

昆虫表皮发育研究进展及展望*

刘晓健** 刘卫敏 赵小明 张建珍*** 马恩波***

(山西大学应用生物研究所, 太原 030006)

摘 要 昆虫体壁(Integument)具有类似高等动物皮肤和骨骼的双重功能,坚硬的表皮限制了躯体生长,因此,虫体生长发育过程中须进行周期性蜕皮。昆虫表皮由皮细胞分泌形成,从外到内可分为外包膜(Envelope)、上表皮(Epicuticle)、外表皮(Exocuticle)、内表皮(Endocuticle)和皮细胞层(Epidermis),外表皮和内表皮统称为原表皮(Procuticle)。脂类物质主要存在于上表皮中,原表皮的主要化学成分为几丁质和蛋白质,在表皮形成过程中上述成分依次分泌并相互作用,最终形成有序排列的表皮结构。近年来基因组学、蛋白质组学、RNA 干扰技术等组学及分子生物学技术的快速发展,极大地促进了昆虫表皮发育的研究进展。本文结合近年来该领域研究成果,就表皮结构和成分、表皮脂代谢、表皮几丁质代谢和表皮蛋白等方面的研究进展进行综述,为深入认识昆虫表皮发育机制提供参考,并为害虫防治提供理论依据。

关键词 表皮;结构和成分;脂代谢;几丁质代谢;表皮蛋白

Progress in the study of insect cuticle development and prospects for future research

LIU Xiao-Jian** LIU Wei-Min ZHAO Xiao-Ming ZHANG Jian-Zhen*** MA En-Bo***

(Institute of Applied Biology, Shanxi University, Taiyuan 030006, China)

Abstract The integument of insects performs the same function as skin and bone in vertebrates. Because the rigid cuticle restricts growth, periodic molting is necessary during growth and development. Cuticle is secreted by epidermal cells, and consists of an outer envelope, an epicuticle, exocuticle, endocuticle and the inner epidermis. The procuticle is subdivided into an upper exocuticle and a lower endocuticle. Lipids are mainly found in the epicuticle whereas the procuticle is composed of chitin and proteins. The above components are secreted in turn and interact with each other, eventually forming the orderly arrangement of the cuticular structure. In recent years, rapid advances in omics and molecular biology, including genomics, proteomics and RNAi, has greatly facilitated research on insect cuticle development. This paper reviews progress in research on cuticle structure and composition, lipid metabolism, chitin metabolism and cuticle proteins with the goal of furthering the understanding of insect cuticle development and providing a theoretical basis for pest control.

Key words cuticle; structure and composition; lipid metabolism; chitin metabolism; cuticular proteins

表皮是昆虫体壁的典型结构,是由真皮细胞分泌产生的细胞外基质。表皮覆盖在虫体表面,提供肌肉附着点,对于维持虫体形状、防止水分散失、抵御微生物侵染和天敌捕食等具有重要作用(Chapman, 2013)。表皮还作为气管、腺管

和感觉器官的导管,以及前、后肠内膜,在虫体呼吸、取食、排泄、渗透调节、水分控制等方面发挥作用。昆虫表皮结构和化学成分的复杂性,使其适应于所处的生态环境(Nation, 2008)。近年来,国内外学者逐步开展了昆虫表皮发育关

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**第一作者 First author, E-mail: xiaojianliu@sxu.edu.cn

***共同通讯作者 Co-corresponding authors, E-mail: zjz@sxu.edu.cn; maenbo2003@sxu.edu.cn

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键基因的鉴定和功能解析等研究工作,如表皮脂代谢关键酶基因 (Chen *et al.*, 2016; Yu *et al.*, 2016b; Li *et al.*, 2019a, 2019b)、表皮几丁质代谢相关基因 (Zhu *et al.*, 2016) 和表皮蛋白基因 (Zhou *et al.*, 2016; Zhao *et al.*, 2017; Pan *et al.*, 2018)。这些关键基因的鉴定加速了昆虫表皮发育的研究进展,为基于表皮的害虫防控提供新的分子靶标。

1 表皮结构和成分

昆虫表皮由三个主要的复合层组成,即外层的外包膜 (Envelope)、中间的上表皮层 (Epicuticle) 和内部的原表皮层 (Procuticle) (图 1), 昆虫表皮发育过程中表皮细胞依次分泌外包膜、上表皮和原表皮,按顺序沉积形成成熟的表皮 (Locke, 2001)。

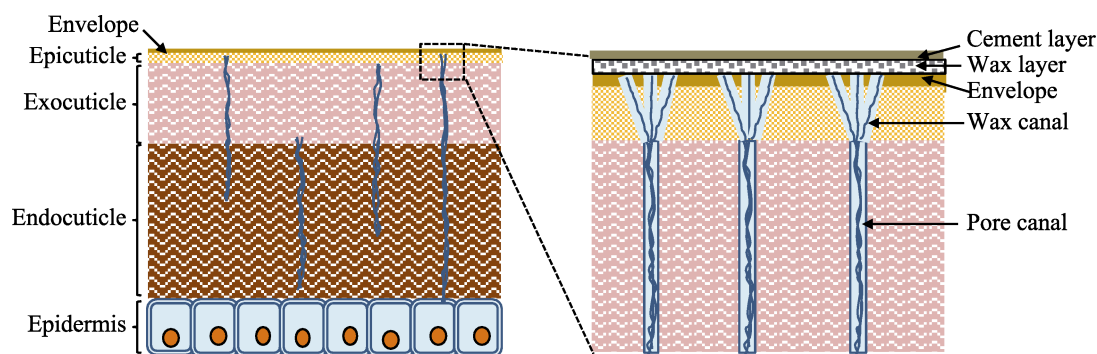


图 1 昆虫表皮结构示意图

Fig. 1 General structure of the insect cuticle

右图为左图方框内的放大图。The right one is the enlarged one in the left one.

