昆虫组蛋白修饰的研究进展

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摘要：组蛋白修饰作为表观遗传修饰的一种主要形式，对基因表达和表型调控具有重要作用。组蛋白修饰的N端尾区可通过乙酰化、甲基化、磷酸化等修饰来改变染色质的状态以及调控基因的表达。与脊椎动物相比，昆虫种类繁多，且有变态发育、表型复杂等特征，可以成为探索动物社会行为、发育调控和毒理作用等表观遗传基础的模型。本文总结了昆虫组蛋白修饰的主要类型（乙酰化和甲基化修饰）及修饰酶的研究进展，对染色质免疫共沉淀测序技术（chromatin immunoprecipitation followed by sequencing，ChIP-seq）、染色质转座酶可及性测序技术（assay for transposase-accessible chromatin with high-throughput sequencing，ATAC-seq）、转录组测序技术（RNA-seq）、组蛋白修饰酶功能验证以及Western blot、免疫细胞化学（immunocytochemistry，ICC）、免疫组织化学（immunohistochemistry，IHC）、酶联免疫吸附测定（enzyme linked immunosorbent assay，ELISA）等免疫学方法进行了介绍，并综述了昆虫组蛋白修饰的功能，以期为进一步研究昆虫组蛋白修饰提供借鉴和参考。同时对加强昆虫组蛋白修饰在种群水平、跨代遗传等方面的研究进行了展望。

关键词：昆虫；表观遗传学；组蛋白修饰

Advances in histone modification in insects

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Abstract: Histone modification is one of the main epigenetic mechanisms, and plays an important role in regulating gene expression and phenotype. Histone modification can change chromatin status and regulate gene expression through modifications at the N-ternimus of histone residue, including acetylation, methylation, phosphorylation, etc. Compared with vertebrate animals, insects have higher species abundance, and unique features such as metamorphic development and complex phenotypes, making it a potential model system for exploring the epigenetic basis of social behavior, developmental regulation, and responses to toxicological substances in animals. In this review, we first provide an overview of recent advances in histone modification in insects and focus on a number of frequently studied covalent changes (mainly histone acetylation and methylation), the enzymes responsible for the covalent changes, research methods and techniques (*e. g.*, chromatin immunoprecipitation followed by sequencing (ChIP-seq), assay for transposase-accessible chromatin with high-throughput sequencing (ATAC-seq), RNA sequencing (RNA-seq), histone modification enzyme function verification, and immunological technique such as Western blot, immunocytochemistry (ICC), immunohistochemistry (IHC), enzyme

基金项目：国家自然科学基金（31772230）

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收稿日期：2020-08-13

linked immunosorbent assay (ELISA)) used in these studies, and the functions of individual covalent changes to provide a reference for further study exploring histone modification in insects. This review also suggests possible future directions for insect histone modification research by making efforts to study histone modification at population level and exploring transgenerational epigenetic effects.

Key words: insect; epigenetics; histone modification

表观遗传学最早由Waddington提出，并在其早期研究中被定义为控制从基因型到表型的机制（Waddington，1942）。几十年后，Holliday（1987）针对表观遗传学提出更新的系统性论断，即由非DNA序列改变而引起的可遗传的基因表达或表型变化。介导表观遗传的分子机制通常有3种形式，即DNA甲基化、组蛋白翻译后修饰（histone post-translational modifications，hPTM）和非编码RNA（Waterland & Michels，2007）。表观遗传修饰可通过调控基因表达影响多种生物学过程，如细胞命运的维持和转变、细胞的增殖和分化、衰老和疾病的发生等，从而增加生物应对因生物及非生物胁迫带来的环境压力的能力甚至进化潜力（Ellstrand & Schierenbeck，2000；Kim et al.，2009）。表观遗传学可以解释某些不能归因于遗传变异的表型变异，既能单独影响表型的可塑性又可以调控遗传编码的可塑性，从而促进对环境变化的响应，这有助于更好地理解具有生态学重要特征的自然变异的潜在机制（Bossdorf et al.，2008；Richards et al.，2010；Duncan et al.，2014）。

昆虫由于其明显的表型分化、丰富的表观遗传多样性以及较小的基因组，逐渐成为研究表观遗传的重要生物学材料（Vieira et al.，2012；Chen et al.，2015；Wang et al.，2015）。对PubMed数据库的检索结果显示在已发表的数百篇昆虫表观遗传学文献中，早在1992年Turner et al.（1992）通过免疫染色证明了果蝇雄虫X染色体上的H4K16ac修饰水平（表示在组蛋白H4上第16位赖氨酸的乙酰化修饰），首次将昆虫中特定的组蛋白修饰与染色质功能联系起来。与模式脊椎动物相比，昆虫具有许多优势，包括动物伦理上的可接受性、较短的繁殖周期，以及用于研究如繁殖力、寿命、性比和对病原体、寄生虫和环境压力抗性等复杂性状的潜力，因此昆虫逐渐成为学者们探索动物的社会行为（Yan et al.，2014）、发育调控（Mukherjee et al.，2015）、毒理作用（Bingsohn et al.，2016）等表观遗传基础的模型（Bonasio，2015）。在昆虫的表观遗传研究中，对于社会性昆虫级型的表观遗传编程研究是最丰富、最活跃的领域之一。因社会性昆虫中多态性的存在非常普遍，幼虫饮食影响其发育成不同级型，社会等级的产生有助于种群中工作和任务的划分（Nijhout，2003；Ernst et al.，2015；Glastad et al.，2015a），因此将社会性昆虫作为研究对象有着独特的前景（Bonasio，2012；2014；Yan et al.，2014）。在表观遗传研究中，评估表观遗传变异受遗传调控的程度是评估表观遗传进化潜力的关键问题。社会性昆虫的遗传背景一致，可为研究不受遗传调控的纯表观遗传变异提供理想的研究材料（Richards，2006；Bossdorf et al.，2008）。

组蛋白修饰在调节基因表达中起着重要作用，可以建立、维持和改变生物体的表型（Jaenisch & Bird，2003）。相比于DNA甲基化修饰，组蛋白修饰的种类多样，在基因组中的定位和功能在不同分类群中也高度保守（Bernstein et al.，2005；Kharchenko et al.，2011；Woo & Li，2012），因此可能比DNA甲基化修饰更能直接地调控生物体的表型可塑性（Simola et al.，2013a；Libbrecht et al.，2016；Jambhekar et al.，2019）。而且有研究表明无脊椎动物基因组的DNA甲基化水平普遍较低，通常整个基因组胞嘧啶甲基化的水平仅在0~3%之间，几乎无法检测到（Feng et al.，2010；Bewick et al.，2016）。这可能暗示了昆虫中DNA甲基化修饰的重要性较低，而组蛋白修饰是其表观遗传调控的主要机制（Glastad et al.，2011；Boffelli et al.，2014；Kozeretska，2017）。

与脊椎动物和植物相比，昆虫的组蛋白修饰研究还相对滞后（Burggren，2017；Glastad et al.，2019）。但随着近几年各种新技术的发展和创新，特别是高通量测序技术的较快发展，目前已有越来越多关于昆虫组蛋白修饰方面的研究报道，如通过对西方蜜蜂 *Apis mellifera*（Dickman et al.，2013；Wojciechowski et al.，2018）、佛罗里达弓背蚁 *Camponotus floridanus*（Simola et al.，2013a，b；2016）、家蚕 *Bombyx mori*（Mon et al.，2011）、冈比亚按蚊 *Anopheles gambiae*（Gómez-Díaz et al.，2014；Ruiz et al.，2019）和飞蝗 *Locusta migratoria*（Guo et al.，2016）等昆虫的研究发现，昆虫中存在大量不同种类的组蛋白修饰现象，且在调控昆虫生长发育中发挥着重要作用。本文重点综述昆虫组蛋白修饰类型及修饰酶、研究方法及功能，以期为未来研究组蛋白修

饰在昆虫中的作用提供新的思路和参考。

1 组蛋白修饰及修饰酶

核小体是组成染色质的基本结构单位，由H2A、H2B、H3和H4各2个分子组成的组蛋白八聚体及缠绕在八聚体上长约146 bp的DNA组成(Talbert & Henikoff, 2010; Bell et al., 2011; Zhou et al., 2011)。其中，组蛋白N端尾区常暴露在核小体之外，容易接触到细胞核内的各种组蛋白修饰酶而被共价修饰，称为组蛋白修饰(Zhou et al., 2011)。组蛋白修饰在基因表达调控方面具有重要作用，其状态决定了转录复合物的靠近程度，调控了染色质在转录活跃和沉默状态之间的转换，影响了基因的表达活性，对DNA和其他蛋白因子的结合产生协同或拮抗效应(Peterson & Laniel, 2004; Greer & Shi, 2012; Badeaux & Shi, 2013)。

