

黑腹果蝇先天免疫研究进展*

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摘 要 先天免疫是昆虫适应复杂环境的关键,也是新型害虫防治的重要研究方向。昆虫通过模式识别受体识别环境中不同的病原物,激活先天免疫系统以清除病原物。昆虫的先天免疫系统主要包括体液免疫与细胞免疫,体液免疫包括免疫信号通路诱导产生抗菌肽、活性氧以及黑化作用等,细胞免疫包括血细胞的吞噬、包裹和凝结。本文将重点总结黑腹果蝇 *Drosophila melanogaster* 在模式识别受体、免疫信号通路和细胞免疫相关方面的研究进展,为进一步研究其他经济昆虫与农业害虫的免疫机制,提高生产经济效益提供参考。

关键词 黑腹果蝇;先天免疫;模式识别蛋白;免疫信号通路

Advances in research on the innate immune system of *Drosophila melanogaster*

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Abstract The innate immune system plays a critical role in the ability of insects to adapt to changing environments, and its underlying mechanism has become a new area of research in pest control. The insect innate immune system is comprised of humoral immunity and cellular immunity, and involves many proteins and molecules. Research on the innate immune system of insects has advanced greatly in recent years, mainly due to research on the fruit fly *Drosophila melanogaster*. *D. melanogaster* is an ideal model organism for studying innate immunity due to its clear genetic background and many available manipulation tools. Pattern recognition receptors (PRRs), antimicrobial peptides (AMPs) and immune signaling pathways have been well investigated in *Drosophila*. In this mini-review, we summarize recent advances in research on the innate immune system of *Drosophila* over the last 20 years. We hope that this review will inspire the study of the innate immune mechanisms of other insect species and provide a theoretical basis for pest control.

Key words *Drosophila*; innate immunity; pattern recognition receptors; immune signaling pathway

昆虫是目前世界上已知种类最多和分布最广的生物类群。为了适应复杂多变的环境,在漫长的进化历程中,昆虫逐渐形成了可以抵御不同病原微生物入侵的先天性免疫系统(Innate immune system)。昆虫对病原微生物的识别依赖于一系列的模式识别受体(Pattern recognition receptors, PRRs),PRRs可以特异性识别来自于

不同病原微生物的病原相关分子模式(Pathogen-associated molecular patterns, PAMPs)从而激活下游信号通路,启动先天免疫系统以清除病原物,使虫体的生命活动得以正常进行。根据作用机制的不同,先天免疫可分为体液免疫(Humoral immunity)和细胞免疫(Cellular immunity)。体液免疫主要通过Toll、免疫缺失(Immune

*资助项目 Supported projects: 国家自然科学基金面上基金项目(31472049)

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收稿日期 Received: 2019-08-10; 接受日期 Accepted: 2019-08-25

deficiency, IMD)、JAK-STAT 等信号通路上调抗菌肽、活性氧和溶菌酶等效应分子 (Effectors) 的表达杀灭病原物。脂肪体、血淋巴、肠道和马氏管是重要的体液免疫器官。细胞免疫主要通过血细胞 (Hemocytes) 参与的细胞吞噬 (Phagocytosis)、包囊 (Encapsulation) 和结节形成 (Nodulation) 等过程清除异物 (Lemaitre and Hoffmann, 2007)。多酚氧化酶 (Prophenoloxidase, PPO) 属于体液蛋白, 激活后引发黑化反应以杀灭病原物, 能同时激活体液免疫和细胞免疫 (Lu *et al.*, 2014; Yi *et al.*, 2014)。

模式生物黑腹果蝇 *Drosophila melanogaster* 的遗传背景清晰、实验操作简便, 其研究成果推动着脊椎和无脊椎动物先天免疫系统研究。在黑腹果蝇中, 对模式识别受体、抗菌肽和免疫信号通路的研究比较深入, 但由于果蝇的血细胞数量比较少, 细胞免疫主要在鳞翅目昆虫中研究的较多。本文将重点总结黑腹果蝇先天免疫的研究进展及其作用机制, 为进一步研究其他昆虫或哺乳动物的免疫机制提供参考。

