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专论与综述

DNA 聚合酶 IV：抗细菌的潜在药靶

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摘　要：病原微生物及其耐药性是全球公共卫生的重要问题。众多人兽共患病原菌可通过食品产业链传播给人，同时耐药性使得感染更难治疗，增加了疾病传播和死亡的风险。从分子水平上研究病原体的变异规律、毒力及其致病机制有助于寻找新的药物靶点、研制新的药物。DNA 聚合酶 IV (polymerase IV，Pol IV)是 γ 家族聚合酶中的重要成员，广泛分布在原核生物、真核生物和古细菌 3 个生命域。Pol IV 具有跨损伤 DNA 合成的能力，不仅在 SOS 反应(SOS response)和 RpoS 调控下响应 DNA 损伤，还参与细菌抗生素抗性及适应性的获得，在细菌中发挥着至关重要的作用。本文综述了近年来细菌 Pol IV 相关研究，回顾了其遗传特征、结构特征、表达调控及对细菌适应性的影响，并且讨论了 Pol IV 作为潜在药物靶点的可行性。

关键词：DNA 聚合酶 IV；抗菌药物；抗生素

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DNA polymerase IV: a potential target of drugs against bacteria

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Abstract: Pathogens and their drug resistance are key issues of global public health. Numerous zoonotic pathogens can be transmitted to humans through the food industry chain. Drug resistance makes infections more difficult to treat, increasing the risk of disease transmission and death. Studying the variation patterns, virulence, and pathogenic mechanisms of pathogens at the molecular level will help us to find new drug targets and developing new drugs. DNA polymerase IV (Pol IV), an important member of the γ family polymerases, is ubiquitous in the three life domains (prokaryotes, eukaryotes, and archaea). This enzyme is involved in the translesion DNA synthesis and plays a critical role in bacteria. It not only responds to DNA damage under SOS response and RpoS regulation but also is involved in the acquisition of antibiotic resistance and adaptation. This paper reviewed the recent studies related to bacterial Pol IV, including the genetic and structural features, expression regulation, and effects on bacterial adaptation, and discussed the feasibility of Pol IV as a potential drug target.

Keywords: DNA polymerase IV; antibacterial drug; antibiotic

近年来，由病原体造成的食品安全事件频发，对人类生命健康构成了严重威胁。研究表明，在中国，食源性疾病的暴发及受影响人群的数量正在上升[1]。沙门菌、副溶血性弧菌、金黄色葡萄球菌、蜡样芽孢杆菌和腹泻性大肠杆菌已被确定为中国食源性疾病暴发最常见的细菌病原体[2]，而且食物加工链的全球化趋势也增加了不同地区食源性病原体暴发的风险[3]。这些病原体大多是人兽共患菌，如沙门菌可在动物上定殖并在食品加工过程中存活下来，通过污染的乳制品、肉制品等途径进入人体[4-6]。众多学者报道了沙门菌在中国地区的动物性食品和家畜家禽中的流行情况[7-13]，表明沙门菌是危害中国公共卫生安全的因素之一。自发现抗生素具有预防疾病和促进生长的作用以来，抗生素作为饲料添加剂在畜禽养殖中得到了广泛的使用。由于大量使用抗生素，使得细菌耐药性不断增加，动物源病原体的耐药基因可通过食物链传递给人，影响正常的肠道环境[14]。此外，来自家畜和家禽的病原体具有独特的抗生素耐药表型[4,13,15-16]，加上复杂的耐药机制，亟须探索新的药物靶点，开发新的抗菌药物。

从分子水平上研究病原体的变异规律、毒力及其致病机制有助于寻找新的药物靶点，以研制新的药物。过去的几十年里，在多个物种体内发现的 DNA 聚合酶数量不断增加。大多数新发现的 DNA 聚合酶不是参与 DNA 复制，而是参与跨损伤 DNA 合成和修复。虽然这些聚合

酶的错误率比高保真复制型聚合酶高，但在内源性和外源性损伤下，它们避免了细胞死亡。γ家族聚合酶是参与 DNA 损伤修复的重要聚合酶家族，该家族聚合酶绕过 DNA 损伤，使细菌得以复制。DNA 聚合酶 IV (polymerase IV，Pol IV)是 γ 家族聚合酶重要成员，广泛分布在原核生物、真核生物和古细菌 3 个生命域[17]。Pol IV 及其同源物具有跨损伤 DNA 合成(translesion DNA synthesis，TLS)的能力，能绕过多种损伤进行 DNA 复制[18-24]，也参与了细菌多个生物进程，一直是研究的热点。近年来，针对生物体聚合酶的药物不断出现[25-26]，表明聚合酶或许是新的疾病治疗突破口。本文综述了细菌 Pol IV 相关研究，回顾了其遗传特征、结构特征、表达调控及对细菌适应性的影响，并且讨论了 Pol IV 作为潜在药物靶点的可行性。

