

结核分枝杆菌酸抗性基因及其调控网络

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摘要: 病原菌在宿主细胞内的持留分子机理是目前研究的热点和难点。病原菌的抗酸能力与此密切相关。结核分枝杆菌感染导致的结核病仍然是全球公共卫生的重大威胁, 这与结核分枝杆菌抗酸并在宿主巨噬细胞内持留有关。结核分枝杆菌抗酸主要通过调控质子进出、代谢调控胞内酸碱平衡和双组份信号系统调控。本文综述了结核分枝杆菌在酸胁迫下的整体调控网络, 阐述了在酸性环境中结核分枝杆菌的具体调控机理, 旨在为持留结核分枝杆菌的治疗提供新的全局性思路, 寻找新的结核病防控靶标。

关键词: 结核分枝杆菌; 酸抗性基因; 持留; 调控网络

Acid-resistant genes in *Mycobacterium tuberculosis* and the underlying regulatory network

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Abstract: The molecular mechanisms of pathogen persistence within host cells are emerging hotspots, and one of the causes of its persistence is the acid resistance of bacteria. Currently, tuberculosis remains a serious threat to global public health and it is caused by *Mycobacterium tuberculosis*. In particular, acid resistance of *M. tuberculosis* and its persistence within macrophages contribute significantly to tuberculosis. Investigations have uncovered three major mechanisms underlying its acid resistance: the control of proton entry, metabolic regulation of intracellular acid-base balance and regulation of the two-component signaling system. In this review, we summarize the overall regulation network of *M. tuberculosis* in the acidic environment, aiming at providing a new overall idea for treating *M. tuberculosis* persistence and exploring new targets for tuberculosis control.

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结核病(Tuberculosis, TB)现已超过艾滋病,成为全球第一大传染病。结核分枝杆菌(*Mycobacterium tuberculosis*, MTB)是结核病的致病菌。世界上 1/3 的人口为结核菌的潜伏感染者,每分钟至少两人死于结核病,2015 和 2016 年每年新增 1040 万结核病例,约 130 万人死于结核病^[2]。多耐药性结核(multidrug-resistant tuberculosis, MDR-TB),甚至广泛耐药结核(extensively drug-resistant tuberculosis, XDR-TB)^[3]的出现使结核病防控形势更加严峻^[4, 5]。结核分枝杆菌能够长期在宿主细胞,尤其是巨噬细胞内滞留^[6],以抵抗或逃避宿主免疫攻击^[7]和药物杀灭^[8]。吞噬体酸化本来是巨噬细胞控制胞内致病菌的主要机制之一^[9~11]。已有的研究显示结核分枝杆菌能够调控宿主细胞酸化进程,抵御吞噬体对自身的限制。因此,识别结核分枝杆菌应对酸性环境的基因及其调控网络有助于深化对结核分枝杆菌致病机理的认识,发现结核病防控的新干预节点。本文总结了结核分枝杆菌的 3 种酸抗性机制以及调控通路。

1 结核分枝杆菌感染致病过程

结核分枝杆菌有效感染如图 1A 所示:(1)微量结核分枝杆菌以气溶胶的形式感染上呼吸道,然后结核分枝杆菌进入下呼吸道的肺泡中。微量的结核分枝杆菌可以避免被宿主模式识别受体如 Toll-like receptor (TLR)识别^[12]。(2)结核分枝杆菌表面的脂质如 phthiocerol dimycocerosate (PDIM)可以干扰或者抵消被激活的巨噬细胞的杀灭功能^[13, 14]。(3)结核分枝杆菌产生的某些糖肽脂(glycopeptidolipid, GPL)可以刺激细胞产生趋化因子如 chemokine (C-C motif) ligand 2 (CCL2),改变被招募来的免疫细胞的组分,进而抵御免疫细胞的杀伤作用。(4)含有结核分枝杆菌的吞噬体的 pH 会降低。含有一般致病菌的吞噬体会与溶酶体融合,进而被溶酶体释放的酶类消灭。但结核分枝杆菌却会阻断吞噬体-溶酶体融合,并进一步酸化吞噬体^[15],最终 pH 甚至可以达 4.5^[16]。

被感染的巨噬细胞可能分泌某些尚未完全了解的分子,招募更多的免疫细胞,形成肉芽肿^[17],限制其中结核分枝杆菌的生长和播散。当宿主免疫功能降低时,肉芽肿中坏死细胞可以作为结核分枝杆菌生长和繁殖所需的底物。随着活动性结核患者的咳嗽而将结核分枝杆菌通过气溶胶形式排入空气,成为感染下一位宿主的源头^[18]。

2 抗酸能力与结核分枝杆菌胞内存活、滞留密切相关

抗酸能力是几乎所有生物都具备的特征,这在大肠杆菌等肠道微生物^[19, 20]中研究比较多^[21, 22]。抗酸能力与结核分枝杆菌的巨噬细胞内存活和滞留密不可分^[23]。结核分枝杆菌面临的酸性环境主要为:

(1) 由于肉芽肿内缺氧环境而作为其中细胞能量主要来源的糖酵解产生的酸性物质更容易在肉芽肿中积累而形成局部的酸性环境。这使得从坏死的巨噬细胞中释放的结核分枝杆菌的生存和繁殖需要直接面对酸性环境;(2) 激活的巨噬细胞内吞噬体的酸化作用加强,其与溶酶体融合后形成的酸性环境直接影响其中的结核分枝杆菌。一般认为,酸性环境会导致包括结核分枝杆菌在内的生物的酸碱失衡,维持生命活动正常进行所需要的酶类失活,蛋白质被水解等,最终导致菌体死亡^[24]。

滞留,指包括结核分枝杆菌在内的很多微生物的代谢非活跃状态,滞留的微生物对绝大多数靶向代谢的药物不敏感^[25, 26],因而滞留结核分枝杆菌已经成为结核病治疗的三大难题之一^[27]。结核分枝杆菌滞留形成与其耐受或抵抗酸性环境的能力密不可分。一旦被巨噬细胞吞噬,结核分枝杆菌就面临着直接导致菌体死亡的酸性胁迫,为了在酸性环境中存活下来,调控许多抗酸机制以应对酸性胁迫,如通过调节体内的代谢水平,调控双组份系统来改变菌体的生长活跃状态,使其在酸性环境生存下来,而这样的生存伴随着菌体进入休眠状态,即其复制、基因表达及体内代谢活动大幅减弱,因此减少了其

对大多数抗结核药物的敏感性,即进入持留状态。结核分枝杆菌抗酸的分子机制及其调控网络研究是结核病新防控措施的重要基础。

3 结核分枝杆菌的抗酸机制

为成功在巨噬细胞内存活下来,结核分枝杆菌会形成一个完整且复杂的酸性调控网络以应对胞内的酸性胁迫,其具体的调控机制主要包括以下几个方面(图 1B)^[28]。

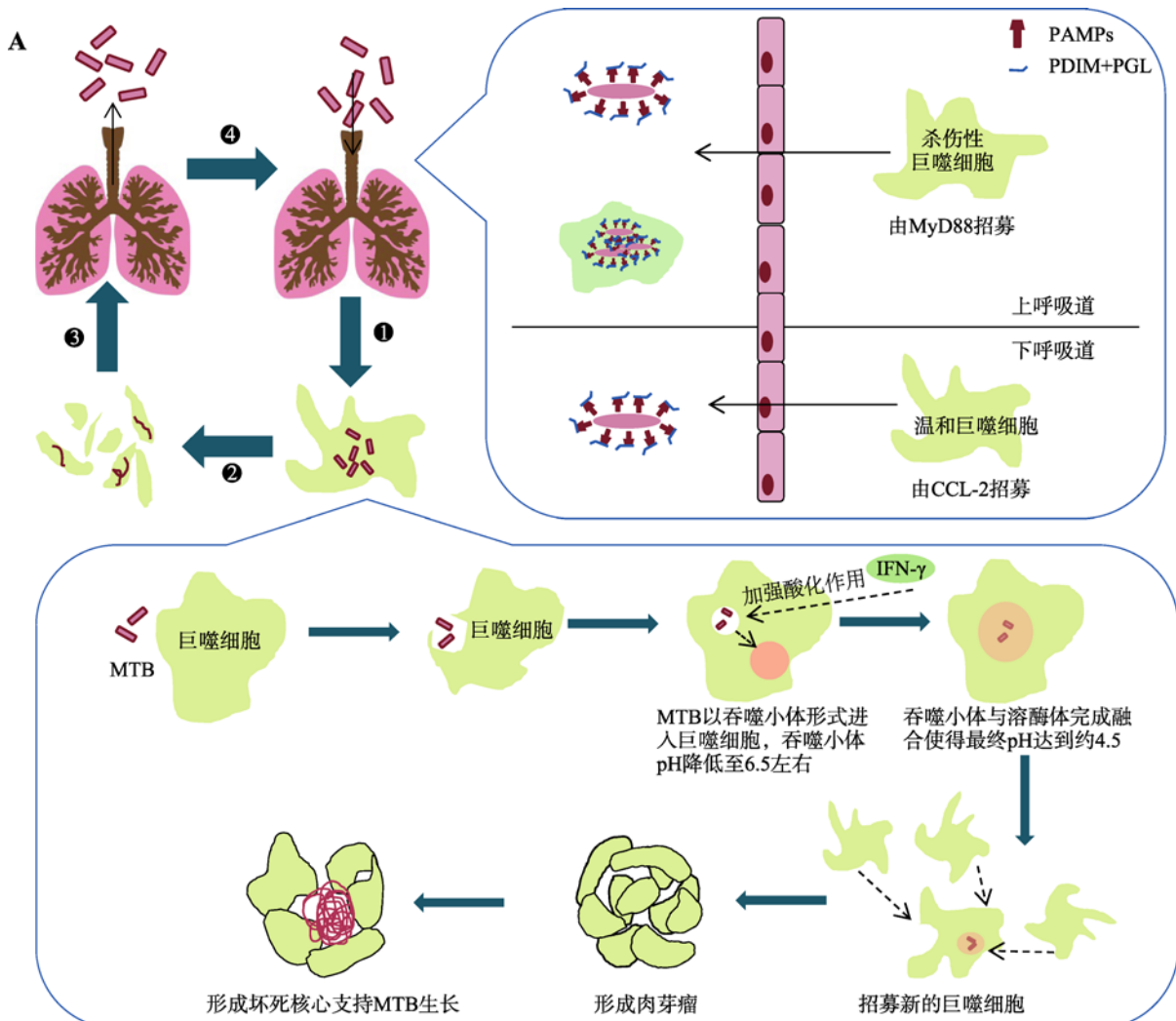
3.1 胞外酸性及其他有害物质的控制

结核分枝杆菌与抗酸相关的变化主要表现在:增加细胞壁的修复能力^[29]、减少胞外酸性物质,以及酸性胁迫下毒性物质的降解能力增加^[30-32]。

3.1.1 细胞壁修复能力提高

宿主产生的攻击致病菌的抗菌肽(antimicrobial peptides, AMPs)的作用可以被结核分枝杆菌的赖氨酰 tRNA 合成酶(lysyl-tRNA synthetase, *lysX*)抵消^[33]。*lysX* (128.208 kDa)是参与肽聚糖(peptidoglycan, PG)合成的膜蛋白,能够增加细胞表面的赖氨酸残基数,降低抗菌肽对细菌表面的损伤^[34]。有研究显示细菌存活率与 *lysX* 的表达水平成正相关^[35]。

