Abstracts: Diptera

Lee *et al.*, 2017, Microbiota, gut physiology, and insect immunity. Adult *Drosophila* contains three distinct domains: foregut, midgut and hindgut. Foregut is at the anteriormost region and is originated from ectoderm. It includes pharynx and oesophagus for passage of ingested food and crop for food storage. Midgut is the region from cardia to midgut-hindgut junction where Malpighian tubules are attached. It is originated from endoderm and functions for food digestion and nutrient absorption. Cardia serves as a valve for food passage regulation. Hindgut is ectoderm-derived, extending to the rectum, and is responsible for absorption of water and ions.

Midgut is a single layer of epithelium and a visceral of muscle layer. The midgut epithelium contains four cell types: enterocytes (ECs), enteroendocrine cells (EECs), ISCs and enteroblasts (EBs). ECs are large polypoid cells secreting digestive enzymes and absorbing nutrients. They are the most abundant in midgut epithelium. EECs secrets hormones. ISCs are dividing progenitor cells. EBs are restricted progenitor cells produced by ISCs differentiation, and further differentiates into ECs or EECs. The lumenal side of midgut is covered by peritrophic matrix, a chitin polymer layer. A mucus layer fills between the epithelium and peritrophic matrix. The peritrophic matrix, mucus layer and epithelium act as physical barrier for immunity.

The midgut is further regionalized into anterior region, copper cell region (CCR) and posterior region. Anterior region functions for food breakdown by secreting enzymes. CCR is for further digestion with its low pH. Posterior region is for absorption of nutrients. When radius of gut is measured, midgut can be divided into six regions (R0-R5). R0 is cardia, R1-R2 is anterior region, R3 is CCR, and R4-R5 is posterior region.

The primary immune systems in midgut of *Drosophila* are DUOX pathway (Ha *et al.*, 2009) and IMD pathway (Tzou *et al.*, 2000). Toll pathway is dispensable in gut epithelium.

IMD pathway includes: (1) recognition of bacterial peptidoglycans; (2) intracellular cascade activating Relish, a member of NF-κB transcription factor family; (3) expression of antimicrobial peptides; (4) negative regulation of IMD pathway.

IMD pathway begins with PGRPs that recognize peptidoglycans. PGRP-LC is a transmembrane receptor recognizes DAP-type peptidoglycan characterized by meso-diaminopimelic acid in peptide chain

(Choe *et al.*, 2002; Gottar *et al.*, 2002; Ramet *et al.*, 2002). PGRP-LE resides in cytoplasm and recognizes DAP-type peptidoglycan, and thus activates IMD pathway (Bosco-Drayon *et al.*, 2012).

After binding to peptidoglycan, PGRP-LC recruits IMD, Dredd and FADD to form a signaling complex (Georgel *et al.*, 2001; Naitza *et al.*, 2002). Dreed cleaves IMD and Relish for their activation. Ultimately, N-terminal cleaved Relish translocates into nucleus for target gene expression (Khush *et al.*, 2001).

Ubquitination and phosphorylation is required for IMD activation. Dredd activation requires K63-ubiquitination by IAP2, a E3-ligase (Meinander *et al.*, 2012). Dredd cleaves IMD, enabling its binding with IAP2. IAP2 generates K63-polyubiquitination, which is required for recruitment of TAK1/TAB2 complex (Vidal *et al.*, 2001). TAB2 binds to K63-polyubiquitination of IMD, and TAK1 is a MAPKKK kinase for activation of IKK complex. IKK complex is composed of IRD5 (catalytic activity) and Kenny (regulatory subunit). Activated IKK complex phosphorylates Relish on multiple sites, which activates its transcription factor activity (Erturk-Hasdemir *et al.*, 2009; Silverman *et al.*, 2000). Relish induces expression of genes involved in non-self recognition, signaling pathways, proteolysis and antimicrobial peptides.