1 病原物的识别

宿主对病原微生物的识别是免疫应答启动的基础, 不同病原微生物的表面都存在保守但异于宿主的成分, 例如革兰氏阴性菌细胞壁中的脂多糖 (Lipopolysaccharide, LPS), 革兰氏阳性菌的磷壁质酸 (Lipoteichoic acid, LTA), 细菌中的肽聚糖 (Peptidoglycan, PGN), 真菌中的 β -1,3 葡聚糖 (β -1,3-glucan) 等, 这些成分统称为病原相关分子模式 (PAMPs) (Kurata, 2014)。针对不同的 PAMPs, 昆虫体内存在多种模式识别受体 (PRRs) 对其进行识别。目前在果蝇中已经发现了多种 PRRs, 包括革兰氏阴性结合蛋白 (Gram-negative binding proteins, GNBP)、 β -1,3-葡聚糖识别蛋白 (β -1,3-glucan recognition proteins, β -GRPs)、肽聚糖识别蛋白 (Peptidoglycan recognition proteins, PGRPs)、清道夫受体 (Scavenger receptors, SRs)、含硫

酯蛋白 (Thioester-containing proteins, TEPs)、唐氏综合症细胞粘附分子 (Down syndrome cell adhesion molecule, Dscam) 和凝集素 (Lectins) 等 (Blandin and Levashina, 2004; Schmucker, 2007; Pal and Wu, 2009; Canton *et al.*, 2013; Kurata, 2014; Hillyer, 2016; Shokal and Eleftherianos, 2017; Vasta *et al.*, 2017; Xia *et al.*, 2018)。此外, 最近的研究发现果蝇的 Toll-1 和 Toll-7 可以直接识别病毒, 也可能具备 PRRs 的特征 (Nakamoto *et al.*, 2012; Moy *et al.*, 2014; Chowdhury *et al.*, 2019a)。

肽聚糖识别蛋白 (PGRPs) 是果蝇中重要的模式识别受体, 根据其分子量大小可分为短型 (S) 和长型 (L) PGRP, 根据是否分解 PGN 又可分为有水解活性和无水解活性的 PGRP (Charroux *et al.*, 2018)。PGRPs 通过识别不同类型的肽聚糖启动相应的免疫信号通路 (Werner *et al.*, 2000; Kurata, 2014)。PGRP-SA、PGRP-SD 和 GGBP1 可以识别革兰氏阳性菌的 Lys-type PGN, 并激活 Toll 信号通路 (Michel *et al.*, 2001; Gobert *et al.*, 2003; Bischoff *et al.*, 2004), PGRP-LC 不同的剪接形式可识别革兰氏阴性菌和少数革兰氏阳性杆菌的 DAP-type PGN (PGRP-LC_x 和 PGRP-LC_a 复合物识别 PGN 单体, PGRP-LC_x 同源二聚体识别 PGN 多聚体), 启动 IMD 免疫信号通路 (Choe *et al.*, 2002; Gottar *et al.*, 2002)。

GNBP3 可识别真菌的 β -葡聚糖 (Gottar *et al.*, 2006), Persephone 蛋白酶能识别细菌和真菌的毒力因子 (Issa *et al.*, 2018), 以上识别均能引发 Toll 信号通路的传递。PGRP-LE、GNBP3、含硫酯蛋白 TEP2 和 TEP4、C-型凝集素 DL2 和 DL3 能激活 PPO 通路 (Takehana *et al.*, 2004; Matskevich *et al.*, 2010; Shokal *et al.*, 2018; Xia *et al.*, 2018)。清道夫受体、TEPs 和 Dscam 主要引发免疫细胞对病原微生物的吞噬反应 (Melcarne *et al.*, 2019a), 凝集素对病原物的识别主要引发血细胞的凝集与黑化反应 (Xia *et al.*, 2018)。