1 Pol IV 的遗传特征

大肠杆菌是重要的模式生物，其 Pol IV 也是较早发现的 γ 家族聚合酶之一[27]，对其遗传特征的研究有助于了解 γ 家族聚合酶表达调控模式。在大肠杆菌 K-12 中，*dinB* 基因表达 Pol IV，其与 *yafP*、*yafN* 和 *yafO* 位于同一个操纵子中[28]。该操纵子会被 DNA 损伤剂诱导表达[29-30]，因为只有 1 个由 LexA 调控的启动子位于 *dinB* 的上游[31]，所以 Pol IV 的表达也会伴随其他 3 个基因的表达[29]。YafO 是一种抑制蛋白质合成的毒素，而 YafN 是其相关的抗毒素[32-33]，两者共同组成一种毒素-抗毒素调节系统[33]。目前，对于 *yafP* 基因知之甚少，推测其表达一个 N-乙酰转移酶，但与 YafN/YafO 调节系统无直接联系[33]。值得注意的是，由于 *yafN* 和 *yafO* 基因在大肠杆菌中不是保守的，操纵子组成不同[34]，表达的基因组合也不同。这种共表达模式的不同也体现在不同物种上。尽管沙门菌与大肠杆菌亲缘很近，但沙门菌的 *dinB* 与 *yafK* 相邻，*yafK* 基因又紧邻 *yafJ* 基因[35]。由于 *dinB* 与 *yafK* 仅间隔 249 bp，这 3 个基因可能在统一调控下。YafK 蛋白参与细菌肽聚糖的生物合成；肽聚糖既是细菌细胞壁重要的组成部分，也参与细胞生物膜的形成[36]。目前对 *yafJ* 参与的生物过程知之甚少，认为它是一个参与谷氨酰胺代谢的谷氨酰胺转移酶(GO：0006541)。目前还不清楚多种共表达模式是否存在进化的选择性，以及不同共表达模式会对生物体造成什么影响。

γ 家族聚合酶在生物界广泛分布，系统进化树可以将其分为 DinB、UmuC、Rev1 和 Rad30 4 个分支[17]。UmuC 家族似乎只存在于原核生物中，在革兰阴性和革兰阳性细菌中有独立的分支；Rev1 和 Rad30 分支的蛋白只在真核生物中发现；DinB 分支分布最广，存在于原核生物、真核生物和古细菌中[17]。理想情况下，抗菌药物应有宽的抗菌谱。在临床上，广谱抗菌药物是针对多个病原体的有效措施之一，在预防性给药中可取得良好的效果[37]。进化树分析结果显示，多个物种的 DinB 相近(图 1)，这种保守性使其成为一个潜在的广谱药物作用靶点。

2 Pol IV 的结构特征

除了遗传特征，对大肠杆菌 Pol IV 结构的研究拓展了人们对γ家族 DNA 聚合酶如何响应 DNA 损伤的认识。大肠杆菌 Pol IV 是一个由 351 个氨基酸组成、相对分子质量约为 39.5 kDa 的单体酶[38]，其三维结构如图 2 所示。大肠杆菌 Pol IV 的 N 端为 DNA 聚合酶结构域，由 5 个基序组成[39-40]，该结构域对 Pol IV 的 TLS 活性至关重要[41]，是 Pol IV 发挥功能的核心结构域。半胱氨酸 66 (Cys-66)和精氨酸 38 (Arg-38)在 Pol IV 进行链式置换时起关键作用[42]，Cys-66

