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综述

嗜肺军团菌的生存策略: 劫持小GTP酶调控宿主囊泡转运

李游智[1,2] 陈涛涛[1,2] 欧阳松应[1,2] 张丹丹[1,2]*
([1]福建师范大学生命科学学院, 福州 350117; [2]福建师范大学南方生物医学研究中心, 福州 350117)

摘要 作为军团菌病的病原体, 嗜肺军团菌侵入宿主细胞后分泌330多个效应蛋白至宿主细胞, 干扰宿主细胞的生命活动, 建立其复制所需的场所——含军团菌吞噬泡(*Legionella*-containing vacuole, LCV)。LCV形成及成熟是嗜肺军团菌在宿主细胞内生存的必要条件。小GTP酶作为分子开关, 参与细胞囊泡转运过程。近十几年来, 大量研究表明小GTP酶在嗜肺军团菌招募宿主囊泡中发挥重要作用。嗜肺军团菌通过多个效应蛋白调控宿主小GTP酶的活性来操纵宿主囊泡运转过程, 进而招募宿主囊泡。该文对小GTP酶、嗜肺军团菌及其调控小GTP酶的分子机制等方面进行了综述, 以期为进一步理解嗜肺军团菌致病机制提供参考。

关键词 嗜肺军团菌; 效应蛋白; 小GTP酶; 囊泡转运

Survival Strategy of *Legionella pneumophila*: Hijacking Small GTPases to Manipulate Host Vesicle Trafficking

LI Youzhi[1,2], CHEN Taotao[1,2], OUYANG Songying[1,2], ZHANG Dandan[1,2]*
([1]*College of Life Sciences, Fujian Normal University, Fuzhou 350117, China*;
[2]*Biomedical Research Center of South China, Fujian Normal University, Fuzhou 350117, China*)

Abstract *L. pneumophila* (*Legionella pneumophila*), as the causative agent of legionellosis, invades host cells and secretes more than 330 effector proteins to interfere with the life activities of host cells, thereby establishing a niche required for its replication, called the LCV (*Legionella*-containing vacuole). The formation and maturation of LCV is essential for the survival of *L. pneumophila* in host cells. The small GTPases, as a molecular switch, participates in the process of cellular vesicle transport. In the past decade, numerous studies have shown that host small GTPase proteins play an important role in the recruitment of host vesicles by *L. pneumophila*. *L. pneumophila* manipulates host vesicle trafficking by its secreted effector proteins involved in modulating the activity of host small GTPase, thus hijacking host vesicles. This study focuses on reviewing the small GTPase, *L. pneumophila*, and the molecular mechanisms by which *L. pneumophila* regulates the small GTPase, providing reference for further understanding of the pathogenesis of *L. pneumophila*.

Keywords *Legionella pneumophila*; effectors; small GTPases; vesicle trafficking

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*通信作者。Tel: 0591-22868199, E-mail: zhangdandan@fjnu.edu.cn
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*Corresponding author. Tel: +86-591-22868199, E-mail: zhangdandan@fjnu.edu.cn

嗜肺军团菌(*Legionella pneumophila*)是一种广泛存在于自然界水环境中的革兰氏阴性条件致病菌, 能够通过气溶胶感染人肺巨噬细胞引起急性肺炎, 即军团菌病[1]。嗜肺军团菌进入细胞后能够迅速建立适宜繁殖的场所——含军团菌吞噬泡(*Legionella*-containing vacuole, LCV), 逃逸宿主细胞的免疫反应[2]。嗜肺军团菌通过掠夺宿主细胞内的膜和脂质为LCV的建立和成熟提供原料。小GTP酶(small GTPase)是宿主细胞内重要的分子开关, 参与并调控多种细胞过程, 特别是囊泡转运。近年来, 大量的研究表明嗜肺军团菌利用其分泌的效应蛋白作用于宿主细胞内不同的小GTP酶来操纵宿主细胞的囊泡转运[3-4], 促进LCV的形成及成熟[5]。为进一步了解宿主小GTP酶在嗜肺军团菌感染过程中的作用, 我们对嗜肺军团菌的效应蛋白调控小GTP酶、操纵宿主囊泡转运的分子机制进行了详细总结, 以期加深对军团菌病的发病机制的了解, 为军团菌病的防御和治疗提供思路, 并为了解病原菌和宿主相互作用提供见解。

