RNAi在害虫防治中的脱靶效应及其规避策略

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摘要： RNA干扰（RNA interference，RNAi）作为一种精准的基因沉默工具，在害虫绿色防控中展现出广阔的应用前景。然而，RNAi的脱靶效应所引发的非靶标生物安全风险，成为制约其推广应用的关键因素。该文系统综述了RNAi脱靶效应的分子机制，将其划分为序列特异性（专一性）与序列非特异性（非专一性）两类，分别涉及序列相似度、碱基匹配特征、基因转录水平、小干扰RNA/双链RNA、微小RNA以及RNA诱导沉默复合体装载保真度等介导的脱靶效应；总结影响脱靶效应的主要因素为双链RNA的结构特征、物种差异及双链RNA施用方式；采取双链RNA设计优化、微小RNA化学修饰以及基于脱靶预测软件和网站的评估方法等措施应对脱靶效应，以期为RNAi技术在害虫防治中的安全应用提供理论依据。

关键词： RNA干扰；脱靶效应；非靶标生物；应对策略；害虫防治

Off-target effects and mitigation strategies of RNA interference in pest control

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Abstract: RNA interference (RNAi), as a precise gene-silencing technology, has shown great potential for application in sustainable pest management. However, the biosafety risks posed non-target organisms by RNAi off-target effects have become a major constraint on its widespread application. This review systematically summarizes the molecular mechanisms underlying RNAi off-target effects, classifying them into sequence-specific and sequence-independent categories. These mechanisms involve sequence similarity, base-pairing characteristics, gene transcription levels, small interfering RNA (siRNA)/double-stranded RNA (dsRNA), microRNA (miRNA), and the loading fidelity of the RNA-induced silencing complex (RISC). The key factors influencing off-target effects, including dsRNA

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structural features, species differences, and dsRNA delivery methods, are also summarized. In addition, current mitigation strategies, such as optimizing dsRNA design, chemically modifying siRNA, and applying off-target prediction software and webbased evaluation tools, are discussed. This review provides a theoretical basis for the safe application of RNAi technology in pest control.

Key words: RNA interference; off-target effect; non-target organism; mitigation strategy; pest control

RNA干扰(RNA interference,RNAi)技术通过引入双链RNA(double-stranded RNA,dsRNA)、小干扰RNA(small interfering RNA,siRNA)或短发夹RNA(short hairpin RNA,shRNA)等分子来精准沉默害虫关键基因,已成为农业绿色防控中一项具有巨大潜力的前沿技术(Palli,2023;Zhao et al.,2024;Wang et al.,2025)。基于RNAi技术的RNA生物农药被誉为"农药史上的第三次革命"(Christiaens et al.,2020;关若冰等,2022)。与传统化学农药相比,RNA生物农药基于序列互补原理,理论上具有高度的靶向性(Willow et al.,2025),并兼具高效性(Tan et al.,2025)、绿色环保(Zhu & Palli,2020)、低残留(Koo & Palli,2025)、不易产生抗药性(张建珍等,2021)、研发周期短(汪芳等,2022)、生态链安全等优点(Zhang et al.,2022),在病虫害防治领域中展现出广阔的应用前景(关若冰等,2025)。