IMD pathway is inhibited by several mechanisms. PGRP amidase (PGRP-LB, -SC1a, -SC1b, -SC2) degrades peptidoglycan and thus inhibits IMD pathway (Bischoff *et al.*, 2006; Guo *et al.*, 2014; Paredes *et al.*, 2011). PIRK is a transcriptional target of IMD pathway and inhibits IMD pathway (Aggarwal *et al.*, 2008; Kleino *et al.*, 2008; Lhocine *et al.*, 2008). It may disrupt IMD signaling as it interacts with PGRP-LC, PGRP-LE and IMD (Aggarwal *et al.*, 2008). Other inhibitors of IMD pathway including Dnr1 for Dredd inhibition (Foley and O'Farrell, 2004; Guntermann *et al.*, 2009); Caspar for Dredd-dependent Relish cleavage inhibition (Kim *et al.*, 2006); Trabid targeting TAK1 (Fernando *et al.*, 2014); CYLD, a deubiquitinating enzyme (Tsichritzis *et al.*, 2007); SkpA, a subunit of SCF-E3 ubquitin ligase targeting Relish (Khush *et al.*, 2002); and transcription inhibitors such as caudal (Ryu *et al.*, 2008) and Nubbin (Dantoft *et al.*, 2013).

IMD pathway is inhibited in gut, and its activation leads to pathologic symptoms including mocrobiota dysbiosis and dysplasia (Bosco-Drayon *et al.*, 2012; Guo *et al.*, 2014; Lhocine *et al.*, 2008; Ryu *et al.*, 2008). For instance, caudal is gut-specific inhibitor of IMD pathway and its knockdown causes gut cell apoptosis, decreased survival rate and change of microbiome (Ryu *et al.*, 2008). Knockdown of PGRP-SC2, an inhibitor of IMD pathway, also leads to mocrobiota dysbiosis and dysplasia (Guo *et al.*, 2014).

DUOX is a member of nicotinamide adenine dinucleotide phosphate oxidase (NOX) family and is responsibe for bacterial-induced reactive oxygen species (ROS) generation. ROS plays an important role in gut immunity, and is degraded by secretory immune-related catalase (IRC) (Ha *et al.*, 2005).

NOX/DUOX family proteins share a catalytic gp91phox domain, and DUOX contains an additional

peroxidase homology domain (PHD). Generally, NOX generates superoxide anion in extracellular space by electron transfer from NADPH in cytoplasm to oxygen across the membrane. Superoxide anion is subsequently converted to H_2O_2, which can be further converted to HClO by myeloperoxidase.

In *Drosophila*, there are one NOX and one DUOX (Ha *et al.*, 2005). The produce of HClO is DUOX-dependent (Ha *et al.*, 2005). DUOX is essential for gut immunity (Ha *et al*, 2005; Ha *et al*, 2009). It is activated only with transient microorganisms, but not with commensals. Uracil acts as a ligand for DUOX activation (Lee *et al.*, 2013). It is secreted by several pathogens, but not by commensals.

DUOX enzymatic activity requires calcium released from ER, and thus is regulated by PLCβ and Gαq (Ha *et al.*, 2005; Ha *et al.*, 2009). At downstream of Gαq, PLCβ is required for generation of 1,4,5-triphosphate, which is recognized by corresponding receptor and enables release of calcium from ER. Transcription of DUOX is up-regulated by (Ha *et al.*, 2009) (1) peptidoglycan-dependent cascade composed of PGRP-LC, IMD, MEKK1, MKK3, p38 and ATF2; (2) uracil-dependent cascade including PLCβ, MEKK1, MKK3, p38 and ATF2. ATF2 is a transcription factor.

Negative regulation of DUOX transcription is mediated by inhibition of peptidoglycan-dependent p38 activation, which requires PLCβ, calcineurin B and MAP kinase phosphatase 3 (MKP3) (Ha *et al.*, 2009). It indicates that activation of DUOX requires certain amount of peptidoglycan, and therefore, DUOX remains inactivated under commensals.