与肽聚糖相关的还有由结核分枝杆菌 *Rv3671c* 基因编码的跨膜丝氨酸蛋白酶(membrane-associated serine protease, MarP)。MarP (40.7124 kDa)位于结核分枝杆菌细胞周质^[36]。MarP 的蛋白水解活性与酸胁迫时结核分枝杆菌胞内 pH 维持有关。MarP 可通过剪切活化 Ras interacting protein (RipA)^[36], RipA



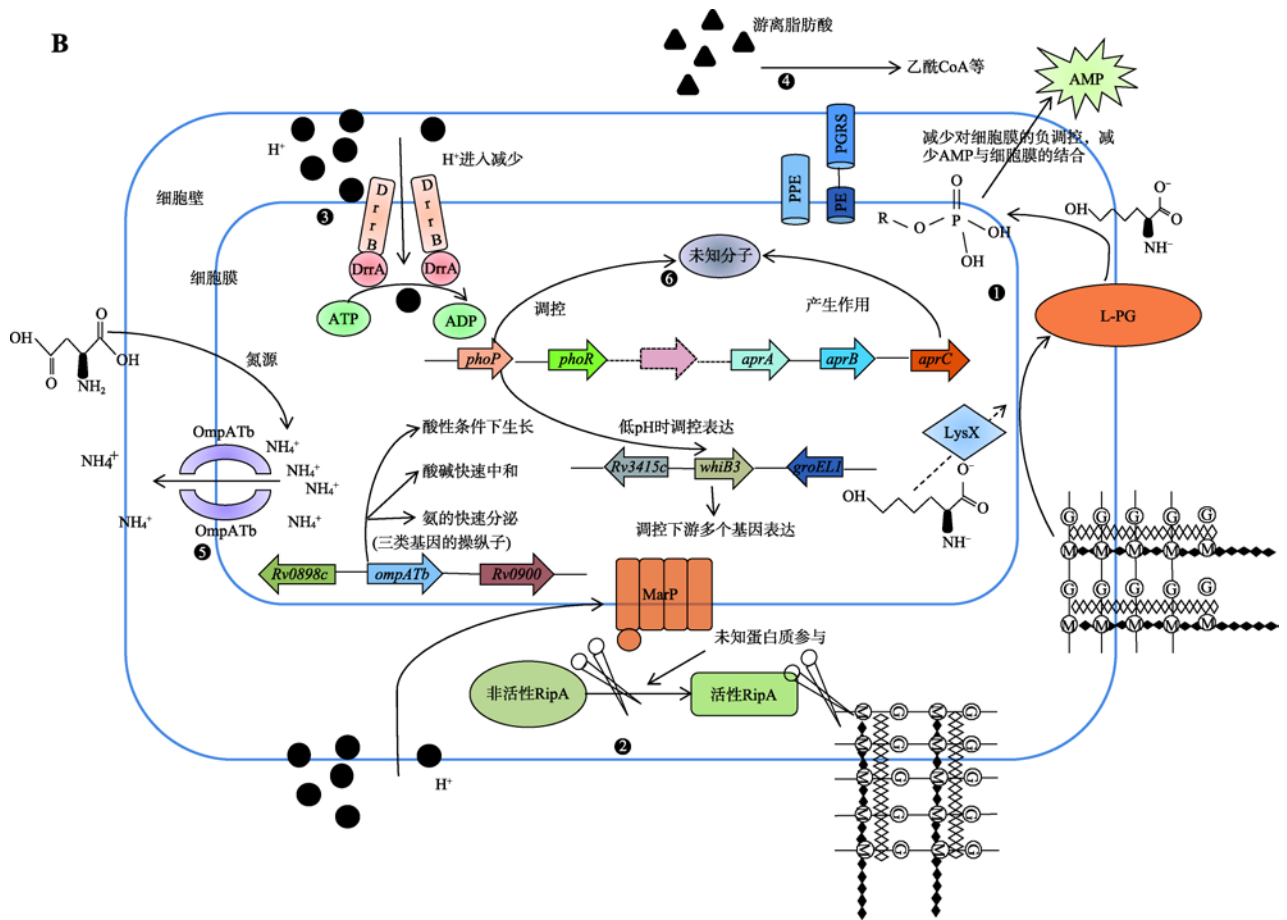


图1 结核分枝杆菌感染途径以及抗酸调控网络

Fig. 1 The infection and the acid resistance network of *Mycobacterium tuberculosis*

A: 结核分枝杆菌感染过程示意图。① 结核分枝杆菌进入巨噬细胞; ② 巨噬细胞裂解释放出结核分枝杆菌; ③ 结核分枝杆菌弥散在肺部; ④ 结核分枝杆菌通过气溶胶形式传至下一个宿主。B: 结核分枝杆菌抗酸通路示意图。① LysX 通过转移赖氨酸残基抵御 AMPs; ② MarP 活化蛋白 RipA, 剪切肽聚糖; ③ ABC 转运蛋白 DrrA/DrrB 减少质子进入细胞; ④ PE/PPE 家族蛋白降解游离脂肪酸等有害物质; ⑤ 蛋白 OmpATb 参与调解胞内氮代谢释放铵根离子; ⑥ 双组份系统 PhoPR 编码基因 *phoP/phoR* 调控 *aprABC* 以及 *whiB3* 的表达。PAMPs: pathogen-associated molecular pattern; PDIM: phthiocerol dimycocerosate; PGL: glycopeptidolipid; AMP: anti-microbial peptides.

(49.8081 kDa)具有肽聚糖水解活性,使细胞壁中的肽聚糖可以正常水解,有利于细胞壁正常的修复过程,可能还参与维持细胞膜的稳定性^[29]。

除此之外, *Rv2136c* 编码的蛋白质对细胞壁的多种成分,如肽聚糖等的形成起关键作用。*Rv2136c* 转座子突变菌株对十二烷基磺酸钠、亲脂性抗生素、高温、氧化压力和活性氮中间物敏感,在小鼠中减缓生长并且降低形成持留的能力^[37]。低 pH 时, *Rv2136c* 转座子突变菌株容易被环境中的 Tween 80 和清蛋白刺激后释放的脂肪酸毒害^[27]。使用磷酸钠缓冲液或其他表面活性剂,如泰洛沙泊,也称四丁酚醛,是烷基芳基聚醚醇型的非离子液体聚合物,

可以有效增溶但不产生脂肪酸等有害物质,可以促进野生型结核分枝杆菌在 pH 为 4.5 环境下的存活事件, *Rv2136c* 转座子突变菌株的存活率仍然降低^[37]。但 *Rv2136c* 同源分子敲出的耻垢分枝杆菌与野生型对酸胁迫的表型相似^[37]。*Rv2136c* 的转座子插入突变可能会导致该基因编码的蛋白功能不完全或者没有蛋白产物,故 *Rv2136c* 转座子突变菌株的表型变化可能与插入导致的下游基因移码突变有关。

3.1.2 减少胞外酸性物质

基因 *drrA/drrB* 编码的是 ABC 超家族柔红霉素运输蛋白(Daunorubicin-transport ATP-binding protein

ABC transporter) DrrA/DrrB。其中 DrrA (35.8182 kDa) 是 ATP 结合蛋白, DrrB (31.1089 kDa) 是膜整合蛋白, 两者结合在一起才具有外排泵活性。DrrA 在膜上的定位取决于 DrrB 的表达情况, 无 DrrA 时, DrrB 对蛋白酶的水解非常敏感^[38]。柔红霉素或者阿霉素促进结核分枝杆菌产生 ATP, 增加底物与转运蛋白结合, 同时改变 DrrA 构象而更易结合 ATP。DrrA/DrrB 缺失或者活性降低导致结核分枝杆菌运输结核分枝杆菌醇二枝菌酸等复杂分子的能力下降, 提示与毒力的密切关系^[38]。酸性环境明显下调 *drrA/drrB* 基因的转录, 该基因编码的产物属于 ABC 超家族, 其对脂质的外排功能已经被大量报导过, 在酸性条件下, 脂质会水解成脂肪酸和甘油, 对细胞产生毒害, 推测结核分枝杆菌在酸性胁迫下, 会通过一定的机制降低转运蛋白 DrrA/DrrB 的活性, 降低脂质的外排量, 从而减少胞外酸性物质, 降低酸性环境对细胞的损伤。

