

## 基因工程的技术飞跃——TALE技术的研究进展

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**摘要:** 类转录激活因子(TALEs)是一类来源于植物致病菌黄单胞菌属(*Xanthomonas*)的III型效应因子。TALE由N端转移结构域、串联重复的DNA结合结构域、C端核定位信号及酸性转录激活结构域组成。TALE的DNA结合结构域经基因工程修饰能够识别并结合任意指定的DNA序列, 从而可以实现对目的基因组位点的调控。目前, 已有多种TALE的快速构建法。因此, TALE以及TALE衍生蛋白在基因工程领域具有广阔的应用前景。本文综述了TALE的研究现状, 对TALE技术的优点和不足进行了简要的阐述, 并对其应用前景做了初步探讨。

**关键词:** TALE分布; TALE结构特征; TALE构建方法; TALE应用前景

### Advances in TALE Research

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**Abstract:** Transcription activator-like effectors (TALEs) are type III effector proteins from plant-pathogenic bacteria of the genus *Xanthomonas*. All TAL effectors are composed of an N-terminal translocation domain, central tandem repeat DNA binding domain, C-terminal nuclear localization signals and an acidic transcriptional activation domain. Central repeats of TALE can be engineered to bind any user-selected DNA targets. Consequently, TALE proteins can be engineered to achieve site-specific modification in the genome. Recently, several rapid TALE assembly approaches have been established. Therefore, TALE and TALE-derived proteins could be widely used for genome engineering. This article reviews recent advances in TALE research.

**Key words:** distribution of TALE; structural features of TALE; TALE assembly approaches; prospect of TALE

类转录激活因子(transcription activator-like effectors, TALEs)是由黄单胞菌属(*Xanthomonas*)的革兰氏阴性植物致病菌产生的一类天然细菌效应因子。该类致病菌侵染植物体时, 能够通过III型分泌系统将TALE等致病因子转移到植物细胞中(Bogdanove等2010)。进入植物细胞后, TALE转运到细胞核中与特异的DNA序列结合, 模仿真核转录因子的作用方式激活某些特异基因的表达(Kay等2007; Romer等2009, 2010)。通常情况下, 此类基因的表达有助于细菌的繁殖和传播。但在某些植物体内, 这类基因的表达能够引发植物的防御反应(图1)(Antony等2010; Boch和Bonas 2010; Christian等2010; Gu等2005, 2009; Sugio等2007; Yang等2006)。

TALE与DNA结合的特异性是由TALE中间区域的DNA结合结构域决定的, 通过基因工程修饰, 该DNA结合结构域能够结合任一指定的DNA靶位点。与核酸酶、甲基化酶或整合酶等功能结构域融合得到的重组TALE能够对基因组的特异性位点进行相关修饰(Mahfouz和Li 2011)。因此, TALE

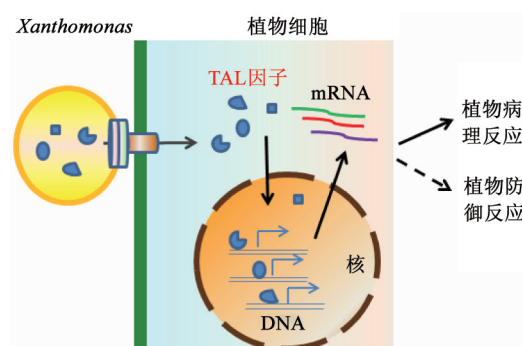


图1 TALE在植物疾病及防御反应中的作用

Fig.1 The roles of TALE in plant disease and defense response

参考Bogdanove等(2010)、Boch和Bonas (2010)、Kay和Bonas (2009)并作修改。TALE通过III型分泌系统进入植物细胞, 然后被转运到植物细胞核中, 识别特异的DNA序列, 激活特异宿主基因的表达, 从而导致植物体产生病理反应。但有些植物能产生防御反应。

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在基因操作相关研究领域具有广泛的应用前景, 对于这一技术的开发和利用具有十分重要的实用价值。