的胞外区结合 PGN (DAP-type) 后促使其胞内区与 IMD 蛋白相互作用, 启动下游的级联反应, 最终激活 NF- κ B 家族转录因子 Relish, 上调抗菌肽等基因的表达 (Takehana *et al.*, 2004; Kaneko *et al.*, 2006)。IMD 蛋白含有死亡结构域 (Death domain, DD), 能招募接头分子 FADD (Fas-associated death domain-containing protein)。FADD 和 caspase 相关蛋白 DREDD (Death-related ced-3/Nedd2-like protein) 结合, 随后募集 TAK1-TAB2 (TGF β -activated kinase 1, TAK1-binding protein 2) 复合物, 并激活由 IRD5 (Kinase immune response deficient 5) 和 Kenny 组成的 IKK 激酶复合体, 使 Relish 发生磷酸化。而后 Relish C-端的 NF- κ B 抑制结构域中 I κ B 序列 (Ankyrin 重复序列) 被 IMD-FADD-DREDD 复合物切割, 解除 I κ B 对 Relish N 端 RHD 结构域 (Rel homology domain) 的抑制, 使其从细胞质转移到细胞核, 结合靶基因启动子上的 κ B-DNA 元件, 上调 *Diptericin*、*Cecropin*、*Defensin* 和 *Attacin* 等抗菌肽基因的表达 (Ferrandon *et al.*, 2007)。其中, Caspar 作为 IMD 通路的负调控因子, 通过阻止 IMD 复合物对 Relish 的切割, 抑制 IMD 通路的活性 (Kim *et al.*, 2006)。另外, 谷氨酰胺转移酶 (TG) 也可以通过影响 Relish 的多聚化, 或将多胺化合物与 Relish 进行整合, 调节 Relish 的转录活性 (Maki *et al.*, 2017)。PGRP-LC 和 Relish 还参与果蝇唾液腺的降解, 其中 Relish 可以通过调节 Atg1 的表达调节细胞自噬的发生, 说明细胞自噬可能也受到 IMD 通路的调节 (Nandy *et al.*, 2018)。

PGRP 家族蛋白是与 IMD 通路最为相关的模式识别受体, PGRPs 可通过结合或者水解 PGN、结合其他的 PGRP 等来调控 IMD 通路的活性。PGRP-LE 可与 PGRP-LC_x 结合, 从而促进 PGRP-LC 与 PGN 的结合; PGRP-SD 结合 PGN (DAP-type), 促进 PGRP-LC 对 PGN 的识别; PGRP-LF 竞争性地结合 PGRP-LC, 从而阻断其对 PGN 的识别 (Maillet *et al.*, 2008; Basbous *et al.*, 2011); 调节型 PGRP-LC (regulatory PGRP-LC, rPGRP-LC) 能结合 PGN, 促进

PGRP-LC 的降解从而降低免疫反应 (Neyen *et al.*, 2016)。PGRP-LB 能水解 PGN 下调 IMD 信号, PGRP-SC 可协同 PGRP-LB 和 Pirk 蛋白一同清除 PGN 从而负调控 IMD 通路 (Zaidman-Remy *et al.*, 2006; Paredes *et al.*, 2011)。

IMD 信号通路还受到翻译后修饰的调节, 其中效应因子的泛素化修饰是一个研究热点。IMD 在泛素化酶 E2 (Ubc5、Ubc13-Uev1a) 和泛素化连接酶 E3 (Diap2) 的作用下, 其 137 号和 153 号赖氨酸残基发生多聚泛素化反应, 从而激活下游 TAK1 活性 (Chen *et al.*, 2017)。与此同时, Diap2 还可以与泛素化酶 LUBEL 协同作用, 促进 Kenny 对 Relish 活性的调节 (Aalto *et al.*, 2019)。另外, IMD 通路中的 IKK β 复合物与 Relish 还涉及抗病毒反应。当核糖酸样病毒 (Picorna-like viruses) 感染果蝇后, IKK β 复合物与 Relish 可以上调 dSTING 以及抗病毒因子 Nazo 的表达。与此同时, dSTING 也可以促进 IKK β 与 Relish 的活性, 更精确地调节免疫反应的进行 (Goto *et al.*, 2018)。

2.3 JAK-STAT 信号通路

JAK-STAT (Janus kinase-signal transducers and activators of transcription) 信号通路主要参与革兰氏阴性菌和病毒的免疫应答。该通路主要由转录因子 STAT92E、JAK 酶 Hopscotch (Hop)、受体 Domeless 和 3 个 Upd 配体组成, 其中 Upd1 和 Upd2 主要负责生长发育, Upd3 主要参与革兰氏阴性菌的免疫应答 (Agaisse *et al.*, 2003; Wright *et al.*, 2011)。Upd 与 Domeless 二聚体结合, 激活胞内的 JAK 酶 Hop, 促使 STAT92E 的酪氨酸残基被磷酸化, 形成同源二聚体, 被转运至细胞核中, 调节下游效应基因的表达。SOCS36E (Suppressor of cytokine signaling 36E) 和 PIAS (Protein inhibitor of activated STAT) 等负调控因子可以调节 JAK-STAT 信号通路 (Kingsolver *et al.*, 2013)。另外, Vago 蛋白可以通过未知受体介导 JAK-STAT 通路对果蝇 C 型病毒 (*Drosophila C virus*, DCV) 的免疫应答。Vago 的表达受 RNAi 通路的 Dicer-2 蛋白调节 (Ruckert *et al.*, 2014), 说明 JAK-STAT 通路可