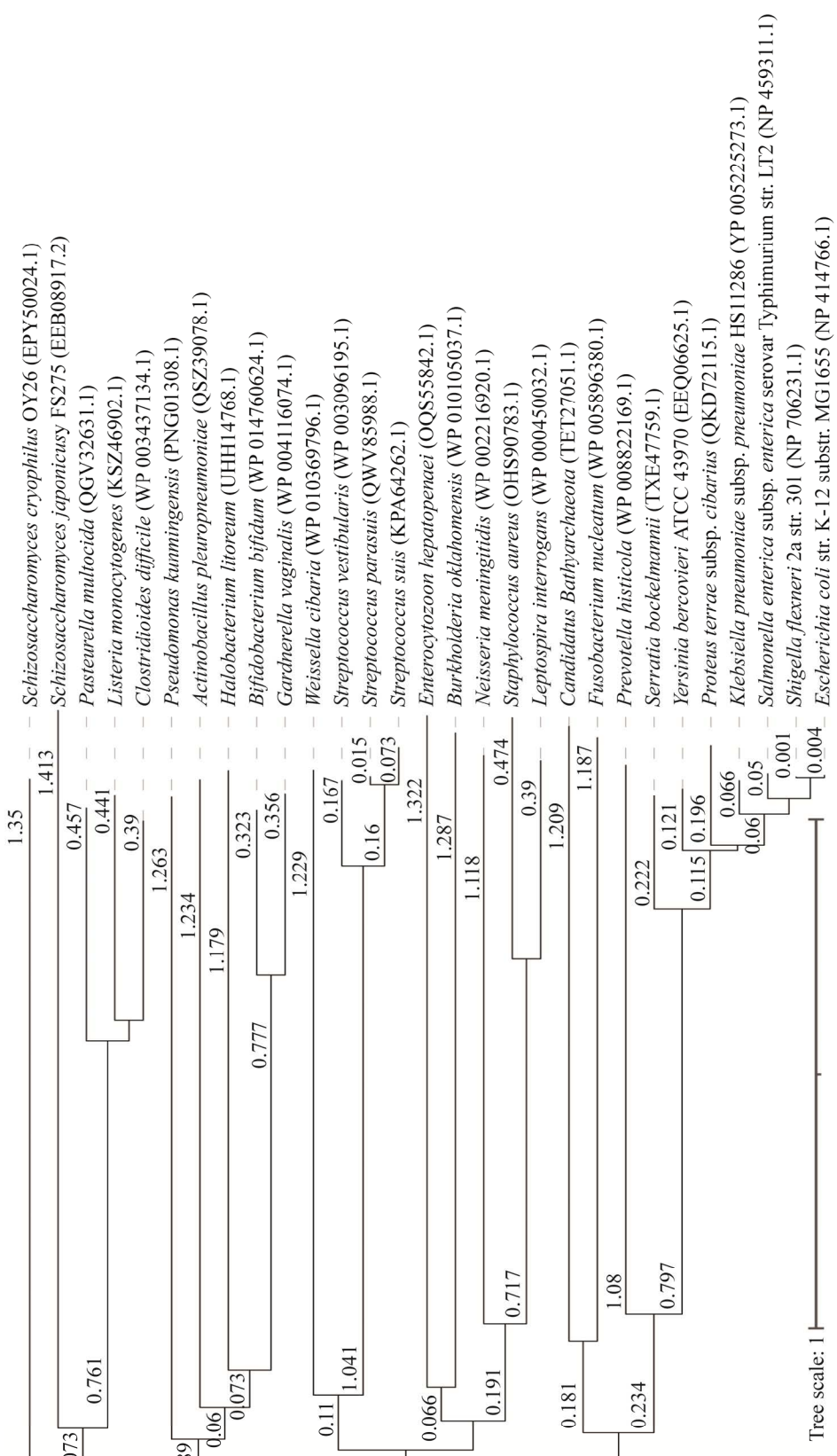

图 1　包括细菌、真菌和古菌在内的 29 个物种基于 DinB 氨基酸序列的系统发育树　图中展示 29 个物种的拉丁名及其 DinB 蛋白序号(括号内)；分支上的数字表示进化分支长度，越短代表进化距离越近

Figure 1　Phylogenetic tree of 29 species including bacteria, fungi and archaea based on amino acid sequencesof DinB. The Latin names of the 29 species and the number of their DinB (in parentheses) were shown; The number on the branch indicates the length of the evolutionary branch, and the shorter indicates the closer the evolutionary distance.

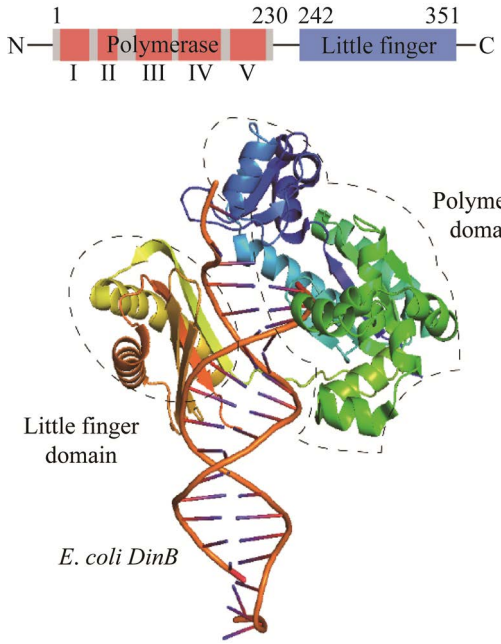

图 2 大肠杆菌 Pol IV 结构图 图中显示了由 5 个基序组成的聚合酶结构域(polymerase domain)(红色矩形)和小指结构域(little finger domain) (蓝色矩形)；Pol IV 与 DNA 相互作用示意图也被展示[改自蛋白质数据库(PDB)：4R8U]

Figure 2 Schematic representation of *Escherichia coli* Pol IV. The figure shows little finger domain (blue rectangle) and polymerase domain which consist of five motifs (red rectangle); schematic representation of Pol IV interacted with DNA is also shown [adapted from protein data bank (PDB): 4R8U].

