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Review 综 述

ppGpp 介导的抗生素胁迫应答机制研究进展

胡晓，沈盆闪，许健萍，余佳琪，音建华*

浙江工业大学生物工程学院，浙江 杭州 310014

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摘　要：抗生素是由微生物在生长发育后期产生的次级代谢产物，具有杀死或抑制细菌生长的能力，因此被广泛应用于细菌感染的临床治疗。在长期的进化过程中，细菌采取多种方式应对环境中抗生素的威胁。除了广为人知的抗生素耐药性(resistance)之外，细菌还能对抗生素产生耐受性(tolerance)和持留性(persistence)，严重影响抗生素的临床疗效。鸟苷四磷酸(guanosine tetraphosphate, ppGpp)和鸟苷五磷酸(guanosine pentaphosphate, pppGpp) (本文统称 ppGpp)是细菌应对营养饥饿等不利环境时产生的“报警”信号分子，其能够在全局水平调控基因的表达，使细菌适应不利的环境。越来越多的研究表明，ppGpp 与细菌应对抗生素胁迫密切相关。基于此，本文综述了细菌中 ppGpp 的合成与水解及其作用机制，并重点阐述了 ppGpp 介导抗生素胁迫应答的分子机制，以期为新型抗生素的开发提供新思路。

关键词：ppGpp；严紧反应；抗生素耐药性；抗生素耐受性；抗生素持留性；胁迫应答

Research progress in ppGpp-mediated antibiotic stress response

HU Xiao, SHEN Penshan, XU Jianping, YU Jiaqi, YIN Jianhua*

College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, Zhejiang, China

Abstract: Antibiotics are secondary metabolites produced by microorganisms during the stationary phase. They are widely used in the clinical treatment of bacterial infections because of their ability to kill bacteria or inhibit bacterial growth. In the long-term evolutionary process,

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*Corresponding author. E-mail: jianhuay@zjut.edu.cn
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bacteria have adopted several strategies to cope with the threats of antibiotics in the environment. In addition to the well-known antibiotic resistance, bacteria can develop tolerance and persistence to antibiotics, which seriously affects the clinical efficacy of antibiotics. Guanosine tetraphosphate (ppGpp) and guanosine pentaphosphate (pppGpp) (herein collectively referred to as ppGpp) are the alarmone signal molecules produced by bacteria in response to unfavorable environmental conditions such as nutritional starvation. ppGpp can regulate transcription globally and enable bacteria to survive in unfavorable conditions. An increasing number of studies have shown that ppGpp is closely related to antibiotic stress response. On this basis, this review summarizes the synthesis, hydrolysis, and mechanism of action of ppGpp in bacteria, with emphasis on the role of ppGpp in antibiotic stress response. This review aims to provide new ideas for the development of novel antibiotics.

Keywords: ppGpp; stringent response; antibiotic resistance; antibiotic tolerance; antibiotic persistence; stress response

自 1928 年弗莱明发现青霉素以来，很多作用机制不同的抗生素被研究者们发现，并被广泛应用于细菌感染性疾病的治疗。抗生素通常是由微生物产生的次级代谢产物，部分细菌逐渐进化出在抗生素胁迫环境中生长的能力，即产生耐药性(resistance)[1-2]。伴随着抗生素的不合理使用甚至滥用，抗生素耐药性问题日益凸显，严重影响抗生素的临床疗效，已成为全球重点关注的公共卫生问题。不仅如此，有些细菌暴露于致死浓度杀菌性抗生素时虽然不能生长，但仍保持存活状态，当抗生素移除后重新恢复生长，这种现象称之为抗生素耐受性(tolerance)，是细菌从敏感性向耐药性进化的一种中间状态[1-4]。此外，有些敏感性细菌群体中部分亚群对抗生素具有高度耐受性，称为抗生素持留性(persistence)[1-4]。由此可见，细菌对抗生素胁迫的应答机制复杂多样。