Envelope: 外包膜; Epicuticle: 上表皮; Exocuticle: 外表皮; Endocuticle: 内表皮; Epidermis: 真皮细胞层;

Cement layer: 黏胶层; Wax layer: 蜡层 (脂质层); Wax canal: 蜡道; Pore canal: 孔道。

外包膜 (厚度 20 nm) 覆盖整个虫体表面和外胚层内陷部分,呈现出电子致密和透明交替排列的三层结构 (Moussian *et al.*, 2006a),由脂类和骨化蛋白组成,可通过共价结合形成蛋白质-醌复合物 (Locke, 1965; Wigglesworth, 1985; Nation, 2008),被醌鞣化后的外包膜十分稳定,对酸水解具有很强的抵抗力 (Locke, 2001)。外包膜起着维持表皮形态和限制昆虫生长的作用,同时还参与体色的形成 (Gillott, 2005)。

大多数昆虫外包膜上覆盖脂质层 (Lipid layer) 或蜡层 (Wax layer),蜡层外有黏胶层 (Cement layer),由真皮细胞腺分泌而成,具有保护蜡层的作用,又称为护蜡层 (Wigglesworth, 1947)。蜡层中最常见的组分是碳氢化合物,可分为正烷烃、饱和甲基支链烷烃和不饱和烃等三大类。脂肪醇、蜡酯和游离脂肪酸含量仅次于碳氢化合物。除此之外,还有酮、醛、醋酸酯、甾醇酯以及酰基甘油等 (Blomquist *et al.*, 2018)。

上表皮主要由蛋白质和脂质共价交联而成 (Moussian, 2013)。蛋白质被酚类化合物及其氧化产物醌鞣化或硬化,使上表皮具有高强度和低透水性。Wigglesworth (1970, 1975) 认为,上表皮的结合脂质由羟基脂肪酸组成。

原表皮主要由蛋白质和几丁质组成。几丁质通过共价键嵌入蛋白质基质中,形成几丁质-蛋白复合物 (Neville, 1975; Gillott, 2005)。原表皮可分为外表皮 (Exocuticle) 和内表皮 (Endocuticle) 两部分。在一些昆虫表皮中,两者之间的边界不清晰,可见中间区域,即中表皮 (Mesocuticle) (Gillott, 2005)。外表皮中的蛋白质经过醌鞣化并与脂质结合,形成坚硬外壳,使其性质稳定,不会受到蜕皮液的降解 (Chapman, 2013)。内表皮不被鞣化,由蛋白质和几丁质通过共价键、氢键以及醌交联,实现其稳定作用 (Nation, 2008)。

孔道 (Pore canal) 是从真皮细胞穿过内表

皮和外表皮的直径为 0.1-0.15 μm 的通道, 终止于外表皮和上表皮的交界处, 在上表皮中分支成蜡道(Wax canal), 直径为 6-13 nm(Locke ,1961 ; Neville *et al.* , 1969)。孔道内有孔道纤维丝, 在真皮细胞和内表皮之间起着加固作用。孔道可能输送脂质和黏胶, 以及其他化学成分到虫体表面(Nation , 2008)。

2 昆虫表皮脂代谢对表皮发育的影响

2.1 昆虫表皮脂的合成

自 20 世纪 70 年代开始, Blomquist 等利用稳定的放射性同位素发现脂肪酸先延伸, 然后去

掉一个羧基碳原子转化为碳氢化合物(Blomquist *et al.* , 2018)。碳氢化合物的生物合成发生在绛色细胞中, 绛色细胞主要存在于昆虫腹部真皮细胞的基底部和基底膜之间, 或分布于血淋巴中脂肪体细胞周围。绛色细胞具有高度发达的光面内质网, 参与碳氢化合物的合成。昆虫碳氢化合物的生物合成可分为四个步骤: (1) 直链和甲基支链脂肪酸前体的形成; (2) 脂肪酸延伸为长链脂肪酰辅酶 A; (3) 长链脂肪酰辅酶 A 转化为醛; (4) 由还原性脱羧将醛转化为碳氢化合物和二氧化碳(Blomquist *et al.* , 2018)(图 2)。

