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昆虫天然免疫反应研究前沿*

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摘 要 昆虫天然免疫反应分为体液免疫和细胞免疫两种,二者共同作用抵御细菌、真菌、病毒等外源病原物的侵染。体液免疫反应主要包括黑色素形成和抗菌肽产生两种机制,细胞免疫反应包括吞噬、集结和包囊等作用类型。在昆虫天然免疫反应中,昆虫模式识别蛋白负责识别并结合外源物表面特有的模式分子,丝氨酸蛋白酶、丝氨酸蛋白酶抑制剂、各种配体、受体等负责级联信号途径的激活和调控,抗菌肽、黑色素等效应分子则负责对入侵物的杀灭和清除。本文根据国外和作者自己的研究,综述了昆虫天然免疫反应的研究进展,并针对该领域最新的研究动态展望了昆虫肠道免疫、昆虫免疫致敏以及不完全变态昆虫免疫学等这些研究前沿。

关键词 昆虫,天然免疫,黑化反应,抗菌肽

Frontiers of research on the innate immune response in insects

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Abstract Innate immunity in insects is divided into humoral immunity and cellular immunity, which function together to defend against infection by various pathogens including bacteria, fungi, viruses, etc. Humoral immunity mainly includes melanin synthesis and antimicrobial peptide production. Cellular immunity relies on phagocytosis, nodulation, and encapsulation. In the insect innate immune response, pattern recognition proteins are responsible for the recognition of specific pathogen-associated molecular patterns in invading pathogens. Serine proteases, serine protease inhibitors, a variety of ligands and receptors are involved in the activation and regulation of signaling pathways. Effectors such as antimicrobial peptides and melanin kill and eliminate the pathogens. This paper describes recent progress in research on the insect innate immune response, and looks at the frontiers of research in this field, including gut immunology, immune priming, and hemimetabolous immunology.

Key words insect, innate immunity, melanization, antimicrobial peptide

无脊椎动物,包括昆虫,都没有脊椎动物所特有的获得性免疫反应能力,但它们拥有高效的天然免疫反应系统。昆虫天然免疫反应系统包括体液免疫(humoral innate immune response)和细胞免疫(cellular innate immune response)两部分,它们协同作用共同抵御细菌、真菌、病毒等外源物的入侵(Ashida and Brey, 1998)。包括吞噬作用(phagocytosis)、集结作用(nodulation)和包囊作用(encapsulation)在内的昆虫细胞免疫反应是由淋巴细胞(hemocytes)介导的;而以黑色素形成(melanin synthesis)和抗菌肽(antimicrobial peptides,AMPs)产生为代表的昆虫体液免疫反应则由脂肪体(相当于哺乳动物的肝脏)和可溶的体液蛋白介导(Glodsmith and Marec,2009)。本文根据国内外和作者自己的研究,综述了昆虫天然免疫反应的研究历史、现状与发展前沿。

1 昆虫天然免疫反应研究的历史

对昆虫细胞免疫反应的研究要追溯于1884年,俄国科学家 Elie Metchnikoff 首次在昆虫中观

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进而再氧化成邻苯醌或对苯醌,最终聚合形成黑色素(An *et al.*,2009;Jiang *et al.*,2010)。