目前已在昆虫中发现160多种组蛋白修饰类型，修饰位点一般位于组蛋白H3和H4的游离N端尾区的赖氨酸或精氨酸残基上，常见的包括乙酰基、甲基、磷酸基和泛素等修饰(Kouzarides, 2007; Bannister & Kouzarides, 2011; Suganuma & Workman, 2011)，其中关于组蛋白乙酰化和甲基化修饰的研究较为广泛。组蛋白乙酰化能降低组蛋白和DNA之间的电荷作用，从而降低其与DNA的亲和力，改变转录因子与模板DNA链的可及性，激活基因转录(Kuo et al., 1996; Zhou et al., 2011; Zentner & Henikoff, 2013)；相反，组蛋白乙酰化的移除则可导致基因抑制和沉默(de Ruijter et al., 2003; Shahbazian & Grunstein, 2007)。组蛋白修饰过程中，甲基化修饰较为复杂，N端尾区有多个修饰位点，每个修饰位点上可发生多种共价修饰，赖氨酸残基可发生一甲基化(me)、二甲基化(me2)或三甲基化(me3)，而精氨酸残基可被一甲基化或二甲基化(Mellor, 2006)。昆虫组蛋白的甲基化修饰主要发生在组蛋白H3的第4、9、27和36位赖氨酸残基上。通常不同类型以及不同位点的甲基化修饰具有不同效应，如H3K4me3、H3K36me3和H3K79me3与基因的转录激活和常染色质有关(Rice et al., 2003; Kouzarides, 2007; Vermeulen et al., 2007)，H3K27me3和H3K9me2/me3多与基因沉默和异染色质相关(Lachner & Jenuwein, 2002; Turner, 2002; Martin & Zhang, 2005)。当基因的启动调控区域同时存在H3K4me3和H3K27me3修饰时，这2种修饰标记相互拮抗，称为组蛋白的二价修饰，在昆虫发育中发挥关键的调控作用(Bernstein et al., 2006)。

组蛋白修饰的添加及去除是由一系列组蛋白修饰酶完成的，且不同残基通常需要不同修饰酶参与介导(Margueron & Reinberg, 2010; Bannister & Kouzarides, 2011)。经修饰的组蛋白可与其他蛋白特异性结合，或被特异性识别而定位于其靶区域(Greer & Shi, 2012; Badeaux & Shi, 2013)。在组蛋白修饰酶中，组蛋白乙酰转移酶(histone acetyltransferase, HAT)负责催化N端尾区的赖氨酸乙酰化，通常与基因表达有关；组蛋白去乙酰化酶(histone deacetylase, HDAC)调控组蛋白脱乙酰基，促进致密核小体的形成，从而导致转录的抑制(Balasubramanyam et al., 2004; Musselman et al., 2012)。组蛋白甲基化由组蛋白甲基转移酶(histone methyltransferase, HMT)催化，并由组蛋白去甲基化酶(histone demethylase, HDM)去除。在所有修饰酶中，HMT最具特异性(Kouzarides, 2007)。Holowatyj et al. (2015)总结了果蝇中13种HDM，并讨论了其在维持基因组功能中的关键作用，如基因沉默、异染色质形成、调控氧化还原过程和调控Notch信号通路等。

2 昆虫组蛋白修饰的研究方法

2.1 染色质免疫共沉淀测序技术

目前研究组蛋白修饰的首选技术是染色质免疫共沉淀(chromatin immunoprecipitation, ChIP)技术，该技术可在染色质水平上真实反映生物体内基因组DNA与蛋白质结合情况的转录调控(Collas, 2010; Galdieri et al., 2012)。过去研究人员常用染色质免疫共沉淀-芯片杂交(chromatin immunoprecipitation followed by chip, ChIP-chip)技术来鉴定组蛋白修饰，但该技术存在一定局限性，仅能作用于探针所及的基因组序列，在序列未知的情况下，芯片技术并不能真正全面地检测基因组中组蛋白修饰的分布(Gilmour & Lis, 1984; Ren et al., 2000; Roh et al., 2004)。随着第二代测序技术的推广应用，染色质免疫共沉淀测序(chromatin immunoprecipitation followed by sequencing, ChIP-seq)技术已成为全基因组范围内检测组蛋白修饰的重要方法(Fields, 2007; Meyer & Liu, 2014; 沈圣等, 2014)。ChIP-seq技术通过特异性抗体结合目标组蛋白，经免疫沉淀后选择组蛋白中包含的DNA片段，经纯化和文库构建后对富集到的DNA片段进行高通量测序(Park, 2009)。在测序深度和范围足够的情况下，ChIP-seq技术可以检测到所有组蛋白修饰所对应的DNA结合位点(Hirst & Marra, 2010; Werner, 2010)。与ChIP-chip相比，ChIP-seq的准确性更高、覆盖范围

更广，且所需样本量更小（Schones & Zhao，2008；Schumacher et al.，2008；Lister & Ecker，2009）。

ChIP技术需要针对目标蛋白的高特异性抗体，因此ChIP-seq中的目标蛋白必须足够保守，或开发新型抗体，抗体的开发往往非常困难而昂贵。但因为组蛋白是动物基因组中最保守的蛋白质之一，因此有可能将在果蝇甚至哺乳动物中开发的抗体用于非模式昆虫全基因组范围的组蛋白修饰测序分析（Malik & Henikoff，2003）。传统的ChIP-seq技术在应用上同样存在一些局限性：ChIP试验中的交联和超声处理会降低目标分子的可及性，且只有少量的基因组可以与靶蛋白结合，效率较低，难以应用于少量甚至微量的昆虫组织研究。针对这些问题，近期已有技术通过文库制备技术的改进从而显著提高ChIP-seq技术效率（Schmidl et al.，2015；Sundaram et al.，2016），例如Simola et al.（2016）通过使用线性DNA扩增获得充足的分析材料使ChIP-seq技术成功应用于单只蚂蚁的大脑研究。Native-ChIP技术也是传统ChIP-seq技术的改进方法，即利用微球菌核酸酶消化未结合的DNA再进行免疫沉淀（O'Neill & Turner BM，2003），该技术的发展进一步促进了使用有限材料进行的研究，可以仅利用昆虫的单个组织开展组蛋白修饰谱的研究（Brind'Amour et al.，2015；Lewis et al.，2016）。

在模式昆虫果蝇中，已通过ChIP-seq技术实现组蛋白H3K4、H3K9、H3K27、H3K36乙酰化、甲基化等多种全基因组修饰图谱的绘制（Yin et al.，2011；Ye et al.，2016；2017）。除此之外，ChIP-seq技术已应用于西方蜜蜂（Shpigler et al.，2017；Wojciechowski et al.，2018）、佛罗里达弓背蚁（Simola et al.，2013b；2016）、家蚕（Shoji et al.，2014；Cheng et al.，2018）、冈比亚按蚊（Ruiz et al.，2019）、艺神袖蝶*Heliconius erato*（Lewis et al.，2016）和昆士兰实蝇*Bactrocera tryoni*（Nagalingam et al.，2018）等昆虫的研究中，为探究组蛋白修饰在转录调节中的作用提供了新的证据。Maleszka（2016）认为，未来的研究需要从测序深度较低的全基因组分析转向细胞类型特定的表观基因组学。

2.2 染色质转座酶可及性测序技术

染色质转座酶可及性测序（assay for transposase-accessible chromatin using sequencing，ATAC-seq）技术利用Tn5转座酶将测序接头整合到DNA开放性增加的区域中，最终对基因组中与开放染色质相对应的序列进行测序。该技术已被用于分析开放染色质或可及性染色质、核小体的定位以及转录因子的结合等研究中（Buenrostro et al.，2013；Davie et al.，2015；Blythe & Wieschaus，2016）。目前，用于研究染色质可及性的方法主要有4种：微球菌核酸酶消解测序（micrococcal nuclease sequencing，MNase-seq）、脱氧核糖核酸酶测序（deoxyribonuclease I-hypersensitive site sequencing，DNase-seq）、甲醛辅助分离调控元件测序（formaldehyde-assisted isolation of reg-ulatory elements sequencing，FAIRE-seq）和ATAC-seq。与其他3种测序方法相比，ATAC-seq有着明显的优势：样品需求量小，最佳细胞量大约为25 000~75 000个；试验操作简单，只需细胞裂解、Tn5转座酶转位和PCR扩增3个步骤即可构建试验所需文库；检测灵敏度高且试验重复性好（Buenrostro et al.，2013；Corces et al.，2017；Doganli et al.，2017）。但ATAC-seq也受到一些限制，其要求试验材料为新鲜样品或经冷冻保存，最好用于单细胞悬液，且容易引入线粒体污染（Corces et al.，2017）。目前ATAC-seq已经成为研究开放染色质的首选技术，并已在果蝇的研究中广泛使用（Blythe & Wieschaus，2016；Meers et al.，2017）。