直链和甲基支链脂肪酸前体的合成原料和所需要的酶存在差异。昆虫取食经中肠和脂肪体消化吸收后形成的短链氨基酸、葡萄糖或脂肪酸

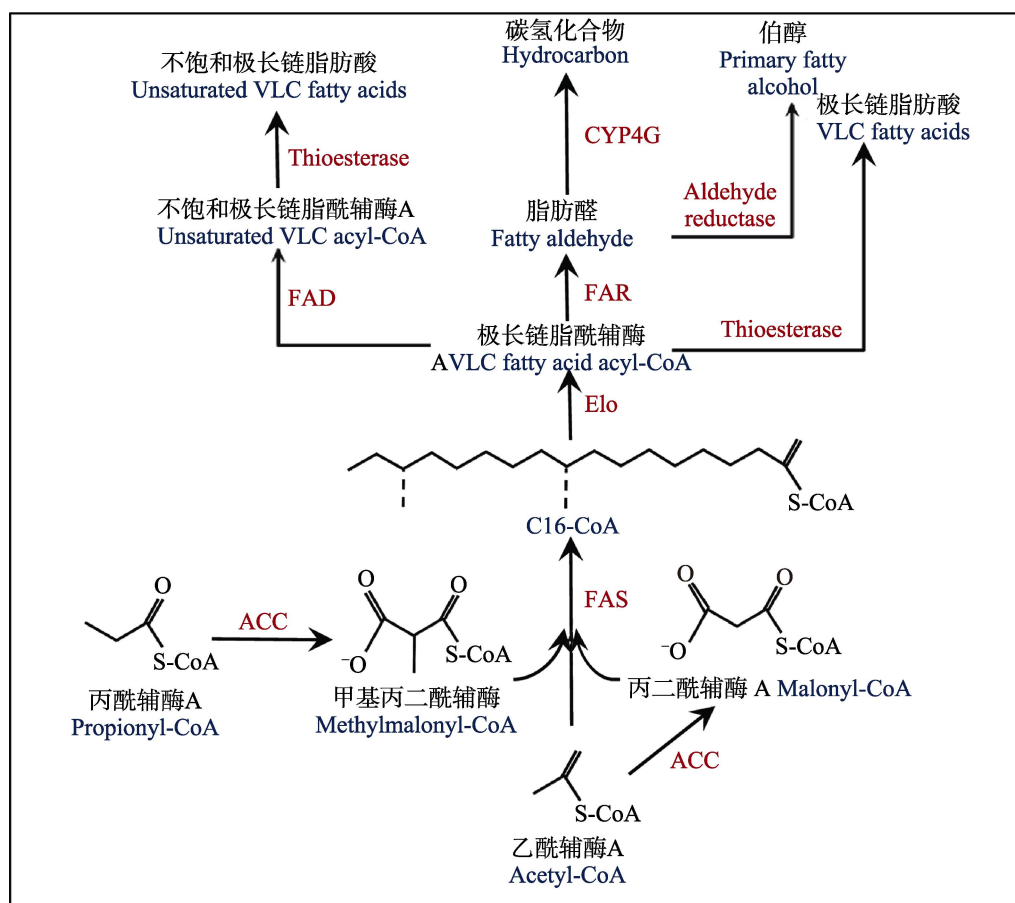


图 2 昆虫表皮脂合成途径图

Fig. 2 Biosynthetic pathways for insect cuticular lipids

ACC: 乙酰辅酶 A 羧化酶; FAS: 脂肪酸合成酶; Elo: 脂肪酸延伸酶; FAR: 脂肪酸还原酶;

FAD: 脂肪酸去饱和酶; CYP: 细胞色素 P450 氧化酶。

ACC: Acetyl-CoA carboxylase; FAS: Fatty acid synthetase; Elo: Elongases; FAR: Fatty Acyl-CoA reductases;

FAD: Fatty acid desaturases; CYP: Cytochrome P450 oxidase.

转化为乙酸盐,以启动脂肪酸前体的合成。乙酸盐与辅酶 A (CoA) 发生缩合反应,提供乙酰辅酶 A,然后在乙酰辅酶 A 羧化酶 (Acetyl-CoA Carboxylase, ACC) 的作用下转化为丙二酰辅酶 A。胞质脂肪酸合成酶 (Fatty Acid Synthetases, FAS) 的多酶系统多次将丙二酰辅酶 A 和乙酰辅酶 A 结合,最终合成 C14, C16, C18 长链脂肪酸前体。缬氨酸、异亮氨酸和甲硫氨酸均可作为合成甲基丙二酰 CoA 的原料,微粒体 FAS 是甲基支链脂肪酸合成的主要酶 (Majerowicz *et al.*, 2017)。黑腹果蝇 *Drosophila melanogaster* 基因组中包含 3 个已注释的 FAS 基因,其中两个在绛色细胞中表达,一个在脂肪体中表达,表明合成碳氢化合物的脂肪酸前体并不完全来源于绛色细胞 (Majerowicz *et al.*, 2017)。

极长链脂肪酰基辅酶 A (Very long chain fatty Acyl-CoAs, VLCFA) 通过脂酰 CoA 延伸酶 (Elongases, Elo) 发生的链延伸而产生。脂酰 CoA 延伸酶催化丙二酰 CoA 单位与脂肪酸 CoA 聚合,延长酶家族基因通常具有组织和底物特异性。在每一次延长反应中还依赖于后续 3 个步骤,分别是 3-酮酰 CoA 还原酶将羰基还原为羟基,3-羟酰 CoA 脱氢酶催化的脱水反应以及烯酰 CoA 还原酶对碳碳双键的还原作用 (Majerowicz *et al.*, 2017)。去饱和酶 (Desaturases, Desat) 则在延长反应中特异性引入双键以生成不饱和极长链脂肪酸 (陈楠, 2018; Blomquist *et al.*, 2018)。极长链脂肪酸前体在脂酰 CoA 还原酶 (Fatty Acyl-CoA reductases, FAR) 作用下转化为醛中间体。Li 等 (2019b) 及王雪庆 (2018) 在白蜡虫 *Ericerus pela* 和褐飞虱 *Nilaparvata lugens* 中研究发现 FAR 参与蜡酯合成和影响表皮碳氢化合物的合成。已有研究表明, FAR 将酯酰 CoA 还原为对应的脂肪醇,其被下游 P450 酶氧化为脂肪醛 (Ginzel and Blomquist, 2016)。脂肪醛发生脱羧反应,在脱羧酶作用下转化为短一个碳原子的脂肪烃,羰基以 CO₂ 形式释放 (Qiu *et al.*, 2012)。在飞蝗 *Locusta migratoria* (Yu *et al.*, 2016b) 冈比亚按蚊 *Anopheles gambiae* (Kefi *et al.*, 2019)。

黄粉虫 *Tenebrio molitor* (Wang *et al.*, 2019) 桃蚜 *Acyrtosiphon pisum* (Chen *et al.*, 2016) 白蜡虫 *Ericerus pela* Chavannes (Hu *et al.*, 2018) 褐飞虱 *N. lugens* (Li *et al.*, 2019b) 等昆虫中的研究表明表皮脂合成关键基因的沉默会影响表皮脂 (或蜡) 的正常沉积,从而导致昆虫表皮发育受阻,虫体水分蒸发加快,防水性减弱,最终死亡。除了绛色细胞外,孔道壁中检测到酯酶的活性,表明孔道可能有助于蜡的合成 (Locke, 1961)。

