

How does a single somatic cell become a whole plant?

植物再生的研究进展

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摘要 再生不仅赋予植物修复受损组织的能力, 更能使植物产生新器官, 实现营养繁殖. 再生能力是植物在严酷环境下能够生存的重要手段, 也被广泛应用于生产实践中. 组织培养、扦插和嫁接等都是基于植物再生能力而开发的农业技术. 再生现象的本质是细胞在受伤或胁迫的环境下命运发生转变的过程. 近年来, 植物再生领域的研究取得了一系列突破性进展, 不仅对植物再生过程中细胞命运转变的谱系有了初步认识, 而且探讨了植物细胞高度可塑性的分子机制. 伤口或胁迫信号、激素、转录因子和表观遗传途径因子形成有序协作的调控通路, 控制着再生过程. 本文将总结种子植物中器官从头发生和体细胞胚发生这两种再生方式的研究进展, 以期从事植物再生研究的工作者提供参考.

关键词 植物再生, 器官从头发生, 体细胞胚发生, 组织培养, 愈伤组织, 细胞可塑性, 细胞全能性, 细胞多能性

植物再生(plant regeneration)是指植物体对受损结构自我修复或替代的过程^[1~3], 这是植物适应环境的重要能力. 植物再生能力的基础是细胞的全能性(totipotency)或多能性(pluripotency), 反映出细胞命运的高度可塑性(flexibility或plasticity). 全能性是指细胞具有分化为完整个体的能力, 多能性是指细胞能够分化成为特定类群组织或器官的能力^[4].

植物再生能力被广泛应用于农业实践中^[5]. 组织培养、扦插和嫁接等技术都是基于植物再生能力而实现的. 组织培养是运用广泛的再生技术之一, 其概念提出已超过一个多世纪^[5]. 1902年, Haberlandt^[6]提出了组织培养(tissue culture)的概念, 设想可以利用体外培养的植物体细胞再生完整植株. 1957年, Skoog和

Miller^[7]提出了组织培养中使用外源激素的核心思路, 即生长素和细胞分裂素浓度的不同配比是植物再生出 不定根和不定芽的关键, 这使得组织培养的技术体系趋于成熟. 1958年, Steward等人^[8]实现了从胡萝卜(*Daucus carota* L.)根的离体韧皮部再生形成新的胚胎进而形成新植株的过程, 证实了植物细胞的全能性.

虽然组织培养技术被广泛而成熟的应用已经长达半个多世纪, 但植物再生过程的细胞谱系和控制再生的分子机制长期以来一直未被揭示. *Science*在创刊125周年时提出的125个科学前沿问题中第9个就是“单个体细胞如何形成完整植物(How does a single somatic cell become a whole plant)”^[9,10]. 对这一问题的解答无疑将极大推动植物再生技术的进步. 本文

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将对这一科学问题的研究进展进行综述,为这一领域的科学研究和技术开发提供参考。

1 植物再生概述

1.1 植物再生现象的归类

种子植物的再生有两种结果^[1]。(i)通过组织再生(tissue regeneration),也称为组织修复(tissue repair),植物体可以将受损的组织修复为受伤前的状态。例如,植物的根尖区域被切除后可以很快再生出新的根尖^[11~13],茎秆的伤口可以很快愈合^[14]。嫁接技术就是利用了植物组织修复的能力,将砧木和接穗的伤口修复在一起^[15]。(ii)植物在受损或受胁迫后,体细胞可以再生为新植株。这种再生结果可以通过器官从头发生(*de novo organogenesis*)和体细胞胚发生(somatic embryogenesis)两种方式实现。它们既可以在自然情况下发生于受伤的植物体,也可以在人为的组织培养条件下发生。本文将主要以模式植物拟南芥(*Arabidopsis thaliana*)的再生现象为例,重点阐述器官从头发生和体细胞胚发生的研究进展。

器官从头发生是指离体或受伤的植物器官上再生出不定根和(或)不定芽的过程^[1,16]。这一现象在自然界非常普遍。例如,景天科观赏植物胧月(*Graptopetalum paraguayense*)的离体叶片可以在伤口处直接再生不定根和不定芽,继而发育成为一棵新的植株(图1(a))。扦插技术就是基于直接的器官从头发生能力实现的。在组织培养中,器官从头发生也可以通过在培养基中添加外源激素间接发生:将离体的植物外植体放置在含有高浓度生长素和低浓度细胞分裂素的愈伤组织诱导培养基(callus-inducing medium, CIM)上首先诱导出一团具有多能性的非胚性愈伤组织(non-embryonic callus),然后将其转移至含有高浓度细胞分裂素和低浓度生长素的芽诱导培养基(shoot-inducing medium, SIM)上诱导不定芽产生,或移至含有低浓度生长素的根诱导培养基(root-inducing medium, RIM)上诱导产生不定根,完成整个植株的再生过程(图1(b))。

体细胞胚发生是指植物体细胞脱分化(dedifferentiate)为胚胎细胞后,通过胚胎发育的方式再生为

完整植株的过程。在组织培养中,体细胞胚发生通常需要利用胁迫条件、激素诱导(如生长素等)或改变基因表达使植物体细胞进行脱分化,直接诱导产生体细胞胚(somatic embryo)(图1(c))或通过胚性愈伤组织(embryonic callus)间接产生体细胞胚(图1(d))^[17]。体细胞胚的再生方式是植物细胞全能性的体现^[1,17,18]。

1.2 愈伤组织的定义

在植物伤口处、病虫害侵染的植物体上或组织培养的外植体上,会产生一团快速分裂的细胞,这些细胞团统称为愈伤组织(callus)^[19]。随着对植物再生机制的深入研究,发现在不同情况下产生的愈伤组织,其细胞学属性差别很大。有些愈伤组织没有再生能力,而有些则展现出不同类型的再生潜能^[2,19]。上文提及的非胚性愈伤组织和胚性愈伤组织是在组织培养中最常见的两种愈伤组织,它们通过不同的再生途径展现出植物细胞的高度可塑性:非胚性愈伤组织出现在器官从头发生过程中¹⁾,具有多能性,可能类似于多能的成体干细胞(adult stem cell),能够再生出不定根和不定芽,但再生过程中并不返回胚胎状态;胚性愈伤组织出现在体细胞胚发生过程中,体现出细胞的全能性,类似于全能的胚胎干细胞(embryonic stem cell),能够模拟出从胚胎到成体的整个发育过程。

