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·专题论坛·

氧化甾醇结合蛋白相关蛋白家族的研究进展

邹文娇[1, 3], 葛磊[2], 予茜[2*]

[1]山东农业大学生命科学学院, 作物生物学国家重点实验室, 泰安 271018; [2]青岛农业大学农学院作物组学中心, 青岛 266109; [3]山东中医药大学, 中医药创新研究院, 济南 250355

摘要 膜脂是细胞膜的主要组分, 也是参与信号转导的重要信号分子。不同脂质分子在细胞膜上的不均等分布需要特殊类型的通道蛋白和运输蛋白来实现。氧化甾醇结合蛋白相关蛋白(ORPs)是一类非常保守的蛋白分子, 能够对磷脂酰肌醇和固醇等脂类分子进行识别并转运, 参与细胞中的许多生理过程, 包括信号转导、囊泡运输、脂类代谢和非囊泡运输等, 对于个体的生长发育具有重要作用。近几年, 关于ORPs在哺乳动物和酵母(*Saccharomyces cerevisiae*)中结构和功能的研究取得了一系列重要进展, 但在植物中相关研究尚少。该文综述了ORPs及其相关蛋白在哺乳动物、酵母和植物中的研究进展, 探讨了植物ORPs的结构及其与哺乳动物和酵母同源蛋白之间的进化关系, 并对植物ORPs未来的研究方向进行了展望。

关键词 氧化甾醇结合蛋白相关蛋白, 脂类运输, 膜结合位点

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细胞膜包括细胞质膜, 以及细胞核、线粒体、质体、内质网(endoplasmic reticulum, ER)、高尔基体、液泡等细胞器膜和胞内运输所需要的各种类型囊泡膜所组成的膜系统。脂质是细胞膜的基本组分, 包含脂肪(fats)和类脂(lipids)。类脂又分为磷脂(phospholipids)、糖脂(glycolipids)、脂蛋白(lipoproteins)和固醇类(steroid)。不同物种、不同类型的细胞以及不同细胞器的细胞膜所含脂质的种类、分布和含量都不尽相同。不同的脂质分子在细胞生命活动过程中起着非常重要的作用。脂质不仅是细胞膜的结构成分, 而且参与能量储存以及维持细胞膜的流动性和通透性, 特别是作为信号转导的关键组分参与生物体的个体发育和生理过程。然而, 膜脂信号在胞内的传送需要脂类运输蛋白(lipid transfer proteins, LTPs)的充分配合。正是由于LTPs在胞内信号转导中具有不可或缺的作用, 其生理功能备受研究者的关注, 相关研究在近几年也取得了重要进展(Pietrangelo and Ridgway, 2018; Balla et al., 2019; Wong et al., 2019)。

氧化固醇结合蛋白相关蛋白(oxysterol-binding protein (OSBP)-related proteins, ORPs)是一类重要的LTPs, 在各类生物中广泛存在, 并含有众多家族成员(Skirpan et al., 2006)。许多研究表明, OSBP/ORPs不仅可以运输脂类, 还参与囊泡运输(Dong et al., 2016)、脂质代谢(Lagace et al., 1997; Beh et al., 2001; Beh and Rine, 2004; Ma et al., 2010)、信号转导(Wang et al., 2005b; Lessmann et al., 2007)、细胞骨架动力学(Johansson et al., 2007; Wyles et al., 2007)和基因表达等生理过程(Yan et al., 2007, 2008)。尽管近2年ORPs相关研究在人类(*Homo sapiens*)和酵母(*Saccharomyces cerevisiae*)中取得了重要进展, 我们对植物中ORPs的生物学功能研究仍不充分, 对其认识较为模糊。揭示植物中ORPs的分子功能将会在生物化学、细胞学、生理学和发育学层面拓宽我们对膜蛋白信号转导的认识, 因此, 本文从8个方面对ORP蛋白家族成员的研究进展进行阐述。

1 膜脂分子的不均一分布及其细胞学功能

磷脂分子作为生物膜磷脂双分子层的主要结构成分, 包括甘油磷脂(glycerophospholipids, GPLs)和鞘磷

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* 通讯作者。E-mail: yuqian@qau.edu.cn