2.2 昆虫表皮脂的转运

碳氢化合物在绛色细胞中合成后需要运输到表皮以发挥其作用。表皮脂质前体以脂滴和晶体状包涵体的形式在绛色细胞中产生,并以载脂蛋白复合物的形式释放到血淋巴中。昆虫的载脂蛋白是一种高密度脂蛋白,其在血淋巴中运输碳氢化合物 (Schal *et al.*, 1994, 2001)。脂质前体在真皮细胞内进行相应的修饰和加工,并通过转运子运输到表皮 (图 3),孔道可能参与进一步的脂质修饰和传递 (Moussian, 2013)。最新研究表明,ABC 转运蛋白参与表皮脂质的运输 (Yu *et al.*, 2017),其沉默会导致表皮脂转运受阻,昆虫保水性降低,最终导致昆虫死亡。表皮蛋白 Sns1 可能参与果蝇表皮脂类经孔道运输的过程 (Zuber *et al.*, 2018)。通过孔道的化学物质可从孔道横向扩散到上表皮。

3 几丁质代谢及其在昆虫表皮发育中的作用

几丁质是昆虫表皮的主要成分之一,在昆虫表皮发育中具有重要作用。几丁质在昆虫体内的合成、修饰、组装、降解和转运是高度复杂的过程。

3.1 几丁质合成

Candy 和 Kilby (1962) 首次提出昆虫几丁质合成通路,该过程始于海藻糖 (Trehalose),终止于 UDP-N-乙酰葡萄糖胺 UDP-N-acetylglucosamine (UDP-GlcNAc)。从 UDP-GlcNAc 到几丁质合成途径于 1963 年在蓖麻蚕

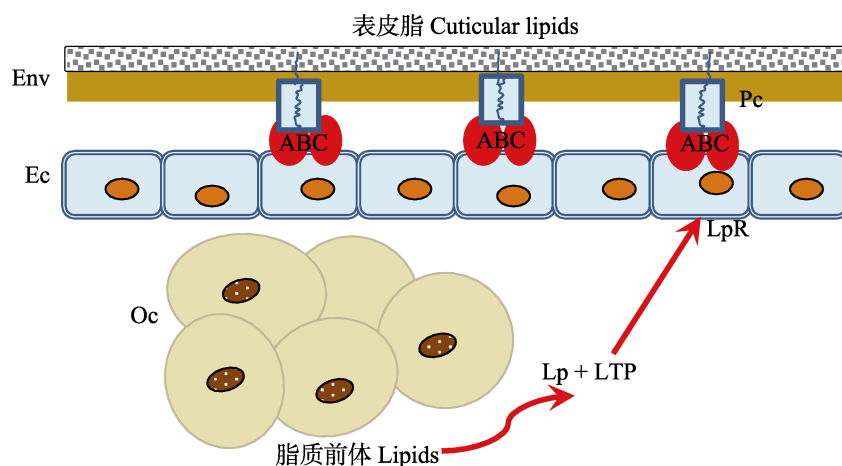


图3 昆虫表皮脂质转运途径图

Fig. 3 Transport pathway of the insect cuticular lipids

Env: 外包膜 Envelope; Ec: 真皮细胞; Oc: 绛色细胞; Lp: 载脂蛋白;

LTP: 脂颗粒转运蛋白; LpR: 载脂蛋白受体; Pc: 孔道。

Env: Envelope; Ec: Epidermal cell; Oc: Oenocyte; Lp: Lipophorin; LTP: Lipid transfer particle;

LpR: Lipophorin receptor; Pc: Pore canal.

Prodenia eridania 中得到证实 (Jaworski *et al.*, 1963)。几丁质合成通路涉及 8 种酶, 依次为海藻糖酶 (trehalase)、己糖激酶 (hexokinase)、葡萄糖-6-磷酸异构酶 (Glucose-6-phosphate isomerase)、谷氨酰:果糖-6-磷酸转氨酶 (Glutamine:fructose-6-phosphate aminotransferase)、葡萄糖-6-磷酸-N-乙酰转移酶 (N-acetylglucosamine-6-phosphate)、磷乙酰氨基葡萄糖变位酶 (Phosphoacetylglucosamine mutase)、UDP-N-乙酰葡萄糖胺焦磷酸化酶 (UDP-N-acetylglucosamine pyrophosphorylase) 和几丁质合成酶 (Chitin synthase, CHS)。其中研究较多且最为重要的是几丁质合成酶 (图 4)。

几丁质合成酶 CHS 是几丁质合成通路的关键酶, 由两个基因编码, 分别为 *CHS1* 和 *CHS2*。两者在基因结构、mRNA 表达特性及功能上存在显著差异: *CHS1* 存在可变剪切, 两个可变外显子编码 59 个氨基酸, 而 *CHS2* 则无剪切现象。*CHS1* 略长于 *CHS2*, 在不同昆虫物种间的保守性也较 *CHS2* 高。*CHS1* 基因只在昆虫体壁和气管等外胚层发育形成的组织中特异表达, 而 *CHS2* 则只在中肠上皮细胞中表达。*CHS1* 在昆虫发育过程中参与表皮和气管等组织中几丁质

的合成, 其沉默会导致昆虫表皮几丁质合成受阻, 昆虫出现蜕皮困难而死亡的表型; *CHS2* 基因仅在昆虫进食期参与中肠围食膜几丁质的合成 (Zhu *et al.*, 2016)。

3.2 几丁质降解

昆虫几丁质降解主要依赖几丁质酶 (chitinases, CHTs) 和 β -N-乙酰己糖胺酶 (β -N-acetylglucosaminidases, NAGs) 的二元酶系统。昆虫 CHTs 属于糖苷水解酶 18 (GH18) 家族, 可从几丁质长链内部随机切割生成几丁质寡糖 (N-acetylglucosamine oligomers), NAGs 属于糖苷水解酶 20 (GH20) 家族, 能够以几丁质寡糖为底物, 从非还原端水解形成单糖 (N-acetylglucosamine, GlcNAc) (图 4)。