1.3 控制植物再生的主要因素

无论哪种形式的再生过程,其本质都是在伤口或胁迫信号指导下的细胞命运转变^[1]。目前的研究揭示了控制再生的4个主要分子类群:伤口或胁迫信号、激素、转录因子和表观遗传途径因子(表观因子)。最早产生的伤口或胁迫信号可能是一些物理或化学信号,但目前并不清楚其本质和行为。在伤口或胁迫信号诱导下,激素行为是控制再生的重要因素^[20],其中生长素(auxin)、细胞分裂素(cytokinin)和脱落酸(abscisic acid)在控制细胞命运方面尤为重要。激素的控制对象通常是细胞命运转变的决定性基因,以转录因子和表观因子为主。转录因子和表观因子相互协作或制约,共同完成对整个基因组基因表达的重

1) 本文所述的“非胚性愈伤组织”,指组织培养中产生的、不具有胚胎属性的、并能够再生不定芽和不定根的愈伤组织。不包括其他情况下的愈伤组织,如树干伤口上产生的用于伤口愈合的细胞团

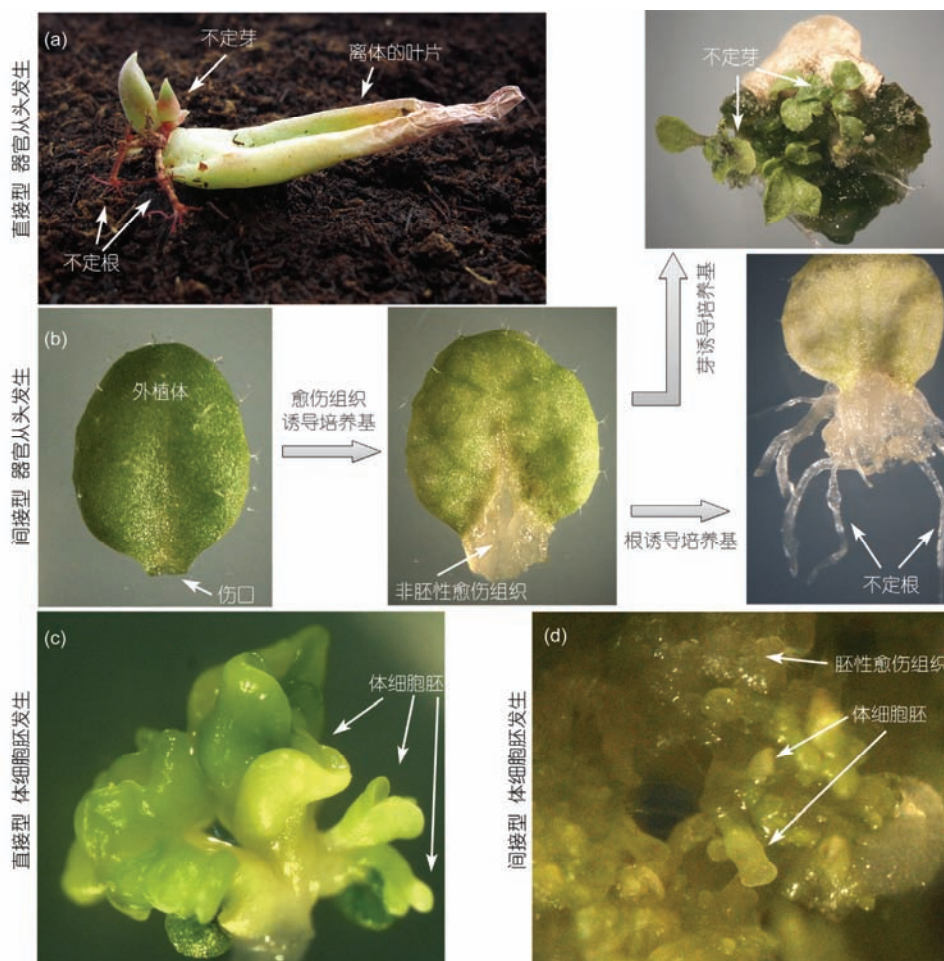


图 1 (网络版彩色)植物再生现象举例. (a) 景天科植物胧月的离体叶片进入直接型器官从头发生; (b) 组织培养中拟南芥叶片外植体进入间接型器官从头发生; (c) 组织培养中拟南芥直接型体细胞胚发生; (d) 组织培养中拟南芥间接型体细胞胚发生

Figure 1 (Color online) Regeneration in plants. (a) Direct *de novo* organogenesis in *Graptopetalum paraguayense*; (b) indirect *de novo* organogenesis in tissue culture of leaf explants from *Arabidopsis thaliana*; (c) direct somatic embryogenesis in tissue culture of *Arabidopsis thaliana*; (d) indirect somatic embryogenesis in tissue culture of *Arabidopsis thaliana*

排, 控制着代谢水平变化、细胞壁组分变化、细胞形态变化、细胞分裂等下游事件, 完成细胞命运的转变. 以上这些控制再生的分子类群之间也具有反馈效应, 形成网络发挥功能.

2 器官从头发生的机制

2.1 根从头发生

从受伤或离体的植物器官上再生不定根的过程, 称为根从头发生(*de novo* root organogenesis)^[1]. 很多离体的植物器官能够在伤口处快速再生出不定根, 保障离体器官吸收水分、维持生存, 这是直接型的根从头发生(图1(a), 图2). 在组织培养中, 将非胚性愈

伤组织移至根诱导培养基上再生出不定根的过程是间接型的根从头发生(图1(b)).

拟南芥离体叶片外植体再生不定根是研究直接型根从头发生的简单实用方法(图2)^[21]. 在这一方法体系中, 培养基中不添加外源激素, 不定根的从头发生完全依赖于叶片外植体的内源激素, 这相当于还原了自然界不定根发生过程^[21]. 通过这个体系, 可以利用不同的分子标记分析细胞命运转变过程的各个阶段. 根据已有实验结果结合已知的侧根发育的模型^[22,23], 不定根的出现大致可以分为4步: 引导(priming)、起始(initiation)、模化(patterning)和显现(emergence) (图3(a)中直接型根从头发生模型).