脂(sphingomyelin) (鞘脂(spingolipids)的一种)。根据与磷酸基团相连基团的不同GPLs可分为磷脂酰胆碱(phosphatidylcholine)、磷脂酰丝氨酸(phosphatidylserine)、磷脂酰甘油(phosphatidylglycerol)和磷脂酰肌醇(phosphatidylinositol, PI)等。其中磷脂酰肌醇的5个羟基位点在特定激酶的作用下可进行单磷酸化、双磷酸化和三磷酸化，产生7种磷酸肌醇(phosphoinositides, PIs)，包括PI3P、PI4P、PI5P、PI(3,4)P2、PI(4,5)P2、PI(3,5)P2和PI(3,4,5)P3。磷酸肌醇主要从3个方面发挥作用：与含有可识别磷脂分子的结构域蛋白结合、影响膜的物理特性以及产生可溶性肌醇磷酸盐(Heilmann, 2016)。能够与磷脂分子结合的蛋白一般含有PH (pleckstrin homology)蛋白结构域、FYVE (Fab1 YOTB Vac1 EEA1)蛋白结构域和PX (phagocytic oxidase)蛋白结构域(Lemmon, 2003)。PIs可以通过诱导膜的弯曲或稳定膜的弯曲来改变膜的生物物理性质，进而影响膜上蛋白的功能(Lundbæk et al., 2010)。PI(4,5)P2在磷脂酶PLC作用下产生2个重要的第二信使，即肌醇1,4,5-三磷酸(Ins(1,4,5)P3)和二酰甘油(DAG)，二者均参与信号调控(Hong et al., 2016)。

磷脂分子参与调控许多生理过程，包括根的发育、花粉发育、微管发育、激素信号、膜脂运输、自噬反应、植物免疫以及一些生物和非生物胁迫反应(Xue et al., 2009; Harayama and Riezman, 2018; Jia et al., 2019; Xing et al., 2021)。在植物中，免疫系统依赖膜脂运输，将防御相关因子转运到特定区域(Wang et al., 2016b)，而磷脂分子对于膜脂运输至关重要(Liu et al., 2013)。例如, PI(4,5)P2是囊泡运输过程中的关键调控因子之一(Martin, 2015; De Craene et al., 2017)。磷酸肌醇也参与细胞内自噬体的形成(Chung, 2019)。自噬相关基因(autophagy related genes, ATG) VPS34 (vacuolar protein sorting 34)影响自噬小体发生部位PtdIns3P的产生。ATG9和ATG18a为植物细胞内质网自噬体形成所必需，ATG9依赖PtdIns3P调控ATG18a在自噬体膜上的转运(Zhuang et al., 2017)。磷酸肌醇也是离子通道的重要调节因子。PI(4,5)P2可通过调节K^+通道调节气孔运动(Ma et al., 2009)。

固醇类也是细胞膜的结构成分之一，作为信号物质在细胞膜或细胞核内对生物体的生长发育起至关重要的作用(Boutté and Grebe, 2009; Ferrer et al., 2017)。有些固醇既存在于动物中，也存在于植物中，如胆固醇、谷甾醇和菜油甾醇(Tarkowská, 2019)。人体细胞内胆固醇水平的变化能够引起心血管疾病、神经性疾病和相关癌症(Luo et al., 2020)。目前植物固醇的分子功能研究相对较少。在植物中，油菜素内酯(brassinosteroids, BRs)是一种重要的植物固醇类激素(Geuns, 1983)，通过与膜受体BRI1蛋白结合，调控细胞的伸长、分裂和分化(Li and He, 2020)。对动物与酵母的研究表明，类固醇分子能够与鞘脂类结合形成一种称为脂筏(lipid rafts)的微区域，介导细胞间的识别、脂膜和蛋白质的细胞内运输、信号转导、逆境反应和极性生长等多种生物学过程(Malinsky et al., 2013; Tapken and Murphy, 2015)。植物中也存在类似的富含固醇和鞘脂的细胞膜微区域，被称为变性剂不溶或者不敏感膜区(detergent-insoluble (resistant) membranes, DIMs/DRMs)，其中所含蛋白质可对外界刺激产生动态应答(Takahashi et al., 2013; Zauber et al., 2014)。由此表明，植物细胞膜上形成的固醇类富集的细胞膜微区域可能对植物发育和环境信号应答具有重要作用。

2 膜脂转运蛋白ORPs家族

脂质在细胞膜上的分布不均一(van Meer et al., 2008)。大多数脂质主要在内质网中合成，随后被运输到各类细胞膜(质膜上的脂类最多)，以维持脂质的特定区域化(Moreau et al., 1998; van Meer et al., 2008)。脂类具有不溶于水的疏水特性。脂质是如何从合成部位(内质网)转移到各种细胞器的？细胞如何维持固醇分子在不同细胞器膜中的不均匀分布？这些都是具有科学意义的重要问题。脂类分子的运输对此过程起到关键作用。胞内脂类运输主要包括3种机制：囊泡运输、非囊泡运输及膜与膜之间的交融扩散。由LTPs参与的非囊泡运输是脂类运输的主要方式。LTPs主要有3方面功能。(1) 作为脂类的运输蛋白，从一种膜撷取脂类运输到另一种膜。(2) 作为脂类的感受器，通过与某种蛋白互作来结合或释放某种脂类。(3) 作为脂类的提供者，如Sec14p (secretion 14 protein)，能够把磷脂酰肌醇运送至磷脂酰肌醇激酶(Schaaf et al., 2008; Raychaudhuri and Prinz,