昆虫基因组中 CHTs 基因的数量从蚜虫的 7 个到赤拟谷盗 *Tribolium castaneum* 的 24 个不等 (Zhu *et al.*, 2004, 2008a; Arakane and Muthukrishnan, 2010; Nakabachi *et al.*, 2010; Zhang *et al.*, 2011; Pan *et al.*, 2012; Tetreau *et al.*, 2015)。最近的研究发现昆虫中共有 11 类 CHTs 和 CHT-like 蛋白 (Tetreau *et al.*, 2015)。其中 8 类 (II、III、VI-X 和 h) 中每个物种均由一个基

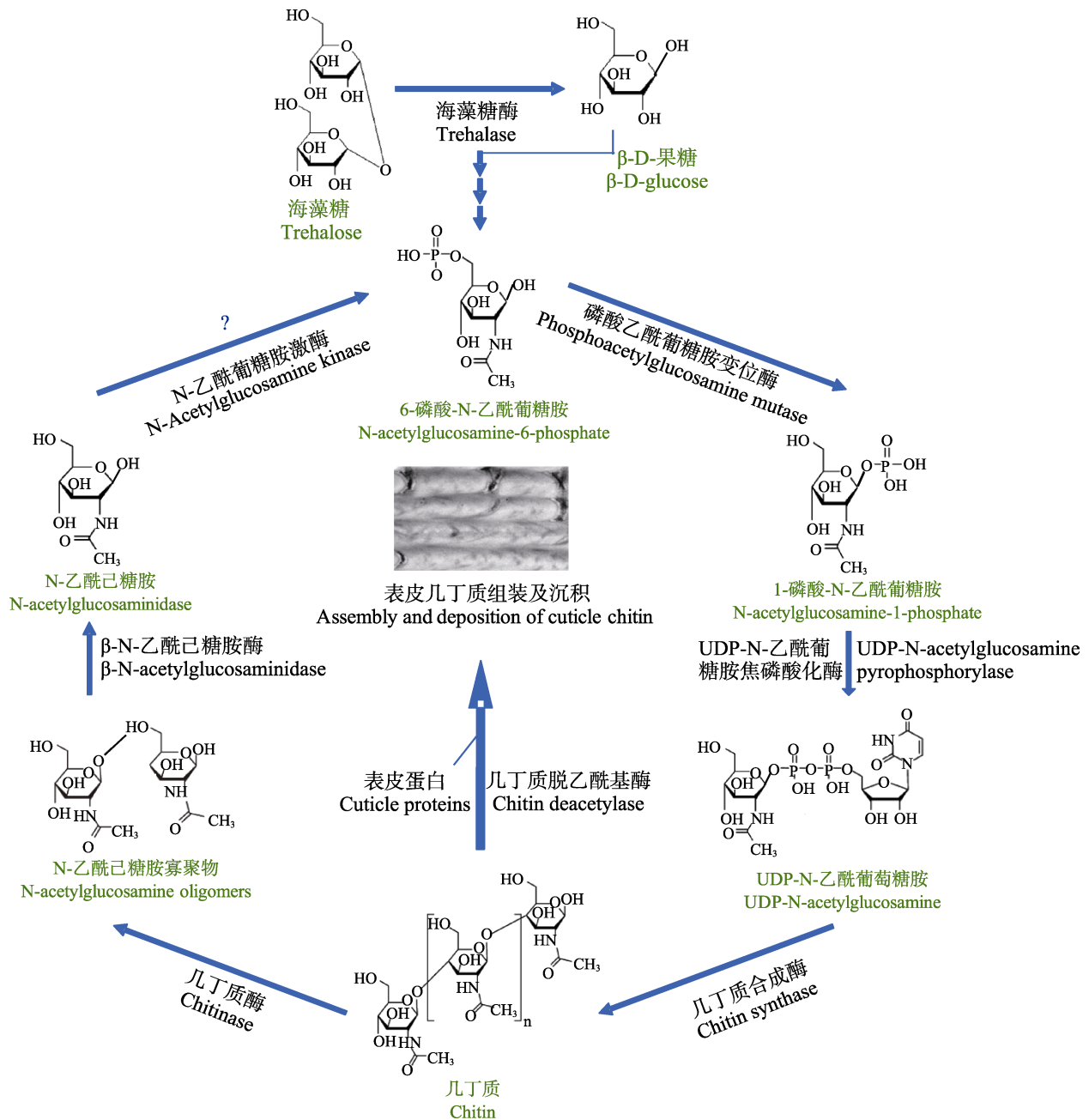


图4 昆虫几丁质代谢关键过程及重要酶和蛋白

Fig. 4 Main processes, key enzymes, and proteins involved in chitin biosynthesis and degradation in insects

因组成,而其余3类(I、IV和V)中则具有多个CHTs和CHT-like蛋白。基于RNAi的功能研究表明,多个几丁质酶基因的沉默均会严重影响表皮几丁质的降解,从而导致昆虫蜕皮受阻死亡(Zhu *et al.*, 2008b; Li *et al.*, 2015; Xi *et al.*, 2015; Omar *et al.*, 2019; Zhu *et al.*, 2019)。最新的研究表明,沉默赤拟谷盗TcCHT7后,不影响旧表皮的降解,昆虫可发育至成虫,但新表皮

几丁质的排列紊乱(Noh *et al.*, 2018)。此外,科学家进一步解析了昆虫几丁质酶的晶体结构,促进小分子药物筛选的研究进展(Jiang *et al.*, 2016; Liu *et al.*, 2017; Chen *et al.*, 2017, 2019)。

根据昆虫NAGs的系统发育和生理功能,NAGs可分为四类,包括NAGs1(I)、NAGs2(II)、NAGs3(III)和NAGs4(IV)(Hogenkamp *et al.*, 2008)。由于不同NAGs的酶学性质存在