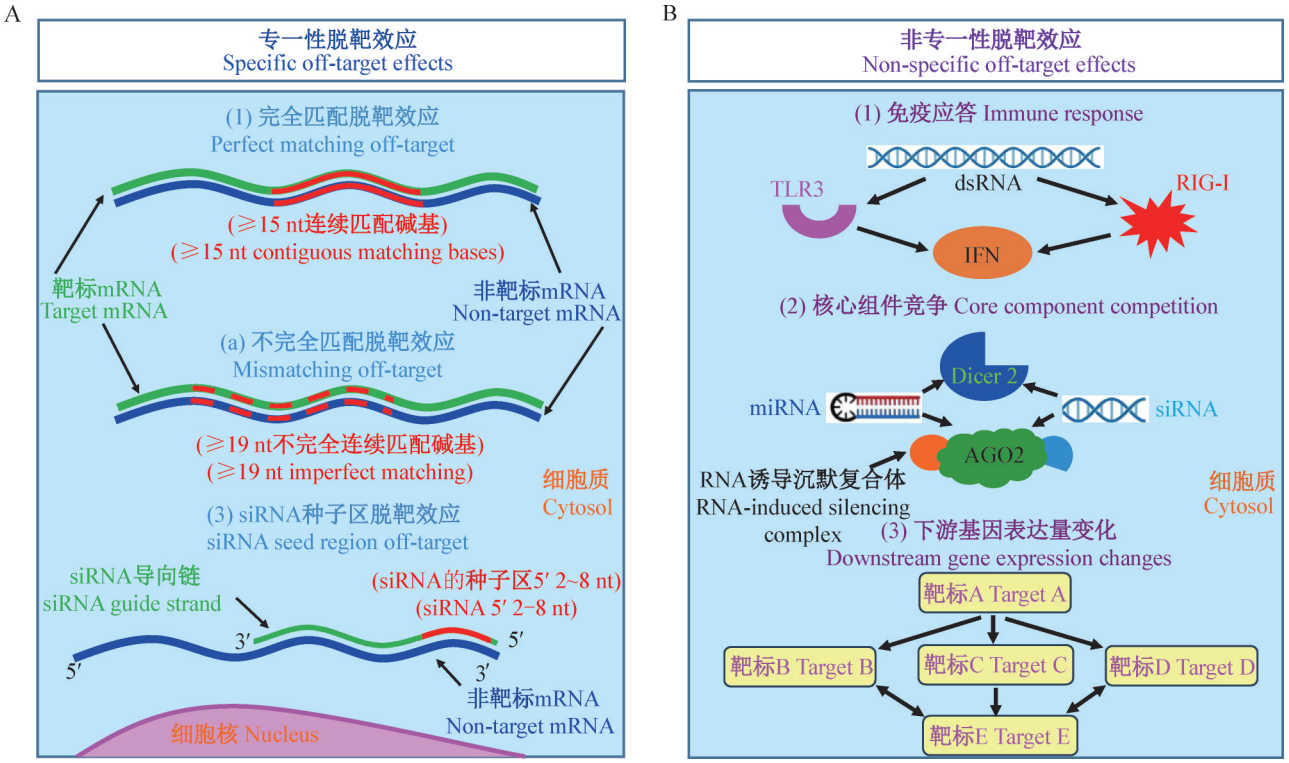
然而RNAi的脱靶效应阻碍了RNA生物农药的广泛应用。在害虫防治中,脱靶效应不仅表现为siRNA/dsRNA与非靶标害虫基因的部分同源沉默,更关键的是可能对有益生物(如天敌昆虫、传粉蜂类及水生非靶标节肢动物)产生非预期毒性,甚至通过食物链传递造成级联生态风险(Chen et al.,2021a;Wang et al.,2023)。农业生态系统中物种多样性高、序列变异复杂,现有的生物信息学预测模型难以准确评估跨物种的脱靶概率,而环境pH、核酸酶降解及生物摄取效率等田间因素更使脱靶效应呈现动态不确定性(Peng et al.,2021;Chen et al.,2025;张建珍等,2025)。因此,本文从RNAi脱靶效应的概念与分离、分子机制、影响RNAi脱靶效应的主要因素、规避RNAi脱靶效应的策略方面进行综述,并对未来研究进行展望,在保证靶向致死效果的同时最大限度降低脱靶风险,以期为RNA生物农药的法规监管和商业化应用提供科学依据。

1 RNAi脱靶效应的概念与分类

根据序列匹配机制,RNAi脱靶效应可分为专一性脱靶效应和非专一性脱靶效应。专一性脱靶效应(图1-A),也称为序列依赖性脱靶效应,由dsRNA、siRNA或微小RNA(microRNA,miRNA)分子诱发的、非预期的、但具有序列特异性的基因沉默现象(Jackson et al.,2003;Jackson & Linsley,2010;Chen et al.,2021b),其特点如下:(1)序列依赖性。严格依赖dsRNA、siRNA或miRNA与非目标序列之间的碱基匹配度(Chen et al.,2021b;Liu et al.,2023)。(2)具有可预测性。通过严谨的生物信息学分析可以预测脱靶效应的强度(Lück et al.,2019;Chen et al.,2025)。(3)具有普遍性。基因仅由4种碱基编码,不同基因随机出现7~8个连续相同碱基序列的概率很大。因此,从序列互补的角度来看,这种脱靶效应普遍存在(Jackson et al.,2003;唐德平等,2012)。