能是联接先天免疫和 RNAi 的桥梁。

2.4 其他信号通路

除了 Toll、IMD 和 JAK-STAT 信号通路之外, JNK 等信号通路也与先天免疫相关。C-Jun N-末端激酶 (JNK) 是丝裂原活化蛋白激酶 (MAPKs) 的家族成员, 果蝇受到免疫刺激, 信号经由上游丝氨酸蛋白酶级联通路逐级传递, 激活 JNK 蛋白 (Sun *et al.*, 2015), 启动下游免疫通路。另一方面, IMD 通路也可以通过 TAK1-TAB2 复合物激活 JNK 通路, 影响果蝇的伤口修复并调节抗菌肽基因的表达 (Stronach *et al.*, 2014)。此外, Hippo 信号通路与 Lic (Licorne)、Pontin/Tip49 和 APLP2 (Amyloid precursor-like protein 2) 等蛋白也可以调节 JNK 信号通路或参与 JNK 信号通路对细胞死亡的调节 (Ma *et al.*, 2017; Wang *et al.*, 2018a, 2018b; Sun *et al.*, 2019)。

鞣化激素 (Bursicon) 是由 α 和 β 两个亚基组成, 形成的异源二聚体与 G 蛋白偶联受体 LGR2 结合, 调节昆虫表皮的鞣化和黑化 (Melanization)。An 等 (2008, 2012) 发现, 当果蝇受到免疫刺激后, 鞣化激素两个亚基可以分别形成同源二聚体, 与未知受体结合, 通过 Relish 调节抗菌肽基因的表达, 类似的结果在埃及伊蚊 *Aedes aegypti* 中也得到进一步的验证 (Zhang *et al.*, 2017)。

2.5 不同信号通路之间的相互作用

上文中已经提到 IMD 通路可以通过调节 JNK 通路活性影响免疫应答, 除此之外, 不同信号通路之间也存在相互作用的情况。例如, Toll-1 受体可以通过 SARM 激活 IMD 通路中 Relish 的表达; Dif、Dorsal 和 Relish 3 种 NF- κ B 转录因子可以形成异源复合物, 共同调节下游基因的表

