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专论与综述

嗜肺军团菌 II 型分泌系统及其底物的研究进展

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摘　要：嗜肺军团菌(*Legionella pneumophila*，*L. pneumophila*)是研究病原菌-宿主相互作用的重要模式菌株之一，其独特的分泌系统及底物效应蛋白的结构与功能是病原微生物领域的研究热点。II 型分泌系统(type II secretion system，T2SS)对促进细菌在环境和人类宿主中的生存至关重要。嗜肺军团菌 *Legionella* secretion pathway(Lsp)系统是革兰氏阴性病原菌中一个典型的 T2SS。本文综述了 *L. pneumophila* 的 T2SS 及其底物效应蛋白的研究进展，重点介绍其结构与功能，为深入了解革兰氏阴性病原菌的 T2SS 功能和作用机制提供参考。

关键词：嗜肺军团菌；II 型分泌系统；效应蛋白；结构与功能

Research progress in the type II secretion system of *Legionella pneumophila* and its substrates

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Abstract: *Legionella pneumophila* (*L. pneumophila*) is a distinct model for pathogen-host interaction research. Its unique secretion systems as well as the structures and functions of their substrate effectors are the research

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hotspots in the field of pathogenic microorganism. Type II secretion system (T2SS) plays a major role in promoting bacterial survival in the environment and in human hosts, and *Legionella* secretion pathway (Lsp) is a typical T2SS in Gram-negative pathogens. This review briefly summarizes the research progress of *L. pneumophila* T2SS and its substrate effectors, emphasizing on their structures and functions, so as to provide in-depth understanding of the function and mechanism of T2SS in Gram-negative pathogens.

Keywords: *Legionella pneumophila*; type II secretion system; effector; structures and functions

革兰氏阴性菌已经进化出至少 7 种类型的分泌系统(I–IX 型)来介导效应蛋白(effector)从细菌输出到细胞外环境或宿主细胞中[1-2]，其中 II 型分泌系统(type II secretion system，T2SS)是一个精巧的多蛋白跨膜组装体，许多革兰氏阴性病原菌通过 T2SS 向细胞外环境和/或宿主细胞中转运多种毒力因子和酶[1,3-4]，有助于细菌适应一系列不同的生境，包括极端的压力、温度和高渗透压环境[5-6]。基因组测序分析表明，典型的 T2SS 主要分布在变形菌门(*Proteobacteria*)，包括 α、β、γ 和 δ 分支[4]。T2SS 输出的底物包括各种毒素、降解酶和其他效应蛋白(包含一些新型未知功能蛋白)，介导的致病过程包括宿主细胞的死亡、组织的降解、固有免疫的抑制、对宿主细胞表面的黏附、生物膜的形成、在宿主细胞中的侵袭和生长、营养物质的同化及宿主离子通量的改变等[4,7]。

1 嗜肺军团菌

1976 年美国费城退伍军人大会中有 221 人突然暴发急性发热性呼吸道疾病，最终共有 34 人死亡，当时引起了人们巨大的恐慌；1977 年，美国疾病预防控制中心(Centers for Disease Control and Prevention，CDC)的科学家从病人的肺组织分离出一种细菌，命名为军团病杆菌(Legionnaires' disease bacterium，LDB)；次年，美国 CDC 将 LDB 正式命名为 *Legionella pneumophila* (*L. pneumophila*)，这种致命性的肺炎被称为军团病(Legionnaires' disease)[8-10]。当含有 *L. pneumophila* 的气溶胶被免疫力低下的人群吸入时，该菌就有机会侵染人肺泡组织的巨噬细胞和上皮细胞[11-12]，引起致命的军团病或一种较温和的流感样疾病——庞蒂亚克热(Pontiac fever)[13-15]。军团病是一种以肺部病变(如化脓性支气管炎、大叶性肺炎伴小脓肿形成)为主的急性全身性疾病，可由肺部迁徙入血引起播散，导致皮肤、肌肉、消化、神经等多系统损伤[16]。庞蒂亚克热是一种非肺炎型军团病，主要表现为急性流感综合征，无明显肺部病变，通常 3−5 d 即可自愈[13]。