鸟苷四磷酸(guanosine tetraphosphate, ppGpp)和鸟苷五磷酸(guanosine pentaphosphate, pppGpp) (本文统称为 ppGpp)是细菌在营养饥饿等不利环境时产生的信号分子，也被称为魔斑(magic spot)或警报素(alarmone)[5-9]。ppGpp 能够触发严紧反应(stringent response)，在全局水平调控基因的表达，通过对细胞中核糖体和代谢类蛋白等资源的重新分配，使细菌在不利的环境中存活下来[10]。近些年的研究表明，ppGpp 与细菌应对抗生素胁迫密切相关，无法合成 ppGpp 的细菌显著降低对抗生素的耐药性、耐受性以及持留性[11-13]。因此，充分了解 ppGpp 的合成与水解、作用机理及其介导的抗生素胁迫应答机制有助于寻找抗生素新靶点，为细菌感染疾病的治疗提供新思路。

1 ppGpp 的合成与水解

当细菌在指数生长时期，细胞中 ppGpp 的含量维持在很低的水平；而当细菌面临营养饥饿以及抗生素胁迫等不利因素时，ppGpp 大量合成，进而触发严紧反应[5-8]。细胞中 ppGpp 的合成与水解由多个代谢酶介导，根据结构和功能的不同可分为 3 种类型：同时具有合成酶与水解酶结构域的 RelA/SpoT 同系物(RelA/SpoT homologue, RSH)家族蛋白、仅具有合成酶结构域的小信号分子合成酶(small alarmone synthetases, SASs)和仅具有水解酶结构域的小信号分子水解酶(small alarmone hydrolases, SAHs)[14]。ppGpp 合成酶将源自 ATP 的焦磷酸(pyrophosphoric acid, PP_i)基团连接至 GDP 或

GTP 的 3′-OH 位置，进而形成 ppGpp 或 pppGpp，同时生成 AMP；而水解酶将 ppGpp 或 pppGpp 水解为 GDP 或 GTP，同时释放 PP_i (图 1A、1B)[6-7]。此外，pppGpp 磷酸水解酶 GppA 能将生成的 pppGpp 快速水解为 ppGpp，因此细胞中 pppGpp 的含量极低。

常见的 RSH 家族蛋白包括大肠杆菌(*Escherichia coli*)等革兰氏阴性菌中的 RelA 和 SpoT，以及结核分枝杆菌(*Mycobacterium tuberculosis*)等革兰氏阳性菌中的 Rel (图 1C)。其中，RelA 虽具有水解酶结构域，但缺少 ppGpp 水解活性，仅具有合成活性；SpoT 同时具有 ppGpp 合成和水解功能，但其合成活性较弱，主要起水解功能；而 Rel 兼具 ppGpp 合成和水解双功能(图 2)。SASs 仅在部分细菌中发现，如霍乱弧菌(*Vibrio cholerae*)和希瓦氏菌(*Shewanella*)等海洋微生物中的 RelV，以及枯草芽孢杆菌(*Bacillus subtilis*)和金黄色葡萄球菌(*Staphylococcus aureus*)等革兰氏阳性菌中的 RelP 和 RelQ 等[6-7,15-17]。SAHs 最初在果蝇等真核生物中发现[18]，近年来在谷氨酸棒杆菌(*Corynebacterium glutamicum*)中找到属于该类型的酶 RelH[19]。