也参与 Pol IV 与 RecA 的相互作用[23-24]。Pol IV 的 C 端为一个小指结构域，这个结构域为 γ 家族聚合酶特有[43]，在 DNA 结合中起作用[44]。Pol IV 的活性位点比高保真 DNA 聚合酶更宽，使其能容纳并绕过损伤[45-46]。灵活的小指结构域可以脱离 DNA 大沟，降低聚合酶与 DNA 之间的相互作用[47]。这种可塑性使 Pol IV 更易与损伤处形成的加合物结合，不过这也使得 Pol IV 无法像高保真 DNA 聚合酶一样进行错配校正[45-46]。Pol IV 在复制时容易出错，大约每复制 10^3–10^4 个碱基会引入 1 个错误碱基，而且倾向产生−1 移码突变[48-49]。

3 Pol IV 的表达调控

Pol IV 可以合成约 1 300 个碱基的 DNA 片段并引入突变[50]，其效率在 β 钳帮助下大大增加[50-51]。而且细胞内的 Pol IV 的基础水平相对较高[48]，过量的 Pol IV 会抑制细菌的生长甚至死亡[38]，所以调节其活性对于维持细菌遗传物质的稳定非常必要。在细菌中，Pol IV 蛋白量受到多种因素的影响，而这些因素主要是通过调控 Pol IV 的表达来发挥作用。目前已知的主要是在转录水平和翻译后水平的调控，转录水平的调节包括 SOS 反应(SOS response)和 RpoS 的调节；翻译后水平的调控是通过蛋白降解来平衡胞内 Pol IV 的量。

SOS 反应是细菌体内一种对 DNA 损伤响应的细胞反应。SOS 反应通过阻遏蛋白 LexA 和 RecA 蛋白酶相互作用来调节反应网络[52]。SOS 盒是一个保守的 DNA 序列[TACTG(TA)$_5$CAGTA][53]，位于基因的启动子区域，是 RNA 聚合酶的结合位点或邻近 RNA 聚合酶结合位点。在正常细胞中，LexA 组成的二聚体与 SOS 盒(SOS boxes)结合，抑制了基因的表达。当细胞发生 DNA 损伤时，游离在细胞中的 RecA 与受损的 DNA 结合(形成 RecA-ssDNA 丝)并刺激 LexA 蛋白的自我裂解(图 3)[54]。随着细胞中 LexA 浓度的降低，细菌基因组不同区域的 40 多个基因相继被激活[55-61]。表达的顺序由 LexA 与每个基因对应的 SOS 盒紧密结合的程度决定。许多较早被诱导的基因，像 *uvrA*、*uvrB*、*uvrD* 和 *recN* 参与无错误的核苷酸切除修复和重组修复[62]。如果细胞内存在大量的 DNA 损伤，精确修复无法实现，细胞会转向跨损伤 DNA 合成途径。在正常细胞中，Pol IV 的浓度约为 250 个分子/细胞，

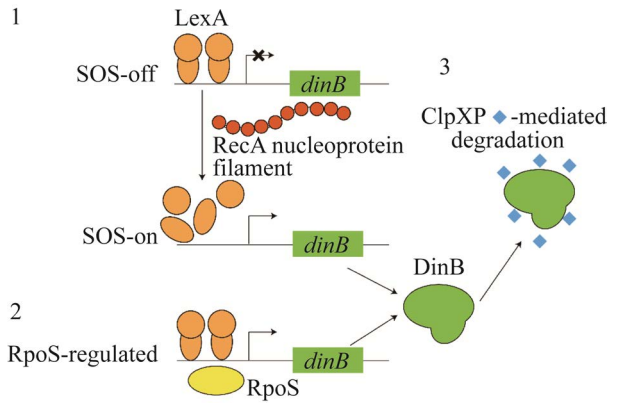

图 3　DinB 表达的多方位调节　1：RecA-ssDNA 核蛋白丝诱导 LexA 发生自溶裂解，导致 SOS-off 切换到 SOS-on，诱导 DinB 的表达；2：*dinB* 的转录也受 RpoS 的调控，RpoS 在静止期绕过 LexA；3：ClpXP 蛋白酶通过降解 Pol IV 来平衡细胞中的 Pol IV 数量

Figure 3　Multifaceted regulation of DinB expression. 1: The RecA-ssDNA nucleoprotein filament induces LexA to undergo autoproteolytic cleavage, which leads to the switch from SOS-off to SOS-on and induces the expression of DinB; 2: Transcription of *dinB* is also regulated by RpoS which bypasses LexA in stationary phase; 3: The ClpXP protease balances the amount of Pol IV in the cell by degrading Pol IV.