RNAi的非专一性脱靶效应,又称为非序列依赖性脱靶效应(图1-B),由dsRNA、siRNA或miRNA引起的、但与dsRNA序列匹配无关的生物学现象(Scacheri et al.,2004;Ge et al.,2010),具有先天免疫激活、RNAi通路饱和、细胞应激与毒性、非特异性表型干扰等特征。先天免疫激活特征:siRNA的双链结构或特定基序(如富含GU/UG的序列)被Toll样受体(Toll-like receptor,TLR)TLR3、TLR7和TLR8或胞质传感器视黄酸诱导基因-I(retinoic acid-inducible gene-I,RIG-I)和黑色素瘤分化相关基因5(melanoma differentiation-associated gene 5,MDA5)识别,导致I型干扰素(type I interferon,IFN-I)、促炎细胞因子肿瘤坏死因子-α(tumor necrosis factor-alpha,TNF-α)、白细胞介素-6(interleukin-6,IL-6)释放,引起炎症反应(Sledz et al.,2003;Meng & Lu,2017)。RNAi通路饱和特征:高浓度siRNA竞争性消耗细胞内有限的RNA诱导沉默复合体组分,如输出蛋白5(exportin-5,XPO5)蛋白、AGO2(argonaute 2)蛋白,干扰内源性miRNA功能,导致大量miRNA靶基因表达全局性失调(Marques et al.,2006;Floris et al.,2016)。细胞应激与毒性特征:激活dsRNA依赖性蛋白激酶(dependent protein kinase,PKR)途径,抑制全局翻译或诱导凋亡(Robbins et al.,2009)。非特异性表型干扰特征:在基因筛选中,细胞增殖抑制或死亡可掩盖目标基因沉默的表型,但细胞增殖抑制或死亡可能源于免疫反应,而非源于靶基因功

能(Liu et al.,2014)；脂质纳米颗粒等递送载体本身可能引起细胞损伤或炎症(何承帅等,2023)。



IFN：干扰素；TLR3：Toll样受体3；RIG-I：视黄酸诱导基因-I；Dicer2：Dicer2蛋白；AGO2：AGO2蛋白。IFN: Interferon; TLR3: Toll-like receptor 3; RIG-I: retinoic acid-inducible gene-I; Dicer2: Dicer2 protein; AGO2: AGO2 protein.

图1　RNAi的专一性(A)和非专一性(B)脱靶效应模式图

Fig. 1　Schematic illustration of sequence-specific (A) and sequence-independent (B) off-target effects of RNAi

2　RNAi脱靶效应的分子机制

2.1　RNAi专一性脱靶效应机制

2.1.1　序列相似度介导的脱靶效应

序列相似度是判断RNAi是否脱靶的核心指标之一。序列相似度介导的脱靶阈值具有显著的物种特异性。当引入的dsRNA与鞘翅目昆虫赤拟谷盗*Tribolium castaneum*中非靶标信使RNA(messenger RNA,mRNA)的序列相似度大于78%时,就可能发生脱靶效应(Tan et al.,2025)；当靶向豌豆蚜*Myzus persicae*的dsRNA与其天敌龟纹瓢虫*Propylea japonica*的同源mRNA序列相似度达到61.7%~77.5%时,该dsRNA可导致龟纹瓢虫同源基因发生显著的脱靶效应(Zhou et al.,2023)；当靶向马铃薯甲虫*Leptinotarsa decemlineata*的ds*β-Actin*与茄二十八星瓢虫*Henosepilachna vigintioctopunctata*的序列相似度超过92%时,即可诱发茄二十八星瓢虫的同源基因*β-Actin*发生脱靶效应。而对于RNAi不敏感的鳞翅目昆虫,引发脱靶效应所需的序列相似度阈值更高。例如,外源dsRNA需与烟草天蛾*Manduca sexta*的同源基因达到96%的相似度才能引起脱靶效应(Poreddy et al.,2017)。不同昆虫诱发脱靶效应的序列相似度存在巨大差异,这主要归因于昆虫对dsRNA的降解能力不同以及靶基因本身对RNAi敏感性不同。具体而言,鳞翅目昆虫中肠液和血淋巴降解dsRNA的速度较快,因此需要更高的序列相似度才能触发脱靶效应(Yao et al.,2022)；而鞘翅目昆虫降解dsRNA速率慢,较低的相似度即可诱发脱靶效应(Chen et al.,2021b)。此外,对RNAi更敏感的基因也更容易发生脱靶效应。