2010)。LTPs是含有许多成员的大家族，根据其脂质结合域可细分为SEC14、PITP (phosphatidylinositol transfer protein)、START (steroidogenic acute regulatory protein (StAR)-related transfer family)、GLTP (glycolipid transfer protein)、SCP-2 (sterol carrier protein 2)和OSBP/ORPs等家族(D'Angelo et al., 2008)。

ORPs家族的第1个成员OSBP最初是作为氧化甾醇的受体被发现，其通过抑制3-羟基-3-甲基戊二酰辅酶A还原酶(HMG-CoA)的活性抑制胆固醇的合成(Kandutsch and Thompson, 1980; Taylor et al., 1984; Taylor and Kandutsch, 1985)。在不同物种中ORPs家族成员的数目不尽相同，但功能域保守性很强。在人类细胞中, ORPs包含12个成员(Jaworski et al., 2001)。老鼠细胞也包含12个成员(Anniss et al., 2002)。在酵母中，ORPs包含7个成员(Beh et al., 2001)。模式植物拟南芥(*Arabidopsis thaliana*)与人类和老鼠一样，其ORPs包含12个成员(Skirpan et al., 2006)。ORPs一般含有结合磷脂酰肌醇的PH蛋白结构域、介导二聚化的coiled-coil蛋白结构域、FFAT基序(two phenylalanine in an acidic tract motif)以及含高度保守的EQVSHHPP序列的可结合脂类的ORD蛋白结构域(OSBP-related domain)。

3 ORPs双膜定位结构域

脂质在2种膜之间的运输需要OSBP/ORPs同时在2个细胞器膜有定位(Gatta and Levine, 2017)。为实现脂质的转运，OSBP/ORPs一般含有双膜靶向序列，将其同时靶向到2个细胞器上。在OSBP/ORPs家族中可以决定膜靶向的结构域或基序主要有PH蛋白结构域、FFAT基序和较稀有的ANK蛋白结构域(ankyrin repeat domain)。

在众多LTPs中，PH蛋白结构域相当保守，且在人类基因组中含量很高。约10% PH蛋白结构域与磷酸肌醇强特异性结合，从而使蛋白结合到特定膜上，其余90% PH蛋白结构域功能还不清楚(Lemmon, 2007)。在人类细胞12个成员中，除了ORP2和ORP9，其余成员均含有PH蛋白结构域(Lehto et al., 2001)。在老鼠细胞12个成员中，7个含有PH蛋白结构域(Anniss et al., 2002)。在酵母7个成员中，Osh1p–Osh3p含有PH蛋白结构域(Beh et al., 2001)。在拟南芥12个成员中，5个成员含有PH蛋白结构域(Skirpan et al., 2006)。

由于PH蛋白结构域与磷酸肌醇结合具有特异性，PH-GFP常用来指示特定磷酸肌醇的定位(Balla et al., 2000; Rusten and Stenmark, 2006)。ORP5和ORP8的PH蛋白结构域通过与N端相邻的多碱基序列识别PI4P和PI(4,5)P2而将其定位于质膜(Ghai et al., 2017)。虽然磷酸肌醇有助于PH蛋白结构域结合到膜上，但是它们之间的定位不特异。例如，Osh1p通过与PI4P互作定位到高尔基体膜，Osh2p通过与PI4P互作不仅定位在高尔基体膜，也定位在质膜(Roy and Levine, 2004; Yu et al., 2004)。因此，ORPs的特异性定位不仅依赖于PH蛋白结构域，还需要多种因子的参与。例如，OSBP定位在高尔基体膜不仅依赖于PH蛋白结构域与高尔基体膜上PI4P的结合，还需要ARF (GTPase ADP-ribosylation factor)蛋白的参与(Levine and Munro, 2002; Godi et al., 2004)。

FFAT基序的氨基酸序列一般为-EFFDAXE-，是内质网定位的决定性区域。人类细胞中除了ORP5和ORP8，其它成员都含有FFAT基序。酵母Osh1–3含有FFAT基序(Raychaudhuri and Prinz, 2010)。FFAT基序能够与VAPs (vesicle-associated membrane protein (VAMP)-associated proteins)蛋白互作，而VAPs是一类内质网定位的囊泡运输相关蛋白(Skehel et al., 1995)，因此FFAT基序能够指导ORPs定位到内质网上(Loewen et al., 2003)。但FFAT基序介导并不是ORPs定位到内质网上的唯一方式。例如, ORP5和ORP8虽然没有FFAT基序，但是可通过C端的跨膜区定位到内质网膜(Yan et al., 2008; Du et al., 2011)。Osh4p没有PH蛋白结构域和FFAT基序，依然在内质网-质膜(plasma membrane) (ER-PM)上定位(Schulz et al., 2009)。目前，在植物界还未见FFAT基序或相似基序的功能报道。