1 嗜肺军团菌概述

1976年, 在美国费城举办的一次退役军人聚会中暴发急性肺炎, 经鉴定导致该肺炎的病原菌是嗜肺军团菌, 该急性肺炎又称军团菌病。随后, 在世界各地也相继报道了不同程度的军团菌病的暴发事件[6]。嗜肺军团菌作为一种胞内寄生菌, 广泛存在于自然水环境中, 其天然宿主是阿米巴原虫。嗜肺军团菌通过气溶胶的形式被人们吸入, 进而感染肺部细胞。嗜肺军团菌感染宿主细胞后, 通过其四型分泌系统(type IV secretory system, T4SS)分泌大量效应蛋白至宿主细胞, 操纵宿主囊泡转运过程, 招募内质网(endoplasmic reticulum, ER)、高尔基来源的囊泡至LCV, 为军团菌提供生存复制的环境[7]。

LCV是一种类似于内质网的囊泡, 是嗜肺军团菌在宿主细胞内生存复制所必需的场所。嗜肺军团菌被宿主细胞吞噬后通过多种机制抑制溶酶体降解途径, 避免LCV与溶酶体的融合。嗜肺军团菌效应蛋白SidK作用于质子泵的亚基VatA抑制ATP水解及质子易位, 进而抑制LCV的酸化[8]。另外, 效应蛋白RavD作为去泛素化酶, 通过结合磷脂酰肌醇-3-磷酸(phosphatidylinositol 3-phosphate, PI3P)锚定至LCV膜上, 特异性去除LCV膜表面的线性泛素链, 进而抑制LCV与溶酶体融合[8-9]。

嗜肺军团菌在宿主细胞内能够干扰宿主内质网高尔基体的分泌途径, 招募内质网及高尔基体来源的囊泡至LCV, 促进LCV形成及成熟[9-10]。效应蛋白SidC是一种新型泛素连接酶, 通过其C-端结合磷脂酰肌醇-4-磷酸(phosphatidylinositol 4-phosphate, PI4P)锚定在LCV膜表面[11], 泛素化修饰宿主小GTP酶及可溶性*N*-乙基马来酰亚胺敏感因子附着蛋白受体(*N*-ethylmaleimide-sensitive factor attachment protein receptors, SNAREs), 促进内质网衍生的囊泡与LCV的膜融合[12]。效应蛋RidL与宿主囊泡蛋白结合蛋白29(vacuolar protein-associated protein 29, Vps29)结合, 抑制内质网–高尔基体的囊泡转运, 有利于嗜肺军团菌生存复制[11]。另外, 嗜肺军团菌通过含有小GTP酶样结构域的效应蛋白, 操纵宿主囊泡转运[13]。嗜肺军团菌效应蛋白LegA15已被证实具有GTP酶活性, 其含有位于N-端的蛋白锚定重复(ankyrin repeat, ANK)结构域及GTP酶结构域。LegA15通过ANK结构域定位于宿主脂滴, 并将宿主囊泡转运因子p115招募至脂滴[14]。p115位于高尔基体, 参与内质网–高尔基体的囊泡转运过程[15], 而LegA15将p115招募至脂滴则干扰宿主内质网–高尔基体物质运输, 促进内质网源性囊泡向LCV的转运。除嗜肺军团菌外, *Legionella longbeachae*也能向宿主细胞释放小GTP酶样的效应蛋白来参与宿主细胞的囊泡转运过程[16]。

不同来源的宿主囊泡含有不同的脂质组成, 如早期内吞体(early endosome)富含PI3P[17], 内质网来源囊泡富含磷脂酰肌醇(phosphatidylinositol, PI)和PI4P[18], 以及高尔基体来源囊泡富含PI4P[19]等。嗜肺军团菌LCV成熟的重要标志是膜脂质组成由PI3P变为PI4P。嗜肺军团菌招募宿主内不同来源的囊泡与LCV融合后, 通过不同方式重塑LCV膜脂质成分, 促使LCV成熟。如嗜肺军团菌通过分泌与脂质代谢相关的效应蛋白重塑LCV膜脂质组成。效应蛋白MavQ具有磷脂酰肌醇-3-激酶(phosphatidylinositol 3-kinase, PI3K)活性, 能够将PI转化为PI3P[20]。效应蛋白LepB具有磷脂酰肌醇-4-激酶活性, 催化PI3P转化为磷脂酰肌醇-3,4-二磷酸[phosphatidylinositol-3,4-bisphosphate, PI(3,4)P_2][21]。PI(3,4)P_2则进一步被磷脂酰肌醇磷酸酶SidF水解产生PI4P[22]。此外, 嗜肺军团菌通过招募宿主的与脂质相关的代谢酶来重塑LCV的脂质组成。如定位于LCV的效应蛋白LpnE通过其Sel1样重复序列(Sel1-like repeats, SLRs)结构域招募

