

# 昆虫miRNAs功能研究进展

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**摘要** miRNA是一类长度在18~25个碱基的具有调控作用的RNA核酸小分子。在各种生物学过程的调控中均起着举足轻重的作用。近年来,随着miRNA研究技术的飞速发展,昆虫miRNA研究取得了许多重要成果。该文主要综述了miRNA在昆虫免疫、生殖、凋亡及神经发育等生物学过程中功能的研究进展,以期为相关研究提供参考。

**关键词** miRNA; 免疫; 生殖; 凋亡; 神经发育

## Advances of miRNAs in Entomology

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**Abstract** MicroRNAs are small non-coding regulatory RNA molecules approximately 18~25 nucleotide in length. Evidences suggested that these molecules impacted a wide range of biological processes. Recently, greater understandings of miRNA have been achieved in insect models. The aim of this short review is to gain insights of miRNA in insect immunology, reproduction, cell apoptosis and neural development.

**Key words** miRNA; immune; reproduction; apoptosis; neural development

微小RNA(microRNAs, 简写miRNA或miR)是一类长度在18~25个碱基的具有调控作用的RNA核酸分子。一般来说,编码miRNA的基因首先在RNA聚合酶II的作用下转录成miRNA初始转录物(pri-miRNAs),然后pri-miRNAs被Drosha和DGCR8组成的复合物切割,形成miRNA的前体结构(pre-miRNAs)<sup>[1]</sup>。接着,具有60~80个碱基和发卡结构的pre-miRNAs被exportin-5转运至细胞质中。在细胞质中pre-miRNAs被Dicer酶和TRBP共同作用切割形成22个碱基左右的双链成熟miRNAs<sup>[2]</sup>。成熟的miRNAs会被组装到由Argonaute和TNRC6蛋白组成的RNA干扰沉默复合体上去,其中的无义链(antisense)会被降解掉<sup>[3]</sup>。在转录后水平上,有

义链(sense)通过碱基互补配对结合到mRNA上,进而调控基因的表达。自从let-7在秀丽隐杆线虫(*Caenorhabditis elegans*)被发现以来<sup>[4]</sup>,目前已经在多种生物中确定了至少3 420种microRNAs,其相关功能也被广泛研究。

在昆虫中miRNA与靶标的结合是不完全互补配对的,主要是通过种子序列与靶标结合,从而达到翻译抑制或者降解mRNA的作用。越来越多的研究表明,miRNA参与了昆虫的多种生物过程,如调节昆虫组织器官的发育<sup>[5]</sup>。此外,已经证实miRNA在昆虫的免疫反应、生殖、凋亡、神经发育、昼夜节律、能量代谢等各种生物学过程中均有重要作用。随着高通量测序技术的发展,越来越多的miRNA被鉴定

收稿日期: 2013-09-25 接受日期: 2013-12-23

国家自然科学基金(批准号: 31171870、30971944)和广东省科技计划项目(批准号: 2012A020100009)资助的课题

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Received: September 25, 2013 Accepted: December 23, 2013

This work was supported by the National Natural Science Foundation of China (Grant No.31171870, 30971944) and the Guangdong Province Science and Technology Plan Project (Grant No.2012A020100009)

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网络出版时间: 2014-03-03 12:01

URL: <http://www.cnki.net/kcms/doi/10.11844/cjcb.2014.03.0310.html>

了出来。特别是在病理和药剂处理下, miRNA表达谱分析已经成为了一种主要的寻找相关miRNA的方法, 这为进一步研究miRNA功能提供了便利。本文综述了近年来在昆虫中miRNA的研究进展, 为人们了解miRNA在昆虫中的功能提供参考。