3.1.3 降解有害物质

PE-PGRS (N-端功能域 PE 含有 110 个氨基酸, C-端 PGRS 功能域编码富含 GC 的多态性重复序列 polymorphic GC-rich repeated sequence) 和 PPE (Pro-Pro-Glu) 家族是分枝杆菌特异性蛋白家族, 具有抗原性^[39, 40]。PPEs 和 PEs 可以活化 T 细胞^[41-43]。PEs 具有类似分子伴侣的功能。一些 PGRS 在 C-端具有某些酶的活性结构域, 如脂酶^[44]。PPEs 和 PEs 经常成对发挥作用。PE/PPE 家族蛋白的分泌涉及 VII 型分泌系统^[45, 46]。酸胁迫改变部分结核分枝杆菌的 PE/PPE 家族蛋白的水平如 PPE50 和 PE34^[47, 48]。据此推测, 该家族蛋白质可能与酸抗性有关, 但具体分子机理仍有待深入研究。

3.2 通过调节代谢维持胞内酸碱平衡

自身的酸碱平衡对于结核分枝杆菌适应酸性环境至关重要。结核分枝杆菌 *Rv0899* 基因编码的外膜蛋白 A (outer membrane protein A, OmpATb) 可以在细胞膜上形成通道^[49], 但无孔蛋白活性^[50, 51]。*ompATb* 是相关酸碱调节基因的操纵子, 这对结核分枝杆菌氨的快速分泌、酸碱快速中和及酸性条件下的生长至关重要。此外 *ompATb* 的表达依赖于 pH^[51]:

低 pH 上调 *ompATb* 的转录^[52], 同时逐步提高培养基 pH 值, 这可能与体外培养时天冬酰胺是结核分枝杆菌的氮源分泌氨有关, 但 *ompATb* 并不调控天冬酰胺的摄取, 而在体内具体情况如何, 仍有待研究^[53]。

3.3 通过双组份系统调控表达

巨噬细胞内的 pH 值约为 5.2, 这种酸性环境足以控制大多数病原微生物。但结核分枝杆菌却仍然能够在巨噬细胞这种酸性条件下存活。结核分枝杆菌可能具有感知吞噬体内 pH 并迅速调整其基因表达的能力。现在已知被酸和吞噬体调控的基因 (Acid and Phagosome Regulated, *apr*) *aprA* (AprA: 7.90905 kDa)、*aprB* (AprB: 5.72264 kDa) 和 *aprC* (AprC: 30.7241 kDa)。该操纵子被双组份信号转导系统 PhoPR 调控: *aprC* 的表达明显依赖于 PhoPR 的存在^[54]。在 *aprABC* 的调控序列中可能有 PhoP (27.5135 kDa) 结合位点^[55, 56]。推测依赖于 *aprABC* 的调控通路最终作用于一种目前未知的分子 X, X 的产生受 *phoP* 调控。缺失 *aprABC* 时, X 的积累可能会影响其他基因的表达; 当 *phoP* 缺失时, X 不会形成, 依赖 *aprABC* 的基因的表达也会被改变^[57]。*aprABC* 缺失的分枝杆菌的脂代谢^[58]也会有缺陷。这提示脂质可能参与结核分枝杆菌酸抗性。

PhoPR 和 *Rv3416* 编码的胞内氧化还原感应分子 WhiB3 (11.6122 kDa) 在结核分枝杆菌酸抗性中发挥重要功能^[59, 60]。低 pH 时, *phoPR* 能直接激活 *whiB3* 的表达。*whiB3* 调控包括 *cpsY* 在内的多个基因的表达, 与结核分枝杆菌毒力和酸抗性有关^[61]。*whiB3* 及其上游基因 *Rv3415c* 的编码序列高度保守。两个基因间的非编码序列可分为两个区域: 非保守区域 1 和相对保守区域 2。结核分枝杆菌的启动子序列位于相对保守区域 2, 表明在分枝杆菌中转录起始保守。非编码区 1 的序列只在结核分枝杆菌和海分枝杆菌中保守, 在耻垢分枝杆菌中并不保守, 这和低 pH 下 *whiB3* 的表达水平差异正好一致^[62]。这提示 *whiB3* 上游的非编码区 1 可能是调控转录起始的关键序列。结核分枝杆菌 PhoP 的 DNA 结合序列也位于 *whiB3* 上游的非编码区 1。该 DNA 片段在结核分枝杆菌和海分枝杆菌中相同, 但在耻垢分枝杆菌中则有差异。结核分枝杆菌 PhoR (52.0164 kDa) 的 H₂₅₉

位点能发生磷酸化, 磷酸基被转运到 PhoP 的 D₇₁ 位点。磷酸化与 *whiB3* 激活相关^[61, 63]。该调控方式目前只在部分致病分枝杆菌中发现。

3.4 其他抗酸机制

结核分枝杆菌的谷氨酸脱羧酶、精氨酸脱羧酶、赖氨酸脱羧酶和鸟氨酸脱羧酶会通过消耗特定氨基酸的氢离子, 最终生成胺类和 CO₂ 来提高胞内 pH 值^[15]。谷氨酸脱羧酶主要是把谷氨酸脱羧生成 γ -氨基丁酸(γ -aminobutyric acid, GABA)并转运到细胞外, 从而消耗酸性环境下进入细胞内的质子, 并将产物运到细胞外, 阻止细胞内 pH 值过度降低^[20, 64]。精氨酸脱羧酶会将精氨酸脱羧并消耗质子, 最终生成胍丁胺和 CO₂, 在精胺反向转运蛋白的作用下, 精胺被运出细胞并同时交换新的精氨酸进入, 再在精氨酸脱羧酶的作用下发生脱羧反应, 如此循环将不断消耗细胞内的质子, 从而维持酸性环境下胞内 pH 稳定。同样, 赖氨酸脱羧酶和鸟氨酸脱羧酶也可以

通过相应氨基酸脱羧维持胞内 pH 稳定。

结核分枝杆菌的分子伴侣可在酸性条件下维持体内蛋白质稳定^[65]。分子伴侣帮助新生蛋白质正确折叠, 也可以促进错误折叠蛋白质的重新折叠, 在酸抗性中也发挥重要作用。pH 中性时, 分子伴侣在结核分枝杆菌胞内主要以无活性的二聚体或多聚体形式存在, 但当细菌处于极端酸性环境中, 其变成有活性的形式, 与其受体蛋白相结合, 使受体蛋白免受酸的伤害或者帮助已经在酸性条件下沉淀的蛋白质恢复活性。一些非编码小 RNA 也参与酸抗性^[66], 小 RNA 可能主要调控一些关键的抗酸基因的表达。

关于结核分枝杆菌的酸抗性基因, 研究还很少。上述基因很多是根据大肠杆菌的信息, 通过比对获得。关于酸抗性, 很多研究以大肠杆菌为模型进行。本实验室在大肠杆菌结果的基础上^[67, 68], 使用生物信息学方法, 通过与结核分枝杆菌基因组进行比对, 预测了结核分枝杆菌其他可能的酸抗性基因(表 1), 为后续在结核分枝杆菌中的验证提供线索。

表 1 结核分枝杆菌潜在的酸抗性基因及其编码产物

Table 1 Possible acid resistance genes and their products in *Mycobacterium tuberculosis*

基因编号	编码产物功能	相关文献
Rv0072	谷氨酰胺以及部分其他分子的跨膜运输	[69]
Rv0247c	参与富马酸盐和琥珀酸的相互转化(有氧呼吸), 催化活性: 琥珀酸+受体=延胡索酸 + 还原性受体	[70]
Rv1032c	双组份系统 trcRS 中的 trcS, 组氨酸激酶	[71~74]
Rv1098c	参与了三羧酸循环, 延胡索酸脱氢酶, 催化活性: (S)-苹果酸 = 延胡索酸 + 水	[75, 76]
Rv1464	半胱氨酸脱硫酶(Cysteine desulfurase, Csd), 催化硫和硒从 L-半胱氨酸, L-胱氨酸, L-硒代半胱氨酸, 和 L-硒代胱氨酸的转移, 产生 L-丙氨酸	[77~79]
Rv1558	功能未知	[80]
Rv1811	可能的镁离子转运 P 型 ATP 酶 C (Mg ²⁺ transport P-type ATPase C, MgtC), 催化活性: ATP + 水 + Mg ²⁺ (膜外) = ADP + 磷酸 + Mg ²⁺ (膜内)	[81~93]
Rv1854c	可能的 NADH 脱氢酶(NADH dehydrogenase, Ndh), 将电子从 NADH 转移到呼吸链	[94~121]
Rv1979c	可能的保守的透性酶, 可能与氨基酸跨膜运输相关	[122~127]
Rv2359	锌指调控蛋白(Zinc uptake regulation protein, Zur), 负调控因子, 以 Zn ²⁺ 作为辅因子	[128~133]
Rv2710	RNA 聚合酶转录起始因子(RNA polymerase sigma factor B, SigB), 可能控制生长稳定期以及一般压力胁迫下的转运调控	[134~143]
Rv2720	基因表达阻遏因子 LexA, 参与核苷酸切除修复和 SOS 反应	[128, 144~150]
Rv2827c	功能未知	[151, 152]
Rv2995c	可能的 3-异丙基苹果酸脱氢酶(3-isopropylmalate dehydrogenase, LeuB)参与了亮氨酸生物合成(第三步), 催化活性: 3-羧基-2-羟基-4-甲基戊酸乙酯+NAD ⁺ = 3-羧基-4-甲基-2-戊酸乙酯+NADH	[153, 154]

续表

基因编号	编码产物功能	相关文献
Rv0477	未知分泌蛋白	
Rv2531c	鸟氨酸/精氨酸/赖氨酸脱羧酶	
Rv3253c	带阳离子氨基酸以及一些底物分子的跨膜转运	
Rv3432c	谷氨酸盐脱羧酶(Glutamate decarboxylase, GadB), 产生谷胱甘肽, 催化活性: L-谷氨酸 = γ -氨基丁酸 + CO ₂	

