基于共生微生物及基因编辑技术对蚊虫防控进展

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摘要：蚊虫是登革热、疟疾等疾病病原的重要传播媒介，因其防控的艰巨性给全球人类健康造成巨大威胁。传统蚊虫防控主要采用化学防控策略，化学药剂造成的环境污染以及蚊虫对化学药剂抗性的产生，亟需探索新的蚊虫防控策略。微生物和基因编辑是很有前景的2种蚊虫防控新策略，两者都可以通过抑制或者修饰蚊虫种群来达到蚊媒病原防控的目的。本文将详细阐述上述2种蚊媒病原防控策略，并基于现有的研究进展阐述其作用机理。

关键词：蚊媒病原；基因编辑；微生物；基因驱动；蚊虫防控

Advances in prevention and control of mosquito based on microorganisms symbiotic and gene editing

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Abstract: Mosquito-borne diseases, such as dengue and malaria, pose significant global health burdens. Chemical control is the most traditional mosquito control strategy. Chemical insecticides cause serious environmental pollution, and mosquitos are evolving drug resistance. Thus, it is urgent to explore new mosquito control strategies. Symbiont and genetic engineering are two promising new strategies. Symbionts and genetic engineering can control mosquito populations either by population suppression or population modification. This review focused on the above two strategies and discussed the latest research progresses.

Key words: mosquito-borne diseases; gene editing; symbiotic microorganisms; gene drive; mosquito prevention and control

以蚊虫为媒介传播的病原被称为蚊媒病原，其危害波及全球，且严重威胁着人类的生存与健康（Gould et al.，2017；Mavian et al.，2018）。诸如，登革热病毒（dengue virus，DENV）（Messina et al.，2019；Xu et al.，2020）、疟原虫*Plasmodium*、基孔肯雅热病毒（chikungunya fever virus，CHIKV）、寨卡病毒（zika virus，ZIKV）、黄热病毒（yellow fever virus，YFV）等蚊媒病原每年能够感染约3.9亿人，其中9 600万人需进行临床诊治（World Health Organization，2011；Steven et al.，2021）。造成这一后果的因素有全球气候的变暖、蚊虫的抗药性、城市化和人类交通及运输业的迅猛发展，不同地域所特有的蚊虫品系也在迅速向新的地域传播，由于外来物种优势更加剧了蚊媒病原在新区域的传播速度和感染范围（Ciota & Kramer，2013；Kolimenakis et al.，2021）。由此，每年蚊媒病原的传播会造成数以亿计的经济损

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失(张友军等,2013;赵志宏等,2018),同时给人们的正常生活带来诸多不便。中国地域辽阔,涵盖了典型的温带、亚热带和热带气候,丰富的气候类型为蚊媒病原的传播提供了有利的条件(莫禹诗等,1990)。中国已被发现的蚊媒病包括疟疾、登革热、流行性乙型脑炎(简称乙脑)、丝虫病和基孔肯雅热(王梦蕾等,2012)。所以,蚊虫的防治不仅仅对于中国有其必要性,对于世界范围内同样有着重要的意义。

传统的病媒控制方法无法安全和全面地解决蚊媒病原的防控,这一事实已逐渐在蚊媒病原的防控中显现(李怡萍等,2009;Scolari et al.,2019)。例如,白斑伊蚊*Aedes albopictus*对化学杀虫剂产生了抗性(Pichler et al.,2017),而白斑伊蚊分布范围之广为蚊媒病原的防控造成了巨大困难(Moyes et al.,2017)。因此,迫切需要开发新颖、环保、易于管理的病媒控制产品或系统,以突破传统的蚊媒病原控制的瓶颈。近年来,随着微生物领域的深入研究和基因编辑技术的迅猛发展,蚊虫微生物防控蚊媒病原和针对蚊虫或微生物的基因编辑技术的蚊媒病原的防控得以快速发展。根据上述进展,基于微生物及基因编辑技术对蚊媒病原防控的研究进展进行总结归纳,为蚊媒疾病防控技术提供新的思路。