ANK蛋白结构域是一种特殊的锚蛋白重复结构域。目前，只发现在人类ORP1和酵母Osh1p–Osh2p中含有ANK蛋白结构域。Osh1p的ANK蛋白结构域通过与核-液泡连接蛋白1 (nuclear-vacuole junction protein 1, NVJ1)互作使其定位在细胞核和液泡之间(Manik et al., 2017)。全长ORP1L (ORP1 large)通过

ANK蛋白结构域与GTP结合将Rab7蛋白定位于晚期核内体与溶酶体之间, 可以干扰内膜的转运过程(Johansson et al., 2005; van der Kant et al., 2013)。ANK结构域在Osh2p中的具体功能还不清楚。

4 膜结合位点

膜结合位点(membrane contact site, MCS)指2个细胞器膜之间距离小于30 nm的区域(Helle et al., 2013)。小分子物质的交换以及信号的传递往往发生于MCS。内质网是细胞内分布最广的细胞器, 能够与多种细胞器形成MCS, 如质膜、高尔基体、核内体、线粒体及脂滴(Levine, 2004; Friedman and Voeltz, 2011; Helle et al., 2013)。其它2种膜之间也可以形成MCS, 如核膜与液泡膜, 线粒体与液泡膜, 溶酶体与过氧化物酶体(Eisenberg-Bord et al., 2016; Saheki and De Camilli, 2017)。

ORPs运输脂类的过程一般发生在MCS。例如, OSBP在内质网-高尔基体之间传递胆固醇与PI4P (Mesmin et al., 2013)。Osh6p、ORP5和ORP8均在内质网-质膜之间传递磷脂酰丝氨酸与PI4P (Maeda et al., 2013; Chung et al., 2015)。目前已报道的ORPs的定位及功能见表1。

5 ORPs运输胆固醇的过程

Mesmin等(2013)提出了OSBP运输胆固醇的详细模型(图1)。OSBP运输胆固醇分4步进行。第1步, 膜的栓系。OSBP通过PH蛋白结构域与PI4P和Arf1GTP结合, 栓系到反式高尔基体网络(*trans*-Golgi network, TGN)上; FFAT基序通过与VAP-A互作, 使OSBP另一边栓系到内质网膜上。第2步, 胆固醇运输。ORD蛋白结构域从内质网膜上捕获胆固醇, 然后运输到TGN上。第3步, PI4P的运输。ORD蛋白结构域释放

表1 动物及酵母中ORPs的亚细胞定位及功能

Table 1 Subcellular localization and function of ORPs in animal and yeast

蛋白	膜结合位点	运输脂类	参考文献
OSBP	ER (endoplasmic reticulum)-*trans*-Golgi	Cholesterol, PI4P	Mesmin et al., 2013
ORP1	ER-late endosome/lysomes	Cholesterol	Rocha et al., 2009; Kobuna et al., 2010; Vihervaara et al., 2011
ORP2	ER-lipid droplet, ER-PM (plasma membrane)	Cholesterol, PI(4,5)P2	Hynynen et al., 2009; Jansen et al., 2011; Weber-Boyvat et al., 2015b; Wang et al., 2019
ORP3	ER-PM	PI4P	Weber-Boyvat et al., 2015a; Gulyás et al., 2020
ORP4	Unknown	Cholesterol	Wyles et al., 2007
ORP5	ER-PM, ER-late endosome?	PS, PI4P, cholesterol, PI(4,5)P_2	Du et al., 2011; Chung et al., 2015; Ghai et al., 2017
ORP6	ER-PM	PI4P	Mochizuki et al., 2018
ORP7	Unknown	Unknown	None
ORP8	ER-PM, ER-mitochondrion	PS, PI4P	Chung et al., 2015; Ghai et al., 2017
ORP9	ER-*trans*-Golgi	Cholesterol, PI4P	Ngo and Ridgway, 2009; Liu and Ridgway, 2014
ORP10	Unknown	PS	Maeda et al., 2013
ORP11	*trans*-Golgi-late endosome	Unknown	Zhou et al., 2010
Osh1p	Nucleus-vacuole junction	Cholesterol, ergosterol, PI4P	Levine and Munro, 2001; Schulz et al., 2009
Osh2p	ER-PM	Cholesterol	Schulz et al., 2009
Osh3p	ER-PM	PI4P, Cholesterol	Schulz et al., 2009; Tong et al., 2013
Osh4p	ER-PM, ER-mitochondrion	Sterol, PI4P, PI(4,5)P_2	Raychaudhuri et al., 2006; Schulz et al., 2009; de Saint-Jean et al., 2011; von Filseck et al., 2015b
Osh5p	Unknown	Cholesterol	Schulz et al., 2009
Osh6p	ER-PM	PS, PI4P	Wang et al., 2005a; Maeda et al., 2013
Osh7p	ER-PM	PS, PI4P	Maeda et al., 2013