4 问题与展望

结核分枝杆菌的抗酸系统是由多个分子组成的相互协调的网络。从对外界环境酸碱性的感应到细胞壁的变化, 内部酸碱代谢平衡及相关基因表达的调控, 最终形成对外界酸性环境高度耐受的状态, 其中的每一个过程都涉及到复杂的调控。这个调控系统中部分基因已经明确, 如细胞壁相关的跨膜丝氨酸蛋白酶 MarP, 酸碱代谢平衡调节相关的外膜蛋白 A (OmpATb)。但是, 对于结核分枝杆菌的耐酸基因的研究仍然颇少。借鉴研究比较深入的如大肠杆菌等肠道微生物耐受肠道酸性环境的基因^[11]及其调控网络^[10]有助于启发结核分枝杆菌的酸抗性机制及其持留机制。

目前认为细胞壁相关基因与酸抗性密切相关, 虽然已经识别了 MarP 等细胞壁成分维持相关基因, 但环境 pH 值变化如何被分枝杆菌所感知并反馈到 MarP 等细胞壁相关基因的改变还不清楚。其他的研究也往往集中在鉴定酸抗性有关基因, 将来需要深入研究鉴定酸抗性基因的作用分子机理, 以及其相互作用网络。这将有助于更全面地揭示结核分枝杆菌持留机制。

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参考文献(References):

- [1] Minch KJ, Rustad TR, Peterson EJR, Winkler J, Reiss DJ, Ma SY, Hickey M, Brabant W, Morrison B, Turkarslan S, Mawhinney C, Galagan JE, Price ND,

- Baliga NS, Sherman DR. The DNA-binding network of *Mycobacterium tuberculosis*. *Nat Commun*, 2015, 6: 5829. [DOI]
- [2] WHO. Global tuberculosis report 2017. 2017. [DOI]
- [3] Sosa ADJ, Byarugaba DK, Amábile-Cuevas CF, Hsueh PR, Kariuki S, Okeke IN. Antimicrobial Resistance in Developing Countries. New York: Springer, 2010. [DOI]
- [4] Cole ST, Eisenach KD, McMurray DN, Jacobs WR, Jr. Tuberculosis and the *Tubercle Bacillus*. Washington, DC: ASM Press, 2005. [DOI]
- [5] Wax RG, Lewis K, Salyers AA, Taber H. Bacterial Resistance to Antimicrobials. 2nd ed. Boca Raton: CRC Press, 2008. [DOI]
- [6] Alvarez-Jiménez VD, Leyva-Paredes K, García-Martínez M, Vázquez-Flores L, García-Paredes VG, Campillo-Navarro M, Romo-Cruz I, Rosales-García VH, Castañeda-Casimiro J, González-Pozos S, Hernández JM, Wong-Baeza C, García-Pérez BE, Ortiz-Navarrete V, Estrada-Parra S, Serafin-López J, Wong-Baeza I, Chacón-Salinas R, Estrada-García I. Extracellular vesicles released from *Mycobacterium tuberculosis*-infected neutrophils promote macrophage autophagy and decrease intracellular mycobacterial survival. *Front Immunol*, 2018, 9: 272. [DOI]
- [7] Da Costa AC, De Resende DP, de P. O. Santos B, Zoccal KF, Faccioli LH, Kipnis A, Junqueira-Kipnis AP. Modulation of macrophage responses by CMX, a fusion protein composed of Ag85c, MPT51, and HspX from *Mycobacterium tuberculosis*. *Front Microbiol*, 2017, 8: 623. [DOI]
- [8] Agnihotri J, Singh S, Wais M, Pathak A. Macrophage targeted cellular carriers for effective delivery of anti-tubercular drugs. *Recent Pat Antiinfect Drug Discov*, 2017, 12(2): 162–183. [DOI]
- [9] Cambier CJ, Falkow S, Ramakrishnan L. Host evasion and exploitation schemes of *Mycobacterium tuberculosis*. *Cell*, 2014, 159(7): 1497–1509. [DOI]
- [10] Wang C, Cui YH, Qu XJ. Mechanisms and improvement

- of acid resistance in lactic acid bacteria. *Arch Microbiol*, 2018, 200(2): 195–201. [DOI]
- [11] Lund P, Tramonti A, De Biase D. Coping with low pH: molecular strategies in neutrophilic bacteria. *FEMS Microbiol Rev*, 2014, 38(6): 1091–1125. [DOI]
- [12] Guo XL, Liu HG. Study on the Effect of Toll-like Receptors in mediation of immune responses in *Mycobacterium tuberculosis* infection. *Medical Recapitulate*, 2015, 21(12): 2142–2145.
郭雪玲, 刘辉国. Toll 样受体在介导结核分枝杆菌感染免疫反应中的作用. *医学综述*, 2015, 21(12): 2142–2145. [DOI]
- [13] Nkwouano V, Witkowski S, Rehberg N, Kalscheuer R, Nausch N, Mayatepek E, Jacobsen M. A novel mycobacterial *in vitro* infection assay identifies differences of induced macrophage apoptosis between CD4⁺ and CD8⁺ T cells. *PLoS One*, 2017, 12(2): e0171817. [DOI]
- [14] Liu YX, Zhang WJ. Progress of interactions between *Mycobacterium tuberculosis* and macrophages. *Chin J Cell Biol*, 2012, 34(6): 617–622.
刘云霞, 张万江. 结核分枝杆菌与巨噬细胞相互作用的研究进展. *中国细胞生物学学报*, 2012, 34(6): 617–622. [DOI]
- [15] Vandal OH, Nathan CF, Ehrt S. Acid resistance in *Mycobacterium tuberculosis*. *J Bacteriol*, 2009, 191(15): 4714–4721. [DOI]
- [16] Huang L, Nazarova EV, Tan SM, Liu YC, Russell DG. Growth of *Mycobacterium tuberculosis* *in vivo* segregates with host macrophage metabolism and ontogeny. *J Exp Med*, 2018, 215(4): 1135–1152. [DOI]
- [17] Queval CJ, Brosch R, Simeone R. The Macrophage: a disputed fortress in the battle against *Mycobacterium tuberculosis*. *Front Microbiol*, 2017, 8: 2284. [DOI]
- [18] Mahamed D, Bouille M, Ganga Y, Arthur CM, Skroch S, Oom L, Catinas O, Pillay K, Naicker M, Rampersad S, Mathonsi C, Hunter J, Wong EB, Suleman M, Sreejit G, Pym AS, Lustig G, Sigal A. Intracellular growth of *Mycobacterium tuberculosis* after macrophage cell death leads to serial killing of host cells. *eLife*, 2017, 6: e22028. [DOI]
- [19] Nicolaou SA, Gaida SM, Papoutsakis ET. A comparative view of metabolite and substrate stress and tolerance in microbial bioprocessing: From biofuels and chemicals, to biocatalysis and bioremediation. *Metab Eng*, 2010, 12(4): 307–331. [DOI]
- [20] De Biase D, Pennacchietti E. Glutamate decarboxylase-dependent acid resistance in orally acquired bacteria: function, distribution and biomedical implications of the *gadBC* operon. *Mol Microbiol*, 2012, 86(4): 770–786. [DOI]
- [21] Ge TD, Feng EL, Yan BJ, Wang HL, Huang LY. Construction of deletion mutant of *Shigella flexneri* acid resistance genes. *Lett Biotechnol*, 2005, 16(5): 488–491.
葛堂栋, 冯尔玲, 晏本菊, 王恒樑, 黄留玉. 痢疾杆菌酸抗性系统相关基因缺失突变体的构建. *生物技术通讯*, 2005, 16(5): 488–491. [DOI]
- [22] Shang L, LiW, Li LJ, Li L, Zhang SH, Li TT, Li YK, Liu L, Guo ZW, Zhou R, Chen HC. The generation of nalidixic acid-resistant strains and signature-tagged mutants of *Actinobacillus pleuropneumoniae*. *Acta Microbiol Sin*, 2008, 48(1): 73–79.
商霖, 李薇, 李良军, 黎璐, 张四化, 李婷婷, 李耀坤, 刘磊, 郭志伟, 周锐, 陈焕春. 胸膜肺炎放线杆菌萘啶酸抗性菌株的选育和信号标签突变株的构建. *微生物学报*, 2008, 48(1): 73–79. [DOI]
- [23] Shi XJ, Wang QL, Gao Q. Current research progress on acid resistance mechanism of *Mycobacterium tuberculosis*. *J Micro Infect*, 2013, 8(3): 192–196.
施旭骏, 王晴岚, 高谦. 结核分枝杆菌耐酸机制的研究进展. *微生物与感染*, 2013, 8(3): 192–196. [DOI]
- [24] Rohde KH, Abramovitch RB, Russell DG. *Mycobacterium tuberculosis* invasion of macrophages: linking bacterial gene expression to environmental cues. *Cell Host Microbe*, 2007, 2(5): 352–364. [DOI]
- [25] Su HB, Zhu SL, Zhu L, Kong C, Huang Q, Zhang Z, Wang HH, Xu Y. *Mycobacterium tuberculosis* latent antigen Rv2029c from the multistage DNA vaccine A39 drives TH1 responses via TLR-mediated macrophage activation. *Front Microbiol*, 2017, 8: 2266. [DOI]
- [26] Brennan PJ, Vissa VD. Genomic evidence for the retention of the essential mycobacterial cell wall in the otherwise defective *Mycobacterium leprae*. *Lepr Rev*, 2001, 72(4): 415–428. [DOI]
- [27] Zhang C, Yang L, Zhao N, Zhao Y, Shi C. Insights into macrophage autophagy in latent *tuberculosis* infection: Role of Heat Shock Protein 16.3. *DNA Cell Biol*, 2018, 37(5): 424–448. [DOI]
- [28] Deghmane AE, Soualhi H, Bach H, Sendide K, Itoh S, Tam A, Noubir S, Talal A, Lo R, Toyoshima S, Av-Gay Y, Hmama Z. Lipoamide dehydrogenase mediates retention of coronin-1 on BCG vacuoles, leading to arrest in phagosome maturation. *J Cell Sci*, 2007, 120 (Pt 16): 2796–2806. [DOI]