差异, 因此其在昆虫发育过程中的功能也不相同。在赤拟谷盗、飞蝗和亚洲玉米螟 *Ostrinia furnacalis* 中, RNAi 沉默 *NAG1* 的表达可导致昆虫蜕皮严重受阻进而死亡 (Hogenkamp *et al.*, 2008; Liu *et al.*, 2012; Rong *et al.*, 2013), 表明 *NAG1* 基因参与昆虫蜕皮过程。目前获得的唯一一个昆虫 NAGs 的晶体结构来自于亚洲玉米螟的 OfHex1, 其参与底物结合的“+1”位点存在一个特殊的三明治结构, 致使对几丁质寡糖具有高度的选择性和水解活性 (Liu *et al.*, 2011)。亚洲玉米螟中 OfHex2 的 RNAi 导致幼虫化蛹异常, 成虫翅、附肢体、触角等异常; 进一步的研究表明 OfHex2 还可以水解以 β 1-3 糖苷键连接的底物 Gb4, 具有较宽的底物谱, 能够水解不同类型的糖复合物 (刘凤翊和杨青, 2013)。目前对第三类和第四类 NAG3s 的研究较少, 对其参与的生理功能尚不完全明确。

3.3 几丁质的修饰及组装

在表皮层状结构组装过程中, 几丁质有序排列起着重要作用, 多种蛋白参与这一精细调控过程。目前已报道参与几丁质排列的蛋白有 3 种, 几丁质脱乙酰基酶 (chitin deacetylase, CDAs)、Knickkopf (Knk) 和 Retroactive (Rtv) (图 4)。

几丁质脱乙酰基酶 CDAs 属于糖酯酶 4 家族的一类修饰酶, 可将几丁质的乙酰基部分脱去, 形成壳聚糖, 壳聚糖的氨基可提供与其他表皮蛋白和围食膜蛋白相结合的位点 (Zhu *et al.*, 2016)。基于序列相似性及功能域的不同, 昆虫 CDAs 可以分为 5 类: 第 I 类 (CDA1 和 CDA2) 和第 II 类 (CDA3) 均含有 3 个功能域, 即几丁质结合域 (Chitin-binding domain, ChBD)、低密度脂蛋白受体 a 类结构域 (LDLa) 和催化域 (CE4), 第 III 类 (CDA4) 和第 IV 类 (CDA5) 含有 2 个功能域, 即 (ChBD 和 CE4), 第 V 类 (CDA6、7、8、9) 仅含有 CE4 功能域。在包括赤拟谷盗 (Arakane *et al.*, 2009) 和飞蝗 (Yu *et al.*, 2016a) 在内的几种昆虫中发现了两个 CDA2 选择性剪切子 (CDA2a 和 CDA2b)。笔者课题组研究发现飞蝗具有第 I 类 (*LmCDA1* 和

LmCDA2)、第 III 类 (*LmCDA4*) 和第 IV 类 (*LmCDA5*) 基因。*LmCDA1* 和 *LmCDA2* 飞蝗蜕皮至关重要且功能存在差异, 分别沉默两个基因均会导致飞蝗蜕皮发育受阻死亡, 其中 *LmCDA1* 主要参与几丁质脱乙酰作用且影响几丁质含量, 而 *LmCDA2* 则负责表皮几丁质排列, 但不影响几丁质脱乙酰程度和几丁质含量 (Yu *et al.*, 2016a, 2018)。*LmCDA4* 和 *LmCDA5* 经 RNAi 后不影响表皮几丁质含量及排列, 飞蝗可正常蜕皮发育 (于荣荣等, 2017)。采用 Gal4/UAS 系统, 通过特异性地干扰果蝇 *DmCDA1* (Serpentine, Serp) 和 *DmCDA2* (Vermiform, Verm) 的表达, 进一步表明证明了 Serp 和 Verm 在果蝇成虫翅表皮发育过程中具有不同的功能。*DmCDA1* 是果蝇成虫翅表皮形成过程中参与几丁质去乙酰化的主要酶, 而 *DmCDA2* 在几丁质层状排列过程中起重要作用 (Zhang *et al.*, 2019)。最近科学家首次解析了家蚕 *Bombyx mori* *BmCDA1* 和 *BmCDA8* 的晶体结构, 并对其生化特征进行了分析 (Liu *et al.*, 2019)。目前鉴定的第 II 类 CDA 仅存在于完全变态昆虫中, 如赤拟谷盗 *TcCDA3* 在肌肉中高表达, 注射该基因的 dsRNA 后昆虫未出现明显可见的异常表型 (Arakane *et al.*, 2009)。不完全变态昆虫如半翅目、无翅目和直翅目等昆虫无 V 类 CDAs (Dixit *et al.*, 2008)。在其他昆虫中, V 类 CDAs 主要在肠道组织中表达, 这表明该类基因可能参与几丁质类物质消化, 而不是内源性几丁质的修饰 (Arakane *et al.*, 2009)。

Knickkopf (Knk) 和 Retroactive (Rtv) 仅在果蝇和赤拟谷盗中有所研究, *DmKnk* 在气管和胚胎表皮几丁质组装中起着重要作用, *Knk* 缺失致使果蝇气管延伸和表皮片层形成受阻, 其中 DOMON 功能域关键氨基酸甲硫氨酸 (Met) 和组氨酸 (his) 对于血红素的结合起关键作用 (Moussian *et al.*, 2006b; Shaik *et al.*, 2014)。*DmRtv* 主要在胚胎的头部外骨骼、气管的螺旋管及皮细胞中表达, *Rtv* 缺失后表皮膨大畸形, 原表皮片层结构消失 (Moussian *et al.*, 2006b)。赤拟谷盗 *TcKnk* 定位于新表皮, 保护新生表皮

免受几丁质酶降解,同时对几丁质片层结构的组装起着重要作用(Chaudhari *et al.*, 2011)。TcRtv 在表皮细胞中表达,沉默 TcRtv 基因后, TcKnk 滞留在上皮细胞中,从而失去了对新表皮的保护作用(Chaudhari *et al.*, 2013)。

