

· 专题论坛 ·

## 植物细胞质膜离子通道研究进展

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**摘要** 多种有机和无机离子作为重要的营养物质、渗透物质、辅酶和信号分子, 参与植物生殖、生长发育和逆境反应等多种生物学过程。离子通道是离子跨质膜和内膜运动的重要渠道和动态调控因子, 直接影响和调控细胞内离子浓度及亚细胞分布的动态变化。目前, 植物尤其是模式植物拟南芥(*Arabidopsis thaliana*)的多个离子通道家族被先后鉴定出来, 其中部分离子通道蛋白定位在细胞质膜上, 其基本生物学功能, 诸如蛋白结构、离子选择性和通透性、门控特点、活性调控机理以及不同离子通道之间的协同关系等均取得重要进展。该文概要介绍近年来植物细胞质膜离子通道方面的研究进展。

**关键词** 离子, 跨膜运动, 离子通道, 细胞质膜, 植物细胞

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多年来, 大量研究揭示了多种植物细胞离子通道的功能和基本特性, 发现大多数离子通道都具有一定的离子选择性和方向性, 即一种离子通道往往只允许一种或一类离子单方向通过, 而不允许其它离子通过, 也不允许或限制离子的反向通过。植物的生殖、生长发育、信号传递和逆境反应等需要多种不同离子物质的频繁跨膜流动, 并精细控制其跨膜流动的动态变化。因此, 对离子需求的多样性决定了植物必须具有多种不同的离子通道, 分别负责介导和调控一种或一类离子的跨膜运输, 并对每一种离子通道的活性进行精细调控。离子通道在植物细胞质膜和内膜系统均有广泛分布, 分别负责调控离子进出细胞和在细胞内的动态分布。因此, 植物细胞离子通道可以根据其亚细胞定位分为内膜离子通道和质膜离子通道; 也可以根据其离子通透性和选择性划分为阳离子通道和阴离子通道。阳离子通道还可以进一步分为单价和二价阳离子通道。在高等植物基因组中, 每一类离子通道都有多个基因家族被鉴定出来, 其部分成员具有重要的生物学功能。本文将概要介绍有关质膜离子通道的研究进展。

### 1 植物细胞质膜K<sup>+</sup>通道

钾是一种重要的植物离子营养物质, 以离子形式(K<sup>+</sup>)

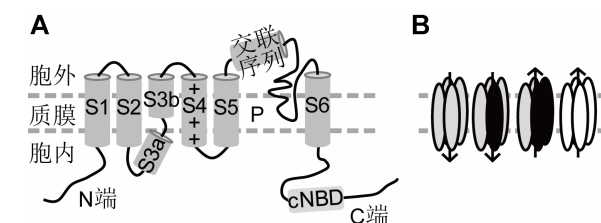
被植物吸收, 并以离子形式在植物细胞内存在。在正常情况下, 植物细胞内K<sup>+</sup>浓度可以达到或超过100 mmol·L<sup>-1</sup> (Clarkson and Hanson, 1980), 有些细胞和细胞器内的K<sup>+</sup>浓度甚至可以达到数百毫摩尔的水平。例如, 处于气孔开放状态的鸭跖草(*Commelina communis*)气孔保卫细胞内K<sup>+</sup>浓度可达300 mmol·L<sup>-1</sup>以上(MacRobbie and Lettau, 1980)。因此, K<sup>+</sup>是植物细胞内最主要的一种阳离子渗透物质, 是各种生化反应得以发生的基础环境条件。植物从土壤中吸收K<sup>+</sup>, 以及K<sup>+</sup>在植物生长发育和逆境反应中被频繁地跨膜运输, 主要依赖于K<sup>+</sup>通道的介导。模式植物拟南芥(*Arabidopsis thaliana*)的质膜K<sup>+</sup>通道主要来自Shaker家族。Shaker家族离子通道首先在果蝇(*Drosophila melanogaster*)中被发现, 因早期发现的一个果蝇突变体颤动表型而得名(Jan and Jan, 1997)。通过序列比对, 科学家在拟南芥中发现了9个Shaker同源基因, 并延续了Shaker的命名。拟南芥Shaker家族每个成员均具有位于质膜内侧的1个自由N端和1个自由C端、6个跨膜结构域、1个定位于第4跨膜结构域的电压感受器、1个位于第5和第6跨膜结构域之间形成离子通道孔道的结构域和1个可能结合环核苷酸的结构域, 其中孔形成结构域包含1个保守的氨基酸序列GYGD/E (Lebaudy et al., 2007;

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Sharma et al., 2013) (图1A)。根据其功能特点, 可以将Shaker家族划分为5个组(Lebaudy et al., 2007)。其中AKT1、AKT5和AKT6/SPIK为第1组; KAT1和KAT2为第2组; AKT2为第3组; AtKC1为第4组; SKOR和GORK组成第5组。除第4组的AtKC1不能单独形成有功能的质膜K<sup>+</sup>通道外, 其它各Shaker成员均可以单独形成有功能的质膜K<sup>+</sup>通道。第1和第2两个组的成员均为强内向K<sup>+</sup> (K<sup>+</sup><sub>in</sub>)通道, 只允许K<sup>+</sup>从胞外向胞内方向流动, 一般不允许K<sup>+</sup>反方向流动。第3组为弱内向性K<sup>+</sup>通道, 主要介导K<sup>+</sup>的跨质膜内流, 但也允许K<sup>+</sup>的缓慢外流。第4组AtKC1虽然不能独自形成有功能的K<sup>+</sup>通道, 但可以作为内向K<sup>+</sup>通道的调控亚基发挥负调控作用(Duby et al., 2008; Jeanguenin et al., 2011)。第5组为典型的质膜外向K<sup>+</sup> (K<sup>+</sup><sub>out</sub>)通道, 只允许K<sup>+</sup>从胞内向胞外跨质膜流动, 不允许K<sup>+</sup>跨质膜内流。动物和植物细胞的Shaker家族组成的K<sup>+</sup>通道(包括内向和外向的K<sup>+</sup>通道)均为四聚体, 且既可以是同源四聚体, 也可以是异源四聚体, 共同围成一个离子通道的孔, 每个亚基的2个自由端在四聚体的形成中发挥重要作用(MacKinnon, 1991; Daram et al., 1997; Dreyer et al., 2004; Lebaudy et al., 2008a, 2008b; Sharma et al., 2013) (图1B)。采用植物基因在非洲爪蟾(*Xenopus laevis*)卵母细胞中的瞬时表达技术, 科学家结合电生理技术对Shaker家族各成员的基本特性作了详细分析, 确定该家族组成的细胞质膜K<sup>+</sup>通道的基本特点和功能, 并发现异源四聚体的亚基组成明显影响K<sup>+</sup>通道的电生理特性(Schroeder et al., 1987; Schroeder, 1988; Schroeder and Hagiwara, 1989; Dreyer et al., 1997; Pilot et al., 2001; Lebaudy et al., 2008a; Geiger et al., 2009; Wang et al., 2010; Jeanguenin et al., 2011)。而拟南芥体内实验也证实, Shaker家族成员在植物体内以异源多聚体的形式发挥作用(Lebaudy et al., 2008b)。拟南芥Shaker家族广泛分布于植物的各个组织器官和细胞类型, 其生物学功能由其组织分布特点和自身的K<sup>+</sup>通道特性共同决定。AKT1在植物根中负责K<sup>+</sup>的吸收和耐盐反应(Fuchs et al., 2005; Xu et al., 2006, 2014; Ardie et al., 2010; Rubio et al., 2010; Zhang et al., 2010; Li et al., 2014)。AtKC1作为1个亚基与AKT1共同组成质膜K<sup>+</sup>通道, 一方面抑制植物对胞外K<sup>+</sup>的吸收, 另一方面也可以抑制AKT1介导的K<sup>+</sup>外