### 1 TALE的分布

最先发现的TALE存在于黄单胞菌属的成员。

1989年, Bonas等在*Xanthomonas campestris* pv. *vesicatoria*中鉴定得到第一个TALE, 命名为AvrBs3 (Bonas等1989)。迄今为止, 已陆续在黄单胞菌属及其致病型中发现将近40种TALE (表1)。研究发现, 在部分罗尔斯通氏菌(*Ralstonia solanacearum*)的突变

表1 黄单胞菌属中已经发现的TALE  
Table 1 The TALE found in *Xanthomonas*

| 致病菌名称                                       | TALE名称                                | GenBank登录号                                 | 重复区结构              | 参考文献   |             |
|---|---------------------------------------|--|--------------------|--|-------------|
| <i>X. campestris</i> pv. <i>vesicatoria</i> | AvrBs3                                | X16130, CAA34257                           | 17.5*34            | Bonas等1989; Kay等2007; Romer等2007                                   |             |
|   | AvrBs4                                | X68781, CAA48680                           | 17.5*34            | Bonas等1993   |             |
| <i>X. campestris</i> pv. <i>armoraciae</i>  | Hax2                                  | AY993937, AAY43358                         | 21.5*35            | Kay等2005   |             |
|   | Hax3                                  | AY993938, AAY43359                         | 11.5*34            | Kay等2005   |             |
|   | Hax4                                  | AY993939, AAY43360                         | 14.5*34            | Kay等2005   |             |
| <i>X. campestris</i> pv. <i>malvacearum</i> | Avrb6                                 | L06634, AAB00675                           | 13.5*34            | De Feyter和Gabriel 1991; De Feyter等1993; Yang等1994                  |             |
|   | PthN                                  | AF016221, AAB69865                         | 13.5*34            | Chakrabarty等1997   |             |
| <i>X. campestris</i> pv. <i>citri</i>       | Apl1                                  | AB021363, BAA37119                         | 17.5*34            | Kanamori和Tsyumu 1998   |             |
|   | Apl2                                  | AB021364, BAA37120                         | 15.5*34            | Kanamori和Tsyumu 1998   |             |
|   | Apl3                                  | AB021365, BAA37121                         | 23.5*34            | Kanamori和Tsyumu 1998   |             |
| <i>X. citri</i>                             | PthA                                  | U28802, AAC43587                           | 17.5*34            | Swarup等1991  |             |
|   | PthB                                  | AY228335, AAO72098                         | 17.5*34            | Brunings和Gabriel 2003  |             |
|   | HssB3.0                               | BAF46269                                   | 17.5*34            | Shiotani等2007  |             |
|   | PthA-KC21                             | BAF46271                                   | 17.5*34            | Shiotani等2007  |             |
| <i>X. axonopodis</i> pv. <i>manihotis</i>   | PthB                                  | AF012325, AAD01494                         | 12.5*34            | Restrepo和Verdier 1997  |             |
| <i>X. axonopodis</i> pv. <i>citri</i>       | PthA1                                 | XACa0022, AAM39226                         | 16.5*34            | Brunings和Gabriel 2003; Da Silva等2002                               |             |
|   | PthA2                                 | XACa0039, AAM39243                         | 15.5*34            | Brunings和Gabriel 2003; Da Silva等2002                               |             |
|   | PthA3                                 | XACb0015, AAM39261                         | 15.5*34            | Brunings和Gabriel 2003; Da Silva等2002                               |             |
|   | PthA4                                 | XACb0065, AAM39311                         | 17.5*34            | Brunings和Gabriel 2003; Da Silva等2002                               |             |
| <i>X. citri</i> pv. <i>citri</i>            | PthA*                                 | ABO77780                                   | 17.5*34            | Al-Saadi等2007  |             |
|   | PthAW                                 | ABO77779                                   | 17.5*34            | Al-Saadi等2007  |             |
| <i>X. citri</i> pv. <i>aurantifolii</i>     | PthB                                  | NP_942641                                  | 17.5*34            | Al-Saadi等2007  |             |
|   | PthC                                  | ABO77782                                   | 17.5*34            | Al-Saadi等2007  |             |
| <i>X. oryzae</i> pv. <i>oryzae</i>          | AvrXa3                                | AY129298, NC_006834, AAN01357, YP_199878.1 | 8.5*34             | Li等2004; Lee等2005  |             |
|   | Avrxa5                                | AY377126, AAQ79773                         | 5.5*34             | Hopkins等1992; Bai等2000   |             |
|   | AvrXa7                                | AF262933, AAF98332, AAF98343               | 25.5*34            | Bai等2000; Hopkins等1992; Vera Cruz等2000; Yang等2000; Yang和White 2004 |             |
|   | AvrXa10                               | U50552, AAA92974                           | 15.5*34            | Hopkins等1992; Zhu等1998   |             |
|   | AvrXa27                               | AY986494, AAY54168                         | 16.5*34            | Gu等2004  |             |
|   | PthXo1                                | ACD58243                                   | 20.5*34            | Yang等2006  |             |
|   | PthXo2                                | AAS46026                                   | 21.5*34            | Yang和White 2004  |             |
|   | PthXo3                                | AAS46027                                   | 21.5*34            | Yang和White 2004  |             |
|   | PthXo6                                | ACD58920                                   | 22.5*34            | Sugio等2007   |             |
|   | PthXo7                                | ACD57198                                   | 21.5*34            | Sugio等2007   |             |
|   | PthXo8                                | ACD60557                                   | 22.5*34            | Yang和White (未发表资料)   |             |
|   | <i>X. oryzae</i> pv. <i>oryzicola</i> | Avr/Pth3                                   | AY875712, AAW59492 | 15.5*34  | Zou等(未发表资料) |
|   |                                       | Avr/Pth13                                  | AY875711, AAW59491 | 5.5*34   | Zou等(未发表资料) |
| Avr/Pth14                                   |                                       | AY875713, AAW59493                         | 19.5*34            | Zou等(未发表资料)  |             |
| <i>X. gardener</i>                          | AvrHah1                               | ABP97430                                   | 17.5*34            | Schornack等2008   |             |

