CRISPR/Cas9 技术: 基因魔剪

汪铭

中国科学院化学研究所, 中国科学院活体分析化学重点实验室, 北京 100190
E-mail: mingwang@iccas.ac.cn

瑞典皇家科学院 2020 年 10 月 7 日宣布今年的诺贝尔化学奖授予美国加州大学教授詹妮弗·杜德纳(Jennifer A. Doudna)和现在德国工作的法国科学家埃曼纽尔·卡彭蒂耶(Emmanuelle Charpentier), 以表彰她们在基因编辑领域作出的突出贡献. 两位科学家合作发展了新型 CRISPR/Cas9 基因编辑方法, 并精准地应用于改变动物、植物、微生物的 DNA. 这项技术对生命科学产生了革命性影响, 有望催生创新性癌症治疗策略, 并可能让治愈遗传性疾病这一人类梦想成真(图 1). (“This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true.”).

随着生物技术的不断发展, 对活细胞 DNA 进行精准操作, 实现碱基或 DNA 片段的插入、删除、替换等, 即基因编辑成为可能. 同时, 运用基因编辑技术可以改变基因的序列和功能, 从而调控细胞的命运和生物特征, 为遗传性疾病的治疗提供新方法. CRISPR/Cas9 基因编辑技术是继“锌指核酸酶(ZFN)”、“类转录激活因子效应物核酸酶(TALEN)”之后出现的新一代基因组定点编辑技术. 与前两代技术相比, CRISPR/Cas9 具有操作简单、快捷高效等优势, 自发现之后迅速发展成为当今最主流的基因编辑方法.

CRISPR 是 clustered regularly interspaced short palindromic repeats(成簇规律间隔短回文重复序列)的首字母缩写. CRISPR 序列是由日本分子生物学家石野良纯(Yoshizumi Ishino)1987 年在大肠杆菌中偶然发现的, 西班牙微生物学家弗朗西斯科·莫伊卡(Francisco Mojica)2003 年进一步发现 CRISPR 中独特的非重复的序列与各种病毒的遗传密码相匹配. 随着研究的不断深入, 研究人员确定了 CRISPR 是一种源自细菌的适应性免疫系统. CRISPR 能够识别(通过 crRNA)入侵细菌的病毒, 并通过一种特殊的核酸酶(Cas9 蛋白)降解入侵病毒的 DNA 序列, 从而保护古细菌免受病毒侵害. Emmanuelle Charpentier 在研究化脓性链球菌(*Streptococcus pyogenes*)的调控性 RNA 过程中发现化脓性链球菌中存在一些新型小 RNA 分子, 其遗传密码与 CRISPR 序列存在部分匹配. 进一步她们发现这些未知的 RNA 分子(后来被称为反式激活的 CRISPR RNA, tracrRNA)可以帮助基因组中的 CRISPR 序列转录产生的长 RNA 分子加工为成熟的、具有活性的 RNA(crRNA), 对

汪铭 中国科学院化学研究所研究员, 主要从事蛋白质递送与化学调控研究. 近期研究包括发展蛋白质化学生物学工具和手段, 实现细胞和活体层次蛋白质递送及功能调控, 并探索其在疾病治疗、神经退行性疾病分子机制研究中的应用.

CRISPR 作用及细菌的免疫系统具有决定性意义.

然而, 第一次全面解析 CRISPR/Cas9 基因编辑的原理则来自于 Emmanuelle Charpentier 和美国分子生物学家 Jennifer Doudna 的合作. 她们发现使用重组 Cas9 蛋白(来自大肠杆菌里表达的化脓性链球菌 *S. pyogenes* 基因)和体外转录的 crRNA 及 tracrRNA 可以在体外切割纯化的 DNA, 并成功编辑了大肠杆菌基因. 此外, 她们证明 crRNA 和 tracrRNA 对 Cas9 发挥作用都是必需的, 两种 RNA 在被融合为单一的导向 RNA(single guide RNA, sgRNA)时也可以在体外发挥作用, 并提到“利用这一系统来进行可设计的基因组编辑的潜力”. 这一结果发表半年之后, 研究人员首次报道可以将 CRISPR/Cas9 基因编辑技术应用于哺乳动物和人类细胞的基因编辑, 从而促进了这一技术的爆发性发展. 此前, 改变细胞基因是一项非常耗时, 甚至是难以完成的工作. 使用 CRISPR 基因编辑方法, 研究人员理论上可以通过设计不同的 sgRNA 引导 Cas9 核酸酶在他们想要的任何基因位点进行基因编辑. 由于这一方法的简单、廉价和高效, CRISPR 迅速发展成为最主流的基因编辑技术, 应用于高效、精确地改变、编辑或替换植物、动物甚至是人类基因, 被称为编辑基因的“魔剪”(图 1).