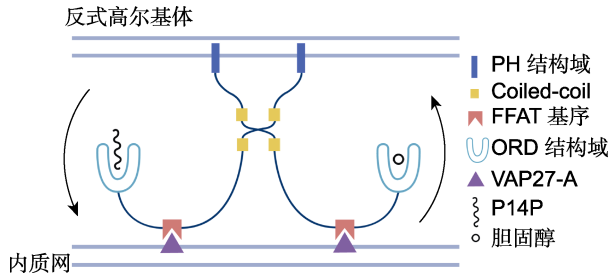

图1 氧化固醇结合蛋白(OSBP)在内质网和高尔基体之间膜结合位点转运胆固醇的功能模式图(改自Mesmin et al., 2013; Pietrangelo and Ridgway, 2018)
OSBP通过FFAT基序与VAP27-A互作，定位于内质网，通过PH蛋白结构域定位在高尔基体。OSBP一般通过Coiled-coil结构域形成二聚体行使功能。ORD蛋白结构域在磷脂酰肌醇-4-磷酸(PI4P)逆行转运的驱动下，将胆固醇从内质网转移到高尔基体。

Figure 1 Working model of oxysterol-binding protein (OSBP) function in cholesterol transportation at membrane contact site (MCS) between endoplasmic reticulum (ER) and *trans*-Golgi (modified from Mesmin et al., 2013; Pietrangelo and Ridgway, 2018)
OSBP is located in ER by FFAT motif interacting with VAP27-A and in *trans*-Golgi by the PH domain. OSBP usually forms dimers through the Coiled-coil domain to play its role. The ORD domain could move cholesterol from the ER to *trans*-Golgi, driven by the retrograde transport of phosphatidylinositol-4-phosphate (PI4P).

胆固醇后，撷取PI4P运输至内质网上。第4步，PI4P水解。PI4P被运至内质网后，被内质网定位的SAC1磷酸酶分解，释放的能量可为此过程提供能量支持。这种水解作用在内质网与TGN之间形成一个持续的PI4P梯度，并促进OSBP在浓度梯度下连续反方向运输胆固醇。阻断OSBP导致的甾醇在内质网/脂滴处积累，从而降低分泌通路的脂质顺序梯度，影响细胞内固醇的分布(Mesmin et al., 2017)。

ORP5和ORP8可以在EPCS区(ER-PM contact site)转运PI4P和磷脂酰丝氨酸(Chung et al., 2015)。研究表明，PI(4,5)P2也可以作为ORP5和ORP8的供体(Ghai et al., 2017)。ORP5和ORP8还定位于内质网-线粒体和内质网-后期囊泡(late endosome)的MCS，调节胆固醇的再分配(Ishikawa et al., 2010; Galmes et al., 2016)。

在酵母细胞中，Osh1–3含有PH和ORD蛋白结构域，Osh4–7只含有ORD蛋白结构域(Raychaudhuri and Prinz, 2010)。Osh1可以通过ANK蛋白结构域定位于细胞核与液泡之间的MCS，转运磷脂和固醇，进而促进细胞内脂质代谢(Levine and Munro, 2001; Manik et al., 2017)。只有ORD蛋白结构域的Osh4p依然可通过形成PI4P浓度梯度在ER-PM之间运输固醇(de Saint-Jean et al., 2011; von Filseck et al., 2015b)。与哺乳动物一样，酵母细胞中固醇的转运也通过PI4P-磷酸酶Sac1水解PI4P形成一定浓度梯度来维持。Osh6p和Osh7p可以将磷脂酰丝氨酸从内质网转运到质膜(Maeda et al., 2013; von Filseck et al., 2015a)。

6 ORPs互作蛋白VAPs

6.1 动物VAPs

VAPs是一类在真核生物中非常保守的囊泡相关膜蛋白(Nishimura et al., 1999)。动物VAPs家族成员众多且分布广泛，主要通过FFAT基序定位在内质网和内质网与其它膜形成的MCS (Murphy and Levine, 2016)。VAPs一般含有MSP蛋白结构域(major sperm protein)、Coiled-coil结构域和C端跨膜结构域(C-terminal transmembrane domain, TMD)。尚未在酵母中发现可以形成二聚体的Coiled-coil结构域或其它类似结构域。VAPs主要与3类蛋白互作：突触囊泡SNAREs (soluble N-ethylmaleimide-sensitive factor attachment protein receptors)、病毒性蛋白和含有FFAT基序的蛋白(Lev et al., 2008)。VAPs与含有FFAT基序的蛋白互作发现于Scs2p与Opi1p相互关系的研究(Loewen et al., 2003)。通过FFAT基序与VAPs互作的脂类运输相关蛋白主要包括ORPs、CERT (ceramide transport protein)和Nirs (PYK2 N-terminal domain-interacting receptors) 3个蛋白家族(Wyles and Ridgway, 2004; Amarilio et al., 2005; Kawano et al., 2006)。除了典型的FFAT基序, ORP3还含有2个FFAT-like基序(HFFSGST和NYSDGSE), 介导与VAPs的互作(Weber-Boyvat et al., 2015a, 2015b)。