L. pneumophila 为兼性需氧的革兰氏阴性病原菌，广泛存在于天然淡水环境和人工水域中，可以在原生动物(如阿米巴原虫)体内寄生和繁殖，并成为生物膜的一部分[17-18]。*L. pneumophila* 入侵宿主细胞后通过形成“含军团菌的泡”(Legionella-containing vacuole，LCV)逃避宿主细胞的内吞噬作用(endocytosis)[19-21]，其在宿主细胞中的生存周期包含两个阶段：复制期(replicative phase，在充足的养分供应下以指数增长为特征)和传播期(transmissive phase，宿主细胞中营养源耗尽时细菌的表型完全改变，具有运动性和细胞毒性，细菌在这一阶段裂解杀死宿主细胞并逃逸，伺机进行新一轮的感染和增殖)[20,22-24]。

L. pneumophila 具有专门的分泌系统作为毒力的基本策略，目前已经鉴定出 *L. pneumophila*

中至少存在 6 种类型的分泌系统：I、II、IVA、IVB、V 和 VI 型分泌系统，其中 II 型分泌系统(T2SS)和 IVB 型分泌系统(T4BSS)与 *L. pneumophila* 的致病性密切相关[4,25]。*L. pneumophila* 的 T4BSS 即 Dot/Icm 分泌系统，负责将 300 多个效应蛋白转运进入宿主胞质溶胶中，对于 *L. pneumophila* 在所有宿主细胞内的生存、复制和致病性必不可少[26]。T2SS 即 *Legionella* secretion pathway (Lsp)，对于促进细菌在环境和人类宿主中的生存方面至关重要，涉及的细胞功能包括促进 *L. pneumophila* 在低温下生长，感染阿米巴原虫和巨噬细胞宿主，损伤肺组织，滑动运动以及逃避免疫系统的追杀[7,27-31]。

2 嗜肺军团菌 T2SS

目前已经鉴定出至少 63 种军团菌，其中 32 种军团菌具有致病性，而且编码 T2SS 的基因在军团菌属中高度保守[3]。嗜肺军团菌 T2SS 由 12 种核心蛋白组成(T2S C、D、E、F、G、H、I、J、K、L、M 和 O) (结构模型见图 1)，可分为 4 个亚复合物[4,32]：(1) 延伸到周质空间的外膜(outer membrane，OM) “分泌素孔”，是由 T2S D 蛋白组成的一个五聚体，为底物穿过外膜提供一个孔道[33]；(2) 一个由 T2S C、F、L 和 M 组成的内膜平台(inner membrane platform，IM)，其中 T2S C 连接 OM“分泌素孔”[34-35]；(3) T2S E 六聚体复合物是一种胞质 ATPase，被招募到 IM 平台上，为分泌过程提供能量[7,36]；(4) 一个跨越细胞周质的假菌毛(pseudopilus)，由小的假菌毛蛋白 T2S H、I、J 和 K 覆盖大假菌毛蛋白 T2S G 组成的螺旋丝状结构，跨越了由 IM 平台与 OM “分泌素孔”相互作用产生的通道内周质[30-31,37]。T2S O 是一种 IM 肽酶，在假菌毛蛋白组装成假菌毛前对其切割并进行甲基化修饰[4,38-41]。T2S G 与 T2S L 相互作用，促进假菌毛的生物发生[42]。