## 1 miRNA在免疫中的作用

近年来, miRNA在自然免疫反应和适应性免疫反应中的作用已经得到了广泛的研究<sup>[6]</sup>, 如淋巴细胞的发育和分化、炎症细胞因子的产生等。而昆虫中只有自然免疫反应, 相关的研究还处在初步探索阶段。Garbuzov等<sup>[7]</sup>研究发现, *let-7*可以抑制抗菌肽(双翅杀菌肽)的翻译, 从而调节果蝇(*Drosophila melanogaster*)的自然免疫反应。钙/钙调素依赖的蛋白激酶II(CaMKII)是一种非常保守的蛋白, 在免疫反应中它可以招募免疫突触并发挥重要作用<sup>[8]</sup>。在果蝇Dicer酶的突变体中内源性miRNAs的产生会有缺陷, 而此时突变体中会产生CaMKII<sup>[9]</sup>。在鼠(Rat)中CaMKII会受到*miR-148a*、*miR-148b*和*miR-152*三种miRNA的负调控<sup>[10]</sup>。随着高通量技术的发展, 人们可以更加便捷地筛选参与了免疫反应的miRNA。如Freitag等<sup>[11]</sup>利用基因芯片的方法筛选了面粉甲虫(*Flour beetle*)应对微生物感染时的miRNA; Etebari等<sup>[12]</sup>利用高通量测序技术研究了小菜蛾(*Plutella xylostella*)在被弯尾姬蜂(*Diadegma semiclausum*)寄生后miRNA表达谱的变化。Fullaondo等<sup>[13]</sup>建立了一种筛选参与免疫反应信号通路的miRNAs的方法, 并在果蝇中利用此方法鉴定了7种极有可能参与了免疫反应调控的miRNA。随着更多免疫相关的miRNA被鉴定出来, 昆虫的免疫反应调控网络会更加清晰。

## 2 miRNA在生殖中的作用

miRNA在昆虫的胚胎发育、生殖细胞自我更新、卵母细胞发育等生殖相关过程中具有重要作用。研究人员通过基因芯片检测了家蚕中的354个miRNA, 发现其中106个在家蚕(*Bombyx mori*)各个龄期都有表达, 248个在卵和蛹中特异表达, 这表明, miRNA对胚胎的产生有着重要意义<sup>[14]</sup>。通过基因芯片检测发现, 家蚕*miR-1*、*bantam*、*miR-29*和*miR-92*与胚胎的生成相关<sup>[15]</sup>。miRNA在生殖干细胞相关的生物学过程中也具有调控作用, 如在果蝇的成虫中*batman*等miRNA对生殖干细胞维持未分化状态起

到重要作用<sup>[16-18]</sup>。而有趣的是, 生殖干细胞的分化也是由miRNA调控的。如Pek等<sup>[19]</sup>发现, 果蝇通过抑制miR-7的表达来确保生殖干细胞系的正常分化。有学者利用蛋白组学的方法分析了Dicer-1缺失型和野生型在蛋白水平上的差异<sup>[20]</sup>, 表明果蝇卵母细胞在成熟过程中受到了miRNA的精细调控。另外, 给棉铃虫(*Helicoverpa armigera* Hubner)饲喂miRNA抑制剂和模拟物表明, *har-miR-2002b*在棉铃虫的繁殖中起着重要作用<sup>[21]</sup>。通过研究miRNA, 可从崭新的角度了解昆虫干细胞分化、生殖机制, 为害虫防治提供新的思路。

## 3 miRNA在凋亡中的作用

在多细胞动物的发育过程中, 需要对细胞的增殖、分化、凋亡进行精细的调控。其中, 凋亡的调控是十分重要的方面, 因为它可以及时清除不必要的细胞。在果蝇中, *hid*基因是一个十分重要的凋亡前基因, 其3'UTR包含了miRNA的潜在结合位点。研究表明, *miR-263a*和*miR-263b*能下游调控促凋亡基因*hid*, 从而保护果蝇刚毛上的机械感受器(mechanosensory bristles)免受凋亡影响<sup>[22]</sup>。*hid*基因是眼癌家族蛋白基因(*rbf*)突变体细胞凋亡的一个调控因子, 而*bantam*可以调控*hid*基因的翻译, 这表明这种miRNA可以间接调控与*rbf*相关的凋亡<sup>[23]</sup>。另外, *bantam* miRNA也参与了成虫器中电离辐射诱导的凋亡和Hippo信号通路调控的细胞分化和凋亡<sup>[24-25]</sup>。Reaper也是细胞凋亡的一个重要影响因子, 其受*miR-14*的调控: *miR-14*的缺失可以促进依赖Reaper的细胞凋亡, 而其异位表达则可以抑制多种刺激引起的细胞凋亡<sup>[26]</sup>。*miR-2*家族和*miR-6*、*miR-11*可以通过调节凋亡前基因*rpr*、*hid*、*skl*来实现对凋亡的调控。研究表明, 它们共同发挥作用可以限制果蝇在胚胎发育中的凋亡水平, 其缺失突变体可以导致胚胎死亡和中枢神经系统的缺陷<sup>[27]</sup>。*miR-9a*可以通过抑制翅发育的转录调控因子LIM-only来实现抑制翅发育中的凋亡, 其缺失可导致果蝇感觉刚毛增多, 翅组织缺失<sup>[28]</sup>。总之, miRNA渗透到了细胞凋亡的各个方面, 对其相关功能进行进一步的研究可以更加清晰地认识凋亡的调控网络。