在 SOS 诱导下会升高 10 倍(图 3)，所以 Pol IV 是大肠杆菌 DNA 损伤时最丰富的聚合酶[48]，是细菌响应 DNA 损伤最主要的聚合酶之一。除了损伤 DNA 的物理和化学因素外，一些生理因素，如 pH[63]和 dNTPs 的改变[64]也能引起细菌的 SOS 反应。大肠杆菌体内 dNTPs 浓度降低导致复制叉暂停，并引发 SOS 应答[65]。SOS 调控的 Pol IV 蛋白可提高细菌在核苷酸饥饿时的存活率[40]。

除了 SOS 反应，RpoS 调控网络对细菌的生存至关重要。宿主细胞内环境[66]、低 pH [67]和生长稳定期的营养限制等因素影响着 RpoS 的表达。RpoS 蛋白作为 RNA 聚合酶的一种 σ 因子，能够调控一组响应压力的特定基因的表达。全基因组表达谱数据表明，大肠杆菌约 10% 的基因受到 RpoS 的直接或间接调控[68]，被认为是大肠杆菌一般压力反应的主要调节器。Pol IV 也受 RpoS 的调节，主要发生在细菌生长稳定期，Pol IV 的分子数因 RpoS 的调控而增加(图 3)[69-71]。虽然 Pol IV 受 LexA 的控制，但在稳定期 RpoS 会绕过 LexA 来调节 Pol IV 的表达[72]。

除了转录水平上的调节，ClpXP 通过降解 Pol IV 来平衡细胞内 Pol IV 含量(图 3)。ClpXP 是一种依赖 ATP 水解产生的能量降解蛋白质的蛋白酶。其底物包括 UmuD′[73]、LexA[74]和 RpoS[75]。Al Mamun 等的实验表明，*clpP* 和 *clpX* 缺失的细胞显示出一种突变的表型，而这种突变依赖于 Pol IV[76]。ClpXP 介导的 Pol IV 蛋白分解是一种重要的翻译后机制，帮助细胞规避有害突变[76]。

除了表达调控外，还有一些蛋白与 Pol IV 相互作用，改变其活性和亚细胞定位以达到调节 Pol IV 功能的效果，如 GroE[77]、Ppk[78]、UmuD[40]等。这些蛋白对于维持细菌遗传物质的稳定性非常必要。

4　Pol IV 对细菌适应性的影响

细菌适应性指的是细菌在不同环境(即体外或体内)中生存和生长的能力。在特定环境下，适应性强的个体在群体中占优势，被视为细菌传播的决定因素之一[79]。Pol IV 赋予的适应性体现于细菌通过突变来减少抗生素和营养限制对自身的影响，也体现于细菌在宿主生态位通过 Pol IV 响应宿主施加的压力。

新达尔文主义者(neo-Darwinists)认为进化是持续和渐进的，因此驱动进化的基因变化也应该如此[80-81]。然而，研究者在对微生物适应性突变研究中发现，细胞可以定向增加突变速

率，产生进化过程所依赖的遗传变异，提高细菌在环境和宿主的生存能力[77]，已经在大肠杆菌、鼠伤寒沙门菌、枯草芽孢杆菌、假单胞菌属、梭状芽孢杆菌属、酿酒酵母和白色念珠菌中报道了适应性突变或相关现象[82-88]。适应性突变对选择压力做出的特异性反应依赖于几个潜在的分子机制[89]。第一，当细胞无法正常复制时，重组启动了产生突变的 DNA 合成；第二，低保真度的聚合酶可能会在压力下增加遗传变异；第三，共轭质粒可能在基因的进化中起到一定作用[89]。大肠杆菌 FC40 (Lac-)是研究适应性突变的常用模型[90]。因为一个+1 的移码突变导致 *lacZ* 基因失活，导致 FC40 菌株不能利用乳糖。乳糖基础营养培养基上大约以每小时每 10^9 个细胞出现 1 个 Lac+回复突变体，并以这种速率持续几天[89]。通过对野生株的比较，Pol IV 的缺失降低了 50%的 Lac+回复突变[91]，该结果确定了 Pol IV 在适应性突变中的作用[69]。值得注意的是，在 DNA 聚合酶 II 缺失下，回复突变菌株更多[91]，这个结果表明两种聚合酶可能存在一定的竞争关系[89]。

此外，Pol IV 也参与了细菌对耐药性的获得。细菌可通过基因突变迅速获得对喹诺酮类和利福平类抗生素的抗性。Cirz 等在体内感染模型中发现干扰 LexA 蛋白裂解阻止了 SOS 反应的诱导，使致病性大肠杆菌无法对环丙沙星或利福平产生耐药性；为了进一步确定 LexA 裂解是如何诱导抗性的，他们研究了 Pol IV 在体外生存和变异中的作用，结果表明，删除 Pol IV 的效果与干扰 LexA 裂解的效果相同[92]。此外，Pol IV 也参与了细菌对 β 内酰胺类抗生素抗性的获得。在一些肠道细菌病原体中，功能缺失突变是导致临床相关 β 内酰胺类抗生素耐药性的常见途径之一[93]。饥饿应激可以促进临床相关模型中由 *ampD* 突变导致的 β 内酰胺类抗药性[94]。在肠杆菌中，*ampD* 基因编码的 AmpD 是一种细胞溶质蛋白，是 β 内酰胺酶表达的负调节因子[95-96]。AmpD 功能缺失突变会导致组成性 AmpC β 内酰胺酶的产生，进而产生 β 内酰胺抗性[97-99]。Pol IV 的缺失影响了 *ampD* 突变的产生，这可能与 Pol IV 参与双链断裂修复有关；研究者也发现在 *ampD* 突变中碱基替换突变超过–1 移码突变，碱基替换突变并不由 Pol IV 产生，在很大程度上依赖于 DNA 聚合酶 V；所以 *ampD* 突变介导的 β 内酰胺抗性需要两种聚合酶共同参与[94]。这种在染色体基因突变中的聚合酶合作与 Lac+回复突变中的竞争明显不同。