宿主的磷脂酰肌醇-5-磷酸酶OCRL(oculocerebrorenal syndrome of Lowe)至LCV, 将LCV膜上的脂质PI(4,5)P_2水解为PI4P[23]。效应蛋白RalF作为ADP核糖基化因子1(ADP ribosylation factor 1, Arf1)的鸟苷酸交换因子(guanine exchange factor, GEF), 通过激活Arf1招募宿主的磷脂酰肌醇4-激酶IIIβ(phosphatidylinositol 4-kinase IIIβ, PI4KIIIβ)至LCV, 催化PI生成PI4P[24]。综上, 通过不同效应蛋白的作用可使得LCV膜表面脂质成分发生改变, 促进LCV的成熟。

2 小GTP酶概述

小GTP酶家族的成员是鸟苷二磷酸(guanosine diphosphate, GDP)/鸟苷三磷酸(guanosine triphosphate, GTP)结合蛋白, 具有分子开关的功能, 参与调节和整合细胞信号转导[25]。这些GTP酶的开启和关闭是通过结合不同核苷酸进行转换的: 结合GDP时处于无活性状态, 而结合GTP时则处于活性状态[26-27]。小GTP酶的活性状态通过两个被称为开关I和开关II的区域传递给相互作用的蛋白, 这两个调节区域在活性和非活性状态之间发生明显的构象变化[28-29]。而GDP和GTP结合形式之间的相互转换需要调节蛋白GEF和GTP酶激活蛋白(GTPase-activating protein, GAP)的参与: GEF特异性催化GTP与GDP的交换, 进而激活小GTP酶; GAP通过激活GTP酶的GTP水解活性, 将GTP水解成GDP进而终止信号的传导, 使其返回到非活性状态。

小GTP酶家族成员包括Ras(rat sarcoma)、Rho、Ran、Arf/Sar和Rab五大类[30]。每类家族参与不同的细胞内信号通路, 如Ras调控细胞分化[31], Rho调控细胞骨架重排[32], Ran参与核蛋白输入[33], Arf/Sar控制膜囊泡形成[34]及Rab协调囊泡转运[35]。值得一提的是, Rab家族几乎参与所有的囊泡转运。如Rab1调控内质网–高尔基体的囊泡转运[36], Rab17、Rab20等能在内吞和外排过程中发挥作用[36], Rab5及Rab7在内吞体与溶酶体的转换过程中发挥作用[37-38], Rab10参与表皮生长因子受体的转运[39]。

囊泡作为细胞内物质运输和交换的重要载体, 通常是病原体如真菌、细菌、立克次氏体和病毒等感染过程中的作用靶标。病原体通过分泌毒力因子劫持宿主的囊泡转运, 是其在宿主细胞内生存的重要手段之一。

结核分枝杆菌(*Mycobacterium tuberculosis*)是一种致病性胞内寄生菌, 每年能导致数以百万计的人死亡。结核分枝杆菌在感染时能够形成含结核分枝杆菌的液泡(*Mycobacterium*-containing vacuole, MCV), 并释放多种毒力因子, 避免MCV被宿主溶酶体降解, 促进其自身在宿主细胞内存活。如毒力因子SapM作为一种磷酸酶通过水解PI3P抑制MCV表面PI3P的积累, 进而阻碍吞噬泡与晚期内吞体(late endosome)的融合[40]。PtpA作为一种去磷酸化酶, 结合宿主质子泵v-ATPase H亚基并对膜融合调节因子VPS33B(vacuolar protein sorting 33B)去磷酸化, 抑制吞噬泡酸化[41], 避免吞噬泡进入溶酶体途径被降解[42-44]。布鲁氏菌(*Brucella*)是一种革兰氏阴性菌, 感染人和动物后会引起严重的传染性疾病。感染宿主细胞后, 布鲁氏菌在内质网处建立含布鲁氏菌的囊泡(*Brucella*-containing vacuole, BCV)调控宿主细胞内吞途径, 抑制布氏小体与溶酶体融合。效应蛋白BspB通过与COG(conserved oligomeric Golgi)作用, 干扰宿主高尔基体膜运输, 招募高尔基体来源的囊泡至BCV, 促进布鲁氏菌复制[45]。沙门氏菌是一类革兰氏阴性菌, 感染宿主后能导致宿主的肠道疾病。在感染早期, 沙门氏菌劫持小GTP酶Rab5和Rab7, 破坏宿主囊泡转运过程, 建立含沙门氏菌的吞噬泡(*Salmonella*-containing vacuole, SCV)。SseK3是一种糖基转移酶, 糖基化修饰宿主小GTP酶Rab1、Rab5和Rab11, 促进沙门氏菌的感染[46]。