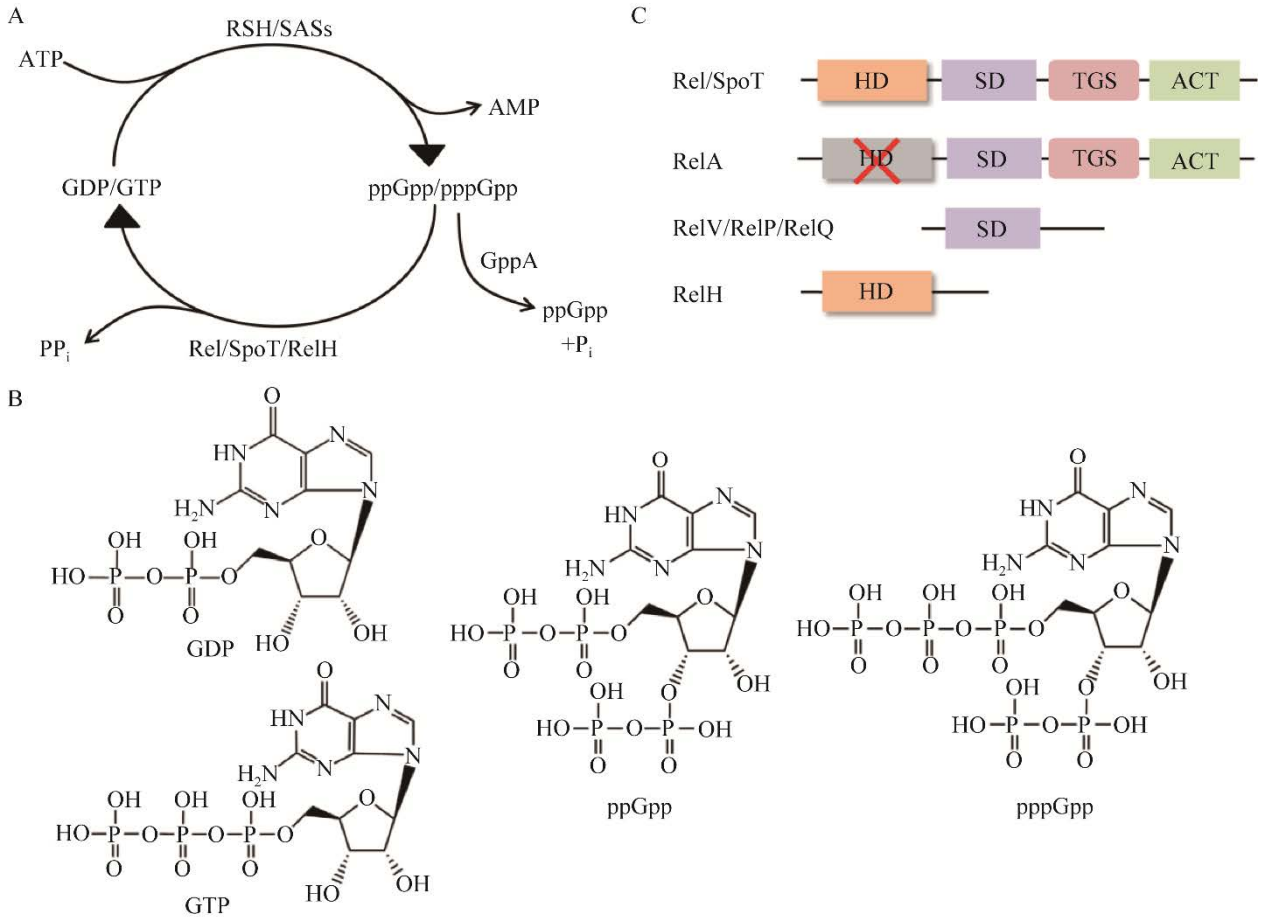

图 1 ppGpp 的合成与水解

Figure 1 Synthesis and hydrolysis of ppGpp. A: Metabolism of ppGpp. B: Chemical structures of GDP, GTP, ppGpp, and pppGpp. C: The domains of the enzymes involved in ppGpp metabolism. The ppGpp hydrolysis activity of RelA is completely absent in the HD domain. RSH: The RelA-SpoT homologue; SASs: Small alarmone synthetases; P_i: Inorganic phosphate; PP_i: Pyrophosphate; HD: ppGpp hydrolysis domain; SD: ppGpp synthesis domain; TGS: ThrRS, GTPase, and SpoT domain; ACT: Aspartokinase, chorismate mutase, and TyrA domain.

这些 ppGpp 代谢酶具有保守的二级结构和特征基序(图 1C)[6-7]。其中，RSH 家族蛋白 N-末端具有负责酶催化反应的水解酶(hydrolase, HD)结构域和合成酶(synthetase, SD)结构域，C-末端具有负责酶活性调节的 TGS (ThrRS, GTPase, and SpoT)结构域和 ACT (aspartokinase, chorismate mutase, and TyrA)结构域。合成酶结构域携带保守的 EXDD 或 RXKD 基序，而水解酶结构域携带保守的 HDXXED 基序，这些特征基序对于 ppGpp 合成和水解功能的发挥至关重要(图 2A)。革兰氏阴性菌中 RelA 的水解酶结构域缺少 HDXXED 特征基序，导致缺少 ppGpp 水解活性(图 2A)[20]。RelV/P/Q 等 SASs 仅具有一个合成酶结构域，其中的保守基序 GYR 和 EXQX 对于 ppGpp 合成活性必不可少(图 2B)[21]。RelH 等 SAHs 仅具有水解酶结构域，其中携带保守的 HDXXED 基序[19]。