- [29] Small JL, O'Donoghue AJ, Boritsch EC, Tsodikov OV, Knudsen GM, Vandal O, Craik CS, Ehrt S. Substrate specificity of MarP, a periplasmic protease required for resistance to acid and oxidative stress in *Mycobacterium tuberculosis*. *J Biol Chem*, 2013, 288(18): 12489–12499. [DOI]
- [30] Banaiee N, Jacobs WR, Ernst JD. LspA-independent action of globomycin on *Mycobacterium tuberculosis*. *J Antimicrob Chemother*, 2007, 60(2): 414–416. [DOI]
- [31] Rampini SK, Selchow P, Keller C, Ehlers S, Bottger EC, Sander P. LspA inactivation in *Mycobacterium tuberculosis* results in attenuation without affecting phagosome maturation arrest. *Microbiology*, 2008, 154 (Pt 10): 2991–3001. [DOI]
- [32] Pathak R, Rathor N, Garima K, Sharma NK, Singh P, Varma-Basil M, Bose M. LspA gene of *Mycobacterium tuberculosis* co-transcribes with *Rv1540* and induced by surface and acidic stress. *Gene*, 2015, 560(1): 57–62. [DOI]
- [33] Maloney E, Stankowska D, Zhang J, Fol M, Cheng QJ, Lun SC, Bishai WR, Rajagopalan M, Chatterjee D, Madiraju MV. The two-domain LysX protein of *Mycobacterium tuberculosis* is required for production of lysinylated phosphatidylglycerol and resistance to cationic antimicrobial peptides. *PLoS Pathog*, 2009, 5(7): e1000534. [DOI]
- [34] Maloney E, Lun SC, Stankowska D, Guo HD, Rajagopalan M, Bishai WR, Madiraju MV. Alterations in phospholipid catabolism in *Mycobacterium tuberculosis* LysX mutant. *Front Microbiol*, 2011, 2: 19. [DOI]
- [35] Montoya-Rosales A, Provvedi R, Torres-Juarez F, Enciso-Moreno JA, Hernandez-Pando R, Manganeli R, Rivas-Santiago B. LysX gene is differentially expressed among *Mycobacterium tuberculosis* strains with different levels of virulence. *Tuberculosis (Edinb)*, 2017, 106: 106–117. [DOI]
- [36] Botella H, Vaubourgeix J, Lee MH, Song NM, Xu WZ, Makinoshima H, Glickman MS, Ehrt S. *Mycobacterium tuberculosis* protease MarP activates a peptidoglycan hydrolase during acid stress. *EMBO J*, 2017, 36(4): 536–548. [DOI]
- [37] Darby CM, Venugopal A, Ehrt S, Nathan CF. *Mycobacterium tuberculosis* gene *Rv2136c* is dispensable for acid resistance and virulence in mice. *Tuberculosis (Edinb)*, 2011, 91(5): 343–347. [DOI]
- [38] Choudhuri BS, Bhakta S, Barik R, Basu J, Kundu M, Chakrabarti P. Overexpression and functional characterization of an ABC (ATP-binding cassette) transporter encoded by the genes *drmA* and *drdB* of *Mycobacterium tuberculosis*. *Biochem J*, 2002, 367(Pt 1): 279–285. [DOI]
- [39] Tian C, Xie JP. Roles of PE_PGRS family in *Mycobacterium tuberculosis* pathogenesis and novel measures against tuberculosis. *Microb Pathog*, 2010, 49(6): 311–314. [DOI]
- [40] Yeruva VC, Kulkarni A, Khandelwal R, Sharma Y, Raghunand TR. The PE_PGRS proteins of *Mycobacterium tuberculosis* are Ca^{2+} binding mediators of host-pathogen interaction. *Biochemistry*, 2016, 55(33): 4675–4687. [DOI]
- [41] Chaitra MG, Shaila MS, Nayak R. Evaluation of T-cell responses to peptides with MHC class I-binding motifs derived from PE_PGRS 33 protein of *Mycobacterium tuberculosis*. *J Med Microbiol*, 2007, 56(Pt 4): 466–474. [DOI]
- [42] Bansal K, Elluru SR, Narayana Y, Chaturvedi R, Patil SA, Kaveri SV, Bayry J, Balaji KN. PE_PGRS antigens of *Mycobacterium tuberculosis* induce maturation and activation of human dendritic cells. *J Immunol*, 2010, 184(7): 3495–3504. [DOI]
- [43] Zhang H, Wang J, Lei J, Zhang M, Yang Y, Chen Y, Wang H. PPE protein (Rv3425) from DNA segment RD11 of *Mycobacterium tuberculosis*: a potential B-cell antigen used for serological diagnosis to distinguish vaccinated controls from tuberculosis patients. *Clin Microbiol Infect*, 2007, 13(2): 139–145. [DOI]
- [44] Ekiert DC, Cox JS. Structure of a PE-PPE-EspG complex from *Mycobacterium tuberculosis* reveals molecular specificity of ESX protein secretion. *Proc Natl Acad Sci USA*, 2014, 111(41): 14758–14763. [DOI]
- [45] Espitia C, Lacleste JP, Mondragón-Palomino M, Amador A, Campuzano J, Martens A, Singh M, Cicero R, Zhang Y, Moreno C. The PE-PGRS glycine-rich proteins of *Mycobacterium tuberculosis*: a new family of fibronectin-binding proteins? *Microbiology*, 1999, 145(Pt 12): 3487–3495. [DOI]
- [46] Mohareer K, Tundup S, Hasnain SE. Transcriptional regulation of *Mycobacterium tuberculosis* PE/PPE genes: a molecular switch to virulence? *J Mol Microbiol Biotechnol*, 2011, 21(3–4): 97–109. [DOI]
- [47] Campuzano J, Aguilar D, Arriaga K, León JC, Salas-Rangel LP, González-y-Merchand J, Hernández-Pando R, Espitia C. The PGRS domain of *Mycobacterium tuberculosis*: PE_PGRS Rv1759c antigen is an efficient

- subunit vaccine to prevent reactivation in a murine model of chronic tuberculosis. *Vaccine*, 2007, 25(18): 3722–3729. [DOI]
- [48] Mitra A, Speer A, Lin K, Ehrt S, Niederweis M. PPE surface proteins are required for heme utilization by *Mycobacterium tuberculosis*. *mBio*, 2017, 8(1): e01720–16. [DOI]
- [49] Raynaud C, Papavinasasundaram KG, Speight RA, Springer B, Sander P, Bottger EC, Colston MJ, Draper P. The functions of OmpATb, a pore-forming protein of *Mycobacterium tuberculosis*. *Mol Microbiol*, 2002, 46(1): 191–201. [DOI]
- [50] Yang YS, Auguin D, Delbecq S, Dumas E, Molle G, Molle V, Roumestand C, Saint N. Structure of the *Mycobacterium tuberculosis* OmpATb protein: a model of an oligomeric channel in the mycobacterial cell wall. *Proteins*, 2011, 79(2): 645–661. [DOI]
- [51] Molle V, Saint N, Campagna S, Kremer L, Lea E, Draper P, Molle G. pH-dependent pore-forming activity of OmpATb from *Mycobacterium tuberculosis* and characterization of the channel by peptidic dissection. *Mol Microbiol*, 2006, 61(3): 826–837. [DOI]
- [52] Song HH, Huff J, Janik K, Walter K, Keller C, Ehlers S, Bossmann SH, Niederweis M. Expression of the *ompATb* operon accelerates ammonia secretion and adaptation of *Mycobacterium tuberculosis* to acidic environments. *Mol Microbiol*, 2011, 80(4): 900–918. [DOI]
- [53] Schiller I, Vordermeier HM, Waters WR, Palmer M, Thacker T, Whelan A, Hardegger R, Marg-Haube B, Raeber A, Oesch B. Assessment of *Mycobacterium tuberculosis* OmpATb as a novel antigen for the diagnosis of bovine tuberculosis. *Clin Vaccine Immunol*, 2009, 16(9): 1314–1321. [DOI]
- [54] Abramovitch RB, Rohde KH, Hsu FF, Russell DG. *AprABC*: a *Mycobacterium tuberculosis* complex-specific locus that modulates pH-driven adaptation to the macrophage phagosome. *Mol Microbiol*, 2011, 80(3): 678–694. [DOI]
- [55] Broset E, Martín C, Gonzalo-Asensio J. Evolutionary landscape of the *Mycobacterium tuberculosis* complex from the viewpoint of PhoPR: implications for virulence regulation and application to vaccine development. *mBio*, 2015, 6(5): e01289–15. [DOI]
- [56] Cao GX, Howard ST, Zhang PP, Wang XS, Chen XL, Samten B, Pang XH. EspR, a regulator of the ESX-1 secretion system in *Mycobacterium tuberculosis*, is directly regulated by the two-component systems MprAB and PhoPR. *Microbiology*, 2015, 161(Pt 3): 477–489. [DOI]
- [57] Baker JJ, Johnson BK, Abramovitch RB. Slow growth of *Mycobacterium tuberculosis* at acidic pH is regulated by *phoPR* and host-associated carbon sources. *Mol Microbiol*, 2014, 94(1): 56–69. [DOI]
- [58] Gannoun-Zaki L, Alibaud L, Kremer L. Point mutations within the fatty acid synthase type II dehydratase components HadA or HadC contribute to isoxyl resistance in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*, 2013, 57(1): 629–632. [DOI]
- [59] Mehta M, Rajmani RS, Singh A. *Mycobacterium tuberculosis* WhiB3 responds to vacuolar pH-induced changes in mycothiol redox potential to modulate phagosomal maturation and virulence. *J Biol Chem*, 2016, 291(6): 2888–2903. [DOI]
- [60] Alam MS, Agrawal P. Matrix-assisted refolding and redox properties of WhiB3/Rv3416 of *Mycobacterium tuberculosis* H37Rv. *Protein Expr Purif*, 2008, 61(1): 83–91. [DOI]
- [61] Steyn AJC, Collins DM, Hondalus MK, Jacobs WR, Jr, Kawakami RP, Bloom BR. *Mycobacterium tuberculosis* WhiB3 interacts with RpoV to affect host survival but is dispensable for *in vivo* growth. *Proc Natl Acad Sci USA*, 2002, 99(5): 3147–3152. [DOI]
- [62] Saini V, Farhana A, Steyn AJC. *Mycobacterium tuberculosis* WhiB3: a novel iron-sulfur cluster protein that regulates redox homeostasis and virulence. *Antioxid Redox Signal*, 2012, 16(7): 687–697. [DOI]
- [63] Banaiee N, Jacobs WR, Jr, Ernst JD. Regulation of *Mycobacterium tuberculosis whiB3* in the mouse lung and macrophages. *Infect Immun*, 2006, 74(11): 6449–6457. [DOI]
- [64] Tsai MF, McCarthy P, Miller C. Substrate selectivity in glutamate-dependent acid resistance in enteric bacteria. *Proc Natl Acad Sci USA*, 2013, 110(15): 5898–5902. [DOI]
- [65] Hong WZ, Wu YE, Fu XM, Chang ZY. Chaperone-dependent mechanisms for acid resistance in enteric bacteria. *Trends Microbiol*, 2012, 20(7): 328–335. [DOI]
- [66] Wu JT, Li YN, Cai ZM, Jin Y. Pyruvate-associated acid resistance in bacteria. *Appl Environ Microbiol*, 2014, 80(14): 4108–4113. [DOI]
- [67] Ferrara F, Di Niro R, D'Angelo S, Busetti M, Marzari R, Not T, Sblattero D. Development of an enzyme-linked immunosorbent assay for *Bartonella henselae* infection