总之, 小GTP酶作为重要的分子开关在囊泡转运过程中扮演不可或缺的作用。而作为胞内病原菌, 嗜肺军团菌在感染过程中通过不同策略操控宿主的小GTP酶家族, 干扰宿主的囊泡转运, 促进LCV成熟, 有利于嗜肺军团菌的感染与生存。

3 嗜肺军团菌对小GTP酶的调控作用

蛋白质组学研究表明嗜肺军团菌LCV膜上的蛋白成分包括560多种宿主蛋白, 其中含有大量的小GTP酶蛋白及其相关蛋白, 这表明宿主小GTP酶蛋白在嗜肺军团菌的致病过程中扮演着重要的角色[47]。嗜肺军团菌通过释放多种效应蛋白至宿主细胞并作用于宿主小GTP酶, 促进嗜肺军团菌在宿主细胞中的繁殖。嗜肺军团菌感染宿主细胞后能够通过多种机制(如翻译后修饰、模拟小GTP酶的功能及充当小GTP酶的调节蛋白等)调控宿主的小GTP酶, 以操纵

宿主囊泡转运, 促进LCV形成及成熟。近年的研究表明多个嗜肺军团菌效应蛋白参与调控小GTP酶[48], 如表1所示。与许多针对小GTP酶蛋白Rho家族的细菌病原体不同[49], 嗜肺军团菌则主要劫持Arf和Rab家族蛋白, 调控宿主囊泡转运, 这对于嗜肺军团菌建立LCV并在细胞内生存至关重要, 如图1所示。

3.1 对宿主Arf1蛋白的调控

ADP核糖基化因子(ADP ribosylation factor, Arf)蛋白家族包括Arf1~Arf6, 调节细胞膜和蛋白质的胞内运输以及囊泡外壳的形成[50]。其中, Arf1对嗜肺军团菌LCV的形成很重要。嗜肺军团菌在侵染初期招募Arf1至LCV膜表面。Arf的细胞定位依赖于嗜肺军团菌Dot/Icm分泌系统分泌的效应蛋白RalF[51]。RalF包含N-端具有GEF活性的Sec7结构域及C-端的加帽结构域[52]。RalF通过其加帽结构域锚定于LCV并招募Arf1至LCV, 其Sec7结构域则负责激活Arf1, 进而招募宿主PI4KIIIβ至LCV, 生成PI4P以促进LCV的成熟[53]。需要注意的是, Arf1在嗜肺军团菌LCV形成的早期阶段富集在LCV上, 但在感染后大约10小时消失, 提示可能有其他的宿主ArfGAP(或嗜肺军团菌自身ArfGAP)将Arf1从LCV去除。