**图1** 拟南芥Shaker家族单个亚基和离子通道的拓扑结构(改自Sharma et al., 2013)

(A) 单个亚基拓扑结构; (B) Shaker离子通道的多种不同四聚体组成形式, 包括内向和外向质膜K<sup>+</sup>通道。S1–S6: 单亚基的第1–6个跨膜区; N/C端: 氨/羧基末端; P: 通道孔区; +: 带正电荷的电压感受器结构域; cNBD: 环核苷酸结合域

**Figure 1** Topological structure of a representative subunit and tetramers of Shaker family from Arabidopsis (modified from Sharma et al., 2013)

(A) Topological structure of a Shaker subunit; (B) Diverse tetramers of K<sup>+</sup> channels composed of Shaker members, including inward and outward K<sup>+</sup> channels. S1–S6: Transmembrane domains 1–6; N/C termini: The amino terminal and carboxyl terminal; P: Pore region; +: With positive charged voltage sensor domain; cNBD: Cyclic nucleotide binding domain.

流和渗漏(Reintanz et al., 2002; Geiger et al., 2009; Wang et al., 2010)。因此, AtKC1的缺失可以促进AKT1介导的胞外K<sup>+</sup>吸收。K<sup>+</sup>通过AKT1被植物吸收后, 通过质膜外向K<sup>+</sup>通道SKOR被装载进入木质部, 进一步被长距离运输到植物的地上部(Gaymard et al., 1998; Liu et al., 2006)。SKOR的离子通道活性受胞内和胞外K<sup>+</sup>浓度的调控, 胞内高浓度K<sup>+</sup>可以有效提高SKOR的K<sup>+</sup>通道活性, 促进K<sup>+</sup>向地上部转运, 而对胞外K<sup>+</sup>的敏感性可以确保其选择性地介导K<sup>+</sup>的外流(Johansson et al., 2006; Liu et al., 2006)。植物以此机制实现对K<sup>+</sup>的吸收及长距离体内运输的联动与精细调控。关于Shaker家族在植物地上部分的生物学功能, 有研究表明AKT6/SPIK在花粉管中表达, 参与花粉管的极性生长(Mouline et al., 2002; Zhao et al., 2013)。另有多Shaker成员被发现作为质膜K<sup>+</sup>通道参与气孔运动的调控。光照等通过激活由多个Shaker家族成员组成的气孔保卫细胞质膜K<sup>+</sup><sub>in</sub>通道及其介导的胞外K<sup>+</sup>内流而诱导气孔开放; ABA和干旱胁迫等通过激活K<sup>+</sup><sub>out</sub>通道及其介导的K<sup>+</sup>外流驱动气孔关闭。研究表明, 5个Shaker家族成员(即KAT1、KAT2、AKT1、

AKT2和AtKC1)都参与拟南芥气孔保卫细胞质膜 $K^+_{in}$ 通道的形成,其中最主要的是KAT1,由于其功能缺失导致拟南芥气孔保卫细胞的全细胞内向 $K^+$ 电流减小50%以上(Szyroki et al., 2001)。同时,KAT1的功能缺失并不会明显影响拟南芥气孔运动。因此,KAT1曾被认为对于气孔开放运动并不重要(Szyroki et al., 2001)。但随后来自多个研究组的研究均证明,Shaker家族多个成员组成的质膜 $K^+_{in}$ 通道及其介导的 $K^+$ 内流为气孔开放运动所必需。KAT1作为最主要的 $K^+_{in}$ 通道组成亚基,在气孔开放运动中的关键作用毋庸置疑(Kwak et al., 2001; Lebaudy et al., 2008b; Laanemets et al., 2013; Wang and Wu, 2013; Zhang et al., 2016)。由于多个Shaker成员的参与,气孔保卫细胞内向 $K^+$ 通道主要以异源四聚体的形式存在(Xicluna et al., 2007; Lebaudy et al., 2010)。但在气孔关闭运动中发挥重要作用的质膜 $K^+_{out}$ 通道的亚基组成形式则比较简单,其原因是该 $K^+_{out}$ 通道仅有GORK一个Shaker成员参与组成,必然为同源四聚体(Ache et al., 2000; Hosy et al., 2003)。