引导是根从头发生的第一个步骤, 通常发生在

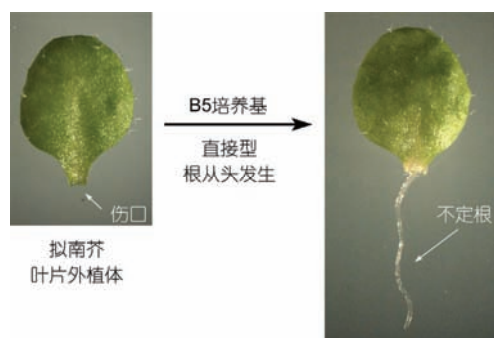


图 2 (网络版彩色)直接型根从头发生的研究体系. 将播种后 12 d 的拟南芥第一对真叶切下, 置于不含外源激素的B5培养基上, 在黑暗(培养基中加糖)或光照(培养基无糖)条件下培养, 可以在培养后 6~12 d 内观察到不定根再生于伤口处^[21]

Figure 2 (Color online) Method to study *de novo* root organogenesis. The first pair of leaves from 12-day-old *Arabidopsis thaliana* were cut and cultured on B5 medium without added hormones in dark conditions (with sucrose) or in light conditions (without sucrose). Adventitious roots formed from leaf explants around 6 to 12 days after culture^[21]

细胞分裂之前, 叶片被剪下后, 伤口快速发出信号, 引发内源生长素在叶肉等细胞中的合成^[24]. 生长素

合成后被极性运输到伤口处维管中的再生潜能细胞(regeneration-competent cell). 再生潜能细胞通常包括维管中的原形成层(procambium)和附近的一些维管薄壁细胞(vascular parenchyma cell)^[25~29]. 生长素在这些细胞中通过信号转导激活 *WOX11* (wuschel-related homeobox 11)和 *WOX12* 转录因子基因的表达, 这标志着再生潜能细胞命运转变为根创始细胞(root founder cell), 或称为根母细胞(root mother cell)^[25]. 抑制 *WOX11*/*WOX12* 基因的功能可以阻断根从头发生, 而增强 *WOX11*/*WOX12* 基因的表达可以剧烈促进根从头发生^[25]. 因此, 引导的结果是产生根创始细胞, 而 *WOX11*/*WOX12* 是标记根创始细胞并控制其发生的重要基因.

起始是指根创始细胞命运转变为根原基细胞(root primordium cell)的步骤, 这一步骤需要细胞分裂. 在根创始细胞中的 *WOX11*/*WOX12* 基因在这一命运转变中逐渐退出表达, 取而代之的是 *WOX5* 和 *LBD16* (lateral organ boundaries domain16)转录因子

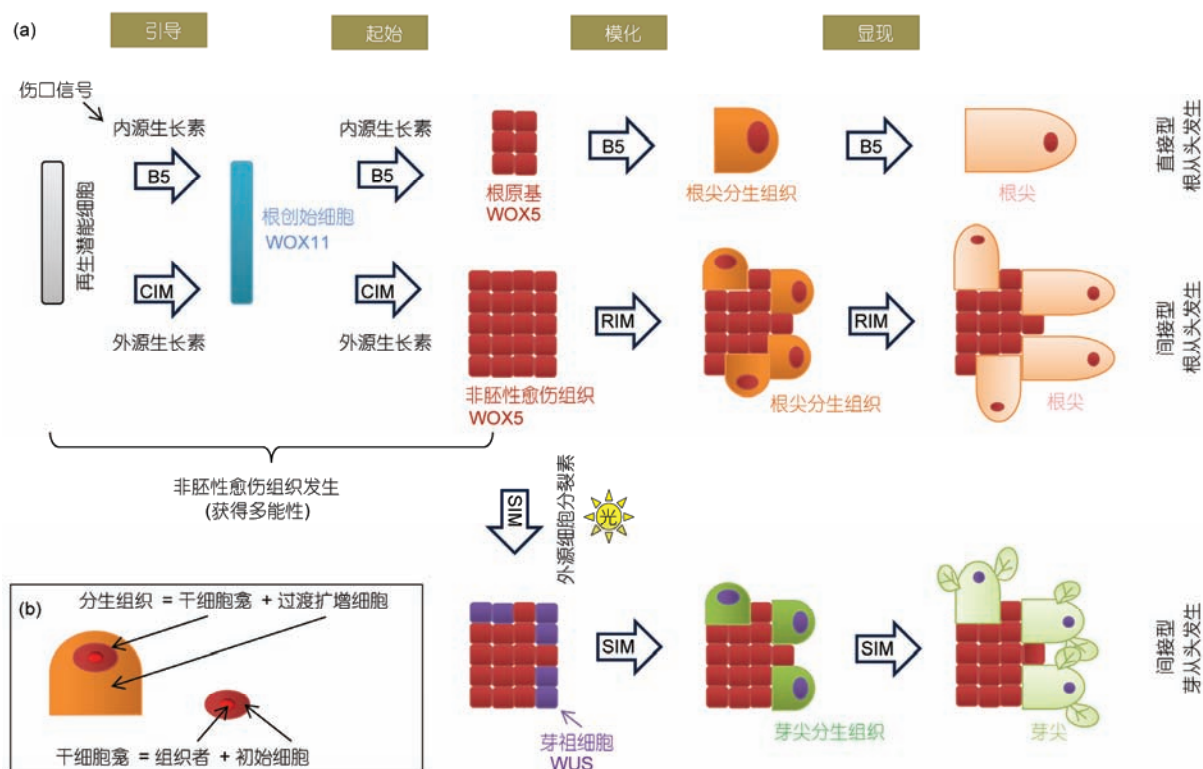


图 3 (网络版彩色)器官从头发生的模型. (a) 直接型根从头发生(B5 培养基)、非胚性愈伤组织发生(CIM)、间接型根从头发生(RIM)和间接型芽从头发生(SIM)的细胞谱系和分子机制模型, 其中RIM和SIM上的细胞谱系是根据推测绘制的; (b) 以根尖分生组织为例的分生组织模型^[31]