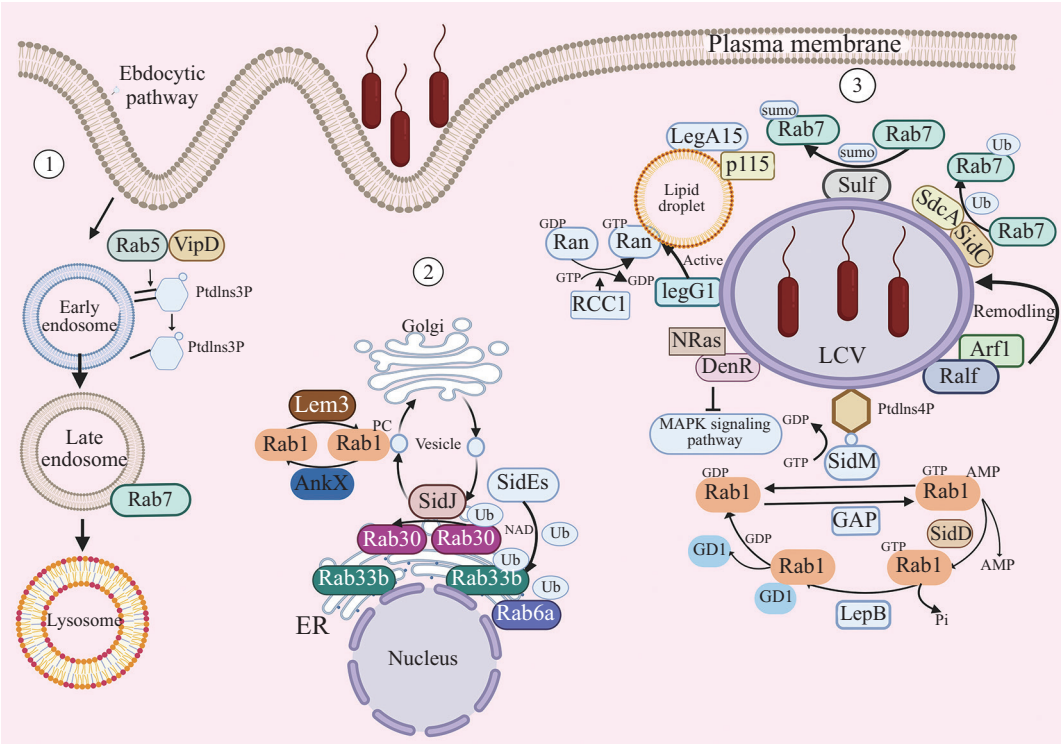
3.2 对宿主Rab蛋白的调控

Rab蛋白家族在真核细胞中有60多个成员, 是小GTP酶中最大的亚家族。作为囊泡转运的主要调控因子, Rab蛋白控制真核细胞内囊泡转运的特异性、方向、时间及协调性[54-56]。在活性状态下, Rabs能够募集各种Rab下游效应因子(分子马达、磷脂调节剂等), 进而调控囊泡转运。控制特定Rab蛋白的定位和活化使得病原体能够操控特定的囊泡转运过程, 以逃逸宿主细胞的防御机制及获取营养物质[57-58]。在感染期间, 嗜肺军团菌分泌的多种效应蛋白能够调节Rab蛋白的活性。Rab1家族包括Rab1A、Rab1B和Rab1C(也称Rab35), 是嗜肺军团菌效应蛋白的特异靶点, 在LCV形成的不同阶段被效应蛋白激活或抑制。Rab1A和Rab1B具有类似的功能, 参与调控内质网–高尔基体间囊泡转运[59], 而Rab1C则定位于网格蛋白包被的囊泡, 调节内吞体的分选[60]。在LCV形成的早期, 宿主内质网衍生的囊泡能够被嗜肺军团

表1 参与小GTP酶调控的嗜肺军团菌效应蛋白

Table 1 *L. pneumophila* effectors involved in regulation of small GTPases

军团菌效应蛋白 *Legionella* effectors	小GTP酶 Small GTPases	功能 Function
Lpg0234 (SidE)	Rab1, Rab6A, Rab30, Rab33b, Rab10	Deubiquitinase; intracellular replication; dynamic regulation of ubiquitin on LCV; recruit endoplasmic reticulum proteins to LCV; endoplasmic reticulum rearrangement
Lpg0393	Rab5, Rab21 and Rab22	Activation of Rab5, Rab21 and Rab22
Lpg0695 (AnkX)	Rab1 and/or Rab35	Rab1 and Rab35 activity regulation
Lpg0696 (Lem3)	Rab1	Rab1 activity regulation
Lpg1950 (RalF)	ARF1	Activation of ARF1; recruit ARF1 to LCV
Lpp1959 (PieG)	Ran	Stable RanGTP; RanGAP1 inactivation
Lpg2224 (PpgA)	Ran	RanGAP1 inactivation
Lpg1976 (LegG1)	Ran	Activate RanBP10
Lpg2153 (SdeC)	Rab1, Rab6A, Rab30 Rab33b, RTN4	Regulate intracellular replication; LCV ubiquitin dynamic regulation; recruit endoplasmic reticulum proteins to LCV; endoplasmic reticulum rearrangement
Lpg2464 (SidM)	Rab1, PtdIns4P	Recruit Rab1 to LCV; regulate the activity of Rab1
Lpg2465 (SidD)	Rab1	Regulate the activity of Rab1
Lpg2490 (LepB)	GAP, phosphoinositide kinase	Regulate the activity of Rab1; generate PtdIns4P
Lpg2510 (SdcA)	Rab7	Ubiquitination of Rab7 with SidC
Lpg2511 (SidC)	Rab7, Rab10	Disrupting the Rab7/RILP axis to inhibit endocytic transport and promote Rab10 polyubiquitination
Lpg2832 (SulF)	Rab7	Recruit Rab7 to LCV
Lpg1909 (DenR)	Nras	Downregulate MAPK signaling
Lpg2831 (VipD)	Rab5, Rab22	Remove PtdIns3P from endoplasmic reticulum membrane