除了帮助细菌产生新的适应性，Pol IV 也对细菌在宿主体内存活有所帮助。DNA 微阵列揭示了巨噬细胞中沙门菌的完整转录谱，确定了许多毒力基因和 SOS 反应基因参与沙门菌与巨噬细胞的相互作用[100]。相比其 DNA 聚合酶，Pol IV 在 12 h 的感染周期中一直高表达(图 4)[100]。自由基是巨噬细胞杀菌、发挥细胞毒作用的主要介质[101]，同时也是细菌 SOS 反应的有效诱导剂[102]。巨噬细胞可以对胞内细菌造成严重的 DNA 损伤[103]，而细菌通过 SOS 反应对 DNA 损伤做出响应以求得生存[104-106]。在早期，细菌会进行相对精确的重组修复和切除修复[64]。如果细胞转向跨损伤 DNA 合成途径，DNA 聚合酶 II、IV 和 V 会代替复制性聚合酶 III 进行复制[45-46,107-115]。虽然 SOS 产生的突变效应可能会引入潜在的有害突变，但至少允许一些细胞存活下来[29,116]。所以高表达的 Pol IV 有利于细菌对抗宿主防御系统，使其在巨噬细胞中形成生态位。不仅如此，这种 Pol IV 赋予的适应性也在动物模型中得到了验证。之前的一项研究表明，尿道致病性大肠杆菌(uropathogenic *Escherihia coli*, UPEC)的 Pol IV 是其在小鼠膀胱感染中的

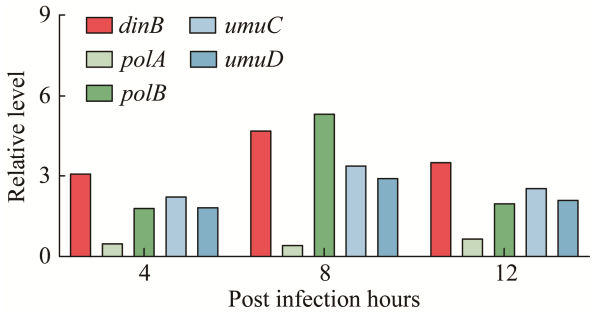

图 4 沙门菌多个聚合酶的表达情况 该图提示巨噬细胞内与体外的细菌聚合酶的相对表达量，是 3 次重复的平均值；*dinB* 编码 DNA 聚合酶 IV；*polA*：DNA 聚合酶 I；*polB*：DNA 聚合酶 II；*umuC* 和 *umuD* 表达产物组成 DNA 聚合酶 V；数据来自文献[100]

Figure 4 Expression of multiple polymerases in *Salmonella*. Data are relative expression of bacterial polymerases in macrophages versus in vitro and are averages of three replicates; *dinB* encodes DNA polymerase IV; *polA*: DNA Polymerase I; *polB*: DNA Polymerase II; Expression products of *umuC* and *umuD* make up DNA polymerase V. Data are from reference [100].

必要条件[117]。与野生型相比，Pol IV 缺失株定殖于宿主膀胱的能力降低[117]。尽管 Pol IV 没有增加体内的诱变，但其可能对 UPEC 在宿主活跃的炎症攻击下的生存起到关键作用。Pol IV 在巨噬细胞和动物感染中的作用为理解病原宿主互作提供了新的启示，这也帮助我们提出新的治疗模式。

5 Pol IV 作为药物靶点的可行性

5.1 干扰 Pol IV 的功能

结核分枝杆菌(*Mycobacterium tuberculosis*)存在 1 种 TLS 聚合酶 DnaE2，其介导的突变依赖于 RecA 的调控；在小鼠感染模型中，*dnaE2* 的缺失降低了菌株的毒力；不仅如此，*dnaE2* 的缺失也限制了利福平耐药菌株的出现[118]。鉴于利福平是一线抗结核治疗药物，这一发现具有重要的临床意义。此外，人类 TLS DNA 聚合酶似乎在抗癌药物的获得性耐药中发挥了相同的作用。虽然目前尚无特异性抑制剂，但最近报道的一种针对人类 TLS 聚合酶 Pol ζ 的小分子抑制剂似乎可以改善化疗的结果[25]。这些研究均为以 TLS 聚合酶作为药物靶点提供了理论依据。鉴于 Pol IV 在细菌适应性中发挥的作用，有望直接针对 Pol IV 发展新药。前文提到，UPEC 的 Pol IV 是小鼠膀胱感染模型中的必要条件[117]，对 Pol IV 的干扰有望降低 UPEC 在膀胱的定殖。