Figure 3 (Color online) Model of *de novo* organogenesis. (a) Model of cell lineages in direct *de novo* root organogenesis (B5), non-embryonic callus formation (CIM), indirect *de novo* root organogenesis (RIM) and indirect *de novo* shoot organogenesis (SIM). Cell lineages in RIM and SIM are predicted based on hypothesis; (b) model of meristem organization in RAM^[31]

基因在根原基中的表达(未发表数据)^[25]。在根原基起始中, *WOX11*/*WOX12*能够激活*LBD16*的表达, 说明引导和起始这两个步骤有着分子上的继承关系^[25]。根原基通常是一个圆顶状、有序排布的多层细胞团; *WOX5*基因在根原基各细胞中较为均匀的表达。由于*WOX5*基因也是根尖干细胞龛(stem cell niche)中的特征基因^[30], 因此根原基本质上可能是处于原始状态的根尖干细胞团。

模化是指根原基分化为根尖分生组织(root apical meristem, RAM)的步骤。在这一步骤中, 根原基细胞团继续分裂, 并逐步明确功能分区, 形成一个新生的、最初的分生组织(meristem)。分生组织是一种高度有序、功能分区和分化程度较低的细胞集群, 其核心为干细胞龛, 它由位于中央的组织者(organizer; 在根中称为静止中心(quiescent center, QC))和围绕在其周围的初始细胞(initial cell)组成。组织者是几个很少分裂的细胞, 能向初始细胞发出信号, 维持初始细胞的干细胞属性(干性, stemness)。初始细胞就是传统意义上的干细胞, 它每次进行细胞分裂后, 其中一个细胞仍维持初始细胞干性(体现出自我维持的属性), 另一个细胞则分化为过渡扩增细胞(transient-amplifying cell) (体现出分化潜能的属性)。过渡扩增细胞能够快速分裂, 并逐步分化为组成各种具体组织的细胞。过渡扩增细胞和干细胞龛共同组成了分生组织(图3(b))^[31]。在模化过程中, *WOX5*基因的表达开始逐渐聚集到干细胞龛中, 而围绕在干细胞龛周围将发育为各成熟组织的过渡扩增细胞逐步形成(未发表数据)。

显现是指在根尖分生组织带动下形成不定根根尖并顶出外植体的步骤。此时, 位于分生组织中的各区域完成了功能群分化, 成熟的根尖分生组织已经构建完成, 各类过渡扩增细胞不断分裂和分化, 形成了根冠、表皮、皮层、内皮层和中柱等结构。这使得根尖开始快速伸长, 顶开周围外植体的细胞, 形成成熟的不定根。这一过程可能需要*NAC1* (petunia NAM (no apical meristem))和*Arabidopsis ATAF1*, *ATAF2*和*CUC2* (cup shaped cotyledon 2))和其同源基因的作用来改变伤口处新生根尖周围的细胞环境, 协助根尖顶出^[32]。

这一连续的4步(引导、起始、模化和显现)反映出直接型根从头发生过程中伤口信号、激素行为、细胞命运连续转变、伤口微环境发生改变的一系列连续

过程(图3(a)中直接型根从头发生模型)。

很多植物在发育过程中也会产生不定根, 如组成水稻(*Oryza sativa*)须根系的冠根、一些木本植物树干上的气生根、拟南芥下胚轴上的不定根等。这些自发产生不定根的发育途径也使用了根从头发生中的重要基因。例如, *WOX11*和*LBD*基因能控制水稻冠根(一种类型的不定根)发生^[33,34]。这说明再生和发育中不定根发生过程可能有类似的分子机制。

2.2 非胚性愈伤组织的发生

在愈伤组织诱导培养基上, 外植体可以被高浓度生长素诱导出非胚性愈伤组织(图1(b))。由于非胚性愈伤组织可以进一步诱导生根和生芽, 但无法产生胚胎结构, 因此非胚性愈伤组织具有多能性。长久以来, 人们对非胚性愈伤组织的认识有误区。传统理论认为, 非胚性愈伤组织是一团脱分化或未分化细胞, 并且每一个体细胞都应该具有起始非胚性愈伤组织的能力^[2]。直到近年来对非胚性愈伤组织的发生部位、转录组、表观组和细胞谱系的分析, 才逐步揭示出非胚性愈伤组织的细胞学本质, 提出了与传统认识不同的新概念: 非胚性愈伤组织的细胞学属性类似于根原基细胞; 并非任何一个体细胞都能被诱导产生非胚性愈伤组织, 它只来源于特定的再生潜能细胞^[2,25,35~38]。在拟南芥中, 起始非胚性愈伤组织的再生潜能细胞包括根中的木质部侧中柱鞘细胞(xylem-pole pericycle cell)和叶中的原形成层与维管薄壁细胞^[25,35~37,39]; 前者是侧根起始的细胞, 而后者是根从头发生过程的再生潜能细胞。因此, 非胚性愈伤组织的发生位置与侧根和不定根的发生位置一致。

通过转录组研究发现, 非胚性愈伤组织的转录组与根尖分生组织中的干细胞龛区域类似^[37]。通过组蛋白H3第27位赖氨酸三甲基化修饰(H3K27me3)的表观组分析发现, 从叶外植体发生非胚性愈伤组织的过程是一个从叶特征转变为根特征的过程^[38]。这些都暗示着非胚性愈伤组织的发生过程可能类似根发生过程。

利用拟南芥根从头发生过程中的根创始细胞特征基因*WOX11*和根原基细胞特征基因*WOX5*进行细胞谱系标记发现, 在再生潜能细胞命运转变为非胚性愈伤组织的过程中, 也需要引导和起始步骤。首先, 培养基中的外源生长素促使叶片外植体中的再生潜能细胞命运转变为由*WOX11*标记的创始细胞,

完成引导步骤;接着,生长素继续促进创始细胞快速分裂,形成由WOX5标记的非胚性愈伤组织,完成起始步骤^[25].非胚性愈伤组织的发生也依赖于多个LBD基因的参与^[40],这也说明该过程与根从头发生过程类似.