①VipD与Rab5结合定位于早期内吞体, 并通过VipD的磷脂酶活性使PtdIns3P失活。②AnkX在顺式–高尔基体中磷酸胆碱化修饰RAB1B, 这种修饰可被Lem3逆转。SidE家族成员能够用一种非经典的泛素化机制来泛素化与ER相关的几种RABGTP酶, 包括Rab6a、Rab30和Rab33b, SidJ能够逆转这种翻译后修饰。③SidM与磷脂酰肌醇-4-磷酸(PtdIns4P)结合, 进而定位于LCV。SidD逆转SidM对Rab1AMP的修饰, 使Rab1·GTP被LepB, 或者宿主GAP结合, 抑制Rab1的活性, 完成Rab1周期性激活。LegG1激活Ran蛋白促进宿主脂滴与LCV相互作用。SidC/SdcA能够招募并泛素化Rab7。SulF招募并sumo修饰Rab7。Ralf招募并激活Arf1来启动磷脂重塑。DenR能够招募NRas来抑制宿主的MAPK途径。RCC1激活Ran。LegA15定位于宿主脂滴, 与宿主囊泡转运因子p115结合并将其招募至脂滴。

① VipD binds to Rab5 and localizes to early endosomes, and inactivates PtdIns3P through its phospholipase activity. ② AnkX phosphorylates and alkalizes RAB1B in a *cis*-Golgi matrix, which is reversed by Lem3. Members of the SidE family are able to ubiquitinate several RABGTPases associated with ER using a non classical ubiquitination mechanism, including Rab6a, Rab30, and Rab33b, SidJ is able to reverse this post-translational modification. ③ SidM is localized to LCV through PtdIns4P (phosphatidylinositol 4-phosphate) binding. SidD reverses the modification of Rab1AMP by SidM, causing Rab1·GTP to be bound by LepB or host GAP, inhibiting Rab1 activity and completing Rab1 periodic activation. LegG1 activates Ran protein to promote the interaction between host lipid droplets and LCV. SidC/SdcA can recruit and ubiquitinate Rab7. SulF recruits and sumo modifies Rab7. Ralf recruits and activates Arf1 to initiate phospholipid remodeling. DenR can recruit NRas to inhibit the host's MAPK pathway. RCC1 activates Ran. LegA15 is located in the host lipid droplet, binds to the host vesicle transport factor p115, and recruits it to the lipid droplet.

图1 嗜肺军团菌操纵囊泡转运相关的小GTP酶蛋白

Fig.1 Manipulation of host small GTPase proteins involved in the vesicular transport by *L. pneumophila*

菌募集到LCV, 该过程依赖于效应蛋白将Rab1募集到LCV[61]。

3.2.1 对宿主Rab1的调控 Rab1在LCV上的募集依赖于嗜肺军团菌分泌的效应蛋白SidM(DrrA)[62]。SidM包含三个不同的功能域: 位于N-端的腺苷酸转移酶域(ATase)、GEF结构域和位于C-端的PI4P结合域[PI(4)P binding module, P4M][63]。P4M对PI4P具有很高的亲和力, 使DrrA有效地定位于LCV。

SidM的GEF结构域具有将Rab1结合的GDP交换为GTP的活性, 这种活性能够特异性地从细胞质Rab1:GDI复合物中竞争性地招募和激活Rab1。Rab1的竞争性招募和激活, 对于其介导的内质网源性的囊泡靶向转运至LCV的意义重大。值得注意的是, SidM的GEF结构域的氨基酸序列和蛋白质结构与其他GEF蛋白质不具有相似性。另外, SidM也通过其腺苷酸转移酶结构域腺苷酸化修饰Rab1的第77位酪氨酸(Tyr77), 抑制Rab1与其相关GAPs的结合, 使Rab1维持在激活状态(AMP-Rab1•GTP)[64]。这种腺苷酸化修饰可以被具有脱腺苷酸活性的效应蛋白SidD去修饰[65], 促进具有GAP活性的效应蛋白LepB与Rab1结合并将Rab1•GTP转变为Rab1•GDP[66]。效应蛋白LidA作为小GTP酶结合蛋白, 通过与PI3P结合定位于LCV膜[67]。LidA优先结合激活状态的Rab1(Rab1•GTP), 募集与膜融合相关的蛋白, 如

GM130或p115, 促进宿主内质网源性囊泡与LCV的融合[14]。总之, 嗜肺军团菌通过SidM、SidD、LepB及LidA维持并调控LCV膜上的Rab1活性, 有利于嗜肺军团菌通过非经典的膜融合方式获取内质网源性囊泡。