1 基于共生微生物对蚊媒疾病的防控

蚊虫的共生微生物种类繁多,且广泛分布于蚊虫的唾液腺、中肠等器官中(Gao et al.,2021)。这些共生体微生物被证实可以参与蚊虫的交配、抗药性和提供营养等功能(Cuthbert et al.,2020),但仅有少部分具有防控病媒病原传播的能力。本部分详细阐述了被研究证实的具有防控蚊媒病原的蚊虫共生微生物的种类和防控机理。本文将蚊虫共生微生物对于病媒病原防控分为以下2种:(1)蚊虫共生微生物对蚊媒病原的控制;(2)蚊虫共生微生物对蚊虫生存和发育的控制。蚊媒病原防控机理如图1所示。

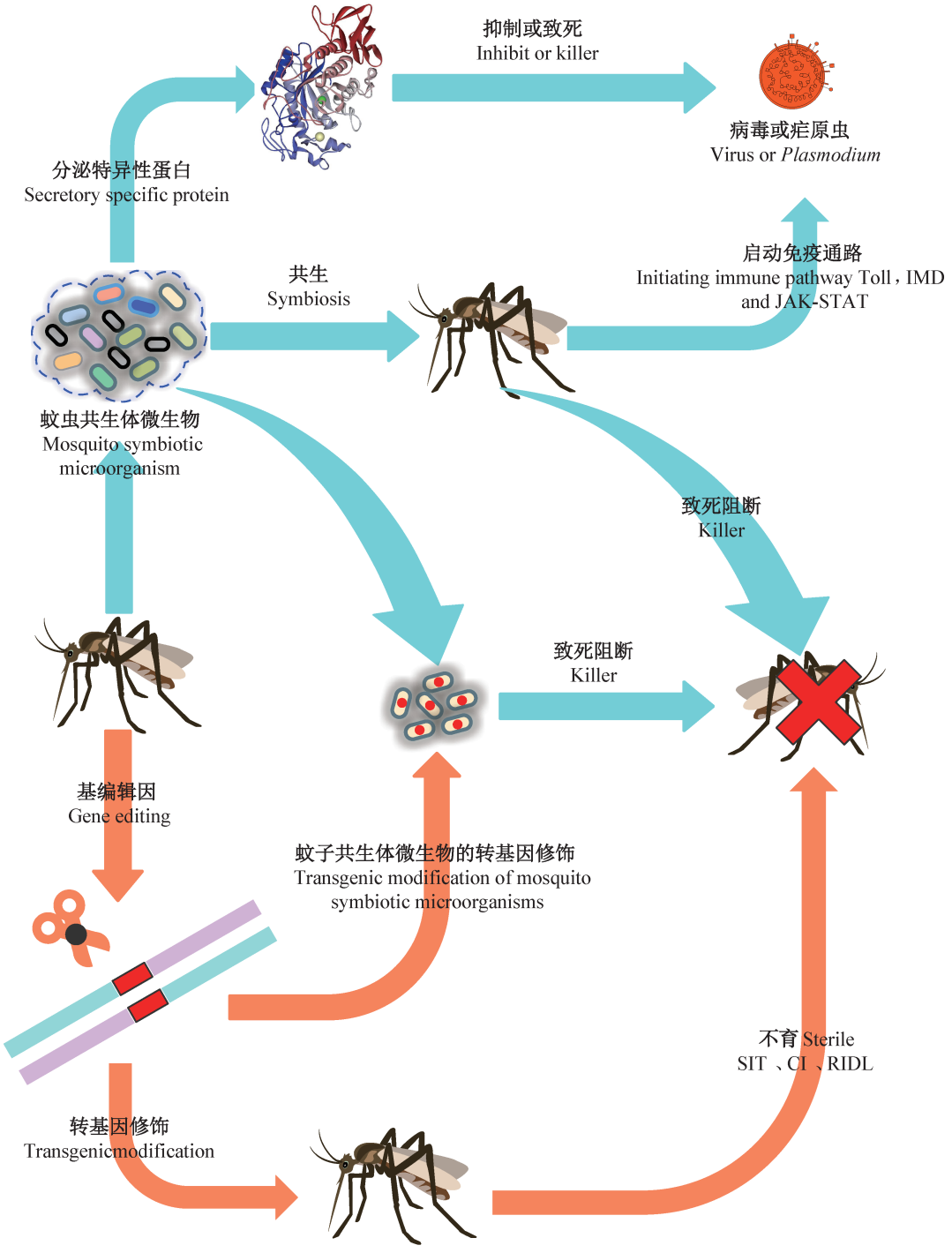
1.1 微生物对蚊媒疾病的控制

据文献报道,可以直接抵御蚊媒病原的蚊虫共生微生物包括解脲沙雷氏菌*Serratia ureilytica* Su_YN1(Gao et al.,2021)、沙雷氏菌属*Serratia* AS1(Wang et al.,2017)、黄病毒属*Flavivirus*(Saraiva et al.,2018a)、异常威克汉姆酵母*Wickerhamomyces anomalus*(Walker,2011;Cappelli et al.,2019)和色杆菌属*Chromobacterium*(Saraiva et al.,2018b)。这些微生物对于病原体的抑制均是先由自身分泌的次级代谢物中的酶、蛋白或多肽来起作用,如解脲沙雷氏菌Su_YN1菌株分泌的抗疟脂肪酶(antimalarial lipase,AmLip)对于疟原虫有直接致死毒性(Gao et al.,2021);黄病毒属产生的氨肽酶则是抑制DENV-2的附着能力,并加快DENV-2的降解速率(Saraiva et al.,2018a);异常威克汉姆酵母产生的致死毒素通过攻击疟原虫表面生物膜表面的葡聚糖成分,导致疟原虫表面膜损伤后致死(Walker,2011;Cappelli et al.,2019)。除此之外,一种超过65%的昆虫体内均含有的细菌引起了研究人员的广泛关注(Hilgenboeker et al.,2010)。该菌称为沃尔巴克氏体*Wolbachia*,其属于立克次体科。