Shaker家族离子通道的活性受多种调控因子的影响。首先,细胞内和细胞外 $K^+$ 浓度对 $K^+_{in}$ 和 $K^+_{out}$ 通道活性均有明显影响。这种影响可能是基于 $K^+$ 从高浓度向低浓度方向流动的基本化学特性。酸性环境可以显著抑制拟南芥花粉原生质体质膜 $K^+_{in}$ 通道活性,而碱性条件则具有一定的激活作用(Fan et al., 2001, 2003)。这种作用可能通过影响 $K^+_{in}$ 通道的某些氨基酸残基电离状态和离子通道蛋白结构变化来实现。其次, $K^+_{in}$ 四聚体的亚基组成形式本身就是一种通道活性调控方式。在非洲爪蟾卵母细胞中,以不同的排列组合共表达2个不同的拟南芥Shaker家族 $K^+_{in}$ 通道基因,可以利用电生理技术记录到具有不同电生理特性的 $K^+_{in}$ 通道电流信号(Xicluna et al., 2007; Lebaudy et al., 2010)。而AtKC1作为抑制亚基,通过重组进入 $K^+_{in}$ 四聚体发挥对 $K^+_{in}$ 通道的抑制作用,是 $K^+_{in}$ 通道的重要负调控因子。 $K^+_{in}$ 四聚体组成形式的变化可能通过影响 $K^+_{in}$ 通道蛋白离子通道孔的结构而影响其离子通道活性。SNARE蛋白SYP121不仅可以影响KAT1等 $K^+_{in}$ 通道的亚细胞定位,而且可使KAT1内化离开细胞质膜,从而抑制全细胞 $K^+_{in}$ 通道的电流大小(Sutter et al., 2006, 2007)。进一步研究表明,SNARE还可以通过与AtKC1互作调控 $K^+_{in}$ 通道的蛋白活性

(Honsbein et al., 2009; Grefen et al., 2010; Jeanguenin et al., 2011)。由此可知,SNARE作为 $K^+_{in}$ 通道的上游调控因子可通过对 $K^+_{in}$ 四聚体组成形式的影响发挥调控作用。蛋白激酶和磷酸酶参与调控多种蛋白的功能,包括内向和外向质膜 $K^+$ 通道,比如CPK (calcium-dependent protein kinase)家族的CPK33对GORK的激活作用和PP2CA对GORK的抑制作用(Lefoulon et al., 2016; Corratgé-Faillie et al., 2017)。 $K^+_{in}$ 通道可以被多种蛋白激酶磷酸化所抑制,包括CPK家族和SnRK (Rnf1-related protein kinase)家族的多个蛋白激酶(Sato et al., 2009, 2010; Ronzier et al., 2014)。此外,AKT1等多个质膜 $K^+_{in}$ 通道的活性还受 $Ca^{2+}$ 信号及其与CBL-CIPK组成的信号链的正调控(Li et al., 2006; Cheong et al., 2007; Zhang et al., 2010; Ren et al., 2013; Behera et al., 2017)。而PP2CA对AKT1的去磷酸化可以与 $Ca^{2+}$ -CBL-CIPK信号链协同调控AKT1的活性(Lan et al., 2011)。有趣的是,ABA甚至可以直接激活GORK的离子通道活性(Ooi et al., 2017)。 $K^+$ 通道的调控因子众多,这些调控因子并非各自单独发挥作用,而是彼此协同发挥作用。AtKC1和 $Ca^{2+}$ -CBL-CIPK23信号链协同调控AKT1就是一个典型的例子(Wang et al., 2016)。

目前,对拟南芥Shaker家族的组织分布和功能研究得较为深入全面;而对包括水稻(*Oryza sativa*)和玉米(*Zea mays*)在内的其它植物Shaker家族的研究相对较少。已有的研究鉴定出多个参与 $K^+$ 吸收、转运和气孔运动的玉米和水稻Shaker同源基因。其与拟南芥同源蛋白相比较有很多类似之处,但也有不同。例如,玉米KZM2通过与KZM3组成异源多聚体,抑制 $K^+_{in}$ 通道的活性和气孔运动(Gao et al., 2017)。而水稻OsKAT3通过与OsKAT2互作抑制OsKAT3的活性(Hwang et al., 2013)。这种 $K^+$ 通道两个同源蛋白之间的互作和调控方式与拟南芥中的AtKC1对AKT1和KAT1等的抑制作用类似(Reintanz et al., 2002; Geiger et al., 2009; Wang et al., 2010)。再比如,水稻KAT2与拟南芥KAT2均作为 $K^+_{in}$ 通道参与光照诱导的气孔开放,但拟南芥气孔保卫细胞质膜 $K^+_{in}$ 通道编码基因数目明显多于水稻(Pilot et al., 2001; Moon et al., 2017)。对于外向 $K^+$ 通道而言,拟南芥SKOR分布于根部,负责 $K^+$ 向木质部的卸载,而GORK则主要参与拟南芥的气孔关闭,二者的组织分布和生物学功能

明显不同。而水稻的同一个Shaker蛋白同时参与气孔关闭和K<sup>+</sup>营养在根部的卸载(Nguyen et al., 2017)。因此, 加强对更多植物, 尤其是重要作物中Shaker家族的研究, 将会促进人们对植物吸收、运输和利用K<sup>+</sup>的分子机理的深入理解。

## 2 植物细胞质膜Ca<sup>2+</sup>通道及其活性调控机制

植物体内存在大量的钙元素。一方面钙作为细胞壁等结构组分发挥作用; 另一方面, 离子形式的钙(Ca<sup>2+</sup>)可以作为信号分子参与各种生物学反应和信号传递过程, 后者则备受关注。胞内Ca<sup>2+</sup>信号以细胞质Ca<sup>2+</sup>浓度的升高和震荡形式出现。一般认为, 胞外Ca<sup>2+</sup>内流是细胞质Ca<sup>2+</sup>信号产生所需Ca<sup>2+</sup>的主要来源, 而质膜内向Ca<sup>2+</sup>通道是胞外Ca<sup>2+</sup>内流的主要渠道。同时, Ca<sup>2+</sup>通道的活性变化通过控制胞外Ca<sup>2+</sup>内流的节奏直接调控胞内Ca<sup>2+</sup>震荡, 发挥Ca<sup>2+</sup>信号上游调控因子的作用。鉴于此, 质膜Ca<sup>2+</sup>通道受到广泛关注, 并成为动植物各信号领域的重点研究对象。科学家首先在动物细胞中发现多个Ca<sup>2+</sup>通道家族, 主要包括受环核苷酸cAMP和cGMP激活的CNG (cyclic nucleotide-gated)家族、受谷氨酸门控的GLR (glutamate receptor)家族、TRPC (transient receptor potential channel)和受胞内Ca<sup>2+</sup>库倒空激活的CRACM1/Orai1 (Zagotta and Siegelbaum, 1996; Vig and Kinet, 2007; Sobolevsky, 2015; Ambudkar et al., 2017)。通过序列比对, 多个动物细胞质膜Ca<sup>2+</sup>通道的同源基因家族先后在植物尤其是模式植物拟南芥基因组中被鉴定出来, 主要包括CNGC (cyclic nucleotide-gated channel)、GLR (glutamate receptor-like)、高渗敏感的OSCA (hyperosmolality-gated calcium-permeable channel)家族和低渗敏感的张力激活离子通道MCA (mechano-sensitive channel) (Mäser et al., 2001; Ward et al., 2009; Iida et al., 2014; Yuan et al., 2014)。