Mid gut is dynamic. Adult *Drosophila* intestinal epithelium is renewed every 1 week (Micchelli and Perrimon, 2006). This gut renewal is dependent on asymmetric division of ISC. The two daughter cells of ISC division, one becomes self-renewed ISC and another one differentiates into EB, which further differentiates into EC or EEC. The fate decision of ISCs after division is dependent on the antagonism of Delta-Notch signaling and BMP signaling (Tian and Jiang *et al.*, 2014). Delta-Notch signaling also plays an important role in differentiation into EC/EEC (Ohlstein and Spradling, 2007; Perdigoto *et al.*, 2011).

Proliferation of ISC is under tightly control to maintain gut homeostasis. Low rate of ISC proliferation leading to reduced replacement of damaged cells, destroying gut integrity and leads to originasm death. High rate of ISC proliferation leads to accumulation of unwanted cells, causing pathology (Biteau *et al.*, 2008). Several signaling pathways are involved in ISC proliferation activation, including JAK/STAT, EGFR, Hippo, JNK and Wingless (Biteau *et al.*, 2008; Cordero *et al.*, 2012; Jiang *et al.*, 2011; Karpowicz *et al.*, 2010; Lee *et al.*, 2009). Myc may be a common downstream of JAK/STAT, EGFR, Hippo and Wingless (Ren *et al.*, 2013). Insulin receptor signaling in ISC is required for ISC proliferation (Amcheslavsky *et al.*, 2009).

Ligands from nearby injured cells are responsible for activation of ISC proliferation. In stressed ECs, JAK/STAT ligand Upd3 and EGFR ligand Keren are expressed under control of JNK and Hippo signaling

(Jiang *et al.*, 2009; Ren *et al.*, 2010; Jiang *et al.*, 2011). EGFR ligands vein and spitz are produced by visceral muscles and progenitors respectively (Jiang *et al.*, 2011). Stressed EBs produce Upd2 through activation of Hedgehog pathway (Tian *et al.*, 2015), and Wingless ligand under control of JNK signaling (Cordero *et al.*, 2012). Hippo signaling in ISC is controled by intracellular interactions of two cadherins, Fat in ISC and Dachsous (DS) in EC (Karpowicz *et al.*, 2010).

IMD pathway may regulate ISC proliferation via controling number of gut bacteria (Buchon *et al.*, 2009). ROS induced by DUOX signaling also accerlates ISC proliferation. ROS may induce ISC proliferation by tissue damaging (Buchon *et al.*, 2009; Karpowicz *et al.*, 2010; Ren *et al.*, 2010; Ren *et al.*, 2013; Shaw *et al.*, 2010; Staley and Irvine, 2010). ROS may also activates ISC proliferation directly by targeting redox-sensitive components of signalings. ROS activates JAK/STAT by redox-sensitive tyrosine phosphatase (Liu *et al.*, 2004), JNK by thioredoxin (Junn *et al.*, 2000) and Wnt by nucleoredoxin (Funato *et al.*, 2006).

2. Husink *et al.*, 2020, Insect-symbiont gene expression in the midgut bacteriocytes of a blood-sucking parasite. Sheep ked *Melophagus ovinus* is a species of wingless, blood-sucking insect that is permanently associated with vertebrate host and transmitted via host interactions. Its primary bacterial symbiont *Arsenophonus melophagi* lives intracellularly in bacteriocytes that assemble into special structure (bacteriome) in midgut.

Sheep ked interacts with its symbiont nutritionally. Transcription analysis shows symbiont high expression of a pathway that converts proline to L-glutamate through the PutA enzyme (EC 1.5.5.2/1.2.1.88) and its subsequent conversion to D -glutamate by the MurI enzyme (EC 5.1.1.3). Proline is almost always the most common amino acid in insect hemolymph (Arrese and Soulages 2010). In insects, proline is generally reserved for energy-demanding activity such as flight. In wingless sheep ked, proline storage might be used for bacterial symbiont for energy metabolism and peptidoglycan synthesis. However, the symbiont expression of B-vitamin synthesis is low, except that of lipoic acid. It is possible that B vitamins are not essential for sheep ked, or are only needed during particular life stage. It is also possible that sheep ked does not rely on symbiont for B vitamins.