2.1.2　序列匹配特征介导的脱靶效应

dsRNA与非靶标mRNA之间的序列匹配特征决定了序列相似度的高低,而完全连续匹配片段长度则是决定RNAi脱靶效应强弱的核心指标。完全连续匹配片段越长,发生脱靶效应的概率越高,沉默效率也随之上升。不同昆虫触发脱靶效应所需的最短完全连续匹配长度差异显著,即呈现鲜明的物种特异性。对于RNAi敏感的物种,较短的完全匹配片段即可诱发脱靶效应。例如,当引入的dsRNA与赤拟谷盗的非靶标mRNA之间仅需15 nt完全连续

匹配即可触发脱靶效应，且匹配长度从15 nt增至21 nt时沉默效率逐步上升（Chen et al.，2021b；Tan et al.，2025）；当引入的dsRNA与龟纹瓢虫、异色瓢虫*Harmonia axyridis*和七星瓢虫*Coccinella septempunctata*的非靶标mRNA之间存在超过21 nt的完全连续匹配片段也能触发脱靶效应（Chen et al.，2023；Liu et al.，2023）。利用二化螟*Chilo suppressalis*的*EF1*基因合成dsRNA干扰赤拟谷盗、直翅目昆虫东亚飞蝗*Locusta migratoria*及黑腹果蝇*Drosophila melanogaster* S2细胞同源基因，当完全连续匹配片段长度分别为32、14和26 nt时均能诱发脱靶效应。而对于RNAi不敏感的物种，则需要更长的完全匹配片段才能诱发脱靶效应（Terenius et al.，2011；Peng et al.，2020）。例如，利用灰飞虱*Laodelphax striatellus*的*Actin*基因合成ds*Actin*，利用合成的ds*Actin*去干扰拟环纹豹蛛*Pardosa pseudoannulata*同源基因*Actin*时，连续完全匹配片段需超过29 nt才能诱发脱靶效应（Zhang et al.，2022）；将玉米根萤甲*Diabrotica virgifera*的液泡H^+-ATP酶亚基A（vacuolar H^+-ATPase subunit A，V-ATPase A）合成ds*DvV-ATPase A*，利用其去干扰鳞翅目黑脉金斑蝶*Danaus plexippus*的同源基因*V-ATPase A*，即使存在大于21 nt完全匹配片段也未诱发黑脉金斑蝶发生脱靶效应（Pan et al.，2017）；同样，利用二化螟*EF1*基因合成的dsRNA干扰棉铃虫*Helicoverpa armigera*与斜纹夜蛾*Spodoptera litura*的同源基因时，当完全连续匹配片段长度分别为35 nt和14 n时也均不能诱发脱靶效应（Chen et al.，2021b）。

除了完全匹配序列长度外，dsRNA与非靶标基因mRNA之间的不完全匹配序列长度同样显著影响脱靶效应的强弱。例如，当引入的dsRNA与赤拟谷盗非靶标基因之间的不完全匹配序列长度大于26 nt时即可诱发脱靶效应（Chen et al.，2021b）。

2.1.3 siRNA介导的脱靶效应

siRNA介导的脱靶效应主要依赖其与非靶标mRNA之间特定的序列匹配模式。Jackson et al.（2003）和Lin et al.（2005）研究结果显示当引入的siRNA与非靶标mRNA之间仅有7~8 nt片段完全匹配时即可诱发脱靶效应。来源于绿色荧光蛋白（green fluorescent protein，GFP）的siRNA与水母的*GFP*之间有8 nt片段完全匹配时也能诱发脱靶效应（Tschuch et al.，2008）。此外，当siRNA向导链5′端第2~8位核苷酸构成的种子区与非靶基因3′UTR互补配对时也能诱发脱靶效应，类似miRNA通过抑制翻译过程或mRNA去稳定化来调控脱靶效应（Birmingham et al.，2006）。

2.1.4 miRNA介导的脱靶效应机制

miRNA介导的脱靶效应指miRNA通过序列匹配非特异性作用于非靶标mRNA的现象。当miRNA 5′端种子区与非靶标mRNA的3′UTR有6~7个碱基连续匹配就能诱发脱靶效应。例如，家蚕的miR-14、miR-279e及miR-281等miRNA通过与蜕皮激素受体*EcR-B*基因3′UTR上的种子区域结合位点结合来抑制其表达。当这些结合位点被定点突变后，miRNA的抑制效果减弱（Jiang et al.，2013）。RNA诱导沉默复合体能容忍miRNA种子区序列与3′UTR之间出现碱基错配，这种错配结合使更多非靶标转录本可能被RNA诱导沉默复合体捕获，从而拓宽了miRNA的潜在脱靶范围。例如，在埃及伊蚊*Aedes aegypti*的RNA诱导沉默复合体种子区即使有1~2个碱基错配，但其仍具有切割非靶标mRNA的活性，这导致miR-8靶向*Toll5B*、*Cactus*等多个免疫基因，从而引发多通路脱靶效应（Cui et al.，2022）。此外，当miRNA种子区与非靶标mRNA错配时，miRNA 3′端可通过非种子区补偿配对来增强与非靶标mRNA的结合，扩大了靶标范围，从而扩大了脱靶效应的基因数量（Mehta & Baltimore，2016）。