沃尔巴克氏体又是一种特殊类型的细菌,其不仅能对昆虫进行生殖操纵,还能直接与虫媒病毒相互作用控制虫媒病原(Zheng et al.,2019;Utarini et al.,2021)。沃尔巴克氏体可以通过与病毒特异性地竞争营养物质或通过调控病毒所必需营养物质的产量减少病毒的复制产量(Kikuchi et al.,2012)。现有的研究证明,沃尔巴克氏体对DENV、CHIKV、YFV、ZIKV和西尼罗河病毒(West Nile virus,WNV)均有显著的防控效果(Moreira et al.,2009;Hoffmann et al.,2011;Ross et al.,2019)。沃尔巴克氏体用于蚊虫防控的研究进展如表1所示。

1.2 微生物对蚊虫的免疫和发育的控制

蚊虫共生微生物对蚊虫的生存和发育的控制分为2种机制:一是通过激活蚊虫自身的免疫系统进而增加蚊虫对蚊媒病原的抵御能力;另一种则是共生微生物能缩短寄主共生蚊虫的繁殖力和寿命等(Allman et al.,2020),进而导致依赖于蚊虫的病原的传播受到限制。蚊虫共生微生物可以调节蚊虫的Toll、免疫缺陷(immune deficiency,IMD)和酪氨酸激酶(Janus kinase-signal transducer,JAK-STAT)信号传导3种先天免疫系统(Xi et al.,2008;Garver et al.,2009;Souza-Neto et al.,2009)。Toll途径对于革兰氏阳性细菌、真菌和病毒有特异的抑制作用(Shin et al.,2006;Souza-Neto et al.,2009;Cirimotich et al.,2010);IMD途径主要是抑制革兰氏阴性菌和恶性疟原虫(Cirimotich et al.,2010);JAK-STAT途径参与病毒防御(Souza-Neto et al.,2009)。能够利用上述原理抑制蚊媒病原的共生微生物有沙雷氏菌属*Serratia* Y1、平沙绿僵菌*Metarhizium pingshaense*、球孢白僵菌*Beauveria bassiana*、沃尔巴克氏体和昆虫特异性病毒(insect-specific viruses,