型个体存在TALE的近缘蛋白, 这些蛋白在致病菌侵染植物体时也是通过III型分泌系统转运到宿主细胞中, 但是否具有转录激活因子的作用尚不清楚(Heuer等2007; Mukaihara等2010)。此外, 甜菜和瞿麦致病菌成团泛菌(*Pantoea agglomerans*)体内的宿主特异性决定因子HsvB和HsvG能够定位于植物细胞核, 结合双链DNA分子, 在酵母中已证实有转录激活活性, 但是在结构上与黄单胞菌属的TALE存在明显差异(Nissan等2006)。

## 2 TALE的结构特征

所有的TALE都由N末端的转移结构域(TS)、中间DNA结合结构域、C末端的核定位信号(NLS)

和酸性转录激活结构域(AD)组成(图2-A) (Bogdanove等2010; Bogdanove和Voytas 2011; Christian等2010; Dean 2011; Heuer等2007; Mahfouz和Li 2011; White等2009)。其中N末端的转移结构域在TALE通过III型分泌系统转移到植物体内的过程中发挥作用(Szurek等2002), 中间串联重复的DNA结合结构域介导TALE与特异序列的DNA结合(Gurlebeck等2005; Herbers等1992; Kay等2007; Mukaihara等2010), C末端的核定位信号和酸性转录激活结构域在TALE转运到细胞核并行转录激活功能的过程中具有重要作用(Van den Ackerveken等1996; Zhu等1998)。