2.3 转录组测序技术

转录组测序（RNA sequencing，RNA-seq）技术是一种对生物细胞内RNA序列、种类和丰度进行分析的技术，是近年来在表观遗传学研究中应用较多的一种测序技术，具有高通量、高灵敏度、高分辨率和不受物种限制等优点（Wang et al.，2009；Costa et al.，2010）。Yocum et al.（2015）通过对苜蓿切叶蜂*Megachile rotundata*滞育及其滞育后状态的转录组进行分析，鉴定了643个滞育后上调的基因转录本和242个滞育后下调的转录本，其中包括介导H3K27me3的PRC2蛋白基因。通过转录组测序筛选出昆虫组蛋白修饰相关的关键基因，通常需要通过实时荧光定量PCR技术快速准确地分析不同处理条件下基因的表达差异（Reynolds & Hand，2009a；Mitaka et al.，2020）。

2.4 组蛋白修饰酶功能验证

对于组蛋白修饰的研究可以通过使用组蛋白修饰酶抑制剂或RNA干扰（RNA interference，RNAi）技术阻断或抑制相关酶的合成来实现，甚至有可能通过基于成簇规律间隔的短回文重复序列及其相关系统的基因编辑（clustered regularly interspaced short palindromic repeats/CRISPR-associated 9，CRISPR/Cas9）技术在特定位点操纵组蛋白修饰。

2.4.1 抑制剂处理

使用组蛋白修饰酶抑制剂导致生物体表型变

异,证明相关组蛋白修饰的功能,增加了目前有限的用于证明表观遗传标记与特定表型直接联系的研究方式(Afshinnekoo & Mason, 2016)。HAT、HDAC、HMT和HDM具有特异性,相应抑制剂处理只会影响某种组蛋白的特异性修饰,如通过HDAC抑制剂TSA和BuA处理果蝇后能引起*hsp70*基因的表达水平升高,表明果蝇的组蛋白乙酰化参与*hsp70*基因的转录调控(Chen et al., 2002; Zhao et al., 2005)。Huang et al.(2012)表明HDAC抑制剂NBM-HD-1能够调节蜂王浆中MRJP3蛋白的比例,从而改变蜂王发育过程中的体型。但是缺乏针对兴趣位点的特异性、高选择性的抑制剂成为了阻碍其进一步发展的重要原因。

2.4.2　RNA干扰技术

在表观遗传研究中必须开发更特异的工具来操纵特定的表观遗传学特征,即通过试验制造特定位点的表观突变(Bonasio, 2012)。一种常规策略是使用RNAi靶向在表观遗传学中起作用的基因,通过向昆虫注射或饲喂外源双链RNA(double-stranded, dsRNA)阻碍特定基因的翻译或转录,引起内源靶标mRNA降解,从而导致靶标基因沉默或表达量明显下调(杨中侠等, 2008)。该技术对靶基因具有高特异性、系统性及易操作性等优点,可介导组蛋白修饰酶关键基因的表达沉默,产生相应的功能缺失表型,从而验证组蛋白修饰的作用。Zhang et al.(2018)在褐飞虱*Nilaparvata lugens*中鉴定到12个HDAC成员,通过RNAi技术介导的基因沉默,表明*NlHdac1*、*NlHdac3*和*NlHdac4*通过调控卵巢或产卵器发育在雌虫生殖中发挥着关键作用。Chou et al.(2019)使用RNAi技术干扰赤拟谷盗*Tribolium castaneum*幼虫中的2种HMT相关基因,表明H3K27me3在赤拟谷盗幼虫变态发育和腿再生中发挥着重要作用。

2.4.3　CRISPR/Cas9基因编辑系统

尽管RNAi技术已在昆虫研究中应用多年,但这种方法存在干扰效率不稳定、脱靶率高、无法同时对多个基因进行干扰等缺点(Jackson et al., 2003)。目前正在探索针对特定位点的创新基因工程工具,例如可以使用CRISPR/Cas在HAT、HDAC、HMT和HDM等相关目标基因中特异地引入突变(包括基因的敲除、敲低和激活)(刘素宁等, 2018),导致特定组蛋白的乙酰化/甲基化水平发生改变,从而对表观基因组进行靶向操作(Bonasio, 2012; Gaj et al., 2013)。Bassett & Liu(2014)和Port et al.(2014)在果蝇中以DNA、RNA或蛋白质的形式将核酸内切酶Cas9注射到胚胎中可以进行遗传操作,根据靶向的方法和基因以25%~100%的传递率遗传给后代。Penke et al.(2018)为了研究组蛋白变体H3.3K9在果蝇发育中的作用,用CRISPR/Cas9基因编辑系统在内源性*H3.3B*位点引入*K9R*取代,然后将回收的*H3.3B*^{K9R}突变等位基因与先前产生的*H3.3A*无效等位基因结合,从而产生了H3.3K9R果蝇个体。利用CRISPR/Cas9基因编辑系统建立的组蛋白修饰酶的突变体或组蛋白N端尾区氨基酸残基的突变将为未来表观基因组学的研究提供前所未有的发展前景,从而避免RNAi和抑制剂处理的弊端。

2.5　免疫学方法

昆虫组蛋白修饰的传统研究方法主要基于免疫学方法,例如Western blot、免疫细胞化学(immunocytochemistry, ICC)、免疫荧光技术(immunofluorescence, IF)、免疫组织化学(immunohistochemistry, IHC)和酶联免疫吸附测定(enzyme linked immunosorbent assay, ELISA)等。在果蝇、蜜蜂和蝗虫等昆虫的研究中,利用传统免疫学方法在组蛋白修饰调控寿命(Zhao et al., 2005)、生长发育(Shanower et al., 2005; Bodai et al., 2012)、记忆(Merschbaecher et al., 2012)和社会等级转换(Spannhoff et al., 2011)等方面展开了一系列研究。但是传统免疫学手段无法获得更高分辨率的组蛋白修饰动态变化图谱,从而阻碍了对其作用机制的理解(卢绪坤等, 2019)。

除了上述研究方法,超高效液相色谱(ultra performance liquid chromatography, UPLC)(Henry et al., 2016)、反相高效液相色谱(reverse phase high performance liquid capillary chromatography, RP-HPLC)(Mon et al., 2011)、高分辨率质谱仪(mass spectrometry, MS)(Ong et al., 2002; Dickman et al., 2013)、液相色谱–质谱串联(liquid chromatograph-mass spectrometer, LC-MS)(Feller et al., 2015; Shen et al., 2015)等技术以及将其与稳定同位素标记技术结合使用,同样在昆虫组蛋白修饰类型和位点的检测上发挥了重要作用。

3　昆虫组蛋白修饰的功能

组蛋白修饰对昆虫基因调控的大多数研究集中于模式昆虫黑腹果蝇*Drosophila melanogaster*,且多为与生态环境无关的遗传学研究,如生长发育、学习、记忆和生殖等过程中组蛋白修饰的调控机理(表1)。因此关于黑腹果蝇组蛋白修饰的研究不是本综述的关注重点,在此主要讨论其他昆虫中组蛋白乙酰化和甲基化修饰在生态和进化学中的功能,包括社会性昆虫级型分化、昆虫滞育、营养状况和病原物对表型影响等内容。