拟南芥CNGC家族由20个成员组成, 其蛋白结构与动物CNG同源蛋白和植物Shaker家族成员颇为类似(图1A), 也具有6个跨膜区, 位于质膜内侧的1个N自由端和1个C自由端, 在第4与第5跨膜区之间有1个孔形成所必需的自由环状结构(Köhler et al., 1999;

Kaupp and Seifert, 2002; Matulef and Zagotta, 2003)。但由CNGC组成的离子通道具体包含几个亚基尚不清楚。最近的研究表明, 百脉根(*Lotus corniculatus*) CNGC组成的离子通道与Shaker家族类似, 也是四聚体, 由4个相同或不同的CNGC亚基共同形成1个有功能的Ca<sup>2+</sup>通道蛋白复合体, 且不同的亚基组成影响植物表型(Chiasson et al., 2017)。考虑到CNGC具有较高的序列相似性, 推测包括拟南芥在内的植物CNGC组成的质膜离子通道可能都是四聚体。关于CNGC家族各成员单独形成的同源四聚体离子通道对离子的选择性和通透性, 早期的研究表明, 拟南芥CNGC1和CNGC2允许K<sup>+</sup>通过, 是K<sup>+</sup>通透性离子通道(Köhler et al., 1999; Leng et al., 1999, 2002)。但最新研究表明, CNGC2也对Ca<sup>2+</sup>具有明显的通透性, 介导Ca<sup>2+</sup>跨质膜内流(Wang et al., 2017)。CNGC10对Ca<sup>2+</sup>和Mg<sup>2+</sup>均有明显的通透能力, 并可能通过二价阳离子进一步调控K<sup>+</sup>的跨膜运输(Guo et al., 2010)。而CNGC7、8、9、10、16和18均为Ca<sup>2+</sup>通透性的二价阳离子通道, 对单价阳离子没有明显的通透性(Gao et al., 2014, 2016)。由此表明, CNGC家族各成员的离子选择性并不一致, 且有些成员对不同离子的选择性较弱。从第1个植物CNGC家族基因被克隆(Schuurink et al., 1998)至今已有20年, 关于其生物学功能也已经有很多报道。在作为质膜Ca<sup>2+</sup>通道发挥生物学功能方面, CNGC2通过调控Ca<sup>2+</sup>内流调控质外体Ca<sup>2+</sup>的动态、叶片衰老和介导茉莉酸信号传递(Ma et al., 2010; Lu et al., 2016; Wang et al., 2017); CNGC2与CNGC4共同参与植物的病原防御反应(Chin et al., 2013); CNGC7、8、9、10、16和18均在成熟花粉和花粉管中表达, 但只有CNGC7、8、16和18被确定参与花粉萌发及花粉管的极性生长与导向调控(Tunc-Ozdemir et al., 2013a, 2013b; Gao et al., 2016; Gu et al., 2017); CNGC2和CNGC6通过介导Ca<sup>2+</sup>内流参与拟南芥的热激反应(Finka et al., 2012; Gao et al., 2012); CNGC10参与拟南芥的耐盐反应(Guo et al., 2008; Jin et al., 2015); CNGC17参与植物磺胺素信号的传递过程(Ladwig et al., 2015); CNGC11/12参与植物衰老和细胞程序性死亡过程(Urquhart et al., 2007, 2011); 而CNGC14参与拟南芥根的重力反应(Shih et al., 2015)。关于拟南芥CNGC家族的更多功能可以参考刘海娇等

(2015)的专题评述。

GLR家族是受多种氨基酸门控的离子通道,但其通道门控特点并非如其名称仅为谷氨酸所门控,而是受多种氨基酸影响。1998年,第1个GLR被克隆出来(Lam et al., 1998),人们开始对GLR进行研究。研究表明,拟南芥GLR1和GLR2的N端位于细胞质膜外侧,C端位于细胞质膜内侧,中间包含4个跨膜区,但第2个跨膜区并不能完整地跨过细胞质膜(Lam et al., 1998) (图2)。这种结构特点明显不同于Shaker和CNGC家族。GLR组成的质膜离子通道的亚基组成、多数GLR离子通道的离子选择性等基本特性,以及GLR离子通道的活性调控机理目前还不甚清楚。但已经解析出动物GLR四聚体的通道蛋白结构(Price et al., 2012)。据此推测,植物GLR组成的通道蛋白结构可能与动物类似。GLR在植物体内具有多种生物学功能,包括参与调控花粉管的极性生长(Michard et al., 2011; Wudick et al., 2018)、铝诱导的微管解聚和质膜去极化(Sivaguru et al., 2003)、离子运输(McAinsh and Pittman, 2009)、碳氮代谢(Kang and Turano, 2003)、ABA合成和耐旱反应(Kang et al., 2004; Lu et al., 2014)以及机械损伤信号的长距离传输(Mousavi et al., 2013)等。总体而言,目前对GLR家族的研究还不够深入。关于植物GLR家族更为详细的介绍可以参阅何明杰等(2016)的综述。