自从 1927 年 Serge Metalnikov 首次观察到大蜡螟淋巴液的黑化现象,经过 80 多年的研究积累,时至今日,科学家们对黑化反应,尤其是烟草天蛾、黄粉虫、家蚕等大体积昆虫体内黑化反应,激活与调控的分子机制,已经有了较为全面的认识。以烟草天蛾为例,至少 7 种丝氨酸蛋白酶参与了烟草天蛾酚氧化酶原的激活。当烟草天蛾幼虫被大肠杆菌或藤黄微球菌或 β-1,3-葡聚糖刺激后,在一条通路中外源入侵信号被 β-1,3-葡聚糖识别蛋白识别,然后丝氨酸蛋白酶 HP14 的酶原(proHP14)被激活(Jiang *et al.*,2004;Wang and Jiang,2006)。激活的 HP14 接着去激活另一种丝氨酸蛋白酶-HP21 的酶原(proHP21),最后酚氧化酶原激活酶 2 和 3(PAP-2,-3)被 HP21 激活(Gorman *et al.*,2007;Wang and Jiang,2007)。随后,烟草天蛾酚氧化酶原将被 PAP-2 和/或 PAP-3 激活进一步导致黑色素的形成,完成黑化反应。在另一条通路中,外源物被识别后,未知的蛋白酶激活丝氨酸蛋白酶 HP6 的酶原(proHP6),HP6 再激活另外一种酚氧化酶原激活酶 1(PAP-1),进而激活酚氧化酶原(图 2)(An *et al.*,2009)。目前在其他昆虫中鉴定到的酚氧化酶原激活酶还包括:黄粉虫的 SPE、冈比亚按蚊的 CLIPB9、棉铃虫的 PPAE1、家蚕的 PPAE、东北大黑鳃金龟的 PPAF1 等(An *et al.*,2011a)。值得注意的是:HP6 承担着双重角色,除了激活 proPAP-1 外,还可以激活烟草天蛾 HP8 酶原(proHP8),进一步导致 Spz 前体的激活,激活的 Spz 再与 Toll 受体结合诱导抗菌肽的产生(An *et al.*,2009)。同样的现象也发生在黄粉虫中,SPE 可以同时激活黄粉虫酚氧化酶原和 Spz 前体(图 2)(Kim *et al.*,2008)。

黑化反应除了能杀灭外源物外,反应过程也会产生一些诸如自由氧基等对自身有害的物质,因此需要被严格地调控。丝氨酸蛋白酶抑制剂(serine protease inhibitors,serpins)是潜在调控丝氨酸蛋白酶的抑制剂,含有 3 个标准的 β-折叠(β-sheet A-C)、9 个 α 螺旋(αhelices)和 1 个暴露在分子表面的位于羧基末端的反应中心环(reactive center loop,RCL)。丝氨酸蛋白酶抑制剂的靶标蛋白酶在其反应中心环中的裂解键(scissile bond)处切割并与之形成紧密的高分子量复合物,从而被相应的丝氨酸蛋白酶抑制剂不可逆地抑制(Gettins,2002)。目前已经在许多昆虫中鉴定到丝氨酸蛋白酶抑制剂的存在,部分丝氨酸蛋白酶抑制剂的生理生化功能在鳞翅目昆虫,尤其是在烟草天蛾体内已有了广泛的研究。从烟草天蛾中鉴定到 7 个丝氨酸蛋白酶抑制剂基因,分别是 serpin1 到 serpin7,其中 serpin1 含有 12 个可变剪切导致的同种型(isoforms):serpin-1K 和 serpin-1Z(Jiang and Kanost,1997;Kanost,1999)。在上述的