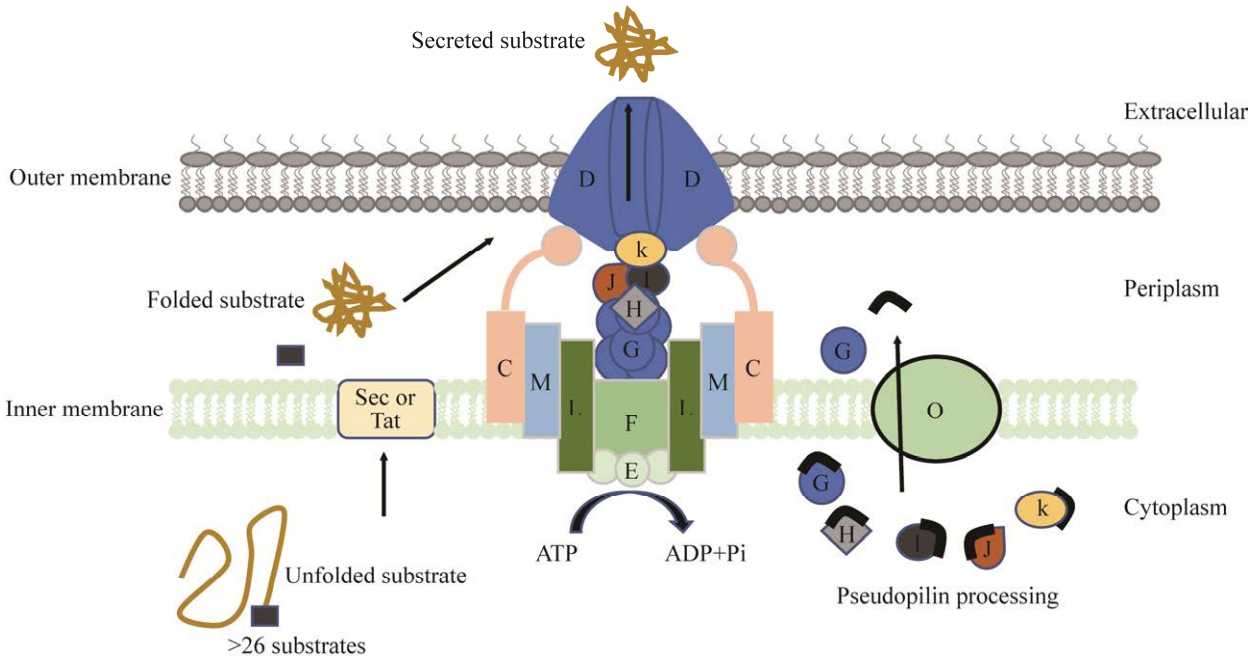

图 1 嗜肺军团菌的 T2SS 结构模型

Figure 1 The structure model of T2SS in *L. pneumophila*.

T2SS 介导的底物转运机制仍不清楚，研究认为 T2SS 的底物与 IM 平台、“分泌素孔”和假菌毛之间的相互作用触发了由假菌毛介导及 ATP 水解驱动向细胞外环境的转运[32,35,38,42]，这一过程可能包含 3 个步骤：分泌的蛋白质首先通过 general secretory pathway (Sec) translocon[43-44]或 twin-arginine translocation (Tat) system 途径[45-46]跨过 IM 并进入细胞的周质空间，信号肽被切掉，然后折叠成三级构象(在某些情况下进行寡聚化)，最后由 T2SS 转运通过细胞 OM “分泌素孔”[7,34]。T2SS 的底物分泌机制可能是周质空间中的蛋白质底物被传送到 T2SS 装置，被 T2S C 和 T2S D 识别，然后利用在 IM 处产生的能量，假菌毛充当活塞或通过阿基米德螺旋(Archimedes screw)推动蛋白质通过 OM “分泌素孔”释放出去[1,7,34,43,47-48]。T2SS 的绝大多数底物蛋白含有典型的信号序列，通过 Sec translocon 转运进入 T2SS[49]，目前仅发现磷脂酶 C (phospholipase C，PLC)家族成员 PlcA (包含一个 twin-arginine motif)和 PPIase (the putative peptidyl-proline cis/trans-isomerase) LirB (包含一个 twin-lysine motif)通过 Tat 途径跨过细胞内膜[50-51]。

3 嗜肺军团菌 T2SS 的底物效应蛋白

L. pneumophila Philadelphia-1 (费城)菌株及其衍生菌株 JR32 基因组的生物信息学分析表明 T2SS 至少有 73 种假定的底物，即含有信号序列并被预测具有细胞外定位的蛋白质。通过对野生株 Philadelphila-1 130b 与 *T2S F* 突变株培养上清的蛋白质组比较分析、酶活测定及二维电泳蛋白质组学研究，证实 Philadelphila-1 130b 菌株中至少有 26 种分泌蛋白/活性依赖于 T2SS[3,28]，见表 1。

3.1 效应蛋白的分类

由于假定底物还需要大量的研究工作来验证其 T2SS 依赖性，因此本文仅讨论已经验证的底物蛋白。根据现有的研究报道，我们将 *L. pneumophila* T2SS 底物蛋白分为三大类。