## 4 miRNA在神经的发育中的作用

研究发现, miRNA在神经分化中起到重要作

用。*dme-miR-34*在果蝇早期神经分化中起作用<sup>[29]</sup>, 而*miR-124*可以通过基因*ana*将神经祖细胞的增殖维持在正常水平<sup>[30]</sup>。*let-7*参与了果蝇大脑胚后发育中神经形态发生的时间调控, 其缺失可以引起蘑菇体的缺失等缺陷<sup>[31-32]</sup>。通过对视黄酸诱导的成神经细胞分化过程中整个基因组miRNA和基因表达水平的分析, 发现*miR-17*家族参与了神经分化的调节。进一步研究表明, 它们参与了促蛋白激酶信号通路、轴突可塑性和神经分化中的凋亡等过程<sup>[33]</sup>。

miRNA在神经突触和神经元的生长发育中也有重要作用。*Bantam* miRNA参与了果蝇幼虫体表树突感觉神经元覆盖网络的发育<sup>[34]</sup>。利用果蝇幼虫的肌肉神经接点作为突触模型来筛选与神经突触生长相关的miRNA, 发现有多种miRNA参与了神经元发育过程的调控, 如轴突的发育、路径选择和生长<sup>[35]</sup>。*miR-9*参与了感觉器官前体的形成, *miR-9*活性的丧失可导致果蝇胚胎和幼虫异位感知神经元形成<sup>[36]</sup>。另外, miRNA也参与了神经元的降解过程, 如*miR-8*阻止神经元的降解<sup>[37]</sup>。这些研究的发现将有助于人们对神经发育网络的调控有一个更加清晰的认识。

## 5 在其他方面的作用

越来越多的研究揭示了miRNA的调控网络渗透到了昆虫整个生命历程的各种生物学过程, 如表型可塑性<sup>[38]</sup>、翅发育<sup>[39-40]</sup>、昼夜节律<sup>[41-43]</sup>等。另外, miRNA也参与了能量代谢<sup>[44]</sup>和蛋白表达等过程。miRNA靶标预测、组织化学和生物化学分析表明, 家蚕中至少有4个miR(*miR-33-190-276-7*)参与了蚕丝蛋白形成的调控<sup>[45]</sup>。我们课题组研究发现, 斜纹夜蛾*miR-14*和*miR-2a*可能在蜕皮激素引起的蜕皮级联反应中起到调控作用<sup>[46-47]</sup>。

## 6 总结与展望

MiRNA的发现和其功能可塑性使得在理解基因转录后的调控时又多了一层复杂的关系。目前的研究表明, miRNA在昆虫的生长发育、免疫反应、生殖调控、细胞凋亡等多种生物学过程中都发挥着十分重要的作用。揭开miRNA在这些生物学过程中的神秘面纱, 无论是对昆虫生命过程的阐释还是对有害昆虫的防治都将大有帮助。

在miRNA研究中, 一个最大的困难是靶标的鉴定。目前, 筛选miRNA靶标的方法主要有两种: 一

是利用生物信息学预测某个miRNA的靶基因, 然后经过试验确定其靶标(如免疫共沉淀法和构建双荧光素酶载体法等); 二是利用高通量测序或基因芯片的方法研究病理或药物处理下miRNA和基因的表达谱, 从中筛选出可能存在调控关系的miRNA和靶基因。值得一提的是, 基因敲除技术已经得到了快速的发展, 广泛应用于基因的功能研究<sup>[48-49]</sup>。这为miRNA功能的研究提供了一种新的思路: 通过基因的缺失和获得来研究miRNA功能。如利用这种方法研究了*miR-1*在果蝇肌肉发育中的作用<sup>[50-51]</sup>。随着miRNA研究技术的发展和完善, miRNA会得到更加充分和广泛的研究。

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