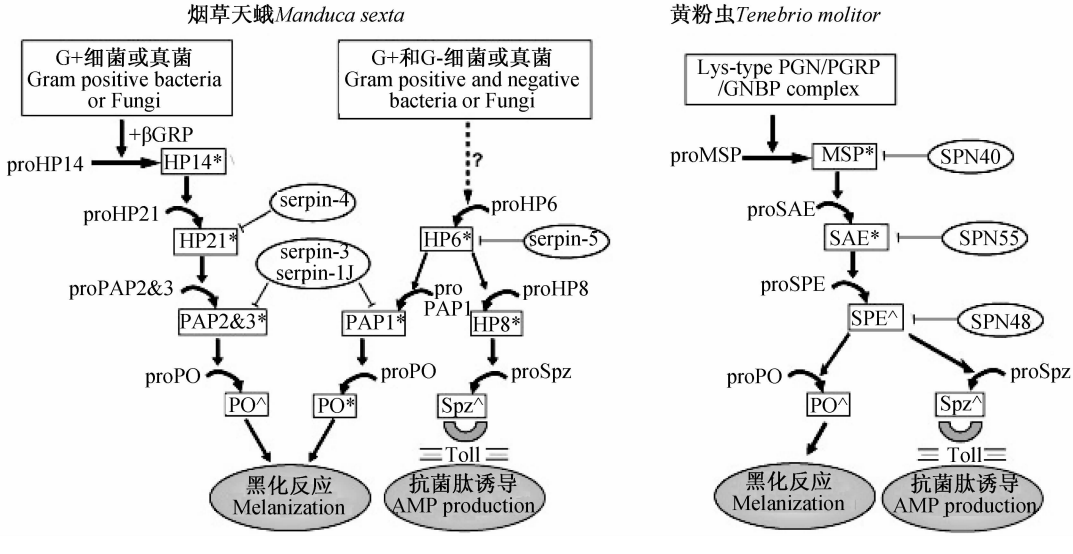

图 2　烟草天蛾和黄粉虫中黑化反应及抗菌肽产生的分子机制

Fig. 2　Molecular mechanisms of melanization and AMP production in *Manduca sexta* and *Tenebrio molitor*

酚氧化酶激活途径中,HP21 被 serpin-4 调控,HP6 被 serpin-5 调控,PAP1-3 均可被 serpin-3 和 serpin-1J 抑制(图 2)(Zhu *et al.*,2003;Tong and Kanost,2005;An and Kanost,2010;An *et al.*,2011b)。

2.3 昆虫细胞免疫反应

昆虫细胞免疫是由血淋巴细胞(hemocytes)介导完成的,目前对昆虫细胞免疫发生机制的研究较少,也主要是因为收集和鉴定血淋巴细胞比较困难。虽然参与细胞免疫反应的血淋巴细胞种类可能不一样,但是细胞免疫反应在不同昆虫中大致都包括吞噬作用、集结作用和包囊作用。

吞噬作用是进化上保守的用于清除入侵病原体和细胞凋亡残体的机制,这个过程由单个细胞完成,包括识别、吞噬、对入侵病原体的破坏和细胞本身的衰竭死亡等步骤(Williams,2007)。首先噬菌细胞表面的受体要被相应的配体激活,进而引发噬菌细胞与病原体之间多重的连续反应,最终病原体被吞噬。目前,在果蝇中已经鉴定出 6 个参与吞噬作用的受体分子(Croquemor、*Drosophila* scavenger receptor dSR-CI、PGRP-LC、Draper、Eater、Nimrod C1)(Stuart and Ezekowitz,2008)和两个配体分子(Pretaporter 和 Calreticulin)(Kuraishi *et al.*,2009)。

集结作用和包囊作用是指很多血淋巴细胞粘附、聚集在细菌等外来物的表面。包囊的对象比集结作用针对的对象体积更大,包括寄生物、原生动物、线虫等(Marmaras and Lampropoulou,2009)。与吞噬作用将外源体陷在单个细胞里不同,集结和包囊作用是血淋巴细胞在外源体的周围层层叠加形成一个“鞘”将异物包围住,被包围在囊中的入侵物就会被昆虫自身产生的自由基 ROS 和 RNS 杀死,或在囊中窒息而死(Nappi and Ottaviani,2000)。在此过程,常伴随着局部黑化反应的产生(Meister and Lagueux,2003)。因此,在集结作用和包囊作用中,细胞免疫反应与体液免疫反应两种类型紧密结合在一起。现有研究结果表明:由受体 Domeless、JAK(Hopscotch)、STAT(Stat92E)和 3 个细胞活素 Unpaired 1(Upd1)、Upd2、Upd3 组成的 JAK/STAT 途径在包囊作用中起了重要作用(Sorrentino *et al.*,2004;Krzemien *et al.*,2007)。