早期的研究中,人们发现植物细胞中存在一类受质膜张力激活的 $\text{Ca}^{2+}$ 信号,从而预测在植物细胞质膜上存在张力激活类质膜 $\text{Ca}^{2+}$ 通道(Crosgrave and Hedrich, 1991; Dutta and Robinson, 2004; Qi et al., 2004; Zhang et al., 2007)。多年来,人们一直没有鉴定到此类受机械或渗透张力激活的离子通道编码基因。近年来,科学家找到了2类对质膜张力敏感的离子通道,其一是受低渗条件激活,通过介导胞外 $\text{Ca}^{2+}$ 内流发挥功能的MCA1和MCA2 (Iida et al., 2014);其二是对高渗胁迫敏感的OSCA家族(Hou et al., 2014; Yuan et al., 2014)。MCA1和MCA2蛋白只有1个跨膜区,其N端较短且位于细胞质膜外侧,而C端较长且位于质膜内侧(图3A)。研究表明,MCA1和MCA2的N端和跨膜区为其离子通道组成及活性所必需,而C端则对其离子通道活性发挥调控作用(Kamano et al., 2015)。MCA1和MCA2组成的离子通道也为四聚体结构,张力和低渗等刺激通过拉伸质膜改

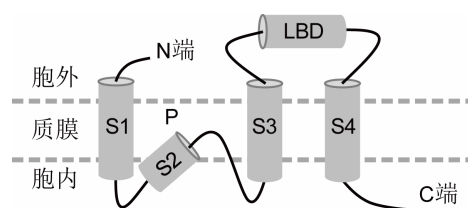


图2 拟南芥GLR亚基的蛋白结构(Lam et al., 1998; Price et al., 2012)

S1–S4: 第1–4跨膜区; LBD: 配体结合结构域; N/C端: 氨/羧基末端

Figure 2 Putative protein structure of an Arabidopsis GLR member (Lam et al., 1998; Price et al., 2012)

S1–S4: Transmembrane domains 1–4; LBD: Ligand binding domain; N/C termini: The amino terminal and carboxyl terminal

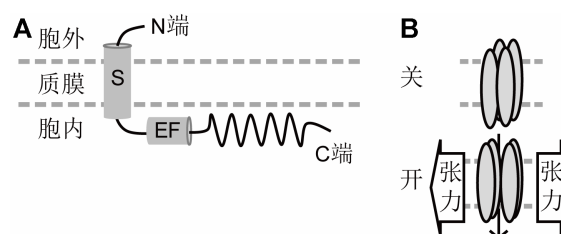


图3 拟南芥MCA1和MCA2通道蛋白结构(A)及张力激活通道活性的分子机理(B) (Kamano et al., 2015)

S: 跨膜区; EF: EF-手型 $\text{Ca}^{2+}$ 结合结构域; N/C端: 氨/羧基端

Figure 3 Protein structure of Arabidopsis MCA1 and MCA2 (A) and a molecular mechanism of how MCA1/2 channels are activated by the tension of plasma membrane (B) (Kamano et al., 2015)

S: The transmembrane domain; EF: EF-hand  $\text{Ca}^{2+}$  binding domain; N/C termini: The amino terminal and carboxyl terminal

变该离子通道蛋白的结构,从而使通道被激活,引起胞外 $\text{Ca}^{2+}$ 内流和细胞质 $\text{Ca}^{2+}$ 水平升高(图3B)。MCA1的拟南芥突变体根无法插入较硬的固体培养基(Nakagawa et al., 2007),说明其在感知机械压力方面发挥作用。最近的研究表明,MCA1和MCA2还作为质膜 $\text{Ca}^{2+}$ 通道介导拟南芥的耐冷反应(Mori et al., 2018)。与MCA1和MCA2相反,OSCA是对高渗敏感的质膜离子通道,可以被细胞外的高渗处理激活(Yuan et al., 2014)。拟南芥OSCA家族的蛋白结构与MCA1和MCA2大不相同,其每个亚基均具有多达9



个跨膜区,在其第8和第9跨膜区之间具有1个孔形成所需的环状结构(Yuan et al., 2014) (图4)。OSCA与MCA1和MCA2类似之处在于其亚基N端位于质膜的胞外侧,而C端则位于质膜胞质侧(Yuan et al., 2014) (图4)。OSCA1允许多种离子通过,其对各种离子的通透能力大小排序为 $K^+ > Ba^{2+} \approx Ca^{2+} > Na^+ = Mg^{2+} = Cs^+$  (Yuan et al., 2014)。目前,人们对质膜张力激活的质膜离子通道的生物学功能了解较少,但其对渗透敏感的特点暗示此类质膜离子通道可能通过对质膜张力变化做出反应。对低渗透敏感的MCA1和MCA2与对高渗透敏感的OSCA家族有可能作为一对质膜张力和渗透敏感元件,协同配合发挥作用,以共同应对渗透环境变化。一方面,其可以通过影响胞内 $Ca^{2+}$ 信号而参与耐盐、耐旱和气孔运动等与细胞渗透和膨压调节密切相关的生物学过程;另一方面,OSCA对 $K^+$ 的通透能力也表明其可能同时介导 $Ca^{2+}$ 和 $K^+$ 的跨膜流动而参与这些生物学过程。

鉴于 $Ca^{2+}$ 信号的广泛存在和重要生物学功能,寻找其上游调控因子一直是这一研究领域的重要任务。其中,质膜 $Ca^{2+}$ 通道是最重要的一大类 $Ca^{2+}$ 信号上游调控因子。循此思路,很多研究组直接从寻找 $Ca^{2+}$ 通道和揭示 $Ca^{2+}$ 通道候选蛋白的生物学功能入手进行研究。但迄今为止依然有很多信号传递过程中 $Ca^{2+}$ 信号和 $Ca^{2+}$ 通道之间无法实现一对一配对,即 $Ca^{2+}$ 信号发挥重要作用的生物学过程或信号传递链中,负责此类 $Ca^{2+}$ 信号产生的 $Ca^{2+}$ 通道编码基因往往并不清楚,且不能阐明一些已知生物学功能的质膜 $Ca^{2+}$ 通道调控 $Ca^{2+}$ 信号的分子机制。因此,这一领域还有诸多科学问题等待解答。