表1 黑腹果蝇的组蛋白修饰功能

Table 1 Histone modification functions of *Drosophila melanogaster*

组蛋白修饰 Histone mark	研究方式 Research method	主要发现 Major finding	参考文献 Reference
H3K4me3, H3K27me3	qRT-PCR, ChIP, ChIP-qPCR	非胁迫条件下H3K4me3富集度更高;干旱胁迫下H3K27me3富集度更高 The enrichment of H3K4me3 is higher under non-stress conditions; H3K-27me3 is more pronounced under stress conditions	Sharma et al., 2017
H3K4me3, H3K27me3	免疫标记、ChIP-qPCR、qRT-PCR Immunostaining, ChIP-qPCR, qRT-PCR	果蝇 Trithorax H3K4 甲基转移酶过表达导致成虫异位附肢的形成 Over-expression of Trithorax H3K4 methyltransferase can induce ectopic adult appendages	Sadasivam & Huang, 2016
H3K4me3, H3K27ac	ISH, qRT-PCR, ChIP	温度调节果蝇*tan*基因增强子的活性,且*tan*启动子上的H3K4me3受温度强烈调节 Temperature modulates the activity of an enhancer of *tan*, and H3K4me3 on the *tan* promoter is strongly modulated by temperature	Gibert et al., 2007; 2016
H3K4me3, H3K9me3, H3K36me3	RNA-seq, ChIP-seq	H3K4me3 和 H3K36me3 的覆盖范围和强度随着进化年龄增长而增加,但H3K9me3则相反 The coverage and intensity of H3K4me3 and H3k36me3 increased with evolutionary age, but H3K9me3 is opposite	Yu et al., 2017
H3K4me2, H3K4me3, H3K79me2	ChIP, DNA microarray	活跃基因的H3和H4被高度乙酰化,且H3K4和H3K79被高度甲基化 Active genes are hyperacetylated for H3 and H4 and hypermethylated at Lys4 and Lys79 of H3	Schübeler et al., 2004
H3K4me3, H3K9ac, H3K27ac	IF, MNase-seq, ChIP-seq, RNA-seq	H3K27ac 和 H3K9ac 在增强子和启动子中的动态变化协同抑制与神经干细胞多能性相关的基因 Dynamic changes of H3K27ac and H3K9ac signals in enhancers and promoters synergistically repress genes associated with neural stem cell-related pluripotency	Ye et al., 2017
H3K4me3, H3K9ac, H3K9me2	ChIP-qPCR, Western blot	H3K9ac、H3K9me2 和 H3K4me3 调控果蝇的节律性转录 H3K9ac, H3K9me2 and H3K4me3 control rhythmic transcription in rhythmic transcription in flies	Taylor & Hardin, 2008
H3K9ac, H3K27me3	MNase-seq, ChIP-seq, IF, RNA-seq	果蝇中顺式调控元件中的H3K27me3信号揭示了神经胶质发育过程中祖细胞的分化潜能;H3K9ac信号在神经胶质分化重要基因组中增加 H3K27me3 signal in the cis regulatory elements reveals the differentiation potential of progenitors during neuroglial development; H3K9ac signals increase in a group of genes important to glial differentiation	Ye et al., 2016; Chen et al., 2019
H3K9ac, H3K18ac, H3K23ac, H4K8ac, H4K12ac	Western blot, MS	在黑腹果蝇的整个衰老过程中,组蛋白乙酰化位点 H4K12、H3K9、H3K9K14 和 H3K23 倾向于变得高度乙酰化,而 H4K8 和 H3K18 位点则低乙酰化 Histone acetylation sites H4K12, H3K9, H3K9K14 and H3K23 tend to become hyperacetylated and the sites H4K8 and H3K18 become hypoacetylated throughout the aging process in *D. melanogaster*	Peleg et al., 2016
H3K9me2, H3K9me3	Western blot, RNAi, ChIP	KDM4A通过去除来自同源启动子的H3K9me2、H3K9me3,作为转录共激活因子起作用 Kdm4A functions as a transcriptional co-activator by removing the repressive histone mark H3K9me2, H3K9me3 from cognate promoters	Tsurumi et al., 2013
H3K9me2	ChIP, Western blot, qRT-PCR	随着年龄的增长,异染色质抑制性相关的H3K9me3、H3K9me2以及异染色质蛋白1的富集度显著降低 Significant decrease in the enrichment of the heterochromatin- repressive H3K9me3, H3K9me2 and heterochromatin protein 1 (HP1) marks have been found with age	Larson et al., 2012
H3K9me2	IHC, Western blot, ChIP-seq, RNA-seq, qRT-PCR, ChIP-qPCR, RNA-FiSH	HMT(EHMT/G9a)调控游离核小体H3K9me2,调控某些合子基因的转录水平,可对学习和记忆进行表观遗传调控 HMT(EHMT/G9a) is responsible for nucleosome free H3K9me2 and controls transcriptional levels of some zygotic genes and orchestrates an epigenetic program featuring classic learning and memory genes	Kramer et al., 2011; Shimaji et al., 2015
H3K9me2	IP, Northern blot, Western blot, DNA microarray	H3K9甲基转移酶dSETDB1对果蝇4号染色体进行表观遗传调控 H3K9 methyltransferase dSETDB1 epigenetic regulation of *Drosophila* chromosome 4	Tzeng et al., 2007

续表1 Continued

组蛋白修饰 Histone mark	研究方式 Research method	主要发现 Major finding	参考文献 Reference
H3K9me2	ChIP, Genome tiling array	果蝇异色基因被整合到富含H3K9me2的结构域中 *Drosophila* heterochromatic genes are integrated into the domain enriched in H3K9me2	Yasuhara & Wakimoto, 2008
H3K9me3	ChIP-chip, RNA-seq, Western blot	随着年龄增加H3K9me3的总体水平有所增加 The overall level of H3K9me3 increases	Wood et al., 2010
H3K9甲基化 H3K9 methylation	Western blot、免疫标记、qRT-PCR Western blot, Immunostaining, qRT-PCR	H3K9甲基转移酶在果蝇精子发生中发挥作用 H3K9 methyltransferases play roles during *Drosophila* spermatogenesis	Ushijima et al., 2012
H3K9、H3.3K9修饰 H3K9, H3.3K9 modification	CRISPR/Cas9, IF, Western blot	H3K9和变体H3.3K9修饰在发育和转录调控中具有重叠的作用 K9 modification of variant and canonical H3 have overlapping roles in development and transcriptional regulation	Penke et al., 2018
H3K18ac	UPLC-MS/MS, QqQ-MS/MS	基于质谱法与发育遗传学,开发了表征果蝇多个发育阶段组蛋白乙酰化模式的系统;γ射线辐射显着提高了H3K18ac水平 Present a methodology that combines MS-based histone analysis with developmental genetics to characterize histone acetylation patterns during multiple developmental stages of *Drosophila*; γ-irradiation dramatically increased the level of H3K18ac	Henry et al., 2016
H3, H4ac	LC-MS, RNAi	基于质谱法,开发了一种普遍适用的精确量化果蝇细胞中H3和H4乙酰化修饰图谱的方式 Devised a generally applicable, MS-based strategy to accurately quantify H3 and H4 combinatorial acetylation motifs in *Drosophila* cells	Feller et al., 2015
H3K23ac	RNA-seq, IP, Western blot, IHC	蜕皮素诱导的基因表达与H3K23ac有关 Ecdysone induced gene expression is associated with H3K23ac	Bodai et al., 2012
H3K27ac, H3K27me3	ChIP-seq	H3K27修饰定义了果蝇复胸基因复合体的片段调控域 H3K27 modifications define segmental regulatory domains in the *Drosophila* bithorax complex	Bowman et al., 2014
H3K27ac	Western blot, RNAi, ChIP-chip, qRT-PCR	CBP介导的H3K27ac拮抗果蝇Polycomb蛋白沉默 CBP-mediated H3K27ac antagonizes *Drosophila* polycomb silencing	Tie et al., 2009
H3K27me3	ChIP-chip, DNA microarray, qRT-PCR	与PcG结合的染色质被H3K27me3修饰,并通常在转录上被沉默 PcG-bound chromatin is trimethylated at histone H3 Lys27 and is generally transcriptionally silent	Schwartz et al.,2006;Tolhuiset al., 2006
H3K27me3	Western blot, qRT-PCR	果蝇成虫中PRC2的2个核心亚基 [H3K27特异性甲基转移酶E(z)及其伴侣H3结合蛋白ESC] 的杂合突变可延长寿命并降低H3K27me3水平 Heterozygous mutations in two core subunits of PRC2, H3K27-specific methyltransferase E(z) and its partner, the H3-binding protein ESC, result in increased longevity and reduced levels of H3K27me3 in adult flies	Siebold et al., 2010
H3K27me3	Western blot, RNAi	低蛋白饮食导致果蝇H3K27me3水平提高及亲代和F_2代寿命缩短 The low-protein diet resulted in higher levels of H3K27me3 and a shortened longevity of F_0 flies as well as their F_2 offspring in *Drosophila*	Xia et al., 2016
H3K27me3	Western blot, CRIPSR/Cas9, ChIP-seq, RNA-seq, qRT-PCR	H3K27me3在衰老过程中的表观遗传漂移将糖酵解与健康寿命关联 Epigenetic drift of H3K27me3 in aging links glycolysis to healthy longevity	Ma et al., 2018
H3K36me	RNA-seq, TAC-seq, ChIP-seq, qRT-PCR, Western blotting, IP	修饰H3K36在调控后生动物基因表达中发挥转录后功能 Modification of H3K36 plays a post-transcriptional role in maintaining metazoan transcriptome fidelity	Meers et al., 2017
H3K36me3	DamID, ChIP, DNA microarray	果蝇中转录活性的2类常染色质H3K36甲基化程度不同 H3K36 methylation of the two types of transcriptionally active euchromatin are different	Filion et al., 2010
H3K36甲基化 H3K36 methylation	RNAi, IP, Western blot	果蝇*dSet2*编码H3K36甲基转移酶,在发育中必不可少 *Drosophila dSet2* encodes a developmentally essential H3K36 methyltransferase	Stabell et al., 2007