3.1.1 酶类

L. pneumophila 中有多种酶具有 T2SS 依赖性，包括锌金属蛋白酶 ProA (Lpg0467)、磷脂酶 PlaA (Lpg2343)和 PlaC (Lpg2837)、脂肪酶如 LipA (Lpg0468)和 LipB (Lpg1157)、核酸酶如 SrnA (secreted ribonuclease A，Lpg2848)、几丁质酶如 ChiA (Lpg1116)、葡聚糖酶如 CelA (Lpg1918)及抗氧化相关的酶 Lpg0406。这些酶(如 ProA 和 SrnA)降解阿米巴中的蛋白质、肽、RNA 和脂质后为 *L. pneumophila* 提供营养物质(氨基酸、核苷酸、磷酸盐、脂肪酸)，促进细菌在宿主细胞内的生长及某些情况下酶介导的 LCV 修饰[30,52]。

3.1.2 类真核蛋白(eukaryotic-like proteins)

水平基因转移(horizontal gene transfer，HGT)可能是 *L. pneumophila* 获得效应蛋白的主要途径，即病原体从真核细胞中获取外来遗传物质，并将其整合进自身的基因组中，而且保留了其原本的部分活性[53]，这类基因被称为类真核基因(eukaryotic-like genes)，对应的蛋白即为类真核蛋白(eukaryotic-like proteins)，其包含的类真核结构域有 ankyrin repeats、leucine-rich repeats、serine/threonine kinase domains、ubiquitin-related domains 等，这类蛋白模仿真核宿主蛋白的功能，操控宿主细胞的多种生命进程，是 *L. pneumophila* 破坏宿主细胞功能的重要武器[54]。*L. pneumophila* T2SS 底物中包含 8 种类真核蛋白：*Lp*Map (Lpg1119)、GamA (Lpg0422)、PlcA (Lpg0502)、PlcB (Lpg1455)、LapA (Lpg2814)、LapB (Lpg0032)、Lcl (Lpg2644)和 LegP (Lpg2999)。

表 1 嗜肺军团菌(费城菌株) T2SS 底物统计

Table 1 Documented T2SS substrates of *L. pneumophila* subsp. *pneumophila* str. Philadelphia-1

T2SS substrates	Activity and role in infection
ProA (Lpg0467)	Zinc metalloprotease; Promotes tissue destruction in lung and growth in Nl and Vv; May promote growth in Ac; Not required for growth in A549, HL-60, U937, Wm, explanted guinea pig macrophages, and murine lung
PlaA (Lpg2343)	Lysophospholipase A; Promotes destabilization of the LCV; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
PlaC (Lpg2837)	Phospholipase A; Promotes growth in Ac, Nl, Vv and Wm; Not required for growth in A549 and U937
LipA (Lpg0468)	Monoacylglycerol lipase; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
LipB (Lpg1157)	Triacylglycerol lipase; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
SrnA (Lpg2848)	Type 2 ribonuclease; Promotes growth in Nl and Vv; Not required for growth in A549, Ac, U937, Wm and murine lung
ChiA (Lpg1116)	Chitinase; Promotes growth in murine lung; Not required for growth in A549, Ac, Nl, U937, Vv and Wm
CelA (Lpg1918)	Endoglucanases; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
Lpg0406	Carboxymuconolactone decarboxylase; Not determined the role in infection
*Lp*Map (Lpg1119)	Eukaryotic-like tartrate-sensitive acid phosphatase; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
GamA (Lpg0422)	Eukaryotic-like glucoamylase; Not required for growth in Ac, Nl, U937, Vv and Wm
PlcA (Lpg0502)	Eukaryotic-like phospholipase C; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
PlcB (Lpg1455)	Eukaryotic-like phospholipase C; Not required for growth in A549, Ac, Nl, U937, Vv and Wm
LapA (Lpg2814)	Eukaryotic-like aminopeptidase; Promotes growth in Ac, not required for growth in A549, Nl, U937, Vv, Wm and murine lung
LapB (Lpg0032)	Eukaryotic-like Lys/Arg aminopeptidase; Not required for growth in A549, Ac, Nl, U937, Vv, Wm and murine lung
Lcl (Lpg2644)	Eukaryotic-like collagen-like protein; Promotes attachment to A549, NCI-H292 and U937; Promotes attachment and invasion of Ac and Vv; Not required for growth in Ac
LegP (Lpg2999)	Eukaryotic-like putative protease; Not required for growth in Ac, Nl, U937, Vv and Wm
AmiA (Lpg0264)	Putative amidases; May promote growth in A549, Ac, U937 and Vv
NttA (Lpg1385)	Predicted novel activity; Promotes growth in Ac and Wm; Not required for growth in Nl, U937 and Vv
NttB (Lpg2622)	Novel C1 family cysteine; Not required for growth in Ac, Nl, U937, Vv and Wm
NttC (Lpg1809)	Predicted novel activity; Promotes growth in Vv and Wm; May promote growth in Ac; Not required for growth in Nl
NttD (Lpg0956)	Predicted novel activity; Promotes growth in Ac; Not required for growth in Nl, U937 and Vv
NttE (Lpg0189)	Predicted novel activity; May promote growth in Ac, Nl, U937 and Vv
NttF (Lpg0873)	Novel activity; May promote growth in Ac
NttG (Lpg1832)	Novel VirK family protein; Not determined the role in infection
LirB (Lpg1962)	Putative peptidyl proline *cis-trans*-isomerase; Not required for growth in Ac and HL-60