### 3 阴离子通道及其活性调控

介导阴离子物质跨膜流动的阴离子通道同样有多个家族。目前被大家广泛熟知的主要包括ALMT (aluminum-activated malate transporter)、SLAC1 (S-type anion channel associate1)、MSL (MscS-like) 和CLC (chloride channel)等,负责介导包括 $NO_3^-$ 、 $Cl^-$ 和苹果酸在内的多种阴离子物质的跨质膜和内膜的流动。其中,CLC家族主要分布于植物细胞内膜系统,本文不做赘述;而ALMT、SLAC1和MSL家族的多数成员主要作为质膜阴离子通道发挥作用。

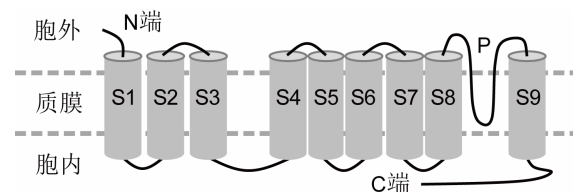


图4 拟南芥OSCA家族通道蛋白结构(改自Yuan et al., 2014)

Figure 4 The putative protein structure of an OSCA member from Arabidopsis (modified from Yuan et al., 2014)

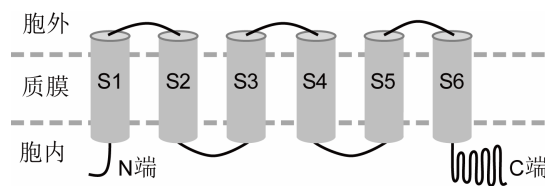


图5 拟南芥ALMT家族通道蛋白结构(改自Sharma et al., 2016)

Figure 5 The putative protein structure of an ALMT member from Arabidopsis (modified from Sharma et al., 2016)

关于ALMT家族,最早被鉴定出来的2个ALMT分别是来自小麦 (*Triticum aestivum*) 和拟南芥的ALMT1,因其均受Al诱导且对苹果酸具有明显的通透能力而得名(Sasaki et al., 2004; Hoekenga et al., 2006)。ALMT虽然被命名为Al诱导的苹果酸转运子 (transporters),但在结构和功能上都是典型的离子通道(图5),在质膜和内膜系统均有分布。ALMT蛋白结构目前尚不清楚。根据现有信息,推测每个ALMT亚基具有6或7个跨膜区,其C端和N端可能均位于质膜胞质侧,其N端和跨膜区为形成有功能的离子通道所必需,而其C端主要对离子通道的活性发挥调控作用(Sharma et al., 2016) (图5)。ALMT的离子选择性也不仅限于苹果酸,而是对苹果酸、草酸、 $NO_3^-$ 和 $Cl^-$ 等多种阴离子物质均有通透能力,且离子流动的方向也不一致(Sharma et al., 2016)。离子通道对离子的选择性及对各种离子的通透能力和通透方向往往由其自身的蛋白拓扑结构及一些关键氨基酸残基决定。由于ALMT组成的离子通道蛋白的拓扑结构还不清楚,目前尚无法从蛋白结构层面详细解释其离子选择性和对各种离子的相对通透能力(Sharma et al., 2016)。ALMT在植物各种组织中广泛分布,其生物学功能多样。其中,分布于植物根细胞质膜的ALMT1向

土壤中分泌苹果酸的作用先后在多种植物根部被观测到,包括小麦、拟南芥、油菜(*Brassica napus*)、玉米、大豆(*Glycine max*)和绒毛草(*Holcus lanatus*)等(Sasaki et al., 2004; Hoekenga et al., 2006; Ligaba et al., 2006, 2012; Fontecha et al., 2007; Chen et al., 2013; Liang et al., 2013)。由植物分泌并进入土壤的苹果酸有2个作用,其一是与土壤中处于离子状态的铝结合,将其螯合和固定,从而使植物免受铝毒伤害;其二是苹果酸使土壤pH值降低,可以有效提升土壤中离子状态磷元素的浓度,从而促进植物对磷营养的吸收。此外,研究表明,豆科植物ALMT1和ALMT4基因在根瘤维管束细胞表达,并受flg22诱导,其蛋白通过介导有机酸的跨膜流动参与植物与微生物的互作(Rudrappa et al., 2008; Lakshmanan et al., 2012; Sukweenadhi et al., 2015; Takanashi et al., 2016)。有意思的是,玉米ALMT1虽然也在根组织中分布,但却对苹果酸等有机酸没有明显的通透能力,而主要介导Cl<sup>-</sup>、NO<sub>3</sub><sup>-</sup>和SO<sub>4</sub><sup>2-</sup>等阴离子营养物质的跨质膜内流和外流(Piñeros et al., 2008)。这明显不同于其它多种植物的ALMT1。在自然情况下,分布于植物根细胞质膜的ALMT编码基因的表达可以同时受多种信号的调控,包括土壤中的磷和铝、土壤酸化、植物-微生物互作、体内激素、活性自由基的产生以及GABA( $\gamma$ -aminobutyric acid)信号的调控(Kobayashi et al., 2013; Liang et al., 2013; Ramesh et al., 2015)。在蛋白水平上,Al和苹果酸可能通过直接与ALMT1通道蛋白结合,提高ALMT1的离子通道活性。在植物地上部,多个ALMT成员在气孔保卫细胞中分布,并参与调控气孔运动。其中,拟南芥ALMT6和ALMT9主要定位于气孔保卫细胞液泡膜上,且AtALMT9的离子通道活性受细胞质苹果酸含量的调控(Kovermann et al., 2007; Meyer et al., 2011; De Angeli et al., 2013b)。苹果(*Malus pumila*)果实细胞液泡膜定位的ALMT6通过介导苹果酸在液泡中的累积进而影响苹果的酸性物质含量和口味;葡萄(*Vitis vinifera*)果实细胞液泡膜定位的ALMT9则通过介导苹果酸和酒石酸的跨液泡膜流动及在液泡中的积累来影响用其所酿酒的品质和产量(Bai et al., 2012; De Angeli et al., 2013a)。而拟南芥ALMT12作为质膜阴离子通道,通过介导苹果酸的外流参与ABA等诱导的气孔关闭运动(Meyer et al., 2010; Sasaki et al., 2010)。除了这