续表1 Continued

组蛋白修饰 Histone mark	研究方式 Research method	主要发现 Major finding	参考文献 Reference
H4K16ac	ChIP, qRT-PCR	组蛋白乙酰化调控果蝇雄虫X染色体基因的转录增强 Global histone acetylation control the transcription enhancement of X-chromosomal genes in *Drosophila* males	Smith et al., 2001
H3K79甲基化 H3K79 methylation	Northern blot, Western blot, ICC	*grappa*编码H3K79甲基转移酶;H3K79甲基化出现与*BX-C*表达阶段吻合,在发育中起特殊作用 *grappa* functions as H3K79 methyltransferase; the appearance of methylated H3K79 coincides with the maintenance phase of *BX-C* expression, having a unique role in development	Shanower et al., 2005
乙酰化 Acetylation	HDACi(TSA, BuA), qRT-PCR, Western blot	HDACi引起果蝇的寿命延长、*hsp*基因表达升高 HDACi causes lifespan extension and elevate *hsp* gene expression in *Drosophila*	Zhao et al., 2005
乙酰化 Acetylation	HDACi(TSA, BuA), IF	组蛋白乙酰化参与*hsp70*基因转录调控 Histone acetylation is involved in *hsp70* gene transcription regulation	Chen et al., 2002
甲基化 Methylation	IHC	HDM(JmjC)可调控果蝇的昼夜节律及睡眠 HDM(JmjC) domain proteins modulate circadian behaviors and sleep in *Drosophila*	Shalaby et al., 2018
甲基化 Methylation	CRISPR, RNAi, Immunostaining, qRT-PCR	敲除果蝇HMT(dSETD3)对生长和繁殖无影响 Complete knock-out of dSETD3 did not affect the proliferation and growth of *Drosophila*	Tiebe et al., 2018
K9BioH3, K18BioH3	RNAi, DNA microarray	羧化全酶合成酶和生物素酶影响果蝇K9BioH3、K18BioH3修饰水平,进而影响寿命及抗逆性 Holocarboxylase synthetase (HCS) and biotinidase (BTD) affect K9BioH3 and K18BioH3 levels, life span and stress resistance in *Drosophila*	Camporeale et al., 2006; Smith et al., 2007

3.1 昆虫组蛋白乙酰化修饰的功能

组蛋白乙酰化修饰在调控社会性昆虫级型方面发挥着重要作用(表2),其中大脑作为重要的中枢神经系统一直以来都是研究的重点(Zayed & Robinson,2012)。Simola et al.(2013a,b)通过对佛罗里达弓背蚁的大脑进行ChIP-seq分析,发现在蛋白质编码基因附近的染色质结构变化有效地区分了2种工蚁级型minors和majors,其中H3K27ac是级型确定最有效的预测因子,且相关乙酰基转移酶CBP(cyclic adenosine monophosphate response element-binding protein(CREB)binding protein)以级型特异性表达(Spannhoff et al., 2011)。不同级型之间H3K27ac修饰水平产生差异的基因大多与肌肉发育、感觉反应和神经元调节有关。H3K27ac还负责调控不同级型的特定行为。当饲喂CBP抑制剂时,工蚁的觅食和搜寻行为会受到干扰(Simola et al., 2016)。与蚂蚁的研究结果类似,在西方蜜蜂中组蛋白乙酰化修饰主要调控雌性幼虫不可逆地分化为蜂后或工蜂这一重要过程,孵化96 h后的幼虫由于蜂王浆引发表观遗传重编程,是个体发育中表观修饰的关键时间点(Dickman et al.,2013)。研究表明蜂王浆中的10-HAD具有HDAC抑制剂活性(Kouzarides,2007;Spannhoff et al.,2011),并通常与DNA甲基转移酶一起发挥作用(Jaenisch & Bird,2003),因此蜂王浆可能通过影响组蛋白乙酰化水平来调控与蜜蜂级型分化有关的基因表达(Foret et al.,2012;Huang et al.,2012)。Wojciechowski et al.(2018)针对蜜蜂的头部分析发现H3K27ac是决定蜜蜂雌性级型的关键组蛋白修饰标记,具有H3K27ac修饰的级型特定区域决定了工蜂级型。而且与工蜂相比,蜂后头部的组蛋白脱乙酰基酶Sirtuin 2(Sir2)表达量更高(管翠等,2012)。营养状况对昆虫的表型变化有着深远的影响,研究表明饮食的品质和数量可以通过诱导组蛋白修饰的变化来调控基因的表达(Maleszka,2008;Cridge et al.,2015;2017)。昆虫中除蜂王浆影响蜜蜂级型分化的研究外,在阔角谷盗*Gnathocerus cornutus*中也开展了相关研究,发现当给其雄虫提供充足的营养时,会长出更大的下颌骨,以此增加繁殖的可能性;而注射HDAC抑制剂或RNA干扰*HDAC*基因则会影响下颌骨的大小,表明组蛋白乙酰化可能与宽角粉甲虫中营养引起的表型可塑性有关(Ozawa et al.,2016),但缺乏营养与组蛋白乙酰化修饰之间联系的直接证据。

大量转录组研究表明组蛋白乙酰化修饰与昆虫滞育相关(Reynolds & Hand,2009a;Hickner et al.,2015;Reynolds et al.,2016)。滞育是一种可塑性反应,通常由环境因素引起,例如光周期和温度(Koštál,2006;Hand et al.,2016)。南部类针蟋*Allonemobius socius*为母系调节滞育,4~6 d的早期胚胎经短日照处理会诱导进入滞育(Reynolds &

Hand，2009b）。在进入滞育之前，诱导为滞育的胚胎中*reptin*（负责编码Tip60组蛋白乙酰化复合体）的表达量上调了2倍（Reynolds & Hand，2009a）。研究表明Tip60复合体通过调控组蛋白H4乙酰化而导致染色质沉默（Qi et al.，2006），可能导致发育停滞。尖音库蚊*Culex pipiens*的4龄幼虫或蛹经短日照处理后会引起雌成虫的生殖滞育（Spielman & Wong，1973）。利用微阵列的转录组谱分析发现短日照条件下饲养的尖音库蚊蛹中涉及染色质重塑过程的基因显著过表达，包括编码NuRD和Sin3染色质重塑复合体组成的部分基因（Hickner et al.，2015）。NuRD是一种ATP依赖的多蛋白复合体，可促进染色质凝聚并抑制基因转录（Clapier & Cairns，2009）；Sin3复合体含HDAC1和HDAC2，具有抑制转录的作用，且研究表明Sin3复合体与滞育相关的生物学过程有关，包括新陈代谢（Liu & Pile，2017）、细胞周期（Swaminathan & Pile，2010）、抗逆性和寿命（Barnes et al.，2014）。组蛋白去乙酰化可能会延长尖音库蚊的寿命，因为滞育尖音库蚊中编码Pax蛋白的基因表达量增长了4倍（Sim et al.，2015）。Pax是尖音库蚊中重要滞育调节因子FOXO的下游靶标（Lechner et al.，2000；Sim & Denlinger，2008），其参与了Sirtuin 1/Sir 2（1种NAD依赖的HDAC）和负责调控寿命延长的沉默蛋白的定位（Tissenbaum & Guarente，2001；Rogina & Helfand，2004；Latham & Dent，2007）。灰麻蝇*Sarcophaga bullata*在卵期或1龄幼虫期进行短日照处理后，在蛹期进入滞育（Denlinger，1972），并伴随着组蛋白H3乙酰化修饰水平减少75%、去乙酰化酶活性显著降低以及相关编码基因*Hdac3*、*Hdac6*、*Sirt1*和*Sirt2*表达量降低（Reynolds et al.，2016）。组蛋白去乙酰化通常与基因沉默相关，因此组蛋白H3乙酰化水平的降低可能导致基因转录下调，这与滞育的特征相符（Denlinger，2002）。今后的研究中应进一步鉴定滞育过程中受组蛋白乙酰化修饰调控的基因，以及组蛋白乙酰化修饰如何调控昆虫的发育轨迹。

表2 其他昆虫的组蛋白乙酰化修饰功能

Table 2 Histone acetylation functions of other insects

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major finding	参考文献 Reference
蜜蜂 Bee	西方蜜蜂 *Apis mellifera*	乙酰化 Acetylation	下咽、下颌腺 Hypopharyngeal, mandibular	Western blot、HDACi、蛋白组学分析、qRT-PCR	蜂王浆具有HDACi活性，可调控蜜蜂级型转变；HDAC基因*sir2*与*hdac1*可能与蜜蜂级型分化有关 HDACi activity in royal jelly might facilitate caste switching in bees; HDAC genes *sir2* and *hdac1* may be related to caste switching in bees	Spannhoff et al.，2011；管翠等，2012；Huang et al.，2012 Spannhoff et al., 2011; Guan et al.，2012; Huang et al., 2012
		H3K27ac	卵巢、头部 Ovary, head	RP-HPLC, ChIP-seq, RNA-seq	蜂后和孵化96 h幼虫的组蛋白修饰模式不同，H3K27ac是决定蜜蜂雌性级型的关键修饰 The histone modifications of queen bee and 96 h old larvae are different; H3K27ac is the key chromatin modification determining the female caste	Dickman et al., 2013;Wojciechowski et al., 2018
		H3K9ac, H3K18ac	脑 Brain	Western blot, ELISA, HDACi (C8, PB, NaB)	乙酰化是蜜蜂记忆形成的双向调节剂，可促进或抑制记忆；广泛参与厌恶嗅觉记忆 Acetylation-mediated processes act as bidirectional regulators of memory formation that facilitate or suppress memory; widely involved in the aversive olfactory memories of bees	Merschbaecher et al., 2012; Lockett et al., 2014
		H3K27ac	蘑菇体 Mushroom body	ChIP-seq	应对社会挑战时染色质可及性几乎没有变化，大多数差异表达基因已“准备好”被激活 Few changes in chromatin accessibility in response to social challenge; most DEGs were ‘ready’ to be activated	Shpigler et al., 2017