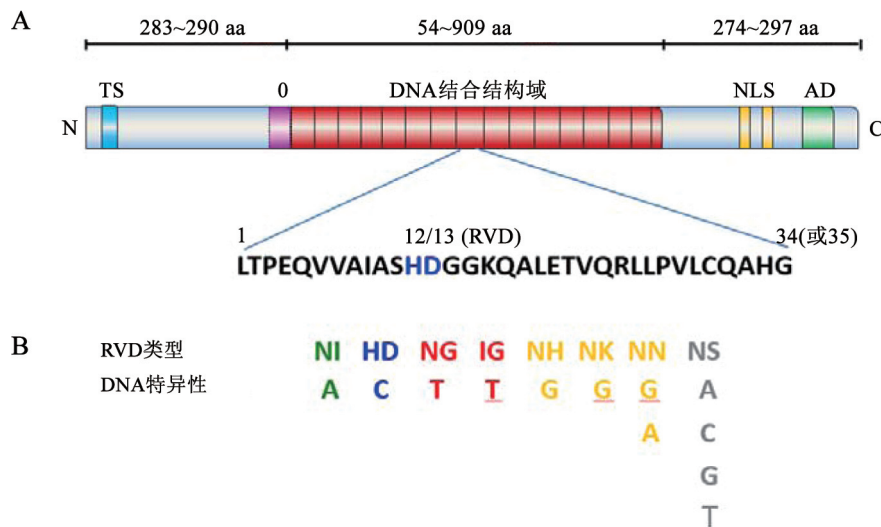


图2 TALE的结构及其靶DNA结合特异性

Fig.2 Structure and DNA-binding specificity of TALE proteins

参考Boch和Bonas (2010)、Scholze和Boch (2011)等并作修改。

TALE的N末端转移结构域以及C末端的核定位信号和转录激活结构域具有高度的保守性, 不同成员之间互换这些功能区域并不影响其正常发挥作用。通过对113个TALE序列进行比对的结果表明, 不同TALE的中间DNA结合结构域的重复数量存在明显差异(Boch和Bonas 2010)。该结构域的重复数量一般为1.5~33.5个, 较为常见的是17.5个(Boch等2009)。具有较短重复区域的TALE很可能没有功能, 因为研究表明6.5个重复序列是诱导基因表达所必需的(Boch等2009)。每个重复序列一般由34或35个氨基酸组成, 具有很高的相似性, 第12和13个氨基酸是可变的, 构成了一个重复可

变双残基对(repeat variable diresidue, RVD), RVD决定了一个重复区域识别一种碱基序列的特异性(Boch等2009; Moscou和Bogdanove 2009; Scholze和Boch 2011)。这种“一重复一碱基”的识别密码已经通过生物信息学和实验手段破译, 并通过体外组装TALE结合预测的DNA靶序列得到了进一步的验证。实验结果表明, HD特异性识别C, NI特异性识别A, NH和NK特异性识别G, NG特异性识别T, NN特异性识别A和G, 而NS能够以同等亲和力结合A、C、G或T(图2-B)(Boch等2009; Boch和Bonas 2010; Bogdanove和Voytas 2011; Cong等2012; Moscou和Bogdanove 2009; Streubel等2012)。自然

环境中的有功能的TALE对DNA的结合位点前均为T (称为 $T_0$ ), 而且TALE分子中与重复区域N端相邻的序列在不同TALE中表现出一定的相似性, 由此表明, 这段序列在TALE与特异DNA序列结合过程中很可能识别T (Bogdanove等2010)。