2 ppGpp 的作用机制

作为细菌严紧反应时最主要的调控因子，ppGpp 在全局水平调控基因表达，进而改变细胞整体生理代谢过程。通常情况下，与 rRNA 和 tRNA 合成以及细胞分裂相关的基因转录水平下调，而与氨基酸生物合成以及毒力等胁迫应答相关的基因转录水平上调[5-8]。革兰氏阴性菌和阳性菌中 ppGpp 调控基因转录的机制具有明显区别(图 3)。

革兰氏阴性菌中，ppGpp 主要通过直接影响 RNA 聚合酶(RNA polymerase, RNAP)活性实现对基因转录的调控[5-6,22]。ppGpp 能够直接结合 RNAP 的核心酶($\alpha_2\beta\beta'\omega$)，产生变构效应改变 RNAP 活性[23]。ppGpp 在 RNAP 核心酶上的结合位点有 2 个：位点 1 位于 β′和 ω 亚基连接界

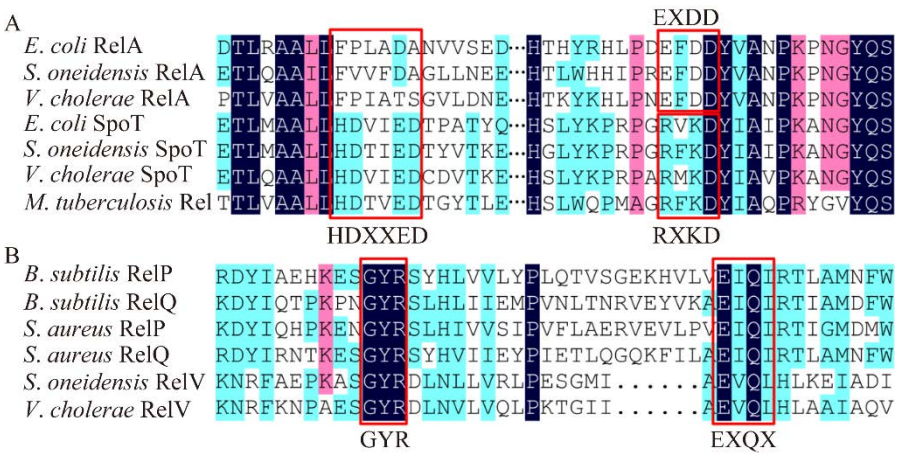

图 2 ppGpp 代谢酶的特征基序比对

Figure 2 Comparison of signature motifs in ppGpp metabolic enzymes. A: Alignment of the RSH family proteins. The HDXXED motif within the hydrolysis domain (HD) is conserved only in SpoT and Rel. Lack of the HDXXED motif in RelA HD results in the absence of ppGpp hydrolysis activity. The synthesis domain (SD) of RelA contains the EXDD motif, while SpoT and Rel contain RXKD motif within SD. B: Alignment of the SAS family proteins. The GYR and EXQX motifs are conserved in the SD of all SASs. Sequence accession number: *E. coli* RelA, NP_417264.1; *S. oneidensis* RelA, AAN56448.1; *V. cholerae* RelA, BBE11625.1; *E. coli* SpoT, NP_418107.1; *S. oneidensis* SpoT, AAN53444.1; *V. cholerae* SpoT, BBE11385.1; *M. tuberculosis* Rel, OHO20527.1; *B. subtilis* RelP, NP_389042.1; *B. subtilis* RelQ, KKJ81461.1; *S. aureus* RelP, OBX95528.1; *S. aureus* RelQ, OBY01590.1; *S. oneidensis* RelV, AAN56822.1; *V. cholerae* RelV, WP_248340448.1.