除上述分子机制外，生物系统中跨界传递和内源性调控网络显著增加了miRNA脱靶效应的复杂性和不可预测性。一方面，跨界RNAi使得miRNA通过细胞外囊泡跨物种传递，引发非预期基因沉默。例如，水稻细胞外囊泡递送的Osa-miR159a.1-1能与灰飞虱的磷脂酶（phospholipase C beta，PLCβ）基因的5′UTR结合，在增强灰飞虱靶基因mRNA稳定性的同时，还会干扰灰飞虱其他基因（Wang et al.，2025）。另一方面，昆虫中内源性诱饵RNA的竞争性结合可间接导致脱靶效应。例如，朱砂叶螨*Tetranychus cinnabarinus*表皮蛋白基因*CPR25*在转录时会同步产生环状RNA—circCPR25，circCPR25可作为分子诱饵吸附并抑制miR-34~317簇的功能，从而解除了miR-34~317簇对*CPR25*的抑制，但也削弱了miR-34~317簇对应激响应基因等其他靶标的调控能力，间接引发脱靶效应（Feng et al.，2025）。

2.2 RNAi的非专一性脱靶效应机制

2.2.1 RNA诱导沉默复合体饱和引发的脱靶效应

当昆虫个体吸收的siRNA/dsRNA浓度过高，超出其细胞内RNA诱导沉默复合体的承载能力时，会

引发RNA诱导沉默复合体饱和效应，即过量的游离siRNA会与非靶标mRNA结合，从而引发脱靶效应（Cooper et al.，2019）。此外，过量的外源siRNA还会与内源性miRNA竞争结合RNA诱导沉默复合体的核心组分AGO2，从而干扰miRNA对靶基因的正常识别与结合，最终造成靶基因表达水平的异常，从而间接诱发脱靶效应。RNA诱导沉默复合体对siRNA向导链与随从链的装配错误，也会诱发脱靶效应。在黑腹果蝇RNAi过程中，Dicer-2/R2D2/siRNA复合物中伴侣蛋白R2D2能非对称地识别siRNA双链中热力学稳定性更高的末端，R2D2优先结合这一稳定末端，引导siRNA以固定的方向加载到AGO2中，从而确保AGO2最终选择5′端热力学稳定性较低的那条链作为向导链用于靶标沉默，另一条链则作为随从链被降解（Aute et al.，2024）。若黑腹果蝇中R2D2蛋白C端CTD结构域中色氨酸W205或dsRNA结合结构域ds*RBD2*中精氨酸R101A发生突变，R2D2对稳定末端的锚定能力便会丧失，导致siRNA双链以随机方向装载AGO2。此时，AGO2可能错误地将原本应作为随从链的稳定末端链选为向导链，进而引导RNA诱导沉默复合体切割与随从链互补的基因mRNA，导致非靶标基因沉默，引发脱靶效应（Yamaguchi et al.，2022）。