CRISPR/Cas9 基因编辑技术改变了我们在不同物种活细胞中操纵基因表达的能力, 短短十几年间便从 CRISPR 机制研究发展到临床试验应用. 与 ZFN 和 TALEN 等基因编辑技术相比, CRISPR/Cas9 在基因编辑和基因治疗等方面具有非常明显的优势: 设计极其简单, 只需要设计一段几十个碱基构成的 gRNA 序列即可实现靶标基因编辑, 而传统方法多需要复杂的分子生物工程设计蛋白质质

别基因组；基因编辑效率高，可以通过设计多个 sgRNA 实现多个基因位点的同时基因编辑；多功能化，随着 CRISPR/Cas9 的不断发展，这一技术不仅可以实现对特定基因组位点的删除、修复、替换等功能，还进一步被应用于基因组成像、表观遗传修饰、核酸检测及疾病诊断(如新冠病毒检测)等方面。

2020 年诺贝尔化学奖颁发给两位从事基因编辑研究的科学家，不仅仅是对她们个人成就的表彰，更是对整个研究领域成果的肯定。当前，CRISPR/Cas9 基因编辑技术已经显示出巨大的生物医学应用价值，但其在生物安全性、基因编辑脱靶效应以及临床应用等方面仍面临很多亟

待解决的问题。如人体可能对细菌来源的基因编辑核酸酶 Cas9 产生免疫反应；高效、安全地将 CRISPR/Cas9 基因编辑系统导入活细胞甚至活体层次至关重要。病毒载体在人类基因治疗中获得了广泛应用，但是其装载容量有限，而常规的 Cas9 蛋白过大，对利用病毒载体实现 CRISPR/Cas9 递送提出了挑战。因此，发展新型 CRISPR/Cas9 递送系统对在体基因编辑及其临床转化应用尤为重要；在降低脱靶效应方面，通过对 Cas9 蛋白进行定点突变或者发展新型基因编辑技术(如碱基编辑等)则有望实现突破。这些研究虽然仍面临众多挑战，但也是未来基因编辑技术发展和其生物医学应用的机遇和突破口。

The CRISPR/Cas9 genetic scissors

When researchers are going to edit a genome using the genetic scissors, they artificially construct a guide RNA, which matches the DNA code where the cut is to be made. The scissor protein, Cas9, forms a complex with the guide RNA, which takes the scissors to the place in the genome where the cut will be made.

图 1 (网络版彩色)CRISPR/Cas9 基因编辑技术示意图(<https://www.nobelprize.org/prizes/chemistry/2020/popular-information/>)

Figure 1 (Color online) Illustration for genome editing using CRISPR/Cas9 (<https://www.nobelprize.org/prizes/chemistry/2020/popular-information/>)

Summary for “CRISPR/Cas9 技术: 基因魔剪”

CRISPR/Cas9: Magic scissors for genome editing

Ming Wang

CAS Key Laboratory of Analytical Chemistry for Living Biosystems, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China
E-mail: mingwang@iccas.ac.cn

The Nobel Prize in Chemistry 2020 was awarded jointly to Emmanuelle Charpentier and Jennifer A. Doudna “for the development of a method for genome editing.” Emmanuelle Charpentier and Jennifer A. Doudna have discovered one of gene technology’s sharpest tools: the CRISPR/Cas9 genetic scissors. Using this strategy, researchers can change the DNA of animals, plants and microorganisms with extremely high precision. This technology has had a revolutionary impact on the life sciences, is contributing to new cancer therapies and may make the dream of curing inherited diseases come true.

The CRISPR sequence was first discovered in *E. coli* by Japanese molecular biologist Yoshizumi Ishino in 1987, and the Spanish microbiologist Francisco Mojica further discovered unique non-repetitions in CRISPR in 2003. Later on, it has been realized that CRISPR is an adaptive immune system derived from bacteria. CRISPR can identify (via crRNA) viruses that invade bacteria, and use a special nuclease (Cas9 protein) to degrade the DNA sequence of the invading viruses, thereby protecting bacteria from viral invasion. Emmanuelle Charpentier discovered that there are some new small RNA molecules in *Streptococcus pyogenes* in the process of studying the regulatory RNA of *Streptococcus pyogenes*, whose genetic code partially matches the CRISPR sequence. Furthermore, they have discovered that these unknown RNA molecules (later known as trans-activated CRISPR RNA, tracrRNA) can help the long RNA molecules produced by the transcription of CRISPR sequences in the genome to be processed into mature, active RNA (crRNA).

The first comprehensive analysis of the principles of CRISPR/Cas9 gene editing came from the collaboration of Emmanuelle Charpentier and Jennifer Doudna. They found that using recombinant *S. pyogenes* Cas9 protein and *in vitro* transcribed crRNA and tracrRNA, either purified DNA or *E. coli* gene could be efficiently edited. In addition, they proved that both crRNA and tracrRNA are necessary for Cas9 to be functional, and that the two RNAs can also function *in vitro* when they are fused into a single guide RNA (sgRNA). Shortly, the research group from MIT reported for the first time that CRISPR/Cas9 gene editing technology can be applied to gene editing in mammalian and human cells, thus promoting the explosive development of this technology. Using the CRISPR gene editing method, researchers can theoretically direct Cas9 nuclease to perform gene editing at any desired gene locus by designing different sgRNAs. Due to the simplicity, low-cost, and efficiency of this method, CRISPR has rapidly developed into the most mainstream gene editing technology, which is used to efficiently and accurately change, edit or replace plant, animal and even human genes.

CRISPR/Cas9 gene editing technology has shown a great potential for biomedical applications, but it still associated with challenges that include biosafety, off-target effects of gene editing. For example, the human body may have an immune response to the gene-editing nuclease Cas9 derived from bacterial; it is very important to efficiently and safely introduce the CRISPR/Cas9 gene-editing system into living cells or *in vivo*. Viral vectors have been widely used in human gene therapy, but their loading capacity for Cas9 is greatly limited. Therefore, the development of new CRISPR/Cas9 delivery systems is particularly important for *in vivo* gene editing and its clinical transformation applications. To reduce off-target effects of CRISPR/Cas9 genome editing, site-directed mutation of Cas9 protein or the development of new gene editing technologies (such as base editing) are highly appreciated for promoting this technology into clinic.

CRISPR/Cas9, genome editing, gene therapy, drug delivery

doi: 10.1360/TB-2020-1514