些有特定组织和亚细胞分布的ALMT外,也有ALMT组织分布比较复杂,同一个蛋白在不同组织中发挥不同的生物学功能。例如,大麦(*Hordeum vulgare*)ALMT1同时分布于气孔保卫细胞、根和种子中,分别参与气孔关闭运动、植物生长发育和种子萌发(Gruber et al., 2010; Xu et al., 2015)。这些研究不仅表明ALMT家族组成的离子通道在离子选择性和方向性方面具多样化,也表现出其组织分布、亚细胞定位和生物学功能的复杂性。

SLAC1家族是较晚被发现的一个植物细胞质膜阴离子通道家族,但植物进化分析表明,SLAC1基因从低等到高等植物均很保守(Lind et al., 2015)。拟南芥SLAC1家族由5个成员组成,包括SLAC1及其同源蛋白SLAH1、2、3和4。其中,SLAC1主要定位在气孔保卫细胞质膜上,通过介导Cl<sup>-</sup>和NO<sub>3</sub><sup>-</sup>的外流驱动气孔关闭,是干旱胁迫及ABA、CO<sub>2</sub>、臭氧和活性氧(reactive oxygen species, ROS)等信号传递途径的关键组分(Negi et al., 2008; Vahisalu et al., 2008)。SLAH3在气孔保卫细胞中分布,与SLAC1一起通过介导NO<sub>3</sub><sup>-</sup>和Cl<sup>-</sup>外流驱动气孔关闭(Geiger et al., 2011),但SLAH3在气孔关闭运动中的作用,相对于SLAC1而言是辅助性的。拟南芥4个SLAH主要分布在根、茎和叶脉等输导组织中(Negi et al., 2008)。其中,SLAH1通过调控SLAH3的活性调控Cl<sup>-</sup>从根向茎的运输(Cubero-Font et al., 2016)。在离子选择性和通透性方面,拟南芥SLAC1和SLAH3均对Cl<sup>-</sup>和NO<sub>3</sub><sup>-</sup>有明显的通透能力,但对NO<sub>3</sub><sup>-</sup>的通透能力明显大于Cl<sup>-</sup>(Geiger et al., 2009; Lee et al., 2009; Brandt et al., 2012)。而SLAH2则为NO<sub>3</sub><sup>-</sup>选择性的阴离子通道,对Cl<sup>-</sup>的通透能力微弱(Maierhofer et al., 2014)。最近的研究表明,来自单子叶植物玉米和水稻的SLAC1,相对于Cl<sup>-</sup>而言,对NO<sub>3</sub><sup>-</sup>的选择性明显大于拟南芥SLAC1(Sun et al., 2016; Qi et al., 2018);而鸭跖草SLAC1则为NO<sub>3</sub><sup>-</sup>激活的Cl<sup>-</sup>通透性的阴离子通道(Müller et al., 2017)。因此,来自不同高等植物的SLAC1,其离子选择性不完全一致,即不同的高等植物可能采用不同的阴离子通道组合来驱动气孔关闭运动。需要注意的是,尽管在几十年前就发现苹果酸在气孔保卫细胞内大量积累,并曾被认为是气孔关闭运动中主要的外流阴离子物质。但实际上,在气孔关闭运动中发挥核心作用的SLAC1和SLAH3均对苹果

酸没有明显的通透能力, 真正通过介导苹果酸外流而参与驱动气孔关闭运动的是来自ALMT家族的ALMT12。因此, 气孔关闭运动应该是由SLAC1、SLAH3和ALMT12等多种质膜阴离子协同作用的结果。每个SLAC1亚基具有10个跨膜区, 其C端和N端均位于质膜的胞质一侧(Vahisalu et al., 2008) (图6A)。3个SLAC1亚基互相结合, 以三聚体的形式形成质膜阴离子通道, 但每个亚基独立形成1个允许阴离子通过的孔(Chen et al., 2010) (图6B)。由此可见, 包括多个SLAHs在内, SLAC1家族形成的阴离子通道可能均为三聚体, 而SLAH1对SLAH3活性的调控作用, 有可能通过与SLAH3组成异源三聚体而实现。SLAC1家族的离子通道亚基组成形式与CNGC和Shaker家族不同, 但它们在亚基组成影响离子通道活性和选择性方面又具有类似之处。SLAC1家族离子通道的激活主要依赖蛋白激酶的磷酸化, 而失活则依赖于磷酸酶的去磷酸化(Geiger et al., 2009, 2011; Lee et al., 2009; Brandt et al., 2012; Maierhofer et al., 2014; Cubero-Font et al., 2016)。其中, 参与SLAC1及其同源蛋白离子通道活性调控的蛋白激酶主要来自SnRK、CPK和CBL-CIPK (calcineurin B-like, CBL; CBL-interacting protein kinase, CIPK) 等蛋白激酶家族及PP2C (protein phosphatases 2C) 磷酸酶家族。

除了SLAC1家族, 植物细胞质膜上还包含张力激活的阴离子通道MSL (mechano-sensitive or stretch-activated channels) (Crosgrove and Hedrich, 1991)。MSL为典型的张力激活的阴离子通道家族。MSL9和MSL10是最早得到电生理鉴定的MSL家族成员, 定位于细胞质膜, 其激活对质膜张力具有依赖性, 主要选择性介导阴离子的跨膜流动(Haswell et al., 2008; Makshev and Haswell, 2012)。随后有研究表明, 拟南芥MSL10参与植物机械损伤信号反应和茉莉酸的累积(Veley et al., 2014; Zou et al., 2016)。此外, 有些拟南芥MSL成员定位在细胞器膜上, 调控细胞器的大小和形状(Veley et al., 2012)。根据氨基酸残基序列预测, MSL蛋白存在多个跨膜区, 具备形成离子通道的基本结构特点。而质膜受到拉伸时对MSL的激活是通过机械拉力直接改变MSL蛋白的结构, 使离子通过的孔被拉开。但这基本上还停留在理论推测阶段, 缺乏有力的直接实验证据支持。总体来