续表2 Continued

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major finding	参考文献 Reference
蚂蚁 Ant	佛罗里达弓背蚁 *Camponotus floridanus*	H3K27ac	整虫 Whole body	ChIP-seq	与激活基因表达相关的hPTM（H3K27ac）在甲基化基因中比例高 The hPTMs that are associated with actively expressed genes (H3K27ac) were highly overrepresented among methylated genes	Glastad et al., 2015b
		H3K27ac	脑 Brain	ChIP-seq, RNA-seq, RNAi	H3K27ac调控蚂蚁级型分化；CREB介导的乙酰化和HDAC介导的去乙酰化调控不同级型的觅食和侦察行为 H3K27ac facilitate caste switching in ants; CBP-mediated acetylation and HDAC-mediated deacetylation of histones control caste-specific foraging and scouting behaviors	Simola et al., 2013a, b; 2016
	长刺痕胸家蚁 *Temnothorax longispinosus*	乙酰化 Acetylation	整虫 Whole body	HATi	组蛋白乙酰化调控蚂蚁昼夜节律 Histone acetylation plays roles in the regulation of circadian rhythm in ants	Libbrecht et al., 2020
蚕 Silkworm	家蚕 *Bombyx mori*	H3修饰 H3 modification	整虫 Whole body	RP-HPLC, TAU-PAGE, MS	鉴定了家蚕全着丝粒染色体组蛋白H3的修饰谱 Identified the modification profiles of histone H3 in holocentric silkworm chromosomes	Mon et al., 2011
		H3K27ac	整虫 Whole body	ChIP-PCR, ChIP-seq, RNA-seq, RNAi, qRT-PCR	通过添加H3K27ac的ChIP-seq数据升级了家蚕BmN4细胞的表观基因组图谱 Upgraded the epigenomic map of BmN4 cells map by adding ChIP-seq data for H3K27ac	Shoji et al., 2014
		H3K27ac	整虫 Whole body	RNA-seq, ChIP-seq	蜕皮激素（20E）处理后富含H3K4me的增强子在H3K27ac中显示出动态变化，并起着调节特定基因转录的作用 Enhancers enriched for H3K4me that showed dynamic changes in H3K27ac after ecdysone (20E) treatment and functioned to regulate the transcription of specific genes	Cheng et al., 2018
白蚁 Termite	黄胸散白蚁 *Reticulitermes speratus*	\	睾丸、卵巢 Testis, ovary	RNA-seq, qRT-PCR	组蛋白修饰基因在蚁王的睾丸和蚁后的卵巢中显著表达 Some histone modification genes are remarkably expressed in the king's testis and queen's ovary	Mitaka et al., 2020
蚊子 Mosquito	冈比亚按蚊 *Anopheles gambiae*	H3K9ac, H3K27ac	中肠 Midgut	ChIP-seq, RNA-seq	人类疟疾寄生虫感染引起冈比亚按蚊组蛋白修饰变化 Infection by the human malaria parasite leads to histone modification changes	Gómez-Díaz et al., 2014; Ruiz et al., 2019
	尖音库蚊 *Culex pipiens*	乙酰化 Acetylation	整虫 Whole body	DNA microarray, qRT-PCR	组蛋白乙酰化修饰是将环境信号整合到尖音库蚊滞育诱导阶段的关键因素 Histone acetylation are key drivers for the integration of environmental signals into the diapause induction phase in mosquitoes	Hickner et al., 2015
果蝇 Fruit fly	黑腹果蝇、海德果蝇 *Drosophila melanogaster*, *D. hydei*	H3、H4修饰 H3, H4 modification	睾丸 Testis	ICC	鉴定了2种果蝇雄虫睾丸的组蛋白修饰模式 Investigated histone modification patterns in testes of *D. melanogaster* and *D. hydei*	Hennig & Weyrich, 2013

续表2 Continued

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major finding	参考文献 Reference
甲虫 Beetle	阔角谷盗 *Gnathocerus cornutus*	乙酰化 Acetylation	头部 Head	RNAi, RNA-seq, qRT-PCR	HDAC调控甲虫下颌骨的表型可塑性 HDAC regulates mandibles phenotypic plasticity in beetle	Ozawa et al., 2016
蟋蟀 Cricket	南部类针蟋 *Allonemobius socius*	乙酰化 Acetylation	整虫 Whole body	RNA-seq, qRT-PCR	组蛋白乙酰化调控蟋蟀滞育 Histone acetylation regulates diapause in the cricket	Reynolds & Hand, 2009b
麻蝇 Flesh fly	灰麻蝇 *Sarcophaga bullata*	H3ac	整虫 Whole body	酶标仪测定、qRT-PCR Measuring with the microplate reader, qRT-PCR	组蛋白乙酰化可调节麻蝇滞育 Changes in histone acetylation as potential mediators of pupal diapause in the flesh fly	Reynolds et al., 2016
蝗虫 Locust	飞蝗 *Locusta migratoria*	H3K9ac	脑 Brain	Western blot, qRT-PCR	蝗虫基因组包含更丰富的组蛋白修饰酶,且在群居和散居蝗虫的大脑存在差异表达模式 The locust genome contains a richer repertoire of histone-modifying enzymes, which exhibit differential expression patterns in brain between solitarious and gregarious locusts	Guo et al., 2016
蝴蝶 Butterfly	艺神袖蝶 *Heliconius erato*	H3K27ac	头部 Head	ChIP-seq	ChIP-seq注释的艺神袖蝶基因组突出了鳞翅目顺式调节进化模式 ChIP-seq-annotated *Heliconius erato* genome highlights patterns of *cis*-regulatory evolution in Lepidoptera	Lewis et al., 016
蜡螟 Wax moth	大蜡螟 *Galleria mellonella*	H3ac	角质层、脂肪体 Cuticle, fat body	全基因组组蛋白H3乙酰化测定试剂盒 Global histone H3 acetylation assay kit	表观遗传机制促进了昆虫中寄生虫抗性的遗传表现 Epigenetic mechanisms facilitate the heritable manifestation of parasite resistance in insects	Mukherjee et al., 2019
粉蚧 Mealybug	柑橘臀纹粉蚧 *Planococcus citri*	H4ac	卵巢 Ovary	免疫标记 Immunolabeling	雄性粉蚧中组蛋白H4乙酰化参与2个单倍体基因组差异活跃状态 Histone H4 acetylation involved in the epigenetic inheritance of different activity states of maternally and paternally derived genomes in the mealybug	Ferraro et al., 2001
飞虱 Planthopper	褐飞虱 *Nilaparvata lugens*	H4ac	卵巢 Ovary	qRT-PCR, RNAi, Western blot, RNA-seq	褐飞虱的HDAC(NlHDAC1)调节雌雄虫的生育能力 HDAC (NlHDAC1) regulates both female and male fertility in the brown planthopper	Zhang et al., 2018
实蝇 Fruit fly	昆士兰实蝇 *Bactrocera tryoni*	H3K27ac	头部 Head	ChIP-seq	首次提供了实蝇科基因组中的组蛋白修饰的证据 Provided evidence for histone modifications in the genome of a tephritid fruit fly for the first time	Nagalingam et al., 2018

通过组蛋白乙酰化修饰调节染色质结构是诱导突触可塑性和形成长期记忆的重要过程。如Merschbaecher et al.(2012)通过HDAC抑制剂TSA改善了蜜蜂的奖励记忆,并发现通过加强训练可以持续更长时间,证明了组蛋白乙酰化在蜜蜂的嗅觉联想奖励记忆中的重要作用。Lockett et al.(2010)发现学习行为可以调控蜜蜂中央脑H3K9ac和H3K18ac修饰水平的改变,由于经训练后Dnmt3也会在相同

的部分上调表达，表明DNA甲基化和组蛋白乙酰化相互作用共同调控蜜蜂的记忆。Lockett et al.（2014）发现使用HDAC抑制剂C8、PB和NaB处理会损伤蜜蜂的厌恶性记忆，但奖励性记忆不受影响，进一步表明组蛋白乙酰化修饰广泛参与蜜蜂的厌恶记忆。