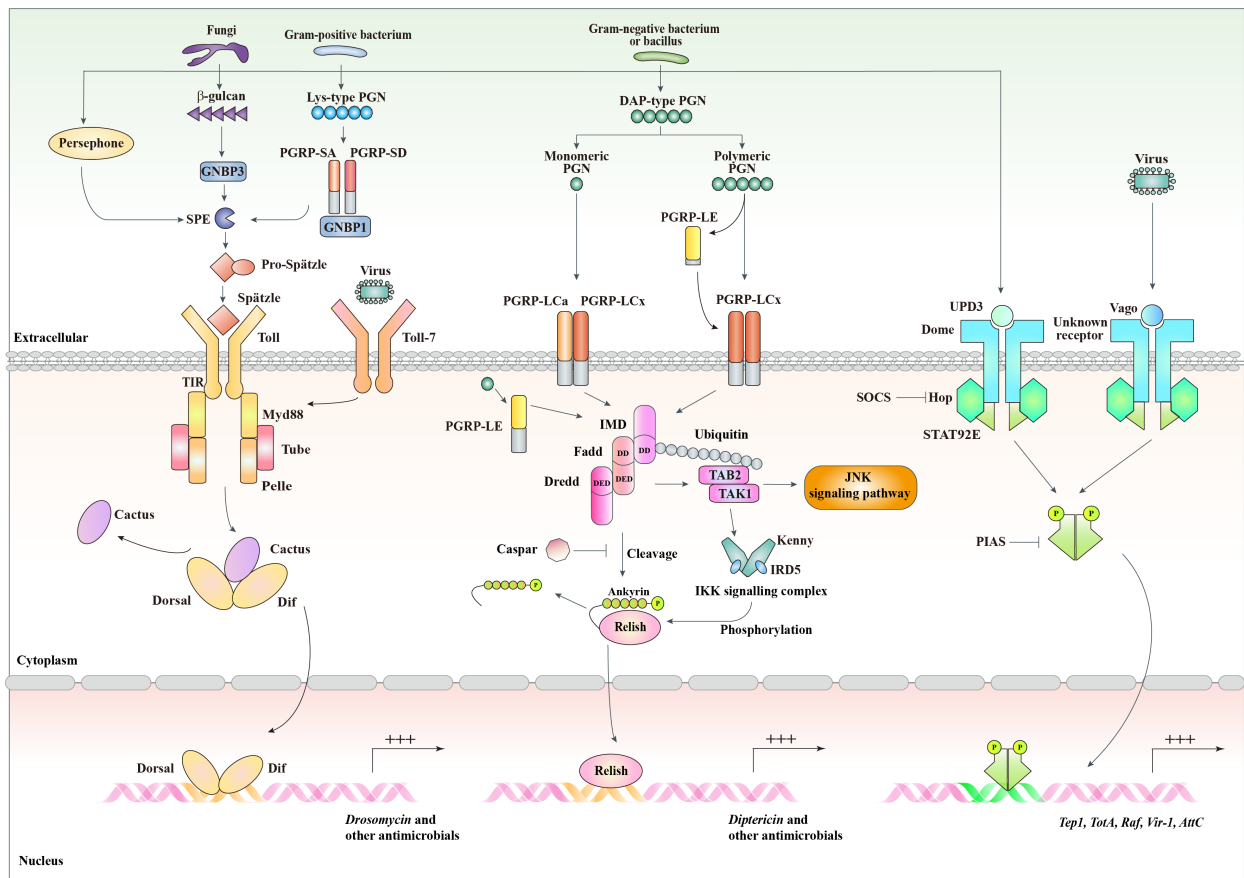


图 1 黑腹果蝇免疫信号通路模式图

Fig. 1 Signaling pathways in *Drosophila* innate immunity

达 (Tanji *et al.*, 2010; Meyer *et al.*, 2014; Chowdhury *et al.*, 2019b)。另外, 当细菌感染上皮气管组织后, IMD 通路可以上调抗菌肽的表达, 与此同时, Spz-2 可以与 Toll-8/Tollo 结合, 激活 Toll-1 下游通路, 拮抗 IMD 通路对抗菌肽的调节, 防止抗菌肽的过度激活 (Akhouchayri *et al.*, 2011)。在被匍滴虫 *Herpetomonas muscarum* 感染的果蝇中, Toll-1、IMD 和 JAK/STAT 通路均参与肠上皮细胞的免疫应答, 而且 Relish 和 STAT 还参与肠道干细胞向肠上皮细胞的分化 (Wang *et al.*, 2019)。这些研究说明不同信号通路之间可以形成复杂的调控网络, 共同参与先天免疫反应。

3 细胞免疫

除了体液免疫外, 细胞免疫也是昆虫先天免疫系统的重要组成部分。血淋巴作为昆虫免疫的重要器官, 其中的浆细胞 (Plasmacytes) 和薄层细胞 (Lamellocytes) 等可通过吞噬作用和包裹作用等方式行使免疫功能。

3.1 细胞吞噬作用

吞噬作用是脊椎动物和无脊椎动物中相对保守的细胞免疫反应, 果蝇的吞噬作用主要由浆细胞负责。当病原体被浆细胞表面的 PRRs 识别后, 胞内肌动蛋白有序地发生聚合, 细胞质膜逐渐包裹靶标物质将其内化形成吞噬体, 吞噬体成熟后, 溶酶体和水解酶等效应因子对病原体进行裂解 (Nakanishi and Shiratsuchi, 2006)。