4 表皮蛋白分类及其在昆虫发育与表皮形成中的作用

4.1 表皮蛋白的分类

表皮蛋白(Cuticular protein, CP)是昆虫体壁的主要成分之一,在昆虫表皮形成和发育中具有重要作用。近年来,随着昆虫基因组学和转录组学的发展,许多物种的表皮蛋白基因被鉴定,这些表皮蛋白基因约占昆虫预测的总基因的1%。昆虫表皮蛋白根据其所含基序不同可划分为13个家族(Willis, 2010; Zhou *et al.*, 2016)。其中最大的为CPR家族,其含有一个Rebers&Riddiford基序(R&R Consensus)(Rebers and Riddiford, 1988)。R&R基序包括一个几丁质结合域(Chitin-binding domain 4, CBD4)(表1),有助于协调几丁质和蛋白质基质之间的相互作用(Rebers and Willis, 2001; Togawa *et al.*, 2004, 2007; Qin *et al.*, 2009)。CPR家族表皮蛋白包含RR-1、RR-2和RR-3三个亚家族,其中RR-1和RR-2表皮蛋白可能与表皮的柔性和

刚性相关(Willis, 2010)。CPRs在不同昆虫中的数量差异很大,如在家蚕、黑腹果蝇、烟草天蛾和褐飞虱中分别具有148、101、207和96个CPRs,而在西方蜜蜂*Apis mellifera*和飞蝗中仅分别有28和51个CPRs(Karouzou *et al.*, 2007; Soares *et al.*, 2007; Futahashi *et al.*, 2008; Zhao *et al.*, 2017; Pan *et al.*, 2018)。

除了CPRs,表皮蛋白还包括CPF家族(具有42-44个氨基酸残基的序列),CPFL家族(类似于CPF,有一个保守的C末端区域),CPT(含有Tweedle基序),CPG(富含甘氨酸),CPAPs,CPH(假设表皮蛋白),CPLC(低复杂度的表皮蛋白,包括CPLCA、CPLCG、CPLCW和CPLCP)(Willis, 2010)。其中CPAPs家族表皮蛋白有1或3个CBD2结构域,不含其他蛋白结构域,具有与几丁质结合的能力(Elvin *et al.*, 1996)。基于CBD2结构域的数量,CPAPs进一步分为具有1个CBD2结构域的CPAP1和3个ChtBD2结构域的CPAP3家族(Jasrapuria *et al.*, 2010, 2012)。

4.2 表皮蛋白特性与表达模式

昆虫表皮蛋白可能参与昆虫内外表皮的形成,目前,已从不同昆虫物种中的蜕皮前和蜕皮后的表皮中鉴定了许多表皮蛋白序列,如在烟粉虱*Tenebrio molitor*中,从蛹中鉴定出5个蜕皮

表1 昆虫表皮蛋白家族及其序列特征
Table 1 The family and its sequence feature of insect cuticular protein

家族 Family	序列特征 Sequence feature	参考文献 References
CPR	含有 Rebers & Riddiford 基序,包括 RR-1, RR-2 和 RR-3 三种亚家族 Containing Rebers & Riddiford motif, including RR-1, RR-2 and RR-3	Rebers and Riddiford, 1988
CPF	具有 42-44 个氨基酸残基的序列 Sequences with 42-44 amino acid residues	Togawa <i>et al.</i> , 2007
CPFL	与 CPF 具有同源性,有一个保守的 C 末端区域 It is homologous to CPF and has a conservative C-terminal region	Togawa <i>et al.</i> , 2007
CPT	含有 Tweedle 基序 Containing Tweedle motifs	Guan <i>et al.</i> , 2006
CPG	富含甘氨酸 GGGX 或 GGXG Glycine-rich GGGX or GGXG	Futahashi <i>et al.</i> , 2008
CPAPs	包括 CPAP1 和 CPAP3,含有 1 个或 3 个 ChtBD2 几丁质结合域 Including CPAP1 and CPAP3, containing one or three chitin binding domains (ChtBD2)	Jasrapuria <i>et al.</i> , 2010, 2012
CPH	假设表皮蛋白 Hypothetical cuticle protein	Pan <i>et al.</i> , 2018
CPLC	低复杂度的表皮蛋白,包括 CPLCA, CPLCG, CPLCW 和 CPLCP Low-complexity epidermal proteins, including CPLCA, CPLCG, CPLCW and CPLCP	Willis, 2010

后表皮蛋白 (Baernholdt and Anderson, 1998; Mathelin *et al.*, 1998) 和 9 个蜕皮前表皮蛋白 (Andersen, 1995; Haebel *et al.*, 1995; Rondot *et al.*, 1996; Andersen *et al.*, 1997)。在飞蝗中, 从成虫表皮中鉴定出两种蜕皮后表皮蛋白 (Talbo *et al.*, 1991; Jespersen *et al.*, 1994) 和 20 种蜕皮前表皮蛋白 (Nohr *et al.*, 1992; Andersen, 1995; Jensen *et al.*, 1998)。