综合上述研究结果,目前的观点趋向于认为非胚性愈伤组织的发生过程借用了根发生的机制,非胚性愈伤组织的细胞学属性是一团在外源激素(主要是高浓度生长素)刺激下快速分裂的类根原基细胞(root primordium-like cells)(图3(a)中非胚性愈伤组织发生)^[25].因此,可以将非胚性愈伤组织理解为大量聚合成簇的类似根原基的细胞团.根原基属性也是非胚性愈伤组织具有多能性的本质(可参考3.3小节芽从头发生).

在理想状态下,高浓度的生长素可能促进非胚性愈伤组织细胞持续分裂且维持在根原基阶段(即停止在模化步骤之前).但在组织培养的实际操作中,由于外植体与培养基接触不均匀、培养基中激素活性降低以及植物体内源发育信号的推动等因素,愈伤组织会进入部分分化状态.通常肉眼可见的非胚性愈伤组织都已部分分化.因此常可以看到根毛状细胞出现在培养较久的非胚性愈伤组织上,有时甚至能看到长出不定根.而且非胚性愈伤组织培养过久或继代后,实现器官从头发生的能力会明显下降.这些现象都说明,当非胚性愈伤组织的根原基属性无法维持时,根从头发生的步骤(模化和显现)将继续推进,多能性逐渐消失(未发表数据).

由于非胚性愈伤组织可以理解成大量拥簇成堆的类根原基细胞团,因此当培养基中撤去高浓度生长素(如放置于不含外源激素的B5培养基)或使用极低浓度的生长素(如放置于根诱导培养基,图1(b)),这堆类似根原基的细胞团就会自发进入模化和显现步骤,最终形成大量的不定根(未发表数据),这就是间接型的根从头发生(图3(a)中间接型根从头发生模型)^[1].

2.3 芽从头发生

芽从头发生(*de novo shoot organogenesis*)是指受伤或离体的植物器官上再生出不定芽的过程,也可以分为直接型(从伤口处直接再生不定芽,图1(a))和间接型(从非胚性愈伤组织上再生不定芽,图1(b))^[1].由于直接型的研究相对较少,本文主要描述间接型

的研究进展.在组织培养中,间接型芽从头发生需要经历两个阶段,分别在愈伤组织诱导培养基和芽诱导培养基上完成.

第一阶段是在愈伤组织诱导培养基上获得具有多能性的非胚性愈伤组织,这一阶段可以称为多能性获得阶段(acquisition of pluripotency,或称为芽发生潜能获得阶段(acquisition of competence for shooting)).在前文中提到,根原基属性是非胚性愈伤组织具有多能性的本质,只有具有这一属性的非胚性愈伤组织才能够再生出不定芽.利用根原基发育缺陷的*plt3 (plethora3) plt5 plt7*三突变体^[41]的研究发现,其外植体产生的非胚性愈伤组织中根原基属性不完整,因此没有再生不定芽的能力^[42].利用根原基诱导不定芽的再生体系也说明,不定芽来源于带有根原基属性的细胞^[43,44].

第二阶段是在芽诱导培养基上再生不定芽.这一阶段通常需要在光下培养,此时非胚性愈伤组织颜色由白(或浅黄)转绿.具有多能性的非胚性愈伤组织在富含细胞分裂素的芽诱导培养基上能够产生不定芽,这说明细胞分裂素是芽从头发生的关键激素.控制植物年龄途径的SPL (squamosa promoter binding protein-like)蛋白会影响细胞分裂素的信号转导通路,因此不同年龄的外植体表现出不同的不定芽再生效率^[45].但细胞分裂素和光信号在不定芽发生中的具体分子功能并不清楚.

第二阶段中关键的分子变化是非胚性愈伤组织的一些区域表达WUS (wuschel)和CUC2基因,形成芽祖细胞(shoot progenitor cell)^[46].WUS和CUC2基因的表达受到其他转录因子和表观遗传修饰的调控.例如,PLT3/PLT5/PLT7可以促进CUC2和其同源基因CUC1的表达^[42],ESR2 (enhancer of shoot regeneration 2)可以促进CUC1的表达^[47],在WUS基因座位上移除抑制型的表观标记也是WUS表达的重要因素^[48].WUS是芽尖干细胞特征基因,类似于WOX5在根尖干细胞的功能^[30,49],因此芽祖细胞可能是处于原始状态的芽尖干细胞团.

芽祖细胞形成后,就开始进入模化步骤,分化为芽尖分生组织(shoot apical meristem, SAM),STM (shoot meristemless)转录因子基因也开始在该区域表达^[46].细胞分裂素和WUS共定位在芽尖分生组织的中央位置,其外围绕生长素的分布及生长素相关基因的表达^[50,51].此时,WUS表达逐步聚集到干细胞

龕的组织者中(在芽中称为组织中心(organizing center, OC)), 芽尖分生组织中各个区域逐渐形成功能分区。但目前并不清楚这些激素和基因在芽尖分生组织功能分区时是如何有序发挥功能的, 这一过程有可能与细胞分裂素和生长素之间的平衡有关。在此之后是显现步骤, 芽尖分生组织继续分化成为成熟的芽尖, 并产生叶片。

综合上述研究, 芽从头发生也是一个细胞命运逐步转变的过程(图3(a)中间接型芽从头发生模型)。芽从头发生过程与根从头发生过程都需要建立具有WOX家族基因(芽从头发生中的WUS和根从头发生中的WOX5)所标记的原始干细胞团, 即芽祖细胞与根原基。有趣的是, 芽祖细胞似乎是在根原基或非胚性愈伤组织的基础上出现的。

芽从头发生中控制细胞谱系发展的机制并不十分清晰, 且各重要基因开启的具体部位、时间及与激素的关系也较为模糊。这可能是因为从非胚性愈伤组织上再生不定芽的过程快速且不易观察。自然界中存在很多直接型芽从头发生的例子(如图1(a)所示的离体叶片再生不定芽, 以及在木本植物树干上发生的不定芽等), 但是直接型芽从头发生的机制和其与间接型之间的关系仍然未知。建立简单而有效的直接型芽从头发生的研究体系可以帮助全面了解不定芽产生的细胞谱系和分子机制。