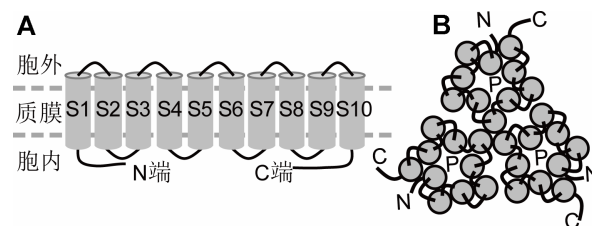


图6 SLAC1亚基(A)和离子通道蛋白结构(B) (Vahisalu et al., 2008; Chen et al., 2010)

**Figure 6** The topological structures of a SLAC1 subunit (A) and the structure of a SLAC1 channel as a trimer (B) (Vahisalu et al., 2008; Chen et al., 2010)

讲, 目前对MSL家族的生物学功能还了解较少, 其组成的离子通道蛋白结构、感知生物膜张力的分子机理和生物学功能等还有待深入研究。

介导阴离子跨质膜运动的离子通道有多种, 且可能并存于相同的组织和细胞类型。在植物生长发育、逆境反应和激素信号传递过程中, 不同的质膜阴离子通道之间是否存在某种关系, 目前还知之甚少。根据这些离子通道的基本电生理特性推测, MSL家族可能主要参与影响细胞内渗透势和膨压的生物学过程; SLAC1和ALMT家族成员的离子通道活性变化则可能与细胞内渗透浓度和膨压的变化关系不大, 而主要依赖于磷酸化/去磷酸化和膜电位的变化。

## 4 离子通道之间的功能偶联和协同

在同一种生物学过程中, 往往有多种同类或不同类型的离子通道同时参与, 从而实现对一种离子跨膜流动和不同离子物质协同跨膜运动的精细调控。例如, 在拟南芥气孔关闭运动中, ALMT12、SLAC1和SLAH3均作为质膜外向阴离子通道参与其中; 而气孔开放过程则由多个Shaker家族成员共同参与胞外 $K^+$ 内流所驱动。植物对单一离子物质跨膜流动的精细调控往往涉及同一家族或不同家族多成员彼此之间的互作关系。而分别介导各类离子跨膜流动的不同类型质膜离子通道之间的功能关联, 则影响不同离子跨膜流动是否可以彼此协调。例如, 气孔开放运动中, 负责渗透离子物质内流的阳离子通道和阴离子通道打开, 同时, 负责渗透离子外流的阳离子通道和阴离子通道关闭; 反之亦然。此外, 离子跨膜流动的调控还涉及内



膜系统离子通道与质膜离子通道之间的功能协同。因此,未来相当长的时间内,相同和/或不同种类的质膜离子通道以及质膜离子通道与内膜离子通道之间的协同关系将是该领域的重要研究内容。随着对单一离子通道研究的增多和相关信息的积累,人们开始关注不同离子转运蛋白之间的功能关联机制,并有一些成功的报道。 $\text{Ca}^{2+}$ 信号对阴离子通道SLAC1的激活作用和对内向 $\text{K}^+$ 通道的抑制作用(Schroeder and Hagiwara, 1989; Vahisalu et al., 2008)可以看作是 $\text{Ca}^{2+}$ 通道与阴离子通道和 $\text{K}^+$ 通道之间的间接功能关联,是一种通过细胞质 $\text{Ca}^{2+}$ 信号实现的间接关联。有意思的是,研究表明,慢阴离子通道SLAC1和SLAH3可以与 $\text{K}^+$ 的亚基KAT1和AKT2互作,直接抑制 $\text{K}^+$ 通道活性和光照诱导的气孔开放运动(Zhang et al., 2016)。进一步分析表明,分布于拟南芥根组织中的阴离子通道SLAHs也可以以类似的方式抑制Shaker家族的内向 $\text{K}^+$ 通道AKT2和KAT2的活性(Yao et al., 2017)。阴离子通道对 $\text{K}^+$ 通道的抑制作用显然是由于不同类质膜离子通道之间直接的功能关联,通过二者之间的直接蛋白互作实现。由此看来,同类或不同类的离子通道之间的功能联系可能普遍存在,但具体分子机理比较复杂,最终都是为了调控一种离子物质和协同不同离子物质的跨膜运动。

## 5 研究展望

离子通道作为离子跨膜运动的重要渠道和调控环节,广泛参与植物生殖、生长发育和逆境反应等过程。因此,研究离子通道的活性调控机制一直是植物学领域的重要课题。经过多年的研究和积累,已经有多个家族上百个植物细胞质膜离子通道编码基因先后被发现,但生物学功能和离子通道活性调控机理得到深入解析的只占其中一小部分。因此,继续逐个深入研究质膜离子通道的生物学功能和活性调控机理依然是本领域的重要研究内容。此外,在部分离子通道蛋白的离子选择性、亚细胞定位、生物学功能及其活性调控机理得到深入解析的今天,阐明不同离子协同跨膜运输的分子机理已经成为可能,并显得尤为重要。其中,既包括介导同一种离子跨膜运动的相同和不同家族质膜离子通道之间的功能关联及介导不同离子跨膜运动的不同类型质膜离子通道之间的功能关联,也

包括质膜离子通道与内膜离子通道之间的关系。最终目的是从更为宏观的层面揭示各种离子物质的跨膜运动及协同参与植物生长发育和逆境反应的分子机理。鉴于此,在未来的研究中,采取两方面并重的研究策略将有利于更快速高效地推进本领域研究,解答众多科学问题。

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## Advances in Plasma Membrane Ion Channels of Plant Cells

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**Abstract** Multiple ions, including cations and anions, are involved in growth, development, reproduction and stress responses as essential nutrients, osmotic components, cofactors of proteins and signaling molecules in plants. Ion channels localized in the plasma membrane and endomembrane of plant cells are essential tunnels and dynamic regulators of ion flux across biological membranes and are involved in the direct regulation of ion homeostasis in plant cells. A large number of ion channels have been identified in diverse plant species, especially in the model plant *Arabidopsis*, in recent years. Many ion channels are localized in the plasma membrane, and their protein structure, ion selectivity and permeability, gating patterns, activity-regulating mechanisms and the functional coordinating mechanisms between different ion channels have been analyzed. In this review, we summarize the research progress on the plasma membrane ion channels of plant cells and discuss the outstanding questions.

**Key words** ions, ion flux across membrane, ion channels, plasma membrane, plant cells

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