Notes: Ac: *Acanthamoeba castellanii*; Ap: *Acanthamoeba polyphaga*; Nl: *Naegleria lovaniensis*; Vv: *Vermamoeba vermiformis*; Wm: *Willaertia magna*; Dd: *Dictyostelium discoideum*; A549 cell line; U937 cell line; HL-60 cell line (the data is mainly from the reference [3]).

3.1.3 新型未知功能蛋白

T2SS 底物中还包含多个未知功能的效应蛋白，命名为 Ntt (novel type two secreted protein)，包括 NttA (Lpg1385)、NttB (Lpg2622)、NttC (Lpg1809)、NttD (Lpg0956)、NttE (Lpg0189)、NttF (Lpg0837)和 NttG (Lpg1832)，以及一些假定的酶如 AmiA (Lpg0264)和 LirB (Lpg1962)。

3.2 效应蛋白的结构与功能

虽然目前已经获得了 10 种 T2SS 底物蛋白的结构信息(图 2)，但是大部分蛋白的结构和具体作用机理仍不清楚。

L. pneumophila 至少编码 3 个不同的 PLAs (phospholipases A)家族(Patatin-like、PlaB-like 和 GDSL 酶家族)中 15 种蛋白，其中 PlaA 和 PlaC 属于 GDSL 酶家族，而且是 T2SS 的底物[55-56]。GDSL 家族酶包含 5 个共有序列(I−V)和 4 个完全保守的催化残基 Ser、Gly、Asn 和 His (分别位于共有序列 I、II、III 和 V)[57]。PlaA 是 *L. pneumophila* 中第一个被鉴定出来的 GDSL 酶，具有分泌性的 LPLA (lysophospholipase A)活性、PLA (phospholipases A)活性和脂肪酶活性[58]。LPLA 活性可以分解具有细胞毒性的 LPC (lysophosphatidyl choline)和 LPG (lysine phosphatidyl glycerin)，这可能有助于细菌在 LPC 环境中的脱毒，对于暴露在 LPC 环境下的细菌生存非常关键[36,55,58]。*plaA* 敲除菌株的培养基上清中分别丧失了 90%裂解 LPC 的活性和 70%−80%的裂解 LPG 的活性，因此 PlaA 承担了 *L. pneumophila* 主要的分泌性 LPLA 的活性[55]。在 LCV 复制增殖阶段，T4BSS 分泌的毒力因子 SdhA 通过抑制 PlaA 的脂肪酶活性，抑制宿主细胞的死亡途径，避免 LCV 膜脂质分子降解，从而维持 LCV 的完整性[20,59-60]。PlaA 的催化三联体(Ser46、Asp295 和 His298)对于促进 LCV 的破裂及宿主细胞的死亡是必不可少的[61]。PlaC 与 PlaA 有 26%的序列一致性(sequence identity)