尽管对TALE的一级结构研究已取得突破性进展, 但是一些人工合成的TALE对靶位点的识别效率很低, 这就要求我们进一步了解TALE的高级结构。Schornack等(2006)对AvrBs3的二级结构预测表明AvrBs3的每个重复包含两个保守的 $\alpha$ -螺旋, 中间由包含RVD的环状结构域连接。这种重复区结构与solenoid蛋白超家族成员结构类似, 该蛋白家族成员也包含折叠成超螺旋的重复结构单元 (Kobe和Kajava 2000)。Murakami等(2010)对最小TALE蛋白PthA的1.5个重复区域进行了核磁共振 (nuclear magnetic resonance, NMR) 结构研究, 并对整个蛋白质结构进行了小角X-射线散射 (small angle X-ray scattering, SAXS) 研究。结果表明, 分离出来的重复区域呈现大的 $\alpha$ -螺旋结构, 全长蛋白分子在与DNA结合过程中其分子结构变得更加紧密。但该研究并未阐明分离的重复结构与全长蛋白分子中的重复结构存在多大差异, 每一个重复与连续的DNA碱基对结合的模式也未能得到解决。最近, Mak等(2012)和Deng等(2012)分别对PthXo1和dHax3进行的晶体结构分析表明, 每个TALE的重复区域包含两个 $\alpha$ -螺旋, 中间由包含RVD的短环状结构域连接, 重复区域自我装配形成一个右手超螺旋结构, 在与DNA相互作用中可以结合DNA双螺旋的大沟。每个重复区域的第12个氨基酸残基负责稳定RVD环, 而第13个氨基酸进行碱基特异性的结合。TALE的DNA结合结构域具有一定的可塑性, 可通过基因工程改变RVD的氨基酸序列, 从而使TALE能够结合任何选定的DNA靶位序列。目前, 越来越多的研究者把按照自己需要构建的TALE作为DNA靶向工具应用到不同的研究领域, 使其得到越来越广泛的应用 (Christian等2010; Li等2011; Mahfouz等2011a, b; Morbitzer等2010; Mussolino等2011; Sander等2011; Zhang等2011)。

### 3 TALE的构建方法

通过重组得到的DNA结合结构域使得TALE

能够特异性识别任何指定的DNA靶位点, 如果在C末端连接特定的结构域如转录抑制结构域、核酸酶结构域等则可以使其具备诸如转录修饰和基因组编辑等多种基因修饰功能 (Christian等2010; Mahfouz和Li 2011)。因此, 人工构建功能性TALE已经逐渐成为生物技术领域的研究热点。目前已经报道的构建方法主要包括基于IIS限制性内切酶法和基于同尾酶对法。其中, 根据具体实验操作的差异, 基于IIS限制性内切酶法又包括利用质粒文库的快速直接构建法、Golden Gate克隆法和PCR与Golden Gate克隆结合的方法。

#### 3.1 基于质粒文库和IIS型限制性内切酶的快速直接构建法

这是一种快速、有效的TALE分子克隆方法, 是基于含有TALE的DNA结合重复序列的100个质粒文库和IIS型限制性内切酶的方法 (图3)。IIS型限制性内切酶的特点是其识别位点和切割位点在空间上是分开的, 可以在与识别位点相隔的特定位点切割产生4 bp的5'粘性末端 (Engler等2008)。因此, 在不同的DNA片段的两端引入这类酶的识别位点, 经酶切后就能引入不同的粘性末端。通过这种设计可以使多个DNA片段根据5'粘性末端按照一定的顺序连接在一起 (Cermak等2011; Engler等2009; Weber等2011a)。研究者通过设计把TALE分子的重复区域分成7个片段, 第1个片段含有1个重复, 每个重复能分别识别A、T、G和C, 由此构建了4种质粒。第2~7个片段分别含有2个重复, 由于每个片段有16种组合, 即16种质粒, 因此, 质粒文库一共包含100种质粒。用指定的IIS型限制性内切酶BsmAI酶切这些质粒, 可以产生7个带有特定5'粘性末端的片段, 这些特定的5'粘性末端使得这些片段按照特定的顺序连接在一起。在第1和第7个片段两端分别是TALE本身含有的PpuMI和SacI酶切位点, 这样就可将克隆的重复区域片段连接到特定的载体骨架中。由于骨架本身在Gateway的Entry clone载体上, 所以, 装配好的TALE可以通过LR反应连接到所需终载体上, 用于细菌、酵母、植物或哺乳动物中的表达。这种方法可以构建包含13个重复序列的TALE。与其他构建方法相比, 这种方法相对简单、快速、直接有效, 一般5 d就可以完成全过程 (Li等2012)。通过这种方