ISV）。上文所述的沃尔巴克氏体也有诸多类似抑制蚊虫的机制，其直接作用于蚊虫的控制方法主要包括遗传雄性的雌性化、孤雌生殖诱导、杀死感染雌性的雄性后代和细胞质不相容（cytoplasmic incompatibility，CI）（Werren et al.，2008）。另外，沃尔巴克氏体也具有调控蚊虫 Toll 和 IMD 途径的能力，从而提高其宿主自身的抵御能力（Bian et al.，2010）。



SIT：昆虫不育技术；CI：细胞质不相容；RIDL：释放携带显性致死基因。 SIT：sterile insect technique；CI：cytoplasmic incompatibility；RIDL：release of insects carrying a dominant lethal.

图 1　基于蚊虫共生微生物及基因编辑的蚊媒病原防控体系

Fig. 1　Control system of mosquito-borne pathogens based on mosquito symbiotic microorganisms and gene editing

表1 沃尔巴克氏体控制蚊媒疾病

Table 1 *Wolbachia*-based control of the mosquito-borne diseases

年份 Year	结论 Conclusion	参考文献 Reference
1980	沃尔巴克氏体与埃及伊蚊存在着显著的共生关系 *Wolbachia* has a significant symbiotic relationship with *Aedes aegypti*	Wright & Barr, 1980
1983	丧失沃尔巴克氏体会使淡色库蚊发育时间显著延长 The developmental duration of *Culex pipiens pallens* was significantly prolonged when it lost *Wolbachia*	Awahmukalah & Brooks, 1983
1998	沃尔巴克氏体在种间具有胞质不相容性,为宿主–共生微生物的共同进化和沃尔巴克氏体在物种形成中的作用研究奠定了良好基础 Cytoplasmic incompatibility induced by *Wolbachia* lays a good foundation for the study of host-symbiotic coevolution and the role of *Wolbachia* in speciation	Bordenstein & Werren, 1998
2000	证实了沃尔巴克氏体在蚊虫中的广泛分布及其在病媒遗传控制中的潜在重要性,首次将沃尔巴克氏体感染按蚊亚群分类,在调查的89种野生蚊虫中检测出28.1%的蚊虫含有沃尔巴克氏体 The widespread distribution of *Wolbachia* in mosquito species and its potential importance in genetic control of disease vectors were confirmed. *Wolbachia*-infected *Anopheles* subpopulations were differentiated for the first time. *Wolbachia* was detected in 28.1% of 89 species of wild mosquitoes	Kittayapong et al., 2000
2002	沃尔巴克氏体作为基因驱动优良品系被证实 *Wolbachia* proved to be an excellent vector for gene drive	Kittayapong et al., 2002
2002	证实沃尔巴克氏体经母系遗传传播并参与调控多个关于其宿主生殖行为 *Wolbachia* transmission is maternally passed, which participate in the regulation of multiple reproductive behaviors in hosts	龚鹏等,2002 Gong et al., 2002
2004	沃尔巴克氏体可以预防蚊媒病原的传播并降低蚊虫种群数量 *Wolbachia* prevents transmission of mosquito-borne pathogens and reduces mosquito populations	Rasgon & Scott, 2004
2005	在埃及伊蚊中建立了稳定的沃尔巴克氏体感染菌群,证实其会产生胞质不相容性 A stable population of *Wolbachia* was established in *A. aegypti*, and the cytoplasmic incompatibility mechanism was confirmed	Xi et al., 2005
2006	沃尔巴克氏体可以转移到媒介物种埃及伊蚊中,也可以作为基因驱动系统对媒介群体进行基因操纵 *Wolbachia* can be transferred into the vector species *A. aegypti* and can also be used as a gene drive system for gene manipulation of vector populations	Ruang-Areerate & Kittayapong, 2006
2009	对沃尔巴克氏体感染的蚊虫进行遗传控制是减少登革热病毒等病原传播的可行策略 A strategy is proven that genetic control based on *Wolbachia* symbiosis with mosquitoes can reduce transmission of dengue and other pathogens	Mcmeniman et al., 2009
2009	沃尔巴克氏体感染直接抑制了一系列病原感染蚊虫的能力。沃尔巴克氏体与蚊虫先天免疫系统的启动有关,这种沃尔巴克氏体介导的病原干扰可能与缩短寿命策略协同工作,为防控病原传播提供了新策略 *Wolbachia* infection of the host directly inhibit the ability of a range of pathogens to infect mosquito. *Wolbachia* is associated with activation of the mosquito innate immune system. This synergistic effect of pathogen interference and mosquito lifespan shortening provide a new insights into the transmission of insect-borne diseases	Moreira et al., 2009
2010	*w*MelPop-CLA感染显著降低了埃及伊蚊的生存能力 The viability of *A. aegypti* was significantly reduced when it infected with *w*MelPop-CLA	Mcmeniman & O'Neill, 2010
2011	沃尔巴克氏体可以入侵并在蚊虫种群中生存,降低成虫寿命,影响蚊虫繁殖并干扰病原复制 *Wolbachia* strains can invade and sustain themselves in mosquito populations, reduce adult lifespan, affect mosquito reproduction and interfere with pathogen replication	Iturbe-Ormaetxe et al., 2011
2012	沃尔巴克氏体可操纵宿主的防御系统,以促进其自身的持续感染,从而降低蚊虫寄生人类病原的能力 The symbiotic bacterium can manipulate the host defense system to facilitate its own persistent infection, resulting in a compromise of the mosquito's ability to host human pathogens	Pan et al., 2012
2013	尽管沃尔巴克氏体感染的细胞系中病毒基因组RNA复制增强,但可以显著抑制分泌病毒 Despite the enhancement of viral genomic RNA replication in the *Wolbachia*-infected cell line the production of secreted virus was significantly inhibited	Hussain et al., 2013
2014	沃尔巴克氏体降低了埃及伊蚊传播病毒的能力。感染了沃尔巴克氏体 *w*MelPop毒株的蚊虫的繁殖力和卵子活力会急剧下降 *Wolbachia* can reduce *A. aegypti*'s ability to transmit viruses. Mosquito fecundity and egg viability decrease sharply when it infected with the *w*MelPop strain of *Wolbachia*	Caragata et al., 2014