表皮蛋白基因在昆虫不同发育阶段或不同组织部位具有不同的表达模式, 意味着其可能具有不同的功能。如在家蚕翅原基中, 测定了 52 个表皮蛋白基因, 根据这些基因的表达峰段, RR-2 表皮蛋白基因在化蛹当天表达, 而 RR-1 表皮蛋白基因在化蛹之前和之后表达, 且 RR-1 表皮蛋白基因的表达持续时间长于 RR-2 表皮蛋白基因, 表明 RR-1 和 RR-2 表皮蛋白基因在家蚕翅原基内外表皮构建中具有不同的功能 (Shahin *et al.*, 2016)。随后, 研究学者进一步研究表明, RR-1、RR-2 和其他类型的表皮蛋白可能参与外表皮的构建, 而 RR-1 和其他表皮蛋白可能参与内表皮的形成 (Shahin *et al.*, 2016, 2018)。通过免疫胶体金标记技术, 学者对几种 RR-1 和 RR-2 表皮蛋白在冈比亚按蚊 *A. gambiae* 表皮中进行了定位, 发现 RR-1 蛋白定位于柔软节间膜的原表皮以及未硬化的软表皮中, 而 RR-2 蛋白则存在于硬表皮中 (Vannini and Willis, 2017)。有研究报道, 表皮蛋白的组氨酸残基用于表皮的硬化 (Schaefer *et al.*, 1987; Hopkins *et al.*, 2000; Andersen, 2010), 因此, 定位于外表皮中的表皮蛋白含有高比例的组氨酸残基。在赤拟谷盗鞘翅中, 发现一个低复杂度的表皮蛋白 TcCP30 通过漆酶 2 与两个 RR-2 表皮蛋白 (TcCPR18 和 TcCPR27) 交联, 但不与 RR-1 表皮蛋白 (TcCPR4) 交联, 而这种交联可能与 RR-2 表皮蛋白 (TcCPR18 和 TcCPR27) 富含组氨酸残基有关, 在鞘翅形成过程中促进表皮的硬化 (Mun *et al.*, 2015)。

4.3 表皮蛋白在昆虫发育和表皮形成中的作用

表皮蛋白存在于表皮中的特定位置, 对于维

持表皮结构完整性具有重要意义。在家蚕中, 编码 RR-1 蛋白的 *BmCPR2* 基因缺失导致表皮几丁质含量显著降低, 使幼虫节间异常分布和折叠, 是造成表皮硬化突变体的主要原因 (Xiong *et al.*, 2017)。在赤拟谷盗中, RNA 干扰 *TcCPR27* 和 *TcCPR18* 可导致鞘翅表皮层状结构和孔道紊乱, 导致鞘翅褶皱, 表明 *TcCPR27* 和 *TcCPR18* 在维持鞘翅的结构完整性中起重要作用 (Arakane *et al.*, 2012)。RNA 干扰褐飞虱中 15 个 CPR 基因可导致致死表型, 表明其在表皮结构形成和发育中具有重要作用 (Pan *et al.*, 2018)。笔者课题组在飞蝗中鉴定出一个 RR-2 亚家族的翅特异表皮蛋白 *LmACP7* (Zhao *et al.*, 2017), 发现 *LmACP7* 定位于成虫翅的外表皮中, 是维持成虫翅表皮正常形态和功能所必需的结构成分 (Zhao *et al.*, unpublished)。

CPAPs 蛋白是含有 CBD2 结构域的表皮蛋白家族。在赤拟谷盗中研究发现, CPAP3 家族蛋白基因在虫体发育和维持表皮的结构完整性方面具有基本功能, 以维持表皮的物理性质 (Jasrapuria *et al.*, 2012)。在黑腹果蝇中, *ObstA* (*TcCPAP3* 家族的直系同源物) 可形成基质支架以协调新沉积的细胞外基质 (ECM) 中蛋白质和酶的运输和定位 (Petkau *et al.*, 2012; Pesch *et al.*, 2015)。CPAP3-C 基因的突变体可导致胚胎致死并且表现出表皮发育缺陷 (Barry *et al.*, 1999; Behr and Hoch, 2005)。Pan 等 (2018) 在褐飞虱中通过 RNAi 干扰 CPAP1 和 CPAP3 家族蛋白基因可导致虫体致死。

研究表明, 黑腹果蝇幼虫表皮结构中包含 Tweedle 蛋白, *TweedleD* 基因突变可以改变虫体形态, 表明其在决定虫体形状中具有重要作用 (Guan *et al.*, 2006)。在家蚕中, 通过转基因技术过表达 *BmCPT1* (*Tweedle* 表皮蛋白) 可导致 *BmRelish1* 的上调表达和诱导两种 *gloverin* 基因的表达, 表明 *BmCPT1* 参与昆虫先天免疫 (Liang *et al.*, 2015)。在冈比亚按蚊中, 已鉴定出 4 种 CPF 基因和一种 CPFL 基因, 这些基因在蛹或成虫蜕皮前表达, 表明其可能参与蛹和成虫外表皮的形成 (Togawa *et al.*, 2007)。在家蚕 *Bo* 突

变体中, 鉴定了一个假设蛋白 BmCPH24 (CPH 家族), RNAi 介导的敲低和 CRISPR/Cas9 介导的 BmCPH24 敲除结果暗示 BmCPH24 可能参与内表皮的形成 (Xiong *et al.*, 2017)。对于 CPLC 家族, Mun 等 (2015) 从赤拟谷盗中鉴定了具有低复杂性序列的表皮蛋白 (TcCP30), RNAi 干扰 TcCP30 的表达不影响幼虫和蛹的生长发育; 然而, 大约 70% 的成虫在羽化期间无法蜕皮而死亡。Lu 等 (2018) 在褐飞虱中鉴定出编码新的未分组表皮蛋白基因 NICP21.92, RNAi 沉默该基因导致昆虫发育异常和致死表型, 分析表明, 该基因参与内表皮的形成。

5 展望

昆虫表皮发育关键基因生物学功能的解析, 对于研发害虫防治分子靶标不仅具有重要的理论意义, 同时也具有重要的应用价值。昆虫表皮脂质对于昆虫生态适应性具有重要意义, 目前, 表皮脂的合成路径尚不十分明确, 表皮脂在真皮细胞和表皮层内如何修饰加工, 怎样运输到上表皮等科学问题尚有待探索。表皮几丁质代谢对于昆虫生长发育至关重要, 然而目前仍有若干科学问题有待深入研究: 如几丁质合成酶如何在质膜上组装并穿过质膜进行转运? 几丁质酶在几丁质降解过程作用部位和作用机制如何? 昆虫表皮蛋白是结构蛋白, 其具有数量众多和结构多样性的特点, 但其编码蛋白的相互作用及其在表皮形成中的作用机制都有待进一步深入研究。

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