嗜肺军团菌也通过不同的机制(如可逆的翻译后修饰)终止或抑制Rab1的活化。效应蛋白AnkX利用胞苷二磷酸胆碱(CDP-胆碱)将磷酸胆碱基团共价转移至Rab1B的第76位丝氨酸(Ser76), 同时释放胞苷单磷酸(cytidine monophosphate, CMP)[35]。Rab1被磷酸胆碱化修饰后活性被抑制, 导致宿主细胞的分泌途径受到抑制[68]。而AnkX介导的抑制作用能够被效应蛋白Lem3解除。Lem3具有去磷酸胆碱化的酶活性, 通过去除AnkX介导的Rab1磷酸胆碱化修饰进而解除对Rab1的抑制作用[69]。Rab1的腺苷酸化和磷酸胆碱化修饰在功能上具有相似性, AnkX能够修饰激活和非激活状态的Rab1, 而DrrA则优先选择修饰激活状态的Rab1。研究发现, AnkX也定位于LCV, 因此AnkX与SidM将Rab1招募至同一区域[70], 使其更容易被Lem3及GEF激活。此外, 在感染期间, 嗜肺军团菌释放一个效应蛋白, SetA, 它是一个含有典型DXD基序($D_{134}XD_{136}$)的葡糖基转移酶, 能够对Rab1的第75位苏氨酸(Thr75)进行葡糖基化修饰, 抑制其与调节蛋白GTP解离抑制剂1(GDP dissociation inhibitor 1, GDI1)的相互作用, 从而干扰宿主膜转运[71]。

3.2.2 *对其他Rab蛋白的调控* 近期的研究表明, 嗜肺军团菌效应蛋白SidE家族能够直接泛素化修饰多个与宿主内质网相关的Rab蛋白, SidE家族催化非经典泛素化, 利用NAD^+对泛素的第42位精氨酸(R42)进行ADP-核糖基化修饰而使其激活。激活的ADPR-Ub随后被具有磷酸二酯酶(phosphodiesterase, PDE)活性的PDE结构域切割产生磷酸核糖基泛素(phospho-ribosylated ubiquitin, PR-Ub), 同时通过磷酸二酯键将其共价连接至Rab家族蛋白如Rab33b、Rab6A和Rab30等的丝氨酸残基上调控GTP酶活性, 参与内质网稳态调控[72]。嗜肺军团菌效应蛋白VipD具有磷脂酶A1活性, 定位于宿主细胞的早期内吞体[73], 并通过其C-端与Rab5/Rab22结合而被激活[74]。VipD与活化的Rab5或Rab22竞争性结合, 从而阻断Rab5或Rab22下游信号转导, 干扰宿主早期内吞体向晚期内吞体的转变, 进而抑制它们与溶酶体的融合, 促进宿主细胞内嗜肺军团菌的存活[74-75]。泛素化修饰Rab7可帮助嗜肺军团菌调控宿主的囊泡转运以及逃逸溶酶体途径。近期研究发现, SulF通过招募sumo化的Rab7到LCV, 并阻断Rab7与GAP的相互作用来抑制其GTPase活性[76]。SidC/SdcA具有E3连接酶活性, 能够招募并泛素化Rab7, 导致晚期内吞体向溶酶体的融合过程被抑制, 进而促进LCV的成熟[12]。

嗜肺军团菌的效应蛋白不仅可直接作用于Rabs, 还能够通过调控与Rabs相关的蛋白的功能来影响Rabs的作用。OCRL具有肌醇5-磷酸酶活性, 是一种调控内吞体动力学的关键性蛋白, 调节早期内吞体和循环内吞体的动力学以及自噬体与溶酶体的融合。OCRL在细胞内与小GTP酶相互作用, 如Rab5和Rab6, 调控内吞体和TGN(*trans*-Golgi network)。嗜肺军团菌的效应蛋白SdhA靶向OCRL, 占据OCRL与小GTP酶互作的结构域, 阻止内吞体和循环内吞体与LCV融合[77]。总之, 嗜肺军团菌进化出一个与宿主相互作用的蛋白质武器库以调控小GTP酶家族的活性。

3.3 对宿主Ran蛋白的调控

Ran家族在核孔移位、有丝分裂后核膜形成和有丝分裂纺锤体组装以及内吞受体运输等许多细胞过程中起着重要作用[78-79]。Ran能够被称为染色体凝聚1调节因子(regulator of chromosome condensation 1, RCC1)的RanGEF激活[80], 而激活的Ran(Ran•GTP)可被RanGAP1和含有Ran•GTP结合结构域的RanBP1失活[81]。嗜肺军团菌编码的效应蛋白LegG1(*Legionella* eukaryotic gene G1)与真核细胞RanGEF RCC1的序列具有高度的相似性[82-83], 能够激活Ran蛋白。与宿主RCC1不同的是, LegG1可能与Ran(Ran•GTP)结合, 并稳定其活性, 或者抑制RanGAP发挥作用, 进而避免Ran失活[84]。LegG1通过其C-端的CAAX基序被宿主戊烯酰化修饰而定位于LCV上[83], 这是第一个被发现的激活Ran蛋白的效应蛋白, 能够促进嗜肺军团菌LCV的移动以及宿主细胞的迁移。此外, 最近的研究表明LegG1对宿主Ran蛋白的激活可促进宿主脂滴(lipid droplet, LD)与LCV的相互作用, 有利于嗜肺军团菌招募宿主脂滴[85]。

