 2011; 221:68–81.
- Toprak U, Harris S, Baldwin D, Theilmann D, Gillott C, Hegedus DD, Erlandson MA. Role of enhancin in *Mamestra configurata* nucleopolyhedrovirus virulence: selective degradation of host peritrophic matrix proteins. *J. Gen. Virol.* 2012; 93:744–753. [PubMed: 22238230]
- Wilkinson T, Ashford D, Pritchard J, Douglas AE. Honeydew sugars and osmoregulation in the pea aphid *Acyrtosiphon pisum*. *J. Exp. Biol.* 1997; 200:2137–2143. [PubMed: 9320049]
- Zoepfel J, Reiher W, Rexer KH, Kahnt J, Wegener C. Peptidomics of the agriculturally damaging larval stage of the cabbage root fly *Delia radicum* (Diptera: Anthomyiidae). *PLoS One.* 2012; 7:e41543. [PubMed: 22848525]

Highlights

- The gut epithelium functions in insect nutrition, immunity and osmoregulation
- Controls over expression of genes for digestive enzymes and transporters shape nutrient acquisition by the gut epithelium
- Gut microbiota is managed by immune functions including reactive oxygen species and antimicrobial peptides
- Breakdown of the gut epithelial barrier function mediates chill coma and is implicated in aging

Fig. 1.

Integration of the digestive and assimilatory function of the gut epithelium into the system-wide regulation of nutrient acquisition and allocation by an insect. White arrows: the multi-way integration of gut epithelium function with feeding and nutrient allocation. Shaded arrows: nutrient allocation to competing fates (here shown as four broad categories) is determined by interaction between nutrient availability and the insect neuro-endocrinal controls.

Fig. 2.

Dynamics of microorganisms associated with the insect gut. Most ingested microbial cells pass through the gut lumen with bulk flow of food or are retained or killed in the gut. Transfer to the hemolymph (dashed line) is generally prevented by physical (peritrophic envelope) and chemical (immune-related) barrier functions. IMD pathway, immune deficiency pathway; AMPs, antimicrobial peptides; ROS, reactive oxygen species.

Fig. 3.

Transport across the gut lumen. Transcellular transport can be mediated by membrane-bound transporters and by transcytosis via endocytic vesicles, and paracellular transport is controlled by septate junctions. Paracellular transport occurs between epithelial cells. Transport may occur from apical to basal surface (shown) or in the reverse direction. EC, enterocyte; EE, endocrine cell; N, nucleus.