Symbiont *Arsenophonus* might be dependent on host for metal ions. High expression of zinc transporters in bacteriocytes and symbionts, together with low expression of zinc protease in bacteriocytes, indicate that symbiont *Arsenophonus* is demanding on zinc. Symbiont zinc-dependent proteins might be protease, or a putative metalo-beta-lactamase. Beta-lactamases are enzymes that provide bacteria with resistance to beta-lactam antibiotics such as penicillin, ampicillin. Metalo-beta-lactamases in particular are well-known for their resistance to a broad spectrum of beta-lactam antibiotics and beta-lactamase inhibitors (Bradford 2001; Drawz and Bonomo 2010). The sheep from which sheep keds are collected

are often treated with beta-lactam antibiotics. Besides, ferritin is highly expressed among whole gut, and transferrin is down-regulated in bacteriocytes. Ferritin sequesters iron from a blood meal and blocks iron ions intracellularly, while transferrin mediates transport of iron through blood plasma. Transferrin can also act as an antimicrobial protein sequestering iron from pathogens (Yoshiga et al. 2001). Down-regulation of transferrin in bacteriome might be a sort of immune privilege.

There is potential immune compromise in sheep kep bacteriome. Immune response genes such as attacin (antimicrobial peptide) and two lysozymes, are down-regulated in bacteriocytes. PGRP-LB, an amidase that degrades peptidoglycan to inhibit immune responses, is highly expressed along whole gut, while GNBP, a pattern recognition receptor, is lowly expressed along gut. Furthermore, sheep ked lacks peritrophic matrix, a physical barrier to protect against pathogens. It is consist with the lack of chitin synthesis in sheep ked gut.

Waterhouse *et al.*, 2007, Immune-related genes and pathways in disease-vector mosquitoes.

Immunity-related genes:

285 *Drosophila melanogaster* (Dm), 338 *Anopheles gambiae* (Ag), and 353 *Aedes aegypti* (Aa) genes from 31 gene families and functional groups implicated in classical innate immunity or defense functions such as apoptosis and response to oxidative stress.

Orthology groups (whole genome):

4951 orthologous trios (1:1:1 orthologs in the three species) and 886 mosquito-specific orthologous pairs (absent from Dm).

Orthology groups (immune-related):

91 trios and 57 pairs, plus a combined total of 589 paralogous genes in the three species.

Immune-related orthology trios are more divergent than that of whole genome:

Phylogenetic distances of genes in each trio is measured by amino acid substitutions. With Dm as reference, immune-related trios of Ag and Am are more divergent (on average) compared with trios of whole genome, and several Ag immunity genes are considerably more divergent than their Aa orthologs.

Large variation exists in different immune families in their proportions of orthologous trios, mosquito-specific pairs and species-specific genes:

(1) Predominantly trio orthologs: apoptosis inhibitors (IAPs), oxidative defense enzymes [superoxide dismutases (SODs), glutathione peroxidases (GPXs), thioredoxin peroxidases (TPXs), heme-containing peroxidases (HPXs)], class A and B scavenger receptors (SCRs).

(2) Rarely trio orthologs: immune effectors, including three antimicrobial peptides.

(3) Intermediately: C-type lectins.

Strong divergent evolution of immune recognition genes:

Fruit fly and mosquito recognition proteins mostly form distinct clades within each gene family.

Bosco-Drayon *et al.*, 2012, Peptidoglycan sensing by the receptor PGRP-LE in the *Drosophila* gut induces immune responses to infectious bacteria and tolerance to microbiota.