3 体细胞胚发生的机制

3.1 体细胞胚发生的部位和诱导因素

通常认为每一个植物细胞都具有脱分化的潜力。

Steward等人^[8]将胡萝卜根的离体韧皮部细胞通过体细胞胚发生的方式获得完整新植株, 这说明与器官从头发生不同, 已高度分化的体细胞也可以实现脱分化。以拟南芥为材料的研究发现, PcG (Polycomb group)的突变体中根毛细胞可以脱分化为胚胎细胞^[52]; 对紫椴(*Tilia amurensis*)的研究发现, 表皮毛也可以脱分化为体细胞胚^[53]。这些研究体系清晰地说明高度分化的表皮细胞也具有发生体细胞胚的潜力。目前, 缺乏证据来说明这些能进行脱分化的细胞之间是否有分子上或细胞学上的共性。如果发现这种共性, 或许可以回答“是否植物的所有体细胞都具有脱分化潜能”这一问题。

环境胁迫和激素诱导是体细胞胚发生的重要因素^[17,54]。在特定环境胁迫和(或)激素诱导条件下, 体细胞可以直接脱分化为体细胞胚(直接型体细胞胚发生, 图1(c)), 也可以先脱分化为胚性愈伤组织后在其基础上诱导体细胞胚的出现(间接型体细胞胚发生, 图1(d))^[17]。目前没有确定这两种发生方式是否利用相同的机制(图4中的问号)。同样, 胁迫信号是什么以及信号发挥功能的分子机制也并不清楚。一种可能性是胁迫信号通过脱落酸或(和)生长素来发挥功能。

3.2 控制体细胞胚发生的激素

体细胞胚的发生过程由多种激素共同控制。植物胚胎细胞和成体细胞的激素水平是不同的: 成体细胞表现出低脱落酸、高赤霉素含量的特征, 而胚胎细胞表现出高脱落酸、低赤霉素含量的特征^[55-63]。因此, 在体细胞胚发生过程中, 细胞内的脱落酸水平需要提高, 而赤霉素的水平则会下降。

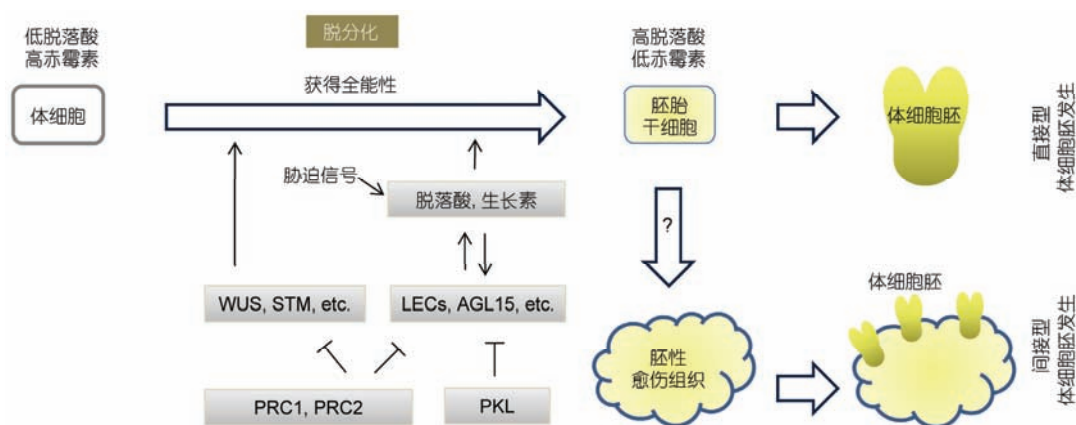


图4 (网络版彩色)体细胞胚发生的模型。根据推测绘制的直接型和间接型体细胞胚发生模型

Figure 4 (Color online) Model of somatic embryogenesis. Predicted processes of direct and indirect somatic embryogenesis

生长素也是促进体细胞胚发生的重要激素。在诱导胚性愈伤组织的培养基中,通常会加入高浓度的生长素类似物2,4-D (2,4-dichlorophenoxyacetic acid)来促发体细胞进行分裂,并进入脱分化过程,形成胚性愈伤组织^[64~73]。将胚性愈伤组织转移到不含生长素的培养基后,在一些区域会出现内源生长素的极性分布,并诱导WUS基因的表达,这是起始胚胎发生的重要步骤^[70]。

另外,乙烯也是参与体细胞胚发生的激素^[67,74]。因此,体细胞胚发生是一个激素平衡发生改变的过程(图4)。但目前对于激素控制体细胞胚发生的分子机制认识仍然比较缺乏,激素信号通路的直接靶基因和激素之间的相互协作和制约都需要进一步解析。

3.3 控制体细胞胚发生的基因

在拟南芥的遗传学研究中,发现了一些参与体细胞脱分化为胚胎细胞的基因。这些基因大致可以分为3类。

(i) 胚胎发育特征基因。这些基因主要包括 *LEC1* (leafy cotyledon1), *LEC2*, *FUS3* (fusca3) 和 *AGL15* (agamous-like15) 等^[63,75~81]。在体细胞中过量表达这些基因都可以引起体细胞向胚胎细胞的转变^[75,76,78]。深入的研究发现,这些基因之间可以相互促进彼此的表达;它们也控制着大量的激素途径基因的表达,如生长素、脱落酸和赤霉素途径的基因,而且这些基因自身的表达也受到激素的控制^[63,82~90]。因此,这些基因与激素之间形成了复杂的网络调控关系。另外, *PGA37/MYB118* (plant growth activator 37/myb domain protein 118) 和 *MYB115*^[91], *AP2* (apetala2) 结构域转录因子基因 *BBM* (baby boom) 和 *EMK* (embryomaker)^[92,93], 以及 B3 结构域基因 *ABI3* (ABA insensitive 3)^[83,89,94] 也都具有促进体细胞胚发生的能力。

(ii) 与芽顶端分生组织相关的基因^[70,95~98]。*WUS* 在根中的异位表达能够促使根部细胞形成体细胞胚^[99], 但至于芽顶端分生组织相关基因如何促使体细胞胚现象出现,其分子机制目前还无法清楚解释。