3 昆虫天然免疫反应研究的前沿展望

对昆虫天然免疫反应的研究从最初只对天然免疫相关现象的简单观察开始,经过一百多年的发展,在昆虫天然免疫反应,尤其是全变态昆虫的天然免疫反应的起始、激活、调控的分子机制方面取得了重大的进展,对哺乳动物的天然免疫研究也有一定的推动作用。未来对昆虫天然免疫反应的研究还会扩大到以下几个方面:

3.1 昆虫肠道免疫(gut insect immunology)

相对于人体肠道中寄生着 500 ~ 1 000 种寄生菌,昆虫(例如果蝇)肠道中的寄生菌种类大为减少,大约有 10 ~ 20 种寄生菌,所以研究昆虫肠道免疫如何容忍寄生菌但同时杀灭食物来源的病原菌相对较为容易,且所获得的知识也有助于帮助理解人体肠道免疫的分子机制。目前,世界上一些研究小组已经开始研究昆虫肠道上皮细胞如何与共生菌和谐共处,而食物来源的病原菌又如何在肠道引发免疫反应从而对其进行清除等这些问题,并且取得了部分研究成果(Ha *et al.*,2009a,2009b)。

现有研究表明昆虫肠道免疫可以被抗菌肽、氧化酶 Duox、活性氧簇(reactive oxygen species,ROS)等介导。Lee 等发现果蝇 NF-κB 依赖的抗菌肽(例如 diptericin 和 cecropin)的产生可以保护和容忍共生菌,同源框转录因子 Caudal(Cad)通过抑制 Imd 途径可调节这些抗菌肽基因的连续性表达从而影响肠道内共生菌的群落结构(Ryu *et al.*,2008)。其他研究者相继发现了其他肠道上皮细胞中 Imd 途径的负调控分子,例如 PGRP-SC,PGRP-LB,PIMS,DAP 型肽聚糖等(Bischoff *et al.*,2006;Lhocine *et al.*,2008)。以果蝇为动物模型的这项研究不仅能理解正常寄生菌对潜在病原微生物集群现象的抑制机制,也暗示着抗生素或抗癌药物以及放射治疗等引起的人体肠道中菌群结构被破坏可能会引起病原菌的感染(Sommer *et al.*,2009)。

此外,氧分子代谢副产物 ROS 一般认为来自吞噬细胞,现在发现也来源于昆虫肠道的上皮细胞,并且昆虫肠道内可能还有一个新的 ROS 产生系统来响应非肽聚糖的微生物组分。Ha 等

(2009a)发现一种氧化酶 Duox 在体内外均可介导 ROS 的产生,Duox 基因的缺陷可导致果蝇所摄入的食物中的微生物过度增殖以至于果蝇对肠道感染高度敏感。Ha 和其他科学家还研究了 Duox 自身的蛋白活性以及在转录水平是如何被调控的,磷脂酶 Cβb(phospholipase Cβb,PLCβ)/1,4,5-三磷酸肌醇(inositol 1,4,5-trisphophate,IP3)介导的钙动员(calcium mobilization)可调控 Duox 的自发激活,而 MEKK1-MKK3-p38 途径则调控 Duox 的基因表达(Ha *et al.*,2009b)。尽管现在已经初步阐述了昆虫肠道免疫的一些机制,但是还有许多问题尚待解决,例如:昆虫肠细胞如何区分共生菌和病原菌? 激活 Duox 的配体结构是什么样的?