2.2.2　siRNA/dsRNA诱发的免疫脱靶效应

外源siRNA/dsRNA可作为配体被模式识别受体识别，从而激活生物体的先天免疫系统，进而引发免疫应答（Olejniczak et al.，2011；2016）。在昆虫中免疫应答主要涉及Toll信号或免疫缺陷（immune deficiency pathway，IMD）等通路的激活，诱导防御素基因*Defensin1*/*Defensin2*和炎症因子基因表达上调，从而间接干扰整体基因表达谱（Yang et al.，2024；Chauhan et al.，2025）。dsRNA可能通过激活昆虫Toll免疫通路诱导抗菌肽等免疫基因的全局性上调，形成高背景转录本干扰，从而削弱靶基因沉默的特异性效果（Swevers et al.，2016；2018）。例如，利用微小扇头蜱*Rhipicephalus microplus*的*Ubiquitin-63E*基因合成长度为594 bp的dsRNA，微小扇头蜱注射该dsRNA后，其包括免疫应答相关基因在内的144个基因表达量上调，136个基因表达量下调（Lew-Tabor et al.，2011）；利用果蝇C病毒（Drosophila C virus，DCV）或短荧光素酶基因合成dsRNA，蜜蜂取食该dsRNA后，其抗菌肽基因以及参与dsRNA运输和参与免疫应答的c-Jun氨基末端激酶通路（c-Jun N-terminal kinase pathway，JNK）的基因表达上调，而部分内吞相关基因表达下调（Brutscher et al.，2017）。跨界RNAi诱发的免疫脱靶效应更复杂。例如，Zhang et al.（2024）研究发现在褐飞虱*Nilaparvata lugens*取食过程中，其唾液中的miR-7-5P被分泌到寄主植物内，并通过沉默宿主免疫基因的表达来削弱植物的防御能力。植物灰霉病病原菌灰葡萄孢菌*Botrytis cinerea*能产生Bc-siRNA，Bc-siRNA通过结合拟南芥*Arabidopsis thaliana*的AGO1蛋白来启动RNAi效应，沉默宿主的免疫基因，降低宿主的防御能力（Weiberg et al.，2013）。此外，感染病毒的蜜蜂注射dsRNA后，其体内Toll、IMD免疫通路相关基因的表达量显著升高，导致蜜蜂体内病毒丰度显著下降，从而共同防御病毒（Brutscher et al.，2017）。

在医学研究中，siRNA/dsRNA诱发的免疫激活可能导致非预期的负面效应。例如，临床药物bevasiranib因激活II型干扰素反应而终止试验（Adams et al.，2018）；在弓形虫*Toxoplasma gondii*中，长链dsRNA可非特异性抑制嘌呤代谢通路基因的表达（余莉等，2008）。除了负面效应，siRNA/dsRNA诱发的免疫效应还有正面效应，免疫刺激siRNA被用于调节免疫反应。例如，免疫刺激siRNA能促进人树突状细胞成熟，诱导分泌IL-6和IL-12两种细胞因子，进而激活$CD4^+$效应T细胞及驱动Th1型免疫应答。此外，靶向免疫抑制酶吲哚胺2，3-双加氧酶（indoleamine 2，3-dioxygenase，IDO）的免疫刺激siRNA，不仅能有效抑制人树突状细胞和单核细胞中*IDO*基因的表达，而且能同时发挥免疫激活作用，实现协同抗肿瘤效应（Sioud，2005；2015）。

2.2.3　siRNA/dsRNA诱发的下游级联效应

siRNA/dsRNA沉默靶标基因后，可能会干扰生物调控网络中关键节点基因（如转录因子或信号枢纽）的功能，引发下游级联式的基因表达紊乱。例如，沉默人类细胞中转录抑制因子肢芽与心脏蛋白（limb-bud and heart，LBH）会异常激活周期蛋白（Cyclin）D1启动子，引起*Cyclin E1*和*Cyclin E2*表达上调，进而驱动视网膜母细胞瘤蛋白（retinoblastoma protein，Rb）过度磷酸化，破坏细胞周期调控（Liu et al.，2015）。同样，沉默人类细胞中多发性内分泌瘤蛋白1（multiple endocrine neoplasia type 1，MEN1）基因，导致肿瘤蛋白（tumor protein 53，TP53）和细胞周期蛋白依赖性激酶抑制剂1A（cyclin-dependent kinase inhibitor 1A，CDKN1A）基因表达下调，表明*MEN1*可能通过染色质重塑复合

体影响全局性的转录程序(Scacheri et al.,2004)。这种下游脱靶效应在昆虫中更普遍。例如,沉默赤拟谷盗胆碱乙酰转移酶(choline acetyltransferase,chat)基因可诱导173个基因差异表达,这些差异表达基因对昆虫的变态发育、繁殖能力及其对杀虫剂的敏感性有显著影响(Gao et al.,2023)。

3 影响RNAi脱靶效应的主要因素

3.1 dsRNA设计参数

dsRNA的长度、浓度和特异性序列等参数会显著影响脱靶效应的发生和脱靶效应的强度。例如,在昆虫中dsRNA与非靶标mRNA的序列相似度越高,连续碱基匹配长度越长,高匹配度的碱基数越多,就越容易发生脱靶效应(Chen et al.,2021b)。长链dsRNA虽然增加了RNAi效率,但也增加了脱靶效应的可能性(Zhang et al.,2017)。RNAi系统核心元件Dicer、AGO2的表达丰度和活性直接影响siRNA的加工精度,从而增加了脱靶的可能性(Li et al.,2015)。靶基因的二级结构可能遮蔽作用位点,导致siRNA无法精确匹配,从而诱发脱靶效应(Bolognesi et al.,2012)。