(iii) 一些表观遗传途径的因子,如 PcG 途径和 ATP 依赖型染色质重塑因子 PKL (pickle)。PcG 途径在拟南芥中由 PRC2 (polycomb repressive complex 2) 和 PRC1 两个蛋白复合体组成,它们分别具有 H3K27me3 和 H2A 单泛素化(H2Aub)的催化活性。这两个蛋白复

合体能够在体细胞中抑制胚胎发育特征基因和芽尖特征基因的表达,因此它们的突变体都会出现在胚后发育阶段的植物器官上产生胚状结构的表型^[100~107]。在 *LEC2* 的启动子上发现了 *RLE* (repressive lec2 element) 顺式作用元件,它被确认为用于招募 PRC2 至 *LEC2* 座位上实现组蛋白 H3K27me3 修饰^[108]。B3 结构域蛋白 VAL1 (VP1/ABI3-LIKE1) 和 VAL2^[109] 能够通过和 PRC1 相互作用后招募 PRC1 抑制胚胎特征基因的表达^[110]。PKL 也具有抑制胚胎发育特征基因的能力,其突变体表现出胚后发育的根部出现胚胎特征^[111~116]。因此,这些表观因子通常是体细胞胚发生的抑制因素,它们的发育学功能是通过抑制胚胎特征基因和芽尖特征基因的表达来维持正常发育植物体上的体细胞特征,防止它们出现脱分化现象。

由此可见,诱导体细胞胚发生是一个从胁迫开始,通过激素、胚胎特征基因和芽尖特征基因组成的调控网络来驱动体细胞向胚胎细胞发生脱分化的过程,表观因子通常是这一过程的抑制因素(图4)。但体细胞胚的发生过程中仍有很多机制尚不清楚,包括胁迫信号的本质是什么、脱落酸和生长素控制细胞脱分化的主效下游通路是什么等问题。此外,体细胞胚发生整个过程的细胞谱系尚不清晰。这些重要问题的解答将能进一步帮助理解植物细胞脱分化和全能性。

3.4 落地生根的无性繁殖

体细胞脱分化为胚胎的现象在自然界中也能发生,例如,景天科落地生根属(*Bryophyllum*)的植物在叶边缘可以无性繁殖出新胚胎,发育成完整的新植株(图5)^[117,118]。对大叶落地生根(*Kalanchoë daigremontiana*)



图5 (网络版彩色)落地生根的无性繁殖
Figure 5 (Color online) Asexual reproduction in *Bryophyllum*

montiana)的研究发现,在这一脱分化过程中,可以检测到两类基因的表达^[117]。(i) *STM*基因,它原本是芽顶端分生组织的特征基因^[119],在胚胎阶段建立芽顶端和胚后阶段维持芽顶端的过程中都有重要功能。当通过RNAi降低*STM*的表达之后,大叶落地生根植株的叶边缘就无法产生小植物。(ii) *LEC1*和*FUS3*基因,它们是胚胎特征基因^[63],这两个胚胎基因的表达标志着胚胎发育的产生。大叶落地生根的这种无性繁殖方式说明植物细胞在自然环境下就具有脱分化为体细胞胚的能力。

4 讨论与展望

4.1 植物干细胞与再生

干细胞是原始未分化的细胞,具有两个重要属性^[120,121]:自我维持,即具有维持未分化状态(干性)的能力;分化潜能,即具有分化为有具体功能细胞的能力。以拟南芥为例,传统上认为干细胞特指干细胞龛中围绕在组织者周围的初始细胞(详见2.1中对根尖分生组织的描述),以及侧生分生组织中的原形成层等细胞^[31,122]。干细胞不仅是植物发育中各组织和器官形成的源头,也是再生的源头。随着植物再生理论的发展,植物干细胞的概念得到不断补充。

在器官从头发生过程中,并非所有的体细胞都有起始愈伤组织、不定根和不定芽的能力,通常它们只起始于再生潜能细胞。Sugimoto等人^[2]设想,这些再生潜能细胞(原文中假设为类中柱鞘细胞, pericycle-like cell)可能类似于动物体内的成体干细胞,因此这类细胞可以视作植物的一种成体干细胞。在动物中,蜗虫(planarians)和蝾螈(salamanders and newts)是研究再生常用的模式生物。蜗虫的很多组织切除后能够再生,蝾螈四肢切除后也可以再生。在这些过程中,体内潜伏的成体干细胞在受伤后快速移动到伤口处,并有序地分化成为各类组织以修复失去的机体,在伤口附近未充分分化的细胞也可能转变为成体干细胞参与修复^[2,123~125]。植物细胞虽然不能移动,但这些潜伏在植物体内的成体干细胞在受伤后能够很快改变各自的命运从而进入再生过程。新生的根原基细胞、非胚性愈伤组织细胞和芽祖细胞也都具有类似于成体干细胞的属性。因此,器官从头发生的前期步骤可能是不同类型成体干细胞之间转分化(transdifferentiation)的过程(再生潜能细胞→根原基

细胞/非胚性愈伤组织细胞→芽祖细胞)^[1,2]。

体细胞胚发生类似于将动物体细胞脱分化为诱导型多能性干细胞(induced pluripotent stem cell, iPS cell)或胚胎干细胞的过程^[126,127]。植物体细胞的这种脱分化比动物体细胞更易进行。动物的体细胞能够在外源导入某些特定的基因或在特定条件下实现脱分化,但频率很低^[126];而植物的体细胞能够在激素或胁迫的诱导下高效地发生脱分化。

因此,与动物再生现象非常类似,植物的再生过程也需要由多种干细胞(或类干细胞)参与。将干细胞概念引入植物再生领域能更好地理解植物再生的机制。

4.2 植物发育与再生

与发育过程不同的是,植物的再生通常是在受伤和胁迫下完成的。但是,再生与发育有着通用的规律和基本原则,再生过程和发育过程共享并互相借鉴了很多激素与分子途径。

以干细胞龛为例可以解释器官从头发生与发育的关系。干细胞龛是干细胞的高级组织形式。在拟南芥正常发育过程中,生长素是维持根尖干细胞龛活性的核心激素^[128],而细胞分裂素是维持芽尖干细胞龛活性的核心激素^[129~135]。因此,器官从头发生过程中利用了生长素促进不定根和非胚性愈伤组织的发生,而利用细胞分裂素促进不定芽的发生。*WOX5*是控制根尖干细胞龛的核心基因^[30],因此不定根从头发生和非胚性愈伤组织发生中需要激活*WOX5*的表达。而*WUS*是控制芽尖干细胞龛的核心基因^[49],因此不定芽从头发生需要激活*WUS*的表达。