续表1 Continued

年份 Year	结论 Conclusion	参考文献 Reference
2015	已感染*w*MelPop的埃及伊蚊具有很强的病毒干扰特性，但其适应性较低，可作为持续控制登革热病毒传播的一种手段 *A. aegypti* infected with *w*MelPop has strong viral interference characteristics. However, *w*MelPop may be used as a means of sustainable control of dengue virus transmission	Nguyen et al., 2015
2017	沃尔巴克氏体和SIT策略都导致蚊虫产生无法存活的后代，能够持续控制蚊媒病原 Both *Wolbachia* and the SIT strategy lead to mosquito infertility, and can persistently control mosquito-borne pathogens	Yakob et al., 2017
2018	基于沃尔巴克氏体的生物防治已成为预防和控制登革热和其他蚊媒传播病原的一种潜在方法。如埃及伊蚊感染沃尔巴克氏体时，其病毒感染和传播能力均会降低 *Wolbachia*-based biological control has recently emerged as a potential approach for the prevention and control of dengue and other mosquito-borne diseases. For example, *A. aegypti* is less able to infect and transmit viruses when it is infected with *Wolbachia*	Campo-Duarte et al., 2018
2019	沃尔巴克氏体能够成功入侵埃及伊蚊，感染率可达80%，显著降低登革热、寨卡病毒等传播风险，增加埃及伊蚊的抗病能力 *Wolbachia* can successfully invade *A. aegypti*, and the high infection rate is up to 80%, which significantly reduces the transmission risk of dengue fever and zika virus, and increases the resistance of *A. aegypti*	Carvajal et al., 2020
2020	沃尔巴克氏体的*w*MelPop菌株显著降低了蚊虫的个体适应度，并使其显著降低了人类宿主中预期的登革热感染数量 The *w*MelPop strain of *Wolbachia* considerably reduces the individual fitness of mosquitoes, and significantly reduces the expected infection incidences of dengue in human hosts	Cardona-Salgado et al., 2020
2020	沃尔巴克氏体对宿主昆虫具有胞质不亲和作用、产雌孤雌生殖作用、雌性化作用或杀雄作用等生殖调控机制，同时可以抑制虫媒病毒在昆虫体内的复制和传播 *Wolbachia* has evolved various mechanisms for manipulating reproduction of their hosts, including induction of reproductive incompatibility, parthenogenesis, feminization, male killing, fecundity or fertility modifying	张治军等，2021 Zhang et al., 2021
2021	沃尔巴克氏体能够降低登革热和寨卡病毒感性效率 *Wolbachia* can reduce the perceptual efficiency of dengue and zika viruses	Dainty et al., 2021