- detection. *Lett Appl Microbiol*, 2014, 59(3): 253–262. [DOI]
- [68] Bernit E, Veit V, La Scola B, Tissot-Dupont H, Gachon J, Raoult D, Harlé JR. *Bartonella quintana* and *Mycobacterium tuberculosis* coinfection in an HIV-infected patient with lymphadenitis. *J Infect*, 2003, 46(4): 244–246. [DOI]
- [69] Kirksey MA, Tischler AD, Siméone R, Hisert KB, Uplekar S, Guilhot C, McKinney JD. Spontaneous phthiocerol dimycocerosate-deficient variants of *Mycobacterium tuberculosis* are susceptible to gamma interferon-mediated immunity. *Infect Immun*, 2011, 79(7): 2829–2838. [DOI]
- [70] Knapp GS, Lyubetskaya A, Peterson MW, Gomes ALC, Ma Z, Galagan JE, McDonough KA. Role of intragenic binding of cAMP responsive protein (CRP) in regulation of the succinate dehydrogenase genes *Rv0249c*–*Rv0247c* in TB complex mycobacteria. *Nucleic Acids Res*, 2015, 43(11): 5377–5393. [DOI]
- [71] Parish T. Two-component regulatory systems of mycobacteria. *Microbiol Spectr*, 2014, 2(1): MGM2–0010–2013. [DOI]
- [72] Pang XH, Cao GX, Neuenschwander PF, Haydel SE, Hou GH, Howard ST. The β -propeller gene *Rv1057* of *Mycobacterium tuberculosis* has a complex promoter directly regulated by both the MprAB and TrcRS two-component systems. *Tuberculosis (Edinb)*, 2011, 91(Suppl. 1): S142–S149. [DOI]
- [73] Haydel SE, Clark-Curtiss JE. The *Mycobacterium tuberculosis* TrcR response regulator represses transcription of the intracellularly expressed *Rv1057* gene, encoding a seven-bladed β -propeller. *J Bacteriol*, 2006, 188(1): 150–159. [DOI]
- [74] Haydel SE, Benjamin WH, Jr, Dunlap NE, Clark-Curtiss JE. Expression, autoregulation, and DNA binding properties of the *Mycobacterium tuberculosis* TrcR response regulator. *J Bacteriol*, 2002, 184(8): 2192–2203. [DOI]
- [75] Mechaly AE, Haouz A, Miras I, Barilone N, Weber P, Shepard W, Alzari PM, Bellinzoni M. Conformational changes upon ligand binding in the essential class II fumarase *Rv1098c* from *Mycobacterium tuberculosis*. *FEBS Lett*, 2012, 586: 1606–1611. [DOI]
- [76] Ruecker N, Jansen R, Trujillo C, Puckett S, Jayachandran P, Piroli GG, Frizzell N, Molina H, Rhee KY, Ehrt S. Fumarase deficiency causes protein and metabolite succination and intoxicates *Mycobacterium tuberculosis*. *Cell Chem Biol*, 2017, 24(3): 306–315. [DOI]
- [77] Chan DSH, Kavanagh ME, McLean KJ, Munro AW, Matak-Vinković D, Coyne AG, Abell C. Effect of DMSO on protein structure and interactions assessed by collision-induced dissociation and unfolding. *Anal Chem*, 2017, 89(18): 9976–9983. [DOI]
- [78] Viswanathan G, Yadav S, Raghunand TR. Identification of novel loci associated with mycobacterial isoniazid resistance. *Tuberculosis (Edinb)*, 2016, 96: 21–26. [DOI]
- [79] Safont M, Angelakis E, Richet H, Lepidi H, Fournier PE, Drancourt M, Raoult D. Bacterial lymphadenitis at a major referral hospital in France from 2008 to 2012. *J Clin Microbiol*, 2014, 52(4): 1161–1167. [DOI]
- [80] Gurumurthy M, Rao M, Mukherjee T, Rao SPS, Boshoff HI, Dick T, Barry III CE, ManjunathaUH. A novel F_{420} -dependent anti-oxidant mechanism protects *Mycobacterium tuberculosis* against oxidative stress and bactericidal agents. *Mol Microbiol*, 2013, 87(4): 744–755. [DOI]
- [81] Belon C, Olvera MR, Vives E, Kremer L, Gannoun-Zaki L, Blanc-Potard AB. Use of the *Salmonella* MgtR peptide as an antagonist of the *Mycobacterium* MgtC virulence factor. *Future Microbiol*, 2016, 11(2): 215–225. [DOI]
- [82] Belon C, Soscia C, Bernut A, Laubier A, Bleves S, Blanc-Potard AB. A macrophage subversion factor is shared by intracellular and extracellular pathogens. *PLoS Pathog*, 2015, 11(6): e1004969. [DOI]
- [83] Belon C, Gannoun-Zaki L, Lutfalla G, Kremer L, Blanc-Potard AB. *Mycobacterium marinum* MgtC plays a role in phagocytosis but is dispensable for intracellular multiplication. *PLoS One*, 2014, 9(12): e116052. [DOI]
- [84] Cabal A, Strunk M, Domínguez J, Lezcano MA, Vitoria MA, Ferrero M, Martín C, Iglesias MJ, Samper S. Single nucleotide polymorphism (SNP) analysis used for the phylogeny of the *Mycobacterium tuberculosis* complex based on a pyrosequencing assay. *BMC Microbiol*, 2014, 14: 21. [DOI]
- [85] Jean-Francois FL, Dai J, Yu L, Myrick A, Rubin E, Fajer PG, Song LK, Zhou HX, Cross TA. Binding of MgtR, a *Salmonella* transmembrane regulatory peptide, to MgtC, a *Mycobacterium tuberculosis* virulence factor: a structural study. *J Mol Biol*, 2014, 426(2): 436–446. [DOI]
- [86] Lee EJ, Pontes MH, Groisman EA. A bacterial virulence