VAPs参与调控许多细胞内的生物过程，如膜运输、脂类代谢和运输以及微管的组织(Soussan et al., 1999; Wyles et al., 2002; Kagiwada and Zen, 2003; Kawano et al., 2006)。Scs2是酵母中很重要的VAPs之一。Scs2p参与脂类感知、磷脂代谢、脂类运输以

及未折叠蛋白反应(unfolded protein response, UPR)和端粒沉默等过程(Lev et al., 2008)。Scs2p可与PI4P和PI(4,5)P2结合，磷酸肌醇含量的增加可抑制Scs2p与Opi1p的结合(Kagiwada and Hashimoto, 2007)。Scs2p功能缺失抑制肌醇-1-磷酸合成酶基因*INO1*的表达，降低磷脂酰肌醇的合成，促进磷脂酰胆碱的合成(Kagiwada and Zen, 2003)。VAPs与LTPs互作介导脂类运输是VAPs的重要功能。Scs2p可与酵母Osh1p、Osh2p和Osh3p互作介导MCS之间脂类的运输(Loewen et al., 2003)。与此类似，人类VAP-A与OSBP蛋白互作使其定位在内质网，介导MCS间胆固醇的运输(Mesmin et al., 2013)。VAPs功能缺失引起高尔基体PI4P水平升高，使高尔基体产生的内吞体增加，最终导致产生非正常溶酶体和自噬体(Mao et al., 2019)。人类VAPs功能缺失可引起一系列神经性疾病。例如，VAP-B^{P56S}的突变引起罕见的遗传性运动神经元疾病脊髓性肌萎缩和肌萎缩侧索硬化(Nishimura et al., 2004; Chen et al., 2010; Kabashi et al., 2013)；VAP-B$^{\Delta V25}$的突变引起帕金森病(Kun-Rodrigues et al., 2015)。VAP-B的突变导致内质网不能栓系到高尔基体上，引发高尔基体PI4P水平升高，继而导致非功能溶酶体的积累，使正常自噬溶酶体的降解速率下降(Mao et al., 2019)。

6.2 植物VAPs

拟南芥VAPs含有10个成员，由于发现的第1个VAP分子量只有27 kDa，故命名该家族为VAP27 (Nishimura et al., 1999)。VAP27分为3个亚家族，亚家族I包括5个成员(VAP27-1、VAP27-3、VAP27-5、VAP27-6和VAP27-7)，亚家族II包括3个成员(VAP27-8、VAP27-9和VAP27-10)，亚家族III包含2个成员(VAP27-2和VAP27-4)。拟南芥VAPs同样含有MSD蛋白结构域、Coiled-coil蛋白结构域和跨膜区。VAP27蛋白主要定位在内质网和EPCS (Wang et al., 2016a)。跨膜区的存在是VAPs定位在内质网的决定性区域。VAP27-1定位在EPCS还需要NET3C的介导(Wang et al., 2014)。NET3C属于植物特异的肌动蛋白结合蛋白NET (networked)家族(Deeks et al., 2012)。NET与肌动蛋白结合影响细胞骨架的运动，因此VAP27蛋白可能通过NET来影响细胞骨架的运动。

在酵母细胞中，VAPs通过与ORPs互作介导脂类运输过程(Mesmin et al., 2013)。但在植物中，关于VAP27的功能及其作用机理的研究相对较少。已有研究表明，拟南芥中鞘氨醇转移蛋白ACD11可以与VAP27-1相互作用(Petersen et al., 2009)。VAP27-1-GFP和VAP27-3-GFP转基因株系和功能缺失RNAi株系在根毛分叉表型上存在缺陷(Wang et al., 2016a)。根毛分叉的表型与肌动蛋白互作蛋白AIP1突变体以及F-Actin2调节蛋白ROP2突变体的表型类似，进一步说明VAP27可能通过调节肌动蛋白来调节根毛的发育(Guimil and Dunand, 2007; Ketelaar et al., 2007)。VAP27-1和VAP27-3通过PIPs与网格蛋白的相互作用调节植物的内吞过程(Stefano et al., 2018)。但是植物VAP27蛋白家族成员能否通过与ORP蛋白家族成员相互结合参与膜脂运输还未见报道。

7 植物ORPs

与动物和酵母相似，植物ORPs家族也非常普遍和保守，但是对其家族成员细胞定位及功能的研究比较匮乏。序列分析表明，拟南芥中ORPs家族含有12个成员，分为4个亚家族，亚家族I包含ORP1A、ORP1B、ORP1C和ORP1D四个成员，亚家族II有ORP2A和ORP2B两个成员，亚家族III包括ORP3A、ORP3B和ORP3C三个成员，亚家族IV含有ORP4A、ORP4B和ORP4C三个成员(Skirpan et al., 2006)。通过对这些蛋白序列进行比对分析，我们发现12个成员均含有ORD蛋白结构域。亚家族I和II都含有PH蛋白结构域，且所有成员中除了ORP1B都含有Coiled-coil蛋白结构域；另两类成员都没有此结构域(图2)。