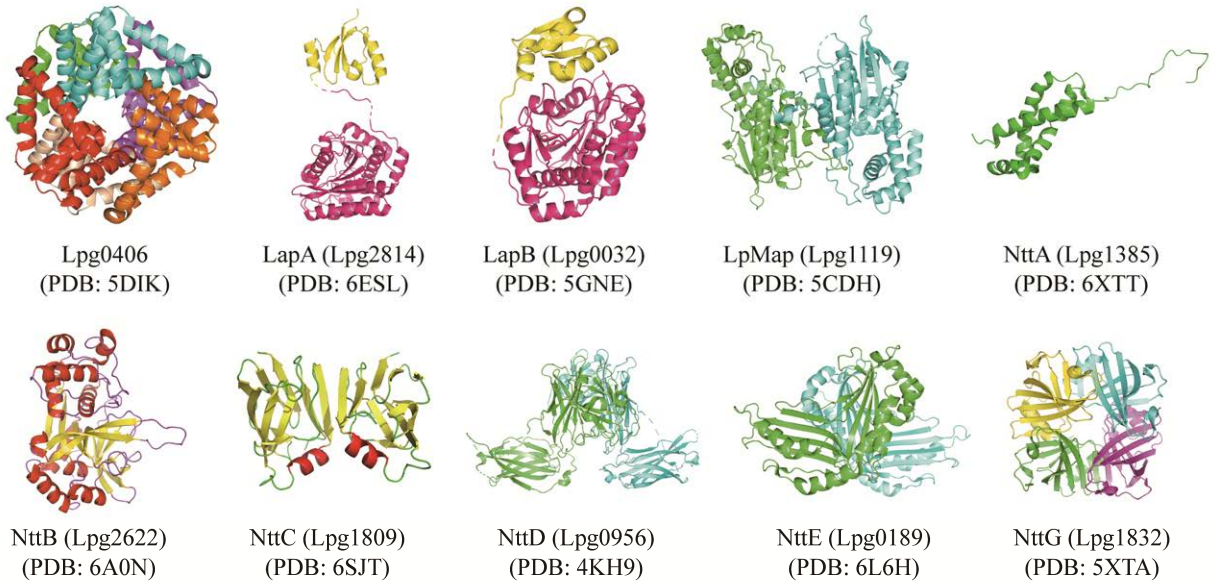

图 2 已经解析的 *L. pneumophila* Philadelphia 1 菌株中 T2SS 底物结构汇总 以上结构数据来源于 protein data bank (http://www.rcsb.org/pdb/)

Figure 2 Structures summary of T2SS substrates of *L. pneumophila* subsp. *pneumophila* str. Philadelphia 1. The structure data comes from protein data bank (http://www.rcsb.org/pdb/).

和46%的序列相似性(sequence similarity)，而且具有分泌性的 LPLA 活性、PLA 活性和 GCAT (glycerophospholipid：cholesterol acyltransferase)活性(即催化长链脂肪酸从二棕榈酰磷脂转移到胆固醇)[30]。*plaC* 敲除菌株的培养基上清中 PLA 分泌减少约 20%，LPLA 分泌减少约 10%，而且完全丧失了 GCAT 活性，因此 PlaC 负责 *L. pneumophila* 中主要分泌性的 GCAT 活性[60]。PlaC 的催化三联体(Ser37、Asp398 和 His401)中的单个氨基酸残基及位于PlaC靠近C末端的4个半胱氨酸残基(Cys343、Cys388、Cys415 和 Cys427)对于其 GCAT 活性和 PLA 活性是必需的[30]。此外，PlaC 的 GCAT 活性依赖于 T2SS 的另一个底物蛋白 ProA (Lpg0467)，其激活机制可能是：在细胞质中，PlaC 和 ProA 以非活性前体形式存在，由 Sec 系统转运通过细菌内膜并伴随信号肽的切割；在周质空间中，PlaC 靠近 C 末端的 4 个半胱氨酸残基可能形成二硫键；ProA 和 PlaC 经 T2SS 输出后，前者通过其 N 端前肽剪切而自我激活成为成熟的 ProA，随后 PlaC 被成熟的 ProA 加工并激活而显示出 GCAT 活性和全部的 PLA 活性[61]。Qu 等完成了 PlaA 的初步晶体学研究[62]，相关的功能研究仍在进行中。

Lpg0406 属于 CMD (carboxymuconolactone decarboxylase)蛋白家族，结构研究显示 Lpg0406 单体包含由 3 个 α 螺旋组成的 CMD 核心基序，单体可形成六聚体环状结构(图 2)，其特征基序 CXXC (C 代表半胱氨酸，X 代表任意氨基酸残基)与 CMD 蛋白家族中 AhpD 蛋白的质子传递系统相似，因此 Lpg0406 在功能上与 AhpD 更相似，可能具有过氧化物酶活性，参与细菌的抗氧化防御[63]。