2 基于基因编辑技术对蚊媒疾病的防控

基因编辑的操作系统由靶向组分和切割组分2部分构成。首先是对宿主细胞的DNA进行靶向敲除，再将外源序列定点敲入。在此过程中使用的人工核酸酶是保证定点基因编辑的关键。现有的人工核酸酶编辑系统包括：锌指核酸酶（zinc finger nuclease，ZFN）、类转录激活因子式核酸酶（transcription activator-like effector nuclease，TALEN）和CRISPR/Cas系统（Koonin et al.，2017；俞珺瑶和杜华平，2018；Noble et al.，2019）。在经过上述基因编辑和转基因后，沃尔巴克氏体之类的细菌或蚊虫个体中整合了目的基因，在其表达过程中可以实现对蚊媒病原的直接控制或对蚊虫自身行为和寿命的调控。

2.1 基于微生物基因编辑对蚊媒疾病的防控

通过基因编辑获得的突变体蚊虫可以导致蚊虫不育，该技术被称为昆虫不育技术（sterile insect technique，SIT）。SIT的应用较为广泛，已成为一种普遍的生物防治方法。SIT通过释放不育雄蚊与野外雌蚊进行交配，导致雌蚊无法繁殖产生不育后代，连续释放可达到种群抑制的效果，该项技术在地中海石蝇*Ceratitis capitata*等害虫防控中均得到了证实（Lloyd et al.，2010）。传统的SIT为自我限定型种群控制策略，但因其不能长时间维持限制作用，研究人员又开发了自我维持型的种群控制策略（徐雪娇等，2019）。典型的SIT维持型技术是基于CI原理的昆虫不相容（incompatible insect technique，IIT）技术。CI是沃尔巴克氏体在昆虫中普遍存在的一种繁殖方式，沃尔巴克氏体诱导雄蚊宿主产生CI，从而出现胚胎期死亡的现象，使感染沃尔巴克氏体的雌蚊与未感染的雄蚊不能正常交配，通过释放感染沃尔巴克氏体的不育雄蚊达到种群抑制或替换的效果（杨翠等，2020）。IIT策略的成功实施依赖于在大规模生产系统中准确分离昆虫雄性和雌性，并将其有效释放（Pagendam et al.，2020）。IIT技术有望成

为一种消除入侵蚊虫种群的方法，可降低DENV、CHIKV等蚊媒传播病原的致病率。但传统的SIT技术也有其严重的弊端，既需要对蚊虫的雌雄进行分离，释放单一性别的不育昆虫才能达到最终的防控目的，这为蚊虫的防控造成了较大的难度。