吞噬细胞表面识别受体是调节吞噬作用的重要因子, 目前在果蝇中已经发现多种识别受体, 负责识别不同类型的病原体。主要包括 I 型清道夫受体 (dSr-CI)、CD36 家族清道夫受体 (Peste 和 Croquemort)、Nimrod 家族蛋白 (Eater、Draper 和 NimC1)、免疫球蛋白超家族蛋白 Dscam、含硫酯蛋白 TEPs (TEP2-4 和 TEP6) 和 PGRP-LC (Ramet *et al.*, 2001; Agaisse *et al.*, 2005; Garver *et al.*, 2006; Mamali *et al.*, 2009; Armitage *et al.*, 2014; Guillou *et al.*,

2016; Shokal *et al.*, 2017; Melcarne *et al.*, 2019b)。果蝇 Wnt 信号通路也可以介导吞噬过程 (Zhu and Zhang, 2013), Fox 蛋白家族成员 Jumu 可以通过介导 NimC1 的表达以及细胞骨架的重塑, 调节细胞吞噬的发生 (Hao *et al.*, 2018), 但吞噬作用的具体分子机制仍不清楚。

3.2 包裹与凝集作用

当病原体体积过大, 浆细胞无法发挥吞噬作用时, 浆细胞会协同血淋巴中的薄层细胞通过包裹作用 (Encapsulation) 清除病原体。例如, 黄蜂将卵寄生于果蝇之后, 浆细胞会对其进行识别并包围。在 Rho GTPase 家族的 Rac1、Rac2 和整联蛋白 (Integrin) 的协助下, 薄层细胞募集到浆细胞外围, 最终杀灭黄蜂卵 (Xavier and Williams, 2011)。有报道指出, 果蝇 C-型凝集素 DL2 和 DL3 可以促进包裹作用的发生 (Ao *et al.*, 2007)。根据病原种类不同, 包裹作用通常伴随着黑化反应 (Williams *et al.*, 2005)。

凝集作用是指血细胞与大量的细菌等病原体凝集在一起, 形成结节 (Nodulation) 并杀灭病原物的作用。与包裹作用相似, 凝集作用一般会伴随黑化反应的发生, 但其具体的机制仍不清楚 (Satyavathi *et al.*, 2014)。

3.3 黑化作用

黑化反应是与表皮硬化、损伤修复、免疫应答等相关的酶促反应。果蝇的黑化反应主要由晶细胞 (Crystal cells) 负责。当 PRRs 识别 PAMPs 之后, 可触发丝氨酸蛋白酶级联途径, 激活前酚氧化酶激活酶, 该酶将前酚氧化酶原 (Prophenoloxidase, PPO) 切割为具有活性的酚氧化酶 (Phenoloxidase, PO)。PO 通过酪氨酸羟基化形成多巴 (Dopa), 从而启动黑色素的产生。丝氨酸蛋白酶抑制剂 Serpin 和部分 C-型凝集素 (C-type lectins, CTLs) 被证明可以抑制酚氧化酶通路的激活 (Osta *et al.*, 2004; Nappi and Christensen, 2005; An *et al.*, 2011), 而有的 CTLs 可以促进酚氧化酶通路的激活 (Shi and Yu, 2012)。

目前在果蝇中鉴定到 3 个酚氧化酶: PPO1、PPO2 和 PPO3, 这三个酶在功能上存在相似性。PPO1-3 都可以参与抗线虫的免疫应答, PPO1 和 PPO2 在抗细菌的免疫反应中起作用, PPO2 和 PPO3 与包囊作用相关 (Dudzic *et al.*, 2015; Cooper *et al.*, 2019)。

4 小结与展望

昆虫与人类社会发展密切相关, 本文系统介绍了模式动物黑腹果蝇先天免疫系统的作用机制, 为进一步研究经济益虫和农业害虫的免疫机制提供参考和理论基础。先天免疫在无脊椎和脊椎动物中相对保守, 果蝇先天免疫机制的研究也有助于更好的探究脊椎动物, 特别是哺乳动物的免疫机制。尽管果蝇先天免疫研究取得了很大的进展, 但有些分子机制仍不清楚。我们利用大肠杆菌 *Escherichia coli* 和金黄色葡萄球菌 *Staphylococcus aureus* 处理果蝇, 发现不同龄期及不同性别果蝇的响应效果不一致 (Wen *et al.*, 2019), 说明果蝇的先天免疫机制远比我们所知道的要复杂。免疫信号通路中新的作用因子、信号通路之间的网络调控、细胞免疫和体液免疫之间的交互作用等是将来研究果蝇先天免疫的方向。

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