硝基呋喃类药物是一种广谱抗菌类药物，可抑制细菌和真菌增殖，曾经被广泛地应用于猪、牛和家禽类的饲养中，用于治疗和预防细菌感染引起的肠道疾病[119]。硝基呋喃类药物主要包括呋喃唑酮、呋喃西林(nitrofurazone，NFZ)、呋喃它酮、呋喃妥因 4 种。研究表明，涂抹 NFZ 药膏在外耳道能有效抑制外耳道细菌增殖，可发挥消毒防腐效果，改善外耳道环境[120]。硝基呋喃类药物主要通过在基因组 DNA 中脱氧鸟苷核苷酸的 N2 原子上形成共价加合物而发挥作用[18,121]。这些加合物干扰了复制进程，导致细菌死亡[18]。然而，像大肠杆菌 Pol IV 可以高效、准确地绕过加合物，这大大降低了硝基呋喃类抗生素的药效。Kottur 等从结构上解析了 Pol IV 降低大肠杆菌对 NFZ 敏感性的机制，在结合和延伸过程中，Pol IV 形成的独特通道和空腔可以容纳加合物，酶和底物的结构不会发生任何扭曲，Pol IV 可以在高催化效率的情况下进行跨损伤合成；因此，抑制 Pol IV 的活性将增强致病菌对硝基呋喃抗生素(如 NFZ)的敏感性，并降低突变产生药物抗性，是一种减少致病菌株耐药性、降低发病率的有效策略[122]。

当然，以 TLS 聚合酶为药物靶点是富有挑战性的。一方面，许多病原体含有多个 DNA 聚合酶，这些聚合酶具有重叠的功能，存在补偿效应；另一方面，评估细菌和人的聚合酶的共同特征为靶向这类蛋白质提供了额外的障碍。不过，随着对 Pol IV 及其类似物研究的深入，解决多物种蛋白质之间的保守性问题，将有助于寻找特异性药物。同时，也要考虑安全性问题。呋喃西林在动物体内的代谢产物氨基脲是一种稳定的残留物质[123]，会在动物体内长期存在，具有潜在的致癌、致畸性[124]，所以呋喃西林只用于外伤治疗。以呋喃西林为母体进行修饰和改造，有望研发安全的呋喃西林替代物。

5.2 干扰 Pol IV 的表达：抗进化疗法

从根本上来看，进化是导致耐药性发展的机制[37]，但目前抗菌药物研发并未着手解决这个问题，而是专注于新的抗生素或变体；但从历史上来看，无论现在药物的性质或效力如何，一段时间后都会产生耐药性，这种耐药性来自于突变或者质粒等进化媒介，像前文提到的菌株通过突变获得环丙沙星、利福平及 β 内酰胺抗性[92,94]。Merrikh 等提供了一个抗进化疗法的框架，评估了一些候选靶标的可行性(表 1)[37]。Pol IV 受多种机制调控，在细菌内表达灵活，而且参与的复制会增加突变频率，在抗生素抗性等进化适应性上发挥作用，因此，干扰 Pol IV 的表达是一种抗进化疗法的途径。

SOS 反应作为细菌响应压力的武器，是其感染宿主和抵抗抗生素必不可少的。通过干扰 SOS 反应可以减少细菌进化适应和药物抗性。一种工程噬菌体可以通过表达 LexA 蛋白来抑制 SOS 反应，进而增强喹诺酮类、氨基糖苷类和 β-内酰胺类药物对大肠杆菌的杀伤力，提高染菌小鼠的生存率；此外，这种减少细菌 DNA 损伤修复的方法也减少抗生素治疗后产生的耐药菌数量[125]。这种噬菌体可与抗生素结合使用，作为抑制 SOS 诱导和使细菌细胞对 DNA 损伤敏感的辅助剂。另一类途径是以 RecA 为靶标实现抑制 SOS 反应。抗菌分子氨基香豆素最近被确定为 RecA 表达的抑制物，它可以阻