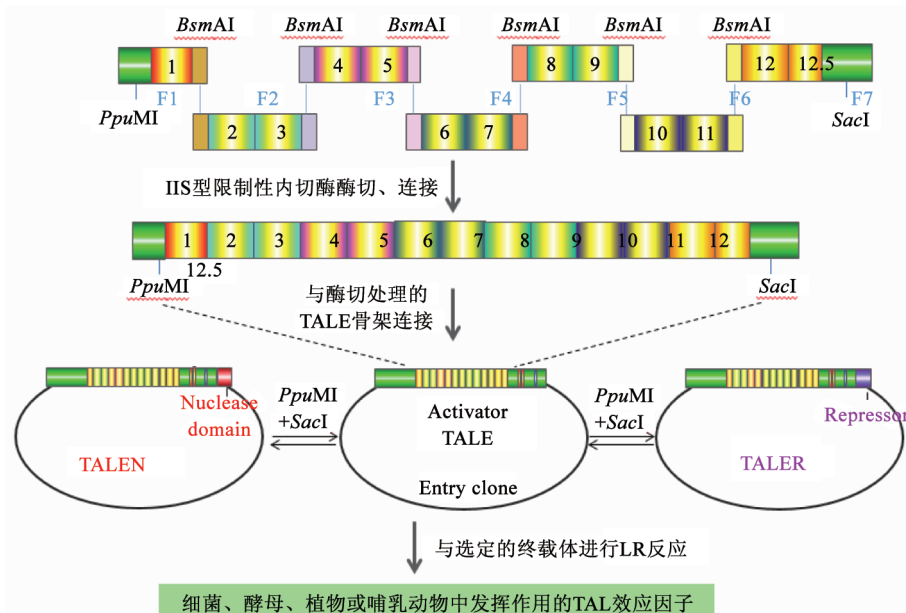


图3 基于质粒文库和IIS型限制性内切酶的快速直接构建法的总体构建策略

Fig.3 Construction strategy of TALE based on plasmid library and type IIS restriction endonuclease

参考Li等(2012)并作修改。

法构建的TALEN核酸酶和TALE-TF转录因子已经在体外实验、酵母、烟草植物的叶片中证明有活性(Li等2012)。

### 3.2 Golden Gate克隆法

Golden Gate克隆法也是基于IIS型限制性内切酶的方法。研究者把能够识别4种核苷酸的重复序列作为模板,通过PCR在其两端引入IIS型限制性内切酶识别位点,经酶切后,识别位点被切除并引入特定的4 bp的5'粘性末端,由此,每个重复单元能够按照特定的顺序进行连接。由于是单个重复序列,为了保证准确性,一般先把几个重复单元连接形成一个片段,再将这些片段按照相同的策略连接成完整的重复区域片段,并装配在TALE骨架上,完成构建(Geissler等2011; Li等2011; Morbitzer等2011; Scholze等2011; Weber等2011b)。Golden Gate克隆方法需要通过PCR克隆引入酶切位点,虽然增多了构建步骤,但不需要质粒文库。通过这种方法构建的TALE因子也具有活性(Geissler等2011; Li等2011; Morbitzer等2011; Scholze等2011; Weber等2011b)。

### 3.3 PCR与Golden Gate克隆结合的方法

与Golden Gate克隆法类似,该方法首先通过

PCR在每个重复单元两侧引入特异的IIS型限制性内切酶识别位点,经酶切后,识别位点被切除并引入特定的4 bp的5'粘性末端。随后,将这些重复片段连接成多聚体,并对多聚体片段通过PCR反应再次引入IIS型限制性内切酶识别位点。然后,将这些扩增产物进行第二次Golden Gate酶切连接,从而将多聚体重复按照特定的顺序组装起来。该方法构建的TALEN和TALE-TF的功能已在人HEK-293FT细胞中得到验证(Sanjana等2012; Zhang等2011)。