为进一步了解植物与动物ORPs的进化关系，我们分析了拟南芥12个成员与人类及酵母中ORPs氨基酸序列的系统进化关系。结果表明，拟南芥ORPs亚家族I、II和人类OSBP及ORP1、2、4处在同一个分支中(图3)，该分支未见酵母ORPs家族成员，暗示植物这2个亚家族成员有可能参与更高级的多细胞个体发育。III和IV亚家族成员与酵母ORPs的亲缘关系较近，它们可能更多参与单细胞或者细胞间的基本功能。进化树分析表明，植物ORPs与动物和酵母ORPs在氨基酸序列，特别是各个功能性蛋白结构域上具有高度同源性，这暗示植物ORPs很可能拥有不同细胞

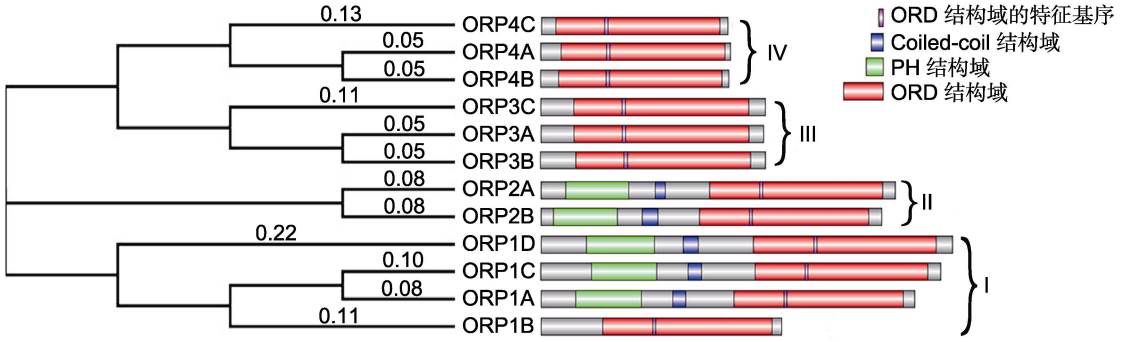

图2 拟南芥ORPs家族成员进化树及蛋白结构域分析

Figure 2 Phylogenetic tree and protein domain analysis of *Arabidopsis* ORPs family

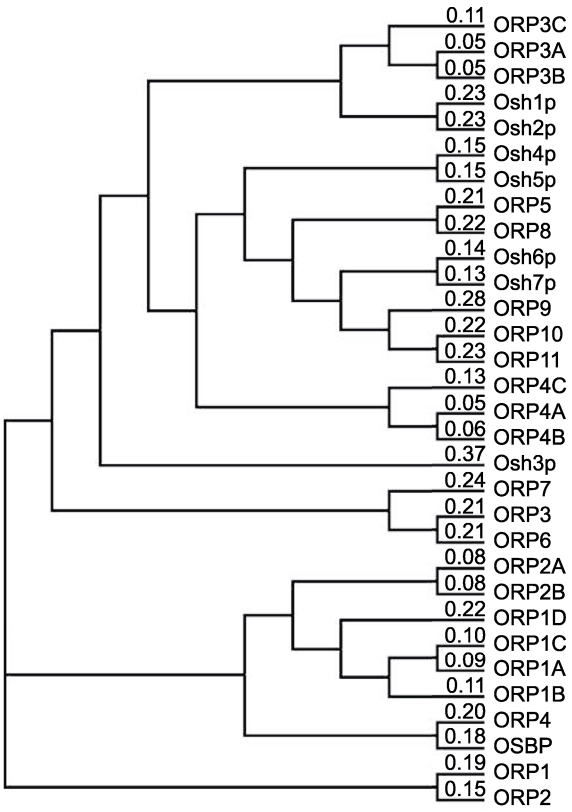

图3 拟南芥、人类和酵母ORPs氨基酸序列的进化分析
拟南芥ORPs包括ORP1A、ORP1B、ORP1C、ORP1D、ORP2A、ORP2B、ORP3A、ORP3B、ORP3C、ORP4A、ORP4B和ORP4C; 酵母ORPs包括Osh1–7p; 人类ORPs包括OSBP和ORP1–11。

Figure 3 Phylogenetic analysis of ORPs in *Arabidopsis thaliana*, human and yeast
Arabidopsis ORPs include ORP1A, ORP1B, ORP1C, ORP-1D, ORP2A, ORP2B, ORP3A, ORP3B, ORP3C, ORP4A, ORP4B, and ORP4C; Yeast ORPs include Osh1–7p; Human ORPs include OSBP and ORP1–11.