寄主与寄生虫之间的相互作用是自然界中最具可塑性和动态性的系统之一，表观遗传修饰可以提供可被寄主和寄生虫选择压力决定的快速、可逆和易于获得的表型变异来源（Bonduriansky & Day，2009；Gómez-Díaz et al.，2012）。Gómez-Díaz et al.（2014）和Ruiz et al.（2019）对感染和未感染疟原虫的冈比亚按蚊进行ChIP-seq和RNA-seq关联分析，表征了组蛋白修饰与功能基因表达的联系：与对黑腹果蝇的研究结果类似，冈比亚按蚊中H3K9ac、H3K4me3、H3K27ac修饰与活性基因的启动子相关，抑制性的H3K9me3修饰与沉默基因相关。其中，感染疟原虫后出现的甲基化或乙酰化差异区域对应冈比亚按蚊多种天然免疫途径所涉及的基因（Gómez-Díaz et al.，2014；Ruiz et al.，2019）。除冈比亚按蚊外，Mukherjee et al.（2019）比较了大蜡螟*Galleria mellonella*的病原真菌抗性种群和敏感种群中组蛋白H3乙酰化修饰水平以及编码HDACs和HATs基因的表达水平。研究发现抗性种群的幼虫角质层和脂肪体中H3乙酰化修饰水平更高，且编码相关修饰酶的基因均上调，证明大蜡螟的组织特异性组蛋白乙酰化差异参与对病原真菌的抗性调控。

3.2 昆虫组蛋白甲基化修饰的功能

组蛋白甲基化修饰在社会性昆虫级型分化中也发挥一定作用（表3）。通过分析佛罗里达弓背蚁中组蛋白H3修饰的全基因组分布，发现雌雄虫之间存在级型特异性的组蛋白甲基化修饰模式差异（Simola et al.，2013b）。跳镰猛蚁*Harpegnathos saltator*中HMT的SET和SMYD家族中几个成员已被鉴定为级型特异性基因，在级型分化中起特定作用（Bonasio et al.，2010）。近年来针对内华达古白蚁*Zootermopsis nevadensis*的级型分化也开展了一定研究，虽然与组蛋白修饰相关的基因并不直接影响白蚁中兵蚁的级型分化，但与组蛋白甲基化和去甲基化酶相关的基因可能参与保幼激素（Juvenile hormone，JH）的合成（Suzuki et al.，2019）。由于JH滴度的变化和随后JH受体的活性对于级型分化至关重要（Masuoka et al.，2015；2018），因此需要进一步的研究来确定组蛋白甲基化修饰在白蚁社会基因组学中的作用。综上，组蛋白甲基化可能直接或间接参与到了社会性昆虫级型分化中。

表3 其他昆虫的组蛋白甲基化修饰功能

Table 3 Histone methylation functions of other insects

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major finding	参考文献 Reference
蜜蜂 Bee	西方蜜蜂 *Apis mellifera*	H3K4me3, H3K36me3	卵巢、头部 Ovary, head	RP-HPLC, ChIP-seq, RNA-seq	蜂后和96 h幼虫的组蛋白修饰模式不同 The histone modifications of queen bee and 96 h-old larvae are different	Dickman et al., 2013; Wojciechowski et al., 2018
蚂蚁 Ant	跳镰猛蚁 *Harpegnathos saltator*	\	整虫 Whole body	比较基因组学分析 Comparative genomic analysis	印度跳蚁中组蛋白甲基转移酶的SET和SMYD家族中几个成员被鉴定为级型特异性基因，在级型确定中起着特定作用 Several members of the SET and MYND domain-containing protein (SMYD) family of histone methyltransferases were identified as caste-specific genes in *H. saltator*	Bonasio et al., 2010
	佛罗里达弓背蚁 *Camponotus floridanus*	H3K4me3, H3K9me3, H3K27me3, H3K36me3	整虫 Whole body	ChIP-seq	与激活基因表达相关的hPTM（H3K4me3和H3K36me3）在甲基化基因中比例高；与抑制基因表达相关的hPTM（H3K27me3和H3K9me3）在甲基化基因中比例低 The hPTM that are associated with actively expressed genes (H3K4me3 and H3K36me3) were highly overrepresented among methylated genes, but repressive hPTM (H3K27me3 and H3K9me3) were underrepresented among methylated genes	Glastad et al., 2015b

续表3 Continued

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major finding	参考文献 Reference
蚕 Silkworm	家蚕 *Bombyx mori*	H3修饰 H3 modification	整虫 Whole body	RP-HPLC, TAU-PAGE, MS	鉴定了家蚕全着丝粒染色体组蛋白H3的修饰谱 Identified the modification profiles of histone H3 in holocentric silkworm chromosomes	Mon et al., 2011
		H3K4me, H3K27me3, H3K36me3	整虫 Whole body	ChIP-PCR, ChIP-seq, RNA-seq, RNAi, qRT-PCR	通过添加H3K4me、H3K27me3和H3K36-me3的ChIP-seq数据升级了家蚕BmN4细胞的表观基因组图谱 Upgraded the epigenomic map of BmN4 cells map by adding ChIP-seq data for H3K4me, H3K27me3 and H3K36me3	Shoji et al., 2014
		H3K4me, H3K4me3	整虫 Whole body	RNA-seq, ChIP-seq	20E处理后富含H3K4me的增强子在H3K27ac中显示出动态变化,并起着调节特定基因转录的作用 Enhancers enriched for H3K4me that showed dynamic changes in H3K27ac after 20E treatment and functioned to regulate the transcription of specific genes	Cheng et al., 2018
白蚁 Termite	内华达古白蚁 *Zootermopsis nevadensis*	H3K27、H3-K36甲基化 H3K27, H3K36 methylation	整虫 Whole body	RNA-seq, RNAi, qRT-PCR	组蛋白修饰基因参与兵蚁分化期间的蜕皮过程 Histone modifying genes are involved in the molting period during soldier differentiation	Suzuki et al., 2019
	黄胸散白蚁 *Reticulitermes speratus*	\	睾丸、卵巢 Testis, ovary	RNA-seq, qRT-PCR	组蛋白修饰基因在蚁王睾丸和蚁后卵巢中显著表达 Some histone modification genes are remarkably expressed in the king's testis and queen's ovary	Mitaka et al., 2020
蚊子 Mosquito	冈比亚按蚊 *Anopheles gambiae*	H3K4me3, H3K9me3, H3K27me3	中肠 Midgut	ChIP-seq, RNA-seq	人类疟疾寄生虫感染引起蚊子组蛋白修饰变化 Infection by the human malaria parasite leads to histone modification changes	Gómez-Díaz et al., 2014; Ruiz et al., 2019
	岸溪摇蚊 *Chironomus riparius*	H3K36甲基化 H3K36 methylation	整虫 Whole body	微孔板分光光度计比色定量 Microplate spectr-ophotometer	暴露在双酚A压力下导致繁殖失败和H3K36甲基化水平明显增加 Exposure to the pressure of BPA resulted in reproduction failure and a significant increase in H3K36 methylation levels	Lee et al., 2018
果蝇 Fruit fly	黑腹果蝇、海德果蝇 *Drosophila melanogaster*, *D. hydei*	H3、H4修饰 H3, H4 modification	睾丸 Testis	ICC	鉴定了2种果蝇雄虫睾丸的组蛋白修饰模式 Investigated histone modification patterns in testes of *D. melanogaster* and *D. hydei*	Hennig & Weyrich, 2013
	缘吸汁果蝇 *Chymomyza costata*	H3K4甲基化 H3K4 methylation	整虫 Whole body	RNA-seq, qRT-PCR	*dpy-30*通过影响H3K4甲基化作为果蝇滞育诱导的调节剂 *dpy-30* acts as the key regulator of the diapause induction by H3K4 methylation	Poupardin et al., 2015
甲虫 Beetle	赤拟谷盗 *Tribolium castaneum*	H3K27甲基化 H3K27 methylation	腿部、翅 Legs, wings	RNAi, qRT-PCR	E(z)和Pc在赤拟谷盗变态和幼虫腿再生中发挥作用 E(z) and Pc play roles in metamorphosis and larval leg regeneration in the flour beetle	Chou et al., 2019
蟋蟀 Cricket	双斑蟋 *Gryllus bimaculatus*	H3K27甲基化 H3K27 methylation	腿部 Legs	RNAi、ISH、免疫标记 RNAi, ISH, Immunostaining	蟋蟀的腿部再生受H3K27甲基化的表观调控 Leg regeneration is epigenetically regulated by H3K27 methylation in the cricket	Hamada et al., 2015