几丁质是一种 N-乙酰氨基葡萄糖的长链聚合物，在环境中非常丰富，可被几丁质酶分解产生碳和氮，为细菌提供营养[64]。*L. pneumophila* 的几丁质酶 ChiA (Lpg1116)是第一个鉴定出来与军团菌毒力密切相关的 T2SS 效应蛋白，其 N 端结构域帮助其分泌后定位于 LCVM (*Legionella*-containing vacuole membrane)的胞质面，这对于细菌在肺组织中存活是必需的[31,65]；C 端的几丁质酶结构域对哺乳动物中类粘蛋白具有新颖的 Zn^{2+}依赖性肽酶活性，因而可通过水解粘蛋白层(mucin layer)增加 *L. pneumophila* 的渗透性[31]。*chiA* 突变株在巨噬细胞中可正常生长，但在肺部的存活率下降且仅在感染后期才表现出来，这说明 ChiA 能够促进细菌在肺部的持续性而不是影响最初的复制[31]。总之，ChiA 是 *L. pneumophila* 中一个重要的多功能蛋白。

L. pneumophila 分泌酸性磷酸酶的活性至少由两种不同的酶引起，其中大部分活性由酒石酸敏感的组氨酸酸性磷酸酶 Lpg1119 决定，因而被命名为 *Lp*Map (major acid phosphatase from *L. pneumophila*)[66]。*Lp*Map 单体结构呈现出由核心结构域(core domain)和帽子结构域(cap domain)组成的典型的 HAP (histidine acid phosphatase)折叠方式，其中核心结构域为 αβα sandwich architecture，包含一个高度保守的组氨酸酸性磷酸酶特征性序列 ^{33}RHGXRXP^{39} (X 代表任意氨基酸残基)，Arg33、His34 和 Arg37 参与 *Lp*Map 与阴离子抑制剂 L(+)-酒石酸结合时磷酰部分的锚定；帽子结构域主要由 α 螺旋组成，介导了 *Lp*Map 的底物特异性[67-69](图 2)。

LapA 和 LapB 是 T2SS 依赖的胞外分泌氨肽酶，可以水解多肽的 N 末端氨基酸残基[70]。其中，LapA 与 *L. pneumophila* 原生动物宿主 *Acanthamoeba castellanii* (*A. castellanii*)的同源蛋白有较高的相似性(41%的序列一致性和 60%的序列相似性)，因此 LapA 可能是通过 HGT 从 *A. castellanii* 获得或二者有着共同的祖先，

而 LapB 是 LapA 复制后获得不同功能的结果[30]。尽管 LapA 和 LapB 有 42%的序列一致性和 59%的序列相似性，但底物偏好有所不同，LapB 倾向于水解带有 Arg 或 Lys 残基的肽键；LapA 属于金属氨肽酶中的 M28 家族，主要偏好水解 N 末端为 Leu、Phe、Tyr 的蛋白质和多肽[71]。生化功能研究发现 LapB 对有机溶剂、碱性环境有一定的耐性，对温度也有很好的适应性，各种有机溶剂(例如乙醇、丙醇和甲醇)大大提高了 LapB 的活性，乙醇浓度为 60%时 LapB 活性最高，而同样环境中 ΔPA-LapB 的活性降低，CD (circular dichroism)实验和结构分析表明 LapB 的 PA 结构域通过覆盖活性位点而降低其催化效率，有机溶剂诱导 PA 结构域构象变化而消除对 LapB 酶活性的抑制作用[71]。这些活性特征表明 LapB 具有潜在的商业价值，经过改造可能成为食品工业中的高效氨肽酶[72-73]。

PLC 属于磷酸二酯酶，水解磷酸二酯酶键生成 1,2-二酰基甘油(1,2-diacylglycerol, 1,2-DG)和磷酸醇[74-75]。*L. pneumophila* PLC 家族包含 3 个成员(PlcA、PlcB 和 PlcC)，三者的活性具有 Zn^{2+}依赖性，其中 PlcA 和 PlcB 是 T2SS 的底物，而且 PlcA 负责了 *L. pneumophila* 约 70%的分泌性 p-NPPC (*para*-nitrophenylphosphorylcholine)水解酶活性[76]。

L. pneumophila 在 *A. astellanii* 中复制增殖期间，GamA (glucoamylase)作为类真核糖化酶被表达且具有糖原和淀粉降解活性，这表明 *L. pneumophila* 能够降解自然环境中广泛存在的外源多糖并利用碳水化合物，从而促进细菌在环境中的生存[77]。