### 3.4 基于同尾酶的单位组合法

该方法是基于同尾酶*NheI*和*SpeI*的一种构建方法。同尾酶是识别位点不同,但能够切割产生相同粘性末端的酶。用同尾酶切割不同的DNA序列得到的产物连接在一起后,则不能再被其中任意一种酶切割。研究者以4种分别识别A、T、G和C的单重复序列为基础,利用PCR的方法在其重复序列两端分别引入*NheI*、*SpeI*和*HindIII*的酶切位点,重新连接在载体上,再对这些载体分别用*NheI*+*HindIII*或*SpeI*+*HindIII*双酶切,接着进行连接反应,*NheI*和*SpeI*产生的粘性末端连接产生双重复单元。然后把双重复单元进行多轮酶切和连接反

应构建DNA结合结构域并连接到骨架上,通过亚克隆转移到终载体上。利用这种方法构建的TALEN已经在斑马鱼胚胎中证明能够正常发挥作用(Huang等2011)。

#### 4 TALE的生物技术应用前景

随着人们对TALE的DNA结合特性的认识和RVD识别核苷酸序列的密码破译以及TALE效应因子构建方法的成熟,TALE被越来越多地用于生物技术领域。

TALE的DNA结合结构域具有高度的DNA识别特异性,重复单元能以任意顺序装配组合,因此可以结合任意序列的DNA靶位点(Szurek等2002)。TALE与不同的功能性结构域融合可以发挥多种功能(图4)。如TALE与核酸酶FokI的切割结构域融合产生嵌合的核酸酶TALEN。在多个系统和细胞类型中,TALEN可结合特定的DNA序列,在特异位点切割产生双链断裂(double-strand breaks, DSBs)(Cermak等2011; Christian等2010; Huang等2011; Li等2011; Mahfouz等2011b; Miller等2011; Sander等2011; Tesson等2011; Wood等2011)。DSB主要通过两种机制修复,即非同源性末端接合(non-homologous end-joining, NHEJ)修复机制和同源性的DNA重组(homologous recombina-

tion, HR)机制(Scholze和Boch 2011)。第一种机制可能伴随着断裂位点处小片段的缺失或插入,引起基因突变(Urnov等2010; Zhang等2010)。对于后一种机制,可利用其原理通过内源序列与外源提供的DNA片段的重组,实现靶位点基因的置换、敲除或堆叠(Cai等2009; Mussolino等2011; Urnov等2010; Weinthal等2010)。TALE可以与甲基化酶等表观遗传学修饰酶类或整合酶等融合,完成基因组中特定位点的修饰(Mahfouz和Li 2011)。另外,TALE也可以与转录抑制因子融合,调控目标基因的表达(Mahfouz等2011a)。人工合成的TALE不仅能特异性地激活植物基因,在其他物种包括人类中也能发挥重要的转录调控作用(Cermak等2011; Cong等2012; Mahfouz等2011a; Morbitzer等2010)。作为DNA结合模块,TALE在生物技术领域有着非常广阔的应用前景。对植物基因组进行定点修饰能大大加快目的基因增加、删除、活化或失活的速度,并且增加用于遗传修饰的植物种类,扩大了可供修饰的性状范围,加快了有利性状的积累(Mahfouz和Li 2011)。除了植物基因组,TALE在修饰其他物种的基因组中也发挥着重要作用。Miller等(2011)和Hockemeyer等(2011)利用TALE技术实现对人类基因组中特定基因的修饰,这对于