续表3 Continued

类群 Group	物种 Species	组蛋白修饰 Histone modification	组织 Tissue	研究技术 Methodology	主要发现 Major findings	参考文献 Reference
蝗虫 Locust	飞蝗 *Locusta migratoria*	H3K4me1, H3K4me2, H3K4me3, H3K9me2, H3K9me3, H3K36me3	脑 Brain	Western blot, qRT-PCR	蝗虫基因组包含更丰富的组蛋白修饰酶,且在群居和散居蝗虫的大脑中存在差异表达模式 The locust genome contains a richer repertoire of histone-modifying enzymes, which exhibit differential expression patterns in brain between solitarious and gregarious locusts	Guo et al.,2016
棉铃虫 Cotton bollworm	棉铃虫 *Helicoverpa armigera*	H3K27me3	脑 Brain	qRT-PCR、Western blot、免疫标记、ChIP-chip、RNAi qRT-PCR, Western blot, Immunostaining, ChIP-chip, RNAi	PRC2蛋白ESC通过介导H3K27me3来调节昆虫的发育时间 PRC2 Protein ESC regulates insect developmental timing by mediating H3K27me3	Lu et al., 2013
切叶蜂 Leafcutting bee	苜蓿切叶蜂 *Megachile rotundata*	H3K27甲基化 H3K27 methylation	整虫 Whole body	RNA-seq	PRC2在苜蓿切叶蜂的滞育中发挥作用 PRC2 plays a role in the diapause of alfalfa leafcutter bees	Yocum et al., 2015
蝴蝶 Butterfly	艺神袖蝶 *Heliconius erato*	H3K4me3	头部 Head	ChIP-seq	ChIP-seq注释的艺神袖蝶基因组突出了鳞翅目顺式调节进化模式 ChIP-seq annotated *Heliconius erato* genome highlights patterns of *cis*-regulatory evolution in Lepidoptera	Lewis et al., 2016
实蝇 Fruit fly	昆士兰实蝇 *Bactrocera tryoni*	H3K4me2, H3K4me3, H3K27me3, H3K36me1, H3K36me3	头部 Head	ChIP-seq	首次提供了实蝇科基因组中组蛋白修饰的证据 Provided evidence for histone modifications in the genome of a tephritid fruit fly for the first time	Nagalingam et al.,2018

关于组蛋白甲基化修饰对滞育的调控作用也开展了大量研究,Koštál et al.(2000)通过比较短日照、长日照条件下饲养的缘吸汁果蝇 *Chymomyza costata* 3龄早期幼虫的转录谱,发现 *dpy-30* 基因响应短日照条件而下调。Simboeck et al.(2013)和Poupardin et al.(2015)研究结果发现 *dpy-30* 基因的缺失会导致与细胞周期、细胞生长和增殖、DNA复制和修复相关的基因下调。*dpy-30* 基因负责编码H3K4特异性组蛋白甲基转移酶Set1C/COM-PASS复合体的1个亚基(Ardehali et al.,2011;Mohan et al.,2011),而H3K4甲基化通常与激活基因转录相关(Kouzarides,2007)。因此该基因的表达可能调控该果蝇的滞育,但还需要进一步的研究来确定H3K4甲基化的变化与 *dpy-30* 表达量降低之间的因果关系。灰麻蝇的滞育也与组蛋白甲基化修饰相关,研究发现滞育前的幼虫期中组蛋白去甲基化酶LSD1和Su(var)3-9分别上调1.5倍和2.5倍,可能在滞育过程中发挥作用(Reynolds et al.,2013;2017)。LSD1是针对H3K4的特异性组蛋白去甲基化酶,LSD1失活可导致H3K4me和H3K4me2修饰水平提高,并导致部分基因上调(Di Stefano et al.,2007),其中H3K4me2与激活转录相关,可充当分子标签或表观遗传占位符以维持发育中重要的转录模式(Li et al.,2007;Katz et al.,2009);Su(var)3-9是针对H3K9的特异性组蛋白去甲基化酶,在其他昆虫中的研究中发现该基因在滞育前的准备阶段可能具有调节脂质代谢的作用(Czermin et al.,2001;Meister et al.,2011)。

果蝇发育中许多基因通过多梳抑制复合物(polycomb repressive complex 2,PRC2)调控H3K27me3而被沉默,使PRC2成为昆虫滞育的候选调控因子(Schwartz et al.,2006;Tolhuis et al.,2006)。在非模式昆虫中也有相关研究分析了组蛋白甲基化修饰该基因与滞育之间的关系:苜蓿切叶蜂的滞育发生在蛹前期,并通过暴露在低温下终止滞育,低温终止滞育后编码PRC2复合体的5个基因 *BMI-1*、*E(Pc)*、*PSC*、*ASX* 和 *SCM* 被上调表达(Yocum et al.,2015)。

由于滞育终止通常与滞育过程中上调基因的快速下调有关(Denlinger,2002),因此这些PRC2复合体基因的上调可能会起到抑制滞育中上调基因表达的作用。PRC2也具有调节棉铃虫*Helicoverpa armigera*滞育的作用,棉铃虫在幼虫期经历短日照和低温作用后,在蛹期进入滞育(Lu et al.,2013)。PRC2通过调控促甲状腺激素(prothoracicotropic hormone,PTTH)的产生来调节棉铃虫进入滞育,其中PTTH是调节蜕皮激素产生的神经肽,而蜕皮激素是昆虫滞育的关键调节剂(Gilbert et al.,2002)。其中PRC2的组成部分Esc(Extra sex combs)的表达受日照长短的调节:长日照(非滞育诱导)条件下饲养的个体大脑中*esc*基因表达水平更高,且H3K27me3修饰水平以及编码PTTH的基因启动子也有所增加。通过dsRNA抑制*esc*基因表达或者利用3-deazaneplanocin A化学制剂抑制PRC2,降低了H3K27me3和PTTH水平,并导致个体进入滞育状态。因此在棉铃虫中H3K27me3修饰促进了PTTH的产生,以阻止其进入滞育状态(Lu et al.,2013)。今后的研究应致力于鉴定滞育终止后被PRC2抑制的基因,并探究其在滞育终止后的发育中的作用。目前大部分昆虫滞育的表观遗传调控信息来自针对转录组的研究,这些研究确定了编码组蛋白修饰的多种基因的转录丰度变化。但这些变化的功能相关性在很大程度上是未知的,表观遗传标记是否直接调节滞育的进入和退出,或者是否仅与滞育所需的表型修饰有关尚不清楚,需要进一步试验来探究这些过程如何导致昆虫发育停滞、代谢抑制、抗逆性增强等特征。

4 展望

随着表观遗传机制研究的迅速发展,表观遗传学在昆虫生物学中发挥的重要作用得到越来越多的认可(Burggren,2014;2016)。值得注意的是,表观遗传修饰的不同机制之间并不相互排斥,并可能存在重要的相互作用(Matzke & Mosher,2014;Matzke et al.,2015;Cuerda-Gil & Slotkin,2016)。昆虫可以通过DNA甲基化、组蛋白修饰和非编码RNA同时调节基因的表达模式(Hunt et al.,2013a, b;Burggren,2017),这表明与单独考虑昆虫组蛋白修饰相比,同时考虑多种表观遗传修饰标记能更好地预测基因表达和表型变化。

目前,大多数的昆虫组蛋白修饰研究集中于组蛋白修饰标记是如何在个体中发生变化,而没有更多关注这种变化在种群和物种水平上的含义,尤其是在动态环境压力源影响的情况下种群和物种适应性发展的趋势(Burggren,2016)。为了更全面地了解组蛋白修饰在促进昆虫表型可塑性和进化中可能发挥的作用,在种群水平上考虑表观遗传过程是很重要的(Johnson & Tricker,2010)。而且关注跨代表观遗传的研究较为少见(Glastad et al.,2019),应开展关于昆虫组蛋白修饰的跨代研究,以确定哪些组蛋白修饰标记是通过世代传递的。从生态学的角度来看,经过环境选择的表观遗传修饰如能跨代遗传,可能会对昆虫后代种群应对环境变化提供帮助,这对于昆虫这类生命周期短的动物尤为重要,是未来昆虫生态学研究的有趣领域。要充分理解昆虫组蛋白修饰的变异和遗传,就必须从生态学的角度来看待这些过程,并针对自然种群研究其产生的原因和后果(Bossdorf et al.,2008)。

由于昆虫的种类丰富、生活环境复杂多样,因此不同种类昆虫的组蛋白修饰水平以及不同修饰类型的发生特点和功能往往有所不同,很难用统一的模式来阐述昆虫组蛋白修饰的特点。但随着越来越多的昆虫尤其是农业害虫和资源昆虫的基因组和组蛋白修饰谱被测定和公开,以及对具体物种组蛋白修饰功能的不断深入研究,如在生长发育、繁殖适应等方面的潜在机理探索,有助于更全面地认识昆虫组蛋白修饰的特点和功能,也可补充和完善组蛋白修饰这种表观遗传修饰对基因表达调控的复杂机制和进化途径。

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