表 1 抗进化策略的理想靶标

Table 1 Ideal targets for anti-evolutionary strategies

Item	Error-prone polymerases	SOS response, RecA	SOS response, LecA	Sigma factor, RpoS
No fitness defect in absence of stress	Yes	No	Yes	Unknown
Synergy with conventional antibiotics	No	Yes	Yes	Partial
High regulatory node governing mutagenesis	No	Yes	Yes	Yes
Discrete/known molecular features to target	Yes	Partial	Yes	Unknown
Existing small molecule inhibitors	Unknown	Yes	Yes	Partial
Uniqueness within pathogens	No	Yes	Yes	Yes
Conservation across pathogens	Yes	Yes	Yes	Yes
Uniqueness relative to host	No	No	Yes	Yes

注：理想情况下，减缓诱变的潜在目标与几个特征相关，其中一些特征与常规抗生素不同(粗体)，而另一些与常规抗生素(非粗体)相同。强调了抗进化治疗的几个候选靶点的拟合度。对于每个目标，评估基于功能失活。在 LexA 的情况下，这是指蛋白水解功能而不是 DNA 结合。改编自文献[37]

Note: Potential targets for mutation reduction are preferably associated with several features, some of which differ from conventional antibiotics (in bold) and others of which are in common with conventional antibiotics (not in bold). Several candidate targets in anti-evolutionary therapy are highlighted. For each target, the assessment is based on functional inactivation. In the case of LexA, this refers to proteolytic function rather than DNA binding. Adapted from [37].

止金黄色葡萄球菌中 SOS 的诱导，并降低突变频率和重组[126]。此外，研究人员也实现了直接通过人工小 RNA 干扰 RecA 的表达[127]。在实验室条件下，使用这种小 RNA 增加了大肠杆菌对氟喹诺酮类药物的敏感性。与 SOS 反应类似，RpoS 也是抗进化药物的理想目标之一。对于细菌来说，RpoS 的失活很可能会影响病原体的生存及对抗生素做出响应，但目前对该靶点的独特性知之甚少，还不清楚如何采用小分子药物来干扰 RpoS。

SOS 和 RpoS 与 Pol IV 之间的联系在于两者是 Pol IV 的上游调节器[48,128]，影响抗生素处理下 Pol IV 参与的突变[129]。鉴于参与压力响应的反应网络复杂，针对网络中像 RpoS、RecA 和 LexA 更高的“节点”可能比针对单独的下游效应蛋白更有优势。从这个层面讲，干扰 RpoS 和 SOS 可以被认为是干扰 Pol IV 表达的全局调控。目前这种全局调控的干扰存在难度。抑制 RecA 需要靶向复杂的蛋白质活性界面，而其 ATP 结合位点在蛋白质之间缺乏唯一性[130]。对于 LexA 来说，靶向分子内自裂解反应本身就是相当大的挑战[131]。RpoS 的失活可能会对病原群体施加强大的选择压力，导致产生抗进化药物的耐药性[37]。此外，人们对靶标的保守性和独特性知之甚少，这可能会对获得具有活性且可以广泛作用的药物构成挑战。

目前抗进化疗法较现实的应用是与常规抗生素联合使用[132]。抗进化疗法不会逆转已经存在的遗传抗性，只是可以预防或至少延缓病原体从头产生和获得耐药性。病原体反复暴露于抗菌治疗的临床病例可能是抗进化治疗应用的主要领域，像囊性纤维化、免疫抑制患者、复杂医疗器械感染或分枝杆菌感染[132]。此外，抗进化策略可能会重新启用之前由于担心耐药性而放弃的候选抗生素[37]。例如，已经开发出靶向亮氨酰-tRNA 合成酶(leucyl-tRNA synthetases)的有效药物，但在 II 期试验中，由于耐药率过高而搁置[133]。此外，这种协同作用允许在较低浓度的抗生素条件下达到杀菌的效果[37]。如果能优化剂量并解决联合毒性，联合治疗将具有非常大的前景[134]。

6 结语和展望

微生物耐药性被认为是一种自然现象，由于其发展和传播的主要驱动因素都是“人为的”，因而，人类正确地干预和准确应对成为关键。对公共卫生来说，解决微生物耐药性的驱动因素和影响都是重大挑战。事实上，理解病原微生物遗传演化，包括针对抗菌药物抗性的变化，一直以来都是重大科学问题。从分子进化水平研究病原微生物的遗传变异规律、毒力演变及其致病机制，将有助于寻找新的药物靶点，改善目前的治疗方式。

自 1980 年首次发现 Pol IV[27]以来，多个物种的 Pol IV 及其同源聚合酶相继被发现[17]，已从结构等层面对其功能机制进行了探究[135]。这些研究极大地拓展了我们对这一保守的 Pol IV 分支 γ 家族聚合酶的认识[17]。外部环境和宿主环境对细菌产生不同的压力[136]。Pol IV 参与的 TLS 和适应性突变对细菌生存非常必要，需要重新关注 Pol IV 参与的生物过程和细胞行为。潜在的抗进化药物和常规杀菌药物协同机制高度相关，抑制抗进化靶标不仅会增强药效，还会减缓对常规药物抗药性的获得，这为病原治疗提供了一种新的方式。

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