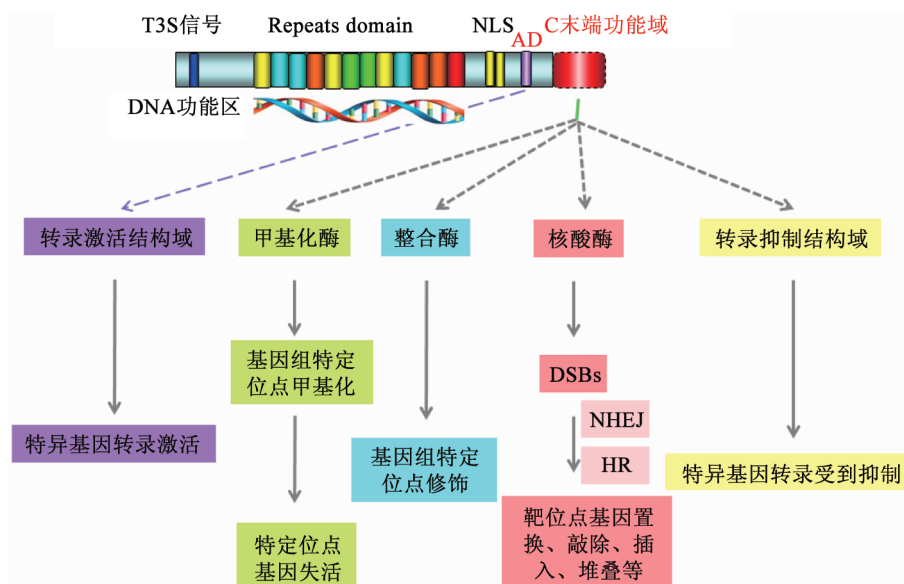


图4 TALE的生物技术应用前景

Fig.4 The biotechnology application prospect of TALE

参考Mahfouz和Li (2011)并作修改。

一些疾病的基因治疗具有重要意义。最近, Hockemeyer等(2011)将TALE技术用于人类胚胎干细胞(embryonic stem cell, ESC)和诱导多能干细胞(induced pluripotent stem cell, iPSC)的基因组修饰以研究相关基因的功能, 这进一步拓展了TALE技术的应用空间。

另外, 研究者们利用TALE的功能特点防御植物致病菌。将几种TALE靶位点序列组合在一个启动子中, 再将这个启动子引入抗性基因如*Bs3*上游, 这样, 转基因植株将会抵抗携带相应TALE的致病菌的侵染(Romer等2009)。

## 5 展望

锌指核酸酶(zinc finger nuclease, ZFN)作为一种特异性的基因组编辑工具已经发展了近15年。ZFN是一种人工改良的核酸内切酶, 由一个DNA识别结构域和一个核酸内切酶结构域组成。DNA识别结构域赋予ZFN特异性, 在特定的位点识别DNA, 而核酸内切酶结构域则可以在该位点进行非特异性剪切, 从而完成对DNA序列的定点断裂(Gabriel等2011; Pattanayak等2011; Urnov等2010)。与TALE识别DNA序列不同, ZFN识别特定碱基序列的三联体核苷酸, 这使其应用灵活性降低, 为设计和构建针对特定核苷酸序列的ZFN增加了难度。与ZFN相比, 以TALE为基础的DNA结合模块具有较高的序列特异性、较低的非靶位点结合能力和较低的细胞毒性。最重要的是, 在设计和构建TALE方面, 可以选择研究者指定的任意序列, 不存在技术上的难度。而且, 通过与核酸酶、转录抑制结构域、甲基化酶或整合酶等功能结构域融合, 由TALE派生形成的因子具有多种功能, 使TALE成为多面手, 应用空间更为广阔。因此, TALE技术受到青睐并迅速发展起来。但是, 由于TALE技术发展时间较短, 在某些方面还需要深入的研究以解决技术上的一些瓶颈问题。例如, 根据相同的RVD密码设计的TALE针对不同的靶位点具有不同的活性, 这很可能是由于存在有利于高效结合的序列依赖性, 也可能是由于存在DNA位点特异性限制因素(例如染色质的状态或DNA的甲基化等)。此外, TALE-DNA复合物的结构研究也留下了一些尚未解决的问题, TALE因子的N和C末端以及含有的结构域的结构尚未阐明。由于两端

结构的混乱, 最小的TALE DNA结合结构域也未能准确地限定。此外, TALE及其嵌合因子对植物细胞是否具有毒性仍需要进一步的实验验证, 目前尚无定论。尽管存在上述问题, 仍然有越来越多的研究者关注TALE技术的开发和利用, TALE技术已经逐步取代ZFN成为更加高效的基因工程技术。

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