器膜和质膜定位，甚至MCS定位，也很可能通过运输脂类物质影响细胞功能，进而作用于个体发育或响应外界环境。

虽然植物中的*ORPs*基因早已被克隆，但关于植物ORPs的功能却少有研究。目前仅有研究表明ORP3A可通过与VAP33家族成员PVA12相互作用而定位于内质网(Saravanan et al., 2009)。BIFC实验证明ORP2A与CPK3相互作用，但在酵母双杂交实验中未检测到二者互作(Berendzen et al., 2012)。PiORP1通过与矮牵牛(*Petunia hybrida*)花粉管质膜上的PRK1受体激酶相互作用参与花粉的生长与发育(Skirpan et al., 2006)。在高盐胁迫条件下大豆(*Glycine max*) *GmOSBP*表达被抑制，而在衰老叶片中表达被诱导，说明GmOSBP可能参与应激反应和细胞衰老过程(Li et al., 2008)。与人类或酵母ORPs的研究相比，植物ORPs影响细胞内脂质转运的机制尚不清楚。

8 研究展望

目前，大量关于人和酵母ORPs功能的研究证实了其在细胞生命活动中发挥重要作用，但是依然存在一些基本问题亟待解决。例如，同一ORPs蛋白如何实现多种定位？PH蛋白结构域实现多种膜定位的机制是什么？同一ORD蛋白结构域如何识别并转运不同脂类？相比于动物，针对植物体中ORPs的研究尚处于起步阶段。近年来，植物细胞学得到了长足发展，已发现众多的细胞器定位标记蛋白(Geldner et al., 2009; Zhu et al., 2020)。植物细胞取材方便，有利于

进行ORPs蛋白家族成员功能研究。目前，我们需要在以下几方面对植物ORPs蛋白的功能进行更深入的探索。

(1) 植物不同ORPs蛋白的细胞定位。相关问题包括植物VAPs是否介导ORPs脂类运输过程、PH蛋白结构域是否具有与磷酸肌醇结合的能力以及ORD蛋白结构域与不同脂类的结合能力分析。

(2) 植物不同ORPs蛋白的生理机制及其在发育过程中的作用。例如，它们是否参与细胞自噬；在动物和酵母中，LTPs在MCS通过介导脂类从内质网至自噬体膜的运输，参与自噬体的形成(Ye et al., 2020)。在低胆固醇条件下，人类ORP1L通过与VAP-A相互作用形成内质网-自噬体接触位点，介导自噬体的运输(Wijdeven et al., 2016)。VAP27-1和VAP27-3已被证明参与植物内吞过程(Wang et al., 2016a; Stefano et al., 2018)。VAP27-1也可以与拟南芥EH蛋白(AtEH1/Pan1和AtEH2/Pan2)互作，调节自噬体的形成(Wang et al., 2019)。然而植物ORPs是否通过VAP27参与细胞自噬过程仍需更多实验验证。

(3) 植物ORPs是否参与植物免疫及对外界环境因子的应激反应。磷脂是磷脂双分子层的重要组成部分，在信号转导中发挥重要作用(Takáč et al., 2019)。磷脂的膜分布可影响膜的弯曲，进而调节膜运输，此过程对植物免疫应答反应至关重要(Wang et al., 2016b; De Craene et al., 2017)。近期有综述阐明基于磷脂的信号转导和膜转运与植物免疫有密切联系(Xing et al., 2021)。因此，探讨脂类运输蛋白ORPs是否通过膜脂运输影响植物应对病原菌侵害和不适环境变化具有重要意义。

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Research Advances in Oxysterol-binding Protein-related Proteins

Wenjiao Zou[1, 3], Lei Ge[2], Qian Yu[2*]

[1]*State Key Laboratory of Crop Biology, College of Life Sciences, Shandong Agricultural University, Tai'an 271018, China*
[2]*Center for Crop Panomics, College of Agronomy, Qingdao Agriculture University, Qingdao 266109, China;* [3]*Innovation Institute of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan 250355, China*

Abstract Membrane lipids not only are important components of cell membranes, but also participate in signal transduction as signal molecules. The uneven distribution of lipid molecules in membranes requires specific types of transport channels and transporters for each lipid type. Oxysterol-binding protein (OSBP)-related proteins (ORPs) are a highly conserved family of lipid transport proteins that recognize and transport phosphoinositides and sterols, which are involved in many physiological processes including signal transduction, vesicle transport, lipid metabolism and non-vesicle transport, and hence play a very important role in the growth and development of individual organism. In recent years, a series of important findings have been made on the structure and function of ORPs in mammals and yeasts, but the advances in plants are relatively slow. In this paper, we review the progress of ORPs research in mammals, yeasts and plants, analyze the structural domains of ORPs in plants and the phylogenetical relationship to their homologs in mammals and yeasts, and also provide perspectives on the directions of plant ORPs research in the future.

Key words oxysterol-binding protein-related proteins, lipid transport, membrane contact site

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* Author for correspondence. E-mail: yuqian@qau.edu.cn

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