- protein promotes pathogenicity by inhibiting the bacterium's own F_1F_0 ATP synthase. *Cell*, 2013, 154(1): 146–156. [DOI]
- [87] Yang YS, Labesse G, Carrère-Kremer S, Esteves K, Kremer L, Cohen-Gonsaud M, Blanc-Potard AB. The C-terminal domain of the virulence factor MgtC is a divergent ACT domain. *J Bacteriol*, 2012, 194(22): 6255–6263. [DOI]
- [88] Abadia E, Zhang J, Dos Vultos T, Ritacco V, Kremer K, Aktas E, Matsumoto T, Refregier G, Van Soolingen D, Gicquel B, Sola C. Resolving lineage assignation on *Mycobacterium tuberculosis* clinical isolates classified by spoligotyping with a new high-throughput 3R SNPs based method. *Infect Genet Evol*, 2010, 10(7): 1066–1074. [DOI]
- [89] Chuang PC, Liu HS, Sola C, Chen YMA, Jou RW. Spoligotypes of *Mycobacterium tuberculosis* isolates of a high tuberculosis burden aboriginal township in Taiwan. *Infect Genet Evol*, 2008, 8(5): 553–557. [DOI]
- [90] Alix E, Godreuil S, Blanc-Potard AB. Identification of a Haarlem genotype-specific single nucleotide polymorphism in the *mgtC* virulence gene of *Mycobacterium tuberculosis*. *J Clin Microbiol*, 2006, 44(6): 2093–2098. [DOI]
- [91] Lavigne JP, O'Callaghan D, Blanc-Potard AB. Requirement of MgtC for *Brucella suis* intramacrophage growth: a potential mechanism shared by *Salmonella enterica* and *Mycobacterium tuberculosis* for adaptation to a low- Mg^{2+} environment. *Infect Immun*, 2005, 73(5): 3160–3163. [DOI]
- [92] Blanc-Potard AB, Lafay B. MgtC as a horizontally-acquired virulence factor of intracellular bacterial pathogens: evidence from molecular phylogeny and comparative genomics. *J Mol Evol*, 2003, 57(4): 479–486. [DOI]
- [93] Buchmeier N, Blanc-Potard A, Ehrt S, Piddington D, Riley L, Groisman EA. A parallel intraphagosomal survival strategy shared by *Mycobacterium tuberculosis* and *Salmonella enterica*. *Mol Microbiol*, 2000, 35(6): 1375–1382. [DOI]
- [94] Korkegian A, O'Malley T, Xia Y, Zhou Y, Carter DS, Sunde B, Flint L, Thompson D, Ioerger TR, Sacchettini J, Alley MRK, Parish T. The 7-phenyl benzoxaborole series is active against *Mycobacterium tuberculosis*. *Tuberculosis (Edinb)*, 2018, 108: 96–98. [DOI]
- [95] Murugesan D, Ray PC, Bayliss T, Prosser GA, Harrison JR, Green K, Soares De Melo C, Feng TS, Street LJ, Chibale K, Warner DF, Mizrahi V, Epemolu O, Scullion P, Ellis L, Riley J, Shishikura Y, Ferguson L, Osuna-Cabello M, Read KD, Green SR, Lamprecht DA, Finin PM, Steyn AJC, Ioerger TR, Sacchettini J, Rhee KY, Arora K, Barry III CE, Wyatt PG, Boshoff HIM. 2-Mercapto-quinazolinones as inhibitors of type II NADH dehydrogenase and *Mycobacterium tuberculosis*: structure-activity relationships, mechanism of action and absorption, distribution, metabolism, and excretion characterization. *ACS Infect Dis*, 2018, doi: 10.1021/acsinfecdis.7b00275. [DOI]
- [96] Harbut MB, Yang BY, Liu RH, Yano T, Vilcheze C, Cheng B, Lockner J, Guo H, Yu CG, Franzblau SG, Petrassi HM, Jacobs WR, Jr, Rubin H, Chatterjee AK, Wang F. Small molecules targeting *Mycobacterium tuberculosis* type II NADH dehydrogenase exhibit antimycobacterial activity. *Angew Chem Int Engl E*, 2018, 57(13): 3478–3482. [DOI]
- [97] Vilchèze C, Weinrick B, Leung LW, Jacobs WR, Jr. Plasticity of *Mycobacterium tuberculosis* NADH dehydrogenases and their role in virulence. *Proc Natl Acad Sci USA*, 2018, 115(7): 1599–1604. [DOI]
- [98] Bainomugisa A, Lavu E, Hiasiri S, Majumdar S, Honjepari A, Moke R, Dakulala P, Hill-Cawthorne GA, Pandey S, Marais BJ, Coulter C, Coin L. Multi-clonal evolution of multi-drug-resistant/extensively drug-resistant *Mycobacterium tuberculosis* in a high-prevalence setting of Papua New Guinea for over three decades. *Microb Genom*, 2018, doi: 10.1099/mgen.0.000147. [DOI]
- [99] Nguyen N, Wilson DW, Nagalingam G, Triccas JA, Schneider EK, Li J, Velkov T, Baell J. Broad activity of diphenyleneiodonium analogues against *Mycobacterium tuberculosis*, malaria parasites and bacterial pathogens. *Eur J Med Chem*, 2018, 148: 507–518. [DOI]
- [100] Tan YJ, Su BY, Zheng HW, Song YY, Wang YF, Pang Y. Molecular characterization of prothionamide-resistant *Mycobacterium tuberculosis* isolates in Southern China. *Front Microbiol*, 2017, 8: 2358. [DOI]
- [101] Enany S, Yoshida Y, Tateishi Y, Ozeki Y, Nishiyama A, Savitskaya A, Yamaguchi T, Ohara Y, Yamamoto T, Ato M, Matsumoto S. Mycobacterial DNA-binding protein 1 is critical for long term survival of *Mycobacterium smegmatis* and simultaneously coordinates cellular functions. *Sci Rep*, 2017, 7: 6810. [DOI]
- [102] Sellamuthu S, Singh M, Kumar A, Singh SK. Type-II NADH Dehydrogenase (NDH-2): a promising therapeutic

- target for antitubercular and antibacterial drug discovery. *Expert Opin Ther Targets*, 2017, 21(6): 559–570. [DOI]
- [103] Hong WD, Gibbons PD, Leung SC, Amewu R, Stocks PA, Stachulski A, Horta P, Cristiano MLS, Shone AE, Moss D, Ardrey A, Sharma R, Warman AJ, Bedingfield PTP, Fisher NE, Aljayyousi G, Mead S, Caws M, Berry NG, Ward SA, Biagini GA, O'Neill PM, Nixon GL. Rational design, synthesis, and biological evaluation of heterocyclic quinolones targeting the respiratory chain of *Mycobacterium tuberculosis*. *J Med Chem*, 2017, 60(9): 3703–3726. [DOI]
- [104] Unissa AN, Subbian S, Hanna LE, Selvakumar N. Overview on mechanisms of isoniazid action and resistance in *Mycobacterium tuberculosis*. *Infect Genet Evol*, 2016, 45: 474–492. [DOI]
- [105] Otchere ID, Asante-Poku A, Osei-Wusu S, Baddoo A, Sarpong E, Ganiyu AH, Aboagye SY, Forson A, Bonsu F, Yahayah AI, Koram K, Gagneux S, Yeboah-Manu D. Detection and characterization of drug-resistant conferring genes in *Mycobacterium tuberculosis* complex strains: a prospective study in two distant regions of Ghana. *Tuberculosis (Edinb)*, 2016, 99: 147–154. [DOI]
- [106] Heikal A, Hards K, Cheung CY, Menorca A, Timmer MSM, Stocker BL, Cook GM. Activation of type II NADH dehydrogenase by quinolinequinones mediates antitubercular cell death. *J Antimicrob Chemother*, 2016, 71(10): 2840–2847. [DOI]
- [107] Rueda J, Realpe T, Mejia GI, Zapata E, Roza JC, Ferro BE, Robledo J. Genotypic analysis of genes associated with independent resistance and cross-resistance to isoniazid and ethionamide in *Mycobacterium tuberculosis* clinical isolates. *Antimicrob Agents Chemother*, 2015, 59(12): 7805–7810. [DOI]
- [108] Boonaiam S, Chaiprasert A, Prammananan T, Leechawengwongs M. Genotypic analysis of genes associated with isoniazid and ethionamide resistance in MDR-TB isolates from Thailand. *Clin Microbiol Infect*, 2010, 16(4): 396–399. [DOI]
- [109] Miller JL, Velmurugan K, Cowan MJ, Briken V. The type I NADH dehydrogenase of *Mycobacterium tuberculosis* counters phagosomal NOX2 activity to inhibit TNF- α -mediated host cell apoptosis. *PLoS Pathog*, 2010, 6(4): e1000864. [DOI]
- [110] Brossier F, Veziris N, Truffot-Pernot C, Jarlier V, Sougakoff W. Molecular investigation of resistance to the antituberculous drug ethionamide in multidrug-resistant clinical isolates of *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*, 2011, 55(1): 355–360. [DOI]
- [111] Shirude PS, Paul B, Choudhury NR, Kedari C, Bandodkar B, Ugarkar BG. Quinoliny pyrimidines: potent inhibitors of NDH-2 as a novel class of anti-TB agents. *ACS Med Chem Lett*, 2012, 3(9): 736–740. [DOI]
- [112] Machado D, Perdigão J, Ramos J, Couto I, Portugal I, Ritter C, Boettger EC, Viveiros M. High-level resistance to isoniazid and ethionamide in multidrug-resistant *Mycobacterium tuberculosis* of the Lisboa family is associated with *inhA* double mutations. *J Antimicrob Chemother*, 2013, 68(8): 1728–1732. [DOI]
- [113] Warman AJ, Rito TS, Fisher NE, Moss DM, Berry NG, O'Neill PM, Ward SA, Biagini GA. Antitubercular pharmacodynamics of phenothiazines. *J Antimicrob Chemother*, 2013, 68(4): 869–880. [DOI]
- [114] Awasthy D, Ambady A, Narayana A, Morayya S, Sharma U. Roles of the two type II NADH dehydrogenases in the survival of *Mycobacterium tuberculosis* *in vitro*. *Gene*, 2014, 550(1): 110–116. [DOI]
- [115] Dunn EA, Roxburgh M, Larsen L, Smith RAJ, McLellan AD, Heikal A, Murphy MP, Cook GM. Incorporation of triphenylphosphonium functionality improves the inhibitory properties of phenothiazine derivatives in *Mycobacterium tuberculosis*. *Bioorg Med Chem*, 2014, 22(19): 5320–5328. [DOI]
- [116] Jagielski T, Bakula Z, Roeske K, Kamiński M, Napiórkowska A, Augustynowicz-Kopeć E, Zwolska Z, Bielecki J. Detection of mutations associated with isoniazid resistance in multidrug-resistant *Mycobacterium tuberculosis* clinical isolates. *J Antimicrob Chemother*, 2014, 69(9): 2369–2375. [DOI]
- [117] Schurig-Briccio LA, Yano T, Rubin H, Gennis RB. Characterization of the type II NADH: menaquinone oxidoreductases from *Staphylococcus aureus* and the bactericidal action of phenothiazines. *Biochim Biophys Acta-Bioenerget*, 2014, 1837(7): 954–963. [DOI]
- [118] Shekar S, Yeo ZX, Wong JCL, Chan MKL, Ong DCT, Tongyoo P, Wong SY, Lee AS. Detecting novel genetic variants associated with isoniazid-resistant *Mycobacterium tuberculosis*. *PLoS One*, 2014, 9(7): e102383. [DOI]
- [119] Verma SC, Venugopal U, Khan SR, Akhtar MS, Krishnan MY. Coupling reporter expression to respiration detects active as well as dormant mycobacteria *in vitro* and in mouse tissues. *Int J Mycobacteriol*, 2014, 3(1): 25–35. [DOI]
- [120] Yano T, Rahimian M, Aneja KK, Schechter NM, Rubin