革兰氏阴性病原菌细胞接触并形成聚集或自聚集的能力与环境和宿主传播密切相关，而且自聚集在病原菌的毒力中起主要作用。Lcl (*Legionella* collagen-like)能够通过二价阳离子依赖性的方式介导 *L. pneumophila* 的自聚集，并参与生物膜的产生及与人细胞的黏附，因此 Lcl 可增强 *L. pneumophila* 接触、附着和感染阿米巴的能力，促进 *L. pneumophila* 与其自然宿主之间的接触和黏附[78-80]。关于几种 Ntt 蛋白，目前的研究进展均十分有限。如图 2 所示：NttA 是一种磷酸肌醇结合蛋白，可促进 *L. pneumophila* 在 *A. castellanii* 和 *Willaertia magna* 中增殖，整体呈现 helical bundle structure，包含一个三螺旋束(three-helix bundle)和一个短螺旋(α2)[81-82]；NttB 是木瓜蛋白酶家族一个新成员，结构和生化分析表明 NttB 呈现与木瓜蛋白酶家族相似的折叠方式，即由 L-domain (主要由 α-helix 组成)和 R-domain (主要由 β-sheet 组成)组成其保守的潜在的催化口袋，进化树分析发现 NttB 与真核组织蛋白酶形成一个独特的新的亚家族[81,83]；NttC 在 *L. pneumophila* 侵染 *Vermamoeba vermiformis* 和 *W. magna* 过程中起着关键的作用[82]，包含由 1 个 α-helix 和 10 个 β-strand 组成的免疫球蛋白样折叠花样，整体结构中间包含一个 breathable internal cavity，可以允许小分子进入，因而有可能结合多种有机分子[84]；尽管已经获得了 NttD 的结构，但是其具体功能仍不清楚；nttE 对于 *A. castellanii* 和 *Hartmannella vermiformis* 的最佳感染及 *L. pneumophila* 的典型菌落形态形成也是必需的[26,82]，其单体结构呈现 L-like/chair-shaped 折叠花样[85]；NttG 是一种 VirK-like 蛋白，结构分析显示一个不对称单位中包含 4 个单体，组成一个紧密的同源四聚体 barrel-shaped 样结构[86]。总而言之，在 26 种底物蛋白中，仅 4 个蛋白(ProA、SrnA、PlaC 和 LapA)对于阿米巴最佳感染是必需的，而且这些效应蛋白的相对重要性因侵染的阿米巴类型而异，这表明 *L. pneumophila* 的 T2SS 及其底物蛋白的进化在一定程度上是为了扩大细菌的宿主范围；7 种

效应蛋白(LapA、NttA、NttC、NttD、PlaC、ProA和SrnA)明显促进了*L. pneumophila*在原生动物宿主内的感染和增殖，但是几乎所有 T2SS 效应蛋白单缺失突变株在巨噬细胞中均能正常生长，表明这些效应蛋白的功能存在显著的冗余性[3]。由于原生动物是 *L. pneumophila* 在天然环境中的宿主，因此 T2SS 对军团菌在环境中生存是必需的。

4 小结与展望

本文主要介绍了嗜肺军团菌的 T2SS 及部分底物蛋白的结构与功能，但是 T2SS 的大部分底物的研究仍然严重滞后，而且效应蛋白功能的冗余性进一步增加了研究难度，因此还需要开展大量的研究工作。嗜肺军团菌 T2SS 底物未来研究的重点包括以下几个方面：(1) 深入地研究 T2SS 表达的调控网络和/或其许多不同的影响因素，揭示效应蛋白的输出和功能是如何根据细菌生长条件的改变而变化；(2) 阐明 T2SS 如何与 T4BSS 及其他分泌系统协同互作，破译 *L. pneumophila* 的整体毒力策略；(3) 已知的 T2SS 底物活性和分子作用模式及这些底物之间更精确的调节机理是未来研究的重点；(4) 未知功能 T2SS 底物的功能和作用机理是研究的难点；(5) 利用所获得的效应蛋白结构和功能信息开发可用于预防或对抗病原菌对人类、动物或植物感染的新策略或试剂。可以预见，*L. pneumophila* 及其 T2SS 的研究成果对于理解其他各种环境和/或医学上的细菌有重要的参考意义，也可能应用于工业生产及疾病诊断、控制和预防方面。

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