In *Drosophila*, peptidoglycan recognition protein (PGRP)-LE senses peptidoglycan, and induces NF-kappaB dependent responses to infectious bacteria, but also tolerance to symbionts via up-regulation of pirk and PGRP-LB, which inhibits IMD signaling. Loss of PGRP-LE-mediated detection of bacteria in the gut results in systemic immune activation, which can be rescued by overexpressing PGRP-LB in the gut.

Wang *et al.*, 2009, Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission.

Tsetse flies have coevolved with mutualistic endosymbiont *Wigglesworthia glossinidiae*. A tsetse peptidoglycan recognition protein (PGRP-LB) is crucial for symbiotic tolerance and trypanosome infection processes. Tsetse *pgrp-lb* is expressed in the *Wigglesworthia*-harboring organ (bacteriome) in the midgut, and its level of expression correlates with symbiont numbers. Adult tsetse cured of *Wigglesworthia* infections have significantly lower *pgrp-lb* levels than corresponding normal adults. RNA interference (RNAi)-mediated depletion of *pgrp-lb* results in the activation of the immune deficiency (IMD) signaling pathway and leads to the synthesis of antimicrobial peptides (AMPs), which decrease *Wigglesworthia* density. Depletion of *pgrp-lb* also increases the host's susceptibility to trypanosome infections. Finally, parasitized adults have significantly lower pgrp-lb levels than flies, which have successfully eliminated trypanosome infections. When both PGRP-LB and IMD immunity pathway functions are blocked, flies become unusually susceptible to parasitism. Based on the presence of conserved amidase domains, tsetse PGRP-LB may scavenge the peptidoglycan (PGN) released by *Wigglesworthia* and prevent the activation of symbiont-damaging host immune responses. In addition, tsetse PGRP-LB may have an anti-protozoal activity that confers parasite resistance.

Martinez *et al.*, 2016, Addicted? Reduced host resistance in populations with defensive symbionts.

Heritable symbionts that protect their hosts from pathogens have been described in a wide range of insect species. By reducing the incidence or severity of infection, these symbionts have the potential to reduce the strength of selection on genes in the insect genome that increase resistance. Therefore, the presence of such symbionts may slow down the evolution of resistance. Here we investigated this idea by exposing *Drosophila melanogaster* populations to infection with the pathogenic Drosophila C

193 virus (DCV) in the presence or absence of *Wolbachia*, a heritable symbiont of arthropods that confers
194 protection against viruses. After nine generations of selection, we found that resistance to DCV had
195 increased in all populations. However, in the presence of *Wolbachia* the resistant allele of *pastrel*—a
196 gene that has a major effect on resistance to DCV—was at a lower frequency than in the symbiont-free
197 populations. This finding suggests that defensive symbionts have the potential to hamper the evolution
198 of insect resistance genes, potentially leading to a state of evolutionary addiction where the genetically
199 susceptible insect host mostly relies on its symbiont to fight pathogens.

200 **You *et al.*, 2014, Homeostasis between gut-associated microorganisms and the immune**
201 **system in Drosophila.**

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203 **Lima *et al.*, 2021, Evolution of Toll, Spatzle and MyD88 in insects: the problem of the**
204 **Diptera bias.**

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206 **Sackton *et al.*, 2017, Rapid expansion of immune-related gene families in the house fly,**
207 ***Musca domestica*.**

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209 **Christophides *et al.*, 2002, Immunity-related genes and gene families in Anopheles**
210 **gambiae.**

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212 **Myllymaki *et al.*, 2014, The *Drosophila* Imd signaling pathway.**

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214 **Kleino Silverman, 2014, The *Drosophila* IMD pathway in the activation of the humoral**
215 **immune response.**

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217 **Zhang Zhu, 2009, Drosomycin, an essential component of antifungal defence in**
218 ***Drosophila*.**

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220 **Ganesan *et al.*, 2010, NF-κB/Rel proteins and the humoral immune responses of**
221 ***Drosophila melanogaster*.**

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223 **The tsetse fly displays an attenuated immune response to its secondary symbiont, *Sodalis***
224 ***glossinidius*.**