2.2 基于蚊虫基因编辑对蚊媒疾病的防控

为了弥补SIT技术的局限性，科研人员对传统技术进行了一些改进，其中一种改进策略就是将致死基因引入昆虫基因，并在特定条件下让其在虫体内表达。即释放携带显性致死基因（release of insects carrying a dominant lethal，RIDL）技术，其主要依靠性别决定系统和转座子的活性进行基因修饰（Klassen，2009）。RIDL技术的调控系统是基于Tet-off系统调控致死基因的表达，导致靶标昆虫特异性死亡。Tet-off系统由转录激活因子（tetracycline-repressible transcriptional activator，Tta）及其相应元件四环素操纵子序列（tetracycline operator，tetO）构成（王玉生等，2015）。当上述基因在虫体中表达时就会导致蚊虫或昆虫的死亡。为确保RIDL技术处理后的昆虫可以正常饲养，需在其饲料中添加四环素及其类似物，这些物质能够结合tetO元件使致死基因不能表达，从而维持RIDL品系昆虫的正常存活。当RIDL品系的昆虫释放到野外后，会在自身死亡前与野生型昆虫交配，并将RIDL品系的特定基因传播到野生昆虫当中，导致其后代无法存活，最终达到了昆虫防控的目的。在蚊虫中的典型案例为埃及伊蚊*Aedes aegypti*的RIDL品系驱动显性致死基因*VP16*的表达后可以使蚊虫失去飞离水体、觅食、寻找配偶及交配的能力（Fu et al.，2010）。

基因修饰后的蚊虫或其共生微生物是基因驱动的技术核心，进而实现基因驱动策略的实现。基因驱动是指特定的性状或基因型在种群中被有目地的遗传给后代的方式（Alphey，2014）。基于CI机制的种群防控策略以及显性不足基因和归巢内切酶基因（homing endonuclease gene，HEG）介导的基因驱动技术均为自我维持型的基因驱动策略（Alphey，2014），而传统的SIT和RIDL技术为自我限定型控制策略。自我限定型种群控制策略遵循孟德尔遗传规律，而自我维持型种群控制策略不遵循孟德尔遗传规律，被称为超孟德尔遗传（徐雪娇等，2019）。基因驱动又可分为全面基因驱动和分离式基因驱动2种系统。全面基因驱动系统可以使改造的性状永久保留在种群当中，使获得此基因性状的个体表达目的性状，进而达到种群控制的目的（Gantz et al.，2015）。该系统包含的基因编辑策略有ZFN、TALEN、CRISPR/Cas9和其*HEG*（Windbichler et al.，2011；Simoni et al.，2014；Gantz & Bier，2015）。其中，CRISPR/Cas9和*HEG*介导的基因驱动具有相同的机理。但CRISPR介导的基因驱动在切割具有原间隔基序（protospacer adjacent motif，PAM）结构位点时只需编辑sgRNA，使其比*HEG*介导基因驱动时需要特定的酶切位点更为便利。全面基因驱动系统的归巢策略强大的不可逆转性可能导致释放编辑的物种全部替换野生型物种的基因，或是直接导致物种灭绝的风险（Grunwald et al.，2019）。基于上述结论，分离式基因驱动系统及其衍生的雏菊式驱动系统弥补了这一潜在风险。它们将编码Cas9和sgRNA的表达盒分离并分别放置在非连锁的几条染色体上。该方法不仅加强了基因的驱动效率，还能使Cas9表达盒所携带的基因遵循孟德尔遗传定律，保证种群基因不被sgRNA表达盒上的基因全部取代（Noble et al.，2019）。至此，分离式基因驱动系统进一步完善了全面基因驱动系统的不足，为基因驱动技术的发展及其推向应用奠定了坚实基础。

3 展望

基于蚊虫共生微生物和基因编辑技术的蚊媒病原的防控在未来必然发挥出其特有成效，但当下仍处于探索阶段。因此，现阶段对于蚊媒病毒的防控还需以传统防控方式为主，逐渐合理地结合新的技术探索出更加合理的防控措施。此外，研究人员也要在探索和研究中高度警惕，防治造成因外源菌引入或外源基因扩散造成的生态失衡，这可能比蚊媒病原传播本身造成的损害更为严重。但随着蚊媒病原控制研究的逐渐成熟，人类不仅能够合理掌握这2种新的蚊媒病原的防控技术；同时，因人工智能的不断发展，也可以通过不断的人工学习模拟这2种方法对自然的负面危害性。虽然蚊媒病原防控的方法在不断更新和完善，但人类自身对于蚊媒病原的防范意识仍不能松懈，仍需提高虫媒病原的日常防范意识。蚊媒病原的防控也不是单一方法就可以实现的，需要多个方法和策略一起协同应用以实现蚊媒病原的防控。

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