3.2　昆虫免疫系统的免疫致敏(immune priming of insect immunology)

传统的观点认为昆虫没有获得性免疫系统,因此也就不会有免疫特异性和免疫记忆性(也称作免疫致敏)。然而,2003 年 Kurtz 和 Franz 在用绦虫 *Schistocephalus solidus* 感染白色大剑水溞 *Macrocyclops albidus* 时发现宿主如果曾经被绦虫感染过再次遇见绦虫时会更有效地激发反应,暗示着无脊椎动物也存在一个有记忆性的特异免疫系统(Kurtz and Franz,2003)。接着在大黄蜂 *Bombus terrestris* 和果蝇中观察到类似现象的存在(Pham *et al.*,2007)。更重要的是 2010 年,Rodrigues 等发现接触过疟原虫 *Plasmodium* 的蚊子再次被感染时,巨噬细胞样(macrophage-like)的昆虫细胞反应会增加 2 到 3.2 倍,这对于疟疾控制和无脊椎动物免疫记忆的理解无疑具有重要的启示意义。

随着多种不同免疫受体在果蝇和蚊子中的发现,人们越来越相信昆虫免疫系统的特异性和致敏性是存在的,并且可能和脊椎动物的免疫机制不一样。Schulenburg 等(2007)曾提出了 3 条假设:(1)昆虫的免疫特异性依赖其病原体识别受体(pathogen recognition receptors)和/或免疫效应分子(immune effectors)的遗传多样性;(2)各种病原体识别受体和/或免疫效应分子协同作用以增加昆虫免疫的特异性;(3)相应受体和/或免疫效应分子的浓度可以极大增强对病原体的识别。但是到目前,昆虫免疫系统致敏的分子机制,与脊椎动物免疫致敏的具体区别等等这些都还几乎一无所知,尚需进一步的研究。

3.3　不完全变态昆虫的免疫研究

迄今为止,昆虫天然免疫系统的研究相当大一部分集中在果蝇、蚊子、家蚕、甲虫等全变态昆虫中,对不完全变态昆虫的免疫系统还知之甚少。2010 年,第一个不完全变态昆虫——蚜虫 *Acyrthosiphon pisum* 的基因组测序工作终于完成(IAGC,2010),科学家开始对不完全变态昆虫的天然免疫系统做初步探讨。

Gerarado 等(2010)通过对蚜虫基因组中免疫和防御相关的基因进行功能注释得到了一些非常有趣的结果:(1)在蚜虫中鉴定到一些参与 Toll 途径的基因(包括 Toll 受体、Spätzle、丝氨酸蛋白酶及丝氨酸蛋白酶抑制剂等),但是 PGRP 这些基因在基因组中没有被发现;(2)蚜虫基因组中缺失参与 Imd 途径的重要基因;(3)蚜虫基因组中有 Domeless、JAK 酪氨酸激酶、STAT92E 转录因子等参与 JAK/STAT 途径的一些同源基因,但是最关键的 JAK/STAT 配体分子 unpaired(upd)却未被发现;(4)蚜虫不表达 Defensin、Cecropin、Drosocin、Diptericin、Drosomycin、Metchnikowin 等全变态昆虫常有的抗菌肽,相反,在蚜虫基因组中鉴定到 6 个类似于 Thaumatin 的有抗真菌活性的植物抗菌肽。所有这些不同都暗示着不完全变态昆虫的免疫反应机制可能在某些方面不同于已知的全变态昆虫天然免疫的分子机制。

4　结语

对昆虫天然免疫反应的研究已经历经百年,现代遗传学、细胞生物学、分子生物学、蛋白质组学、系统生物学、RNAi 等新技术的出现更是帮助在此领域内取得了许多有价值的研究成果。因为昆虫天然免疫反应与哺乳动物有很高的同源性,这些研究成果也为理解更复杂的高等动物天然免疫反应提供了线索。此外,对昆虫天然免疫反应的基础研究也为发展新型药物和临床诊断试剂盒提供了可能,近来针对黄粉虫 Toll 信号途径的分子机制正在开发一种新的检测试剂盒检测血浆等生物制品中可能的细菌污染。相信随着对昆虫天然免疫反应越来越深入的研究,对其各种分子机制的理解会越来越清楚,而对其可能的理论和实际运用也会越来越广泛。

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