3.2 基因转录水平

脱靶效应受基因转录水平的严格调控。在昆虫中转录水平较高的基因对RNAi更敏感,因而更易成为脱靶效应的对象。例如,在赤拟谷盗5龄幼虫中*CYP6BK13*的转录本分子数高达14 350,远高于*CYP6BQ9*的转录本分子数(1 760);尽管合成的ds*CYP6BQ6*与*CYP6BK13*的序列相似度仅为71%,低于与*CYP6BQ9*的序列相似度(86%),由于*CYP6BK13*的转录水平较高,其沉默效率仍达80%,表现为显著的脱靶效应;相反,*CYP6BQ9*因转录水平过低,即使序列高度相似,也未检测到脱靶(Chen et al.,2024)。此外,在东亚飞蝗和黑腹果蝇S2细胞中也观察到转录水平较高的持家基因*EF1*更容易发生脱靶效应(Chen et al.,2021a)。

3.3 昆虫对RNAi敏感性的差异

不同目昆虫对RNAi的敏感性差异明显,这是影响脱靶效应的关键因素(Han et al.,2025)。鳞翅目昆虫中肠高碱性环境(pH 9~11)、肠道微生物、核酸降解酶及血淋巴液对外源dsRNA的快速降解,因此对RNAi普遍不敏感,通常不诱发脱靶效应(王聪等,2018;张秋朗等,2021)。与之相反,鞘翅目昆虫如马铃薯甲虫和赤拟谷盗拥有高效的系统性RNA干扰跨膜蛋白1(systemic RNA interference defective protein 1,SID1)及StaufenC双链RNA结合蛋白介导的dsRNA递送系统(Shih & Hunter,2011;Cappelle et al.,2016;Koo & Palli,2024),因此对RNAi高度敏感,且dsRNA降解速率较慢,故其脱靶效应发生概率显著高于鳞翅目昆虫(Papadopoulou et al.,2020)。

3.4 dsRNA的递送方式

鉴于dsRNA的递送方式不同,诱发的脱靶效应也存在差异。转基因植物表达的dsRNA靶向精准,不易扩散,脱靶风险较低。而喷雾法递送易导致dsRNA扩散,脱靶风险显著增加;此外,喷雾法目前常依赖纳米材料进行包裹递送,这些纳米材料本身也可能对非靶标生物有潜在危害(付淑笙等,2021)。

4 规避RNAi脱靶效应的策略

4.1 优化dsRNA设计

优化dsRNA设计可显著降低脱靶效应。首先,设计靶标基因dsRNA时,避开同源基因高度保守区域,尤其是完全匹配长度超过15 nt或高匹配度区域超过26 nt的序列(Chen et al.,2024);避开已知会诱发序列脱靶效应的特殊siRNA碱基序列,如5′-UGUGU-3′、UGGC和GU(Meng & Lu,2017);避免使用长siRNA,因为长度超过30 nt的siRNA会诱发人宫颈癌细胞(human cervical cancer cell,HeLa)S3细胞系产生强烈免疫反应(Persengiev et al.,2004)。其次,降低siRNA/dsRNA的使用浓度或剂量也能显著降低脱靶效应,但同时也可能会削弱对靶基因的沉默能力(Semizarov et al.,2003)。使用siRNA池设计可以弥补低浓度siRNA/dsRNA导致的沉默能力不足(Jackson & Linsley,2010)。最后,优化dsRNA分子设计本身也能提升递送效率,降低脱靶效应。例如,siRNA自折叠技术可使单链siRNA在体内或递送系统中自发折叠,形成更稳定的类似dsRNA的结构,从而显著增强siRNA的稳定性,延长作用时间,大幅降低所需外源siRNA的初始用量,从而降低环境中siRNA残留引发的非特异性效应和脱靶效应(McRae et al.,2023;Pang et al.,2026)。

4.2 化学修饰siRNA/dsRNA

化学修饰能提高siRNA/dsRNA的稳定性,减少dsRNA用量,从而降低脱靶效应(Eberle et al.,2008;Nomura et al.,2024)。例如,在dsRNA合成过程中引入修饰的2′-O-甲基核苷三磷酸,在高温、紫外辐射以及dsRNA核酸降解酶等条件下,经过修饰的dsRNA的稳定性均显著提升,其沉默效率和致死