- H, Scott CP. *Mycobacterium tuberculosis* type II NADH-menaquinone oxidoreductase catalyzes electron transfer through a two-site ping-pong mechanism and has two quinone-binding sites. *Biochemistry*, 2014, 53(7): 1179–1190. [DOI]
- [121] Jagielski T, Bakula Z, Roeske K, Kamiński M, Napiórkowska A, Augustynowicz-Kopeć E, Zwolska Z, Bielecki J. Mutation profiling for detection of isoniazid resistance in *Mycobacterium tuberculosis* clinical isolates. *J Antimicrob Chemother*, 2015, 70(12): 3214–3221. [DOI]
- [122] Zimenkov DV, Nosova EY, Kulagina EV, Antonova OV, Arslanbaeva LR, Isakova AI, Krylova LY, Peretokina IV, Makarova M, Safonova SG, Borisov SE, Gryadunov DA. Examination of bedaquiline- and linezolid-resistant *Mycobacterium tuberculosis* isolates from the Moscow region. *J Antimicrob Chemother*, 2017, 72(7): 1901–1906. [DOI]
- [123] Xu J, Wang B, Hu MH, Huo FM, Guo SC, Jing W, Nuermberger E, Lu Y. Primary clofazimine and bedaquiline resistance among isolates from patients with multidrug-resistant tuberculosis. *Antimicrob Agents Chemother*, 2017, 61(6): e00239–17. [DOI]
- [124] Zhang S, Chen JZ, Cui P, Shi WL, Zhang WH, Zhang Y. Identification of novel mutations associated with clofazimine resistance in *Mycobacterium tuberculosis*. *J Antimicrob Chemother*, 2015, 70(9): 2507–2510. [DOI]
- [125] Kozak R, Behr MA. Divergence of immunologic and protective responses of different BCG strains in a murine model. *Vaccine*, 2011, 29(7): 1519–1526. [DOI]
- [126] Kozak RA, Alexander DC, Liao RL, Sherman DR, Behr MA. Region of difference 2 contributes to virulence of *Mycobacterium tuberculosis*. *Infect Immun*, 2011, 79(1): 59–66. [DOI]
- [127] Cockle PJ, Gordon SV, Lalvani A, Buddle BM, Hewinson RG, Vordermeier HM. Identification of novel *Mycobacterium tuberculosis* antigens with potential as diagnostic reagents or subunit vaccine candidates by comparative genomics. *Infect Immun*, 2002, 70(12): 6996–7003. [DOI]
- [128] Van Dam JCJ, Schaap PJ, Martins dos Santos VAP, Suárez-Diez M. Integration of heterogeneous molecular networks to unravel gene-regulation in *Mycobacterium tuberculosis*. *BMC Syst Biol*, 2014, 8: 111. [DOI]
- [129] Schuessler DL, Cortes T, Fivian-Hughes AS, Loughheed KE, Harvey E, Buxton RS, Davis EO, Young DB. Induced ectopic expression of HlgB toxin in *Mycobacterium tuberculosis* results in growth inhibition, reduced abundance of a subset of mRNAs and cleavage of tmRNA. *Mol Microbiol*, 2013, 90(1): 195–207. [DOI]
- [130] Serafini A, Boldrin F, Palù G, Manganelli R. Characterization of a *Mycobacterium tuberculosis* ESX-3 conditional mutant: essentiality and rescue by iron and zinc. *J Bacteriol*, 2009, 191(20): 6340–6344. [DOI]
- [131] Maciąg A, Piazza A, Riccardi G, Milano A. Transcriptional analysis of ESAT-6 cluster 3 in *Mycobacterium smegmatis*. *BMC Microbiol*, 2009, 9: 48. [DOI]
- [132] Lucarelli D, Russo S, Garman E, Milano A, Meyer-Klaucke W, Pohl E. Crystal structure and function of the zinc uptake regulator FurB from *Mycobacterium tuberculosis*. *J Biol Chem*, 2007, 282(13): 9914–9922. [DOI]
- [133] Maciąg A, Dainese E, Rodriguez GM, Milano A, Provvedi R, Pasca MR, Smith I, Palù G, Riccardi G, Manganelli R. Global analysis of the *Mycobacterium tuberculosis* Zur (FurB) regulon. *J Bacteriol*, 2007, 189(3): 730–740. [DOI]
- [134] Pisu D, Provvedi R, Espinosa DM, Payan JB, Boldrin F, Palù G, Hernandez-Pando R, Manganelli R. The Alternative sigma factors SigE and SigB are involved in tolerance and persistence to antitubercular drugs. *Antimicrob Agents Chemother*, 2017, 61(12): e01596–17. [DOI]
- [135] Yang SS, Hu YB, Wang XD, Gao YR, Li K, Zhang XE, Chen SY, Zhang TY, Gu J, Deng JY. Deletion of *sigB* causes increased sensitivity to *para*-aminosalicylic acid and sulfamethoxazole in *Mycobacterium tuberculosis*. *Antimicrob Agents Chemother*, 2017, 61(10): e00551–17. [DOI]
- [136] Pettersson BM, Das S, Behra PR, Jordan HR, Ramesh M, Mallick A, Root KM, Cheramie MN, de la Cruz Melara I, Small PL, Dasgupta S, Ennis DG, Kirsebom LA. Comparative sigma factor-mRNA levels in mycobacterium marinum under stress conditions and during host infection. *PLoS One*, 2015, 10(10): e0139823. [DOI]
- [137] Sharma AK, Chatterjee A, Gupta S, Banerjee R, Mandal S, Mukhopadhyay J, Basu J, Kundu M. MtrA, an essential response regulator of the MtrAB two-component system, regulates the transcription of resuscitation-promoting factor B of *Mycobacterium tuberculosis*. *Microbiology*, 2015, 161(6): 1271–1281. [DOI]

- [138] Hu YB, Morichaud Z, Perumal AS, Roquet-Baneres F, Brodolin K. *Mycobacterium* RbpA cooperates with the stress-response σ^B subunit of RNA polymerase in promoter DNA unwinding. *Nucleic Acids Res*, 2014, 42(16): 10399–10408. [DOI]
- [139] Datta P, Shi LB, Bibi N, Balázsi G, Gennaro ML. Regulation of central metabolism genes of *Mycobacterium tuberculosis* by parallel feed-forward loops controlled by sigma factor $E(\sigma^E)$. *J Bacteriol*, 2011, 193(5): 1154–1160. [DOI]
- [140] Dutta NK, Mazumdar K, Dastidar SG, Karakousis PC, Amaral L. New patentable use of an old neuroleptic compound thioridazine to combat tuberculosis: a gene regulation perspective. *Recent Pat Anti-Infect Drug Discov*, 2011, 6(2): 128–138. [DOI]
- [141] MacArthur I, Parreira VR, Lepp D, Mutharia LM, Vazquez-Boland JA, Prescott JF. The sensor kinase MprB is required for *Rhodococcus equi* virulence. *Vet Microbiol*, 2011, 147(1–2): 133–141. [DOI]
- [142] Giovannini D, Cappelli G, Jiang LN, Castilletti C, Colone A, Serafino A, Wannenes F, Giaò L, Quintiliani G, Fraziano M, Nepravishta R, Colizzi V, Mariani F. A new *Mycobacterium tuberculosis* smooth colony reduces growth inside human macrophages and represses PDIM Operon gene expression. Does an heterogeneous population exist in intracellular mycobacteria? *Microb Pathog*, 2012, 53(3–4): 135–146. [DOI]
- [143] Mustyala KK, Malkhed V, Potlapally SR, Chittireddy VR, Vuruputuri U. Macromolecular structure and interaction studies of SigF and Usfx in *Mycobacterium tuberculosis*. *J Recept Signal Transduct Res*, 2014, 34(3): 162–173. [DOI]
- [144] Olivencia BF, Müller AU, Roschitzki B, Burger S, Weber-Ban E, Imkamp F. *Mycobacterium smegmatis* PafBC is involved in regulation of DNA damage response. *Sci Rep*, 2017, 7: 13987. [DOI]
- [145] Yan SQ, Xu MM, Wang R, Li QM, Yu ZX, Xie P. Overexpression of Rv2788 increases mycobacterium stresses survival. *Microbiol Res*, 2017, 195: 51–59. [DOI]
- [146] Nautiyal A, Patil KN, Muniyappa K. Suramin is a potent and selective inhibitor of *Mycobacterium tuberculosis* RecA protein and the SOS response: RecA as a potential target for antibacterial drug discovery. *J Antimicrob Chemother*, 2014, 69(7): 1834–1843. [DOI]
- [147] Smollett KL, Smith KM, Kahramanoglou C, Arnvig KB, Buxton RS, Davis EO. Global analysis of the regulon of the transcriptional repressor LexA, a key component of SOS response in *Mycobacterium tuberculosis*. *J Biol Chem*, 2012, 287(26): 22004–22014. [DOI]
- [148] Forse LN, Houghton J, Davis EO. Enhanced expression of *recX* in *Mycobacterium tuberculosis* owing to a promoter internal to *recA*. *Tuberculosis (Edinb)*, 2011, 91(2): 127–135. [DOI]
- [149] Chandran AV, Prabu JR, Manjunath GP, Patil KN, Muniyappa K, Vijayan M. Crystallization and preliminary X-ray studies of the C-terminal domain of *Mycobacterium tuberculosis* LexA. *Acta Cryst*, 2010, 66(Pt 9): 1093–1095. [DOI]
- [150] Dawson LF, Dillury J, Davis EO. RecA-independent DNA damage induction of *Mycobacterium tuberculosis* *ruvC* despite an appropriately located SOS box. *J Bacteriol*, 2010, 192(2): 599–603. [DOI]
- [151] Janowski R, Panjkar S, Eddine AN, Kaufmann SHE, Weiss MS. Structural analysis reveals DNA binding properties of Rv2827c, a hypothetical protein from *Mycobacterium tuberculosis*. *J Struct Funct Genomics*, 2009, 10(2): 137–150. [DOI]
- [152] Janowski R, Eddine AN, Kaufmann SH, Weiss MS. Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of Rv2827c from *Mycobacterium tuberculosis*. *Acta Crystallogr Sect F Struct Biol Cryst Commun*, 2006, 62(Pt 8): 753–756. [DOI]
- [153] Singh RK, Kefala G, Janowski R, Mueller-Dieckmann C, Von Kries JP, Weiss MS. The high-resolution Structure of LeuB (Rv2995c) from *Mycobacterium tuberculosis*. *J Mol Biol*, 2005, 346(1): 1–11. [DOI]
- [154] Han MY, Son MY, Lee SH, Kim JK, Huh JS, Kim JH, Choe IS, Chung TW, Choe YK. Molecular cloning of the *leuB* genes from *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis*. *Biochem Mol Biol Int*, 1997, 41(4): 657–663. [DOI]

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