3.4 对宿主Rho蛋白的调控

Rho蛋白家族是一种信号转导蛋白, 在触发多种免疫功能中起重要作用, 参与细胞骨架动力学、细胞极性以及免疫细胞的运输和增殖的调节[86]。嗜肺军团菌对宿主细胞的附着依赖于β1整合素和

E-钙黏蛋白激活的下游信号，可导致Rho-GTPases依赖性的细胞肌动蛋白相关蛋白2/3(actin-related protein 2/3, Arp2/3)和mDia(mammalian diaphanous-related)的激活，进而在细胞表面介导包裹丝状细菌的初期LCV膜的形成。之后，嗜肺军团菌的效应蛋白VipA介导初期LCV的快速延伸，促进肺表皮细胞吞噬嗜肺军团菌[32]。

Cdc42是Rho家族蛋白之一、肌动蛋白动力学的关键调节因子，调控细胞的迁移[87]，Cdc42通过WASP(Wiskott-Aldrich syndrome protein)的效应蛋白和TOCA(transducer of Cdc42-dependent actin assembly)家族能够将多种信号与肌动蛋白聚合联系起来。此外，WASP家族还能够直接将Cdc42与肌动蛋白成核子Arp2/3复合物偶联[88]。研究发现，嗜肺军团菌的群体响应因子1(*Legionella* autoinducer-1, LAI-1)能够抑制这种作用。LAI-1通过降低ARHGEF9的活性抑制GTP与Cdc42结合，从而降低Cdc42的活性，破坏细胞骨架的稳定，抑制宿主细胞迁移[89]。

3.5 调节Ras蛋白

Ras蛋白家族在多个物种中高度保守，在吞噬、细胞生长及增殖等细胞活动中发挥重要作用。Ras主要分布于高尔基体和细胞质膜，广泛参与下游信号的传导。NRas是Ras家族的成员之一，主要存在于高尔基体和细胞质膜中，控制众多下游信号，如经典的MAPK信号通路。嗜肺军团菌效应蛋白DenR(Lpg1909)定位于LCV表面，招募NRas至LCV。DenR将NRas招募至LCV抑制MAPK信号通路的转导，促进嗜肺军团菌生存[90]。Rap1属于Ras蛋白超家族，参与细胞内多种信号转导途径，能够响应各种细胞内外信号而被激活。嗜肺军团菌侵染宿主过程中，招募Rap1至LCV，从而促进嗜肺军团菌在细胞内的生存与复制[91]，然而嗜肺军团菌通过何种方式招募Rap1尚未可知。

4 总结与展望

小GTP酶介导的囊泡转运在嗜肺军团菌感染过程中具有重要的作用。嗜肺军团菌侵入细胞后释放效应蛋白调控小GTP酶，干扰宿主的囊泡转运，促进自身的生存和复制。当前的研究从酶学、生物化学和蛋白质结构水平进行阐述，为全面地认识嗜肺军团菌提供扎实的科学基础。嗜肺军团菌分泌多达330多种效应蛋白，虽然它们对嗜肺军团菌在宿主细胞中生存与复制的生理意义依然未能被完全掌握，但随着研究的深入，效应蛋白的功能逐渐清晰。在本文中我们重点整理当前关于嗜肺军团菌劫持宿主小GTP酶参与宿主膜重塑、囊泡转运，促进LCV形成的相关事件，发现效应蛋白靶向宿主小GTP酶通过翻译后修饰、直接作用或者模拟宿主小GTP酶的功能来调控宿主的囊泡转运，这加深了我们对嗜肺军团菌致病机制的理解，也为我们认识病原菌效应蛋白与宿主靶标的相互作用提供了参考。未来，微生物学、生物化学和蛋白质组学等方面的努力并结合多种新兴技术手段也会逐渐阐明嗜肺军团菌与小GTP酶相互作用的方式，并阐明宿主与病原菌相互攻防的机制，为病原菌的治疗与防御提供参考。

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