脱落酸和赤霉素的比例变化和胚胎特征基因的沉默是植物从胚胎状态经历发芽过程发育成为成体植株的重要事件。胚胎细胞含有高水平脱落酸和低水平赤霉素。发芽后,正常发育的体细胞中含有低水平脱落酸和高水平赤霉素^[63]。因此在体细胞胚发生过程中,就需要提高体细胞中的脱落酸含量,使其命运转变为胚胎细胞。从分子角度看,将特异控制胚胎的重要基因异位表达在体细胞中,也能促使体细胞胚的发生^[63]。

这些例子都说明,再生过程和发育过程可能在进化上有着类似的起源,并有共同遵循的分子机制。大叶落地生根通过体细胞胚发生进行无性繁殖和拟南芥下胚轴上产生不定根过程都是正常的发育过程,

而这些过程采用的分子机制又与再生过程的机制极为相似。目前尚不知道这些类似再生的发育过程是否需要植物体自发模拟伤口或胁迫信号。

4.3 各物种间再生能力的差异

在组织培养中,不同种类的植物表现出再生能力的巨大差异。例如,双子叶模式植物拟南芥、烟草(*Nicotiana tabacum*)和番茄(*Solanum lycopersicum*)等,表现出非常强大的器官从头发生能力,但是单子叶禾本科的模式植物水稻、小麦(*Triticum aestivum*)和玉米(*Zea mays*)等的成熟组织难以进行组织培养^[136~145]。拟南芥再生过程中的细胞谱系和分子机制是否在其他物种中具有保守性?各物种间再生能力的差异是因为物种本身发育方式的不同,还是它们对伤口信号和激素具有不同的响应能力?对这些有趣的现象

进行研究将会完善对植物再生机制的理解。

4.4 植物再生技术革新的展望

在组织培养中,提高植物再生能力的传统方法是改变培养基中激素和营养物质的配方。研究人员期望了解植物再生的细胞谱系和分子机理,将其应用于控制植物的再生能力。例如,过量表达 *WOX11* 和 *LBD* 基因可以促进非胚性愈伤组织的发生^[25,40],因此这些控制细胞命运转变的基因可能会是组织培养中定向控制植物外植体再生的分子工具。最新的研究发现,长链脂肪酸也具有调节非胚性愈伤组织发生的能力,也为组织培养的应用提供了新思路^[146]。将这些新方法运用到植物再生技术中,或许可以让植物细胞的多能性和全能性在植物活体上得以体现,从而使无性繁殖技术有新的突破。

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Recent progress on plant regeneration

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For survival from severe natural conditions, plants have evolved powerful regenerative abilities, conferring specific cells with totipotency or pluripotency. The regenerative abilities have been widely exploited in agricultural production, and the commonly used technologies include cuttage, engraft and the propagation through plant tissue culture.

In seed plants, regeneration usually results in two different consequences. Firstly, damaged tissues can be repaired by tissue regeneration; Secondly, certain somatic cells can be used as a source to regenerate a whole plant via *de novo* organogenesis or somatic embryogenesis. In this review, we mainly summarize the recent advances in *de novo* organogenesis and somatic embryogenesis in seed plants, and intend to provide useful information to the plant scientists, especially those who are interested in the improvement of agricultural applications of plant regeneration.

De novo organogenesis refers to the formation of adventitious roots or shoots from the regeneration-competent cells in wounded or detached plant organs. During *de novo* organogenesis, the regeneration-competent cells, such as procambium, pericycle or other parenchyma cells in the vasculature of various plant tissues, do not experience a dedifferentiation process backward to the embryo-stage state. *De novo* organogenesis may occur directly from the regeneration-competent cells in cultured explants, or progress indirectly from the non-embryonic callus. Interestingly, non-embryonic callus formation from different plant organs follows a common mechanism and appears to be the ectopic activation of a root development program. Therefore, unlike previously believed, non-embryonic callus consists of a group of root primordium-like cells.

During somatic embryogenesis, differentiated cells change their fates to become embryonic cells via dedifferentiation. The somatic embryos can be formed either directly from somatic cells or indirectly from embryonic callus. Plant hormones, genes involved in embryo development and shoot apical meristem maintenance, and some epigenetic factors play key roles in either direct or indirect somatic embryogenesis.

The underlying theme of plant regeneration is the cell fate transition upon wounding or stress. In recent years, our knowledge about cell lineage during the fate transition in plant regeneration and molecular mechanism that directs the cell fate transition has been greatly improved. These benefit our understanding of the plant cell flexibility significantly. Wound and stress signals, actions of phytohormones and functions of transcription factors and epigenetic factors were explored in different types of plant regeneration. It becomes clear that wound and stress signals induce phytohormone actions at the earliest stage of regeneration. While auxin is required for *de novo* root organogenesis and callus formation, cytokinin triggers *de novo* shoot organogenesis. In somatic embryogenesis, auxin and abscisic acid play key roles in cell dedifferentiation. The phytohormone actions usually result in expressional changes of many key transcriptions factors, which act together or coordinate with epigenetic factors to control changes of transcriptomes in the cells for their fate transition.

Despite the very rapidly progresses, many questions still remained unanswered in the regulation of plant regeneration. What is the molecular basis of wound and stress signals? Can any kind of cells in the plant undergo dedifferentiation to form somatic embryo? What is the common molecular basis for cells to acquire the regeneration competence? What are the molecular mechanisms guiding actions of phytohormones, transcription factors and epigenetic factors? What is the cell lineage during fate transition of different plant cells in regeneration? All these questions need to be further addressed in the future.

plant regeneration, *de novo* organogenesis, somatic embryogenesis, tissue culture, callus, cell flexibility, cell totipotency, cell pluripotency

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