率显著增加（Zhang et al.，2026）。此外，硫代磷酸、2′-氟以及5′-羟甲基修饰后的dsRNA能抵御昆虫唾液中dsRNA核酸酶的降解，从而增加了dsRNA的稳定性（Howard et al.，2022）。

4.3 采用siRNA/dsRNA递送系统

高度特异性的siRNA/dsRNA递送系统能提高RNAi的精准靶向，从而降低对非靶标生物的危害。例如，在转基因植物中特异性表达针对害虫的dsRNA，目标害虫通过取食摄入dsRNA，可有效避免传统喷洒造成的雾滴扩散，进而降低对传粉昆虫等有益生物的危害（Liao et al.，2025）。利用基因工程改造共生菌或病毒载体，使植物组织特异性表达dsRNA，从而显著提高递送精度（唐前君等，2010；Jiang et al.，2022）。例如，Chen et al.（2026）从桑树中分离出解淀粉芽胞杆菌 *Bacillus amyloliquefaciens* SH-F8菌株，通过基因工程改造使该菌株表达靶向美国白蛾 *Hyphantria cunea* 的 *V-ATPase* 基因的dsRNA，美国白蛾幼虫取食该dsRNA后，蛹畸形，从而有效降低因dsRNA喷雾带来的扩散危害。此外，Mysore et al.（2025）首次将RNAi酵母与靶向糖诱饵技术结合，针对斑翅果蝇 *Drosophila suzukii* 开发出特异性诱饵递送系统，该系统有效提高了dsRNA递送精准度。除了转基因植物和工程菌外，壳聚糖、层状双氢氧化物纳米黏土、全肽纳米材料、苝酰亚胺纳米载体和星状阳离子聚合物等纳米材料均被证实能有效保护dsRNA，避免其被降解，从而减少dsRNA的初始用量，提高RNAi的应用安全性（Yan et al.，2020；付淑笙等，2021；Liu et al.，2025）。

4.4 利用软件预测RNAi脱靶效应

利用生物信息学工具开发针对RNAi脱靶效应的预测网站和软件。如dsCheck、siRNAScan、siRNADirect和siRNA-Finder（Senthil-Kumar & Mysore，2011，Naito & Ui-Tei，2013；Lück et al.，2019）软件通过序列匹配特征预测脱靶效应，这些软件的预测结果可能存在假阳性，预测精度较低，仍需通过检测基因的沉默效率和蛋白含量来确定是否真实脱靶。近期，Chen et al.（2025）开发的在线脱靶预测网站dsRNAEngineer显著提升了脱靶效应的预测精度。Lyu et al.（2025）开发了dsOMG在线平台，该平台收录了29 964个物种的序列信息，大幅提高了dsRNA的设计和脱靶效应预测精度。此外，甜菜夜蛾 *Spodoptera exigua* 与家蚕 *Bombyx mori* 分别注射siRNA后，其体内差异表达基因的变化与siRNA序列连续匹配的碱基数并不成正比，因此Ma et al.（2022）提出利用转录组熵值模型和跨物种脱靶数据库RNAimmuno可进一步提高脱靶效应的预测精度。

5 展望

目前，利用RNAi技术防治害虫仍面临脱靶风险、昆虫敏感性差异、dsRNA不稳定、生产成本高、制剂研发难、非靶标与残留风险、登记审批壁垒等问题。其中，脱靶效应是最令公众担忧的问题。严重的脱靶效应不仅会导致基因功能研究偏差、潜在毒副作用、疾病风险及治疗失败（Du et al.，2005；Ju et al.，2021），还可能对非靶标有益生物造成不利影响，威胁生态平衡。完全消除脱靶效应既不现实也无必要，关键在于将脱靶风险控制在可接受的生态学阈值范围内。因此，建立完善的脱靶效应评估与监控体系是RNAi技术应用中不可或缺的环节（Chen & De Schutter，2024；Gao et al.，2024）。未来结合生物信息学、SpyCLIP技术（Zhao et al.，2019；Zhang et al.，2020）及人工智能等新兴手段，提高脱靶效应预测的准确性和可靠性，将脱靶效应从RNAi技术应用的技术瓶颈转变为可控变量，为RNAi技术的安全、高效应用奠定基础。

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