面的中心；位点 2 位于 β′亚基，ppGpp 与该位点的结合需要转录调控因子 DksA 的辅助(图 3A)。其中，DksA/ppGpp 复合体与位点 2 的相互作用对于调控 RNAP 活性更加重要，不仅能够实现对 rRNA 基因等的转录抑制(约 20 倍)，还能实现对胁迫相关基因的转录激活[24]。这种调控上的差异主要源自被调控基因启动子区序列的差别，转录抑制的基因启动子通常携带富含 GC 区域(GC-rich discriminator)，而转录激活的基因启动子携带富含 AT 区域(AT-rich discriminator)。与位点 2 不同的是，ppGpp 与位点 1 的相互作用仅能起到转录抑制作用，并且抑制程度较低(约 2 倍)。ppGpp 与位点 1 的亲和力更高，可能在 ppGpp 浓度较低时(如细菌处于严紧反应的起始阶段)发挥作用[22]。综合来看，ppGpp 与 RNAP 中两个位点的结合有利于细菌在复杂的环境中对基因表达进行精细的调控。

土拉弗朗西斯菌(*Francisella tularensis*)中 ppGpp 对基因转录的调控机制与其他革兰氏阴性菌不同。该菌中致病岛(pathogenicity island, FPI)以及其他毒力基因的表达受 ppGpp 调控，同时依赖于 MglA、SspA 和 PigR 等 3 个转录调控蛋白。ppGpp 不与 RNAP 直接结合，而是与 MglA 和 SspA 形成的异二聚体复合物(MglA-SspA)结合，并促进它们与 PigR 和 RNAP 的相互作用，进而调控基因的转录[25]。

革兰氏阳性菌中，ppGpp 无法直接结合 RNAP，主要通过间接调控的方式影响基因转录(图 3B)[6-7,22]。研究表明，ppGpp 可以抑制 GTP 的水平，而 GTP 不仅是一种能量储存的载体，更是细菌生长相关基因(如 rRNA 基因)起始转录的核苷酸。一方面，GTP 是合成 ppGpp 的前体，当细菌处于不利环境时因 ppGpp 合成增加致使 GTP 含量降低；另一方面，ppGpp 能够与 GTP 合成相关的酶[包括鸟苷酸激酶(guanylate kinase, GMK)、次黄嘌呤磷酸核糖基转移酶(hypoxanthine phosphoribosyltransferase, HPRT)和黄嘌呤磷酸核糖转移酶(xanthine phosphoribosyltransferase, XPRT)]竞争结合活性位点，从而抑制 GTP 合成相关酶的活性，减少 GTP 的产生[7]。此外，ppGpp 的合成需要 ATP 提供焦磷酸基团，而 ATP 是细胞中主要的能量储存库，因此 ppGpp 的合成将减少细胞中 ATP 的供应，进而影响生物合成过程中的多种酶促反应[23]。

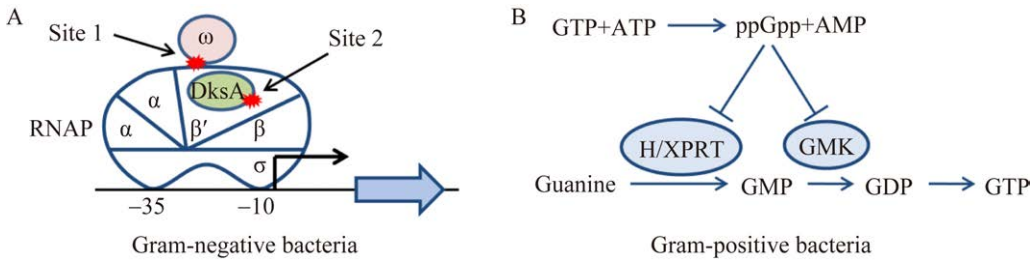

图 3 ppGpp 调控基因转录的作用机制

Figure 3 Regulation of transcription by ppGpp. A: ppGpp regulation through direct binding to RNAP in most Gram-negative bacteria. ppGpp binds RNAP at two sites. “Site 1” is at the interface between the β′ and ω subunits. “site 2” is located at the β′ subunit in coordination with the regulator DksA. B: Indirect regulation of transcription by ppGpp in Gram-positive bacteria. HPRT: Hypoxanthine phosphoribosyltransferase; XPRT: Xanthine phosphoribosyltransferase; GMK: Uanylate kinase.

3 ppGpp 介导的抗生素胁迫应答机制

在长期的进化过程中，细菌已经逐渐发展出一系列应答抗生素胁迫的策略，从而提高存活能力。根据抗生素存在时细菌存活能力的强弱，将细菌应对抗生素胁迫的方式分为 3 种：耐药性、耐受性和持留性(图 4A)[1-2]，这些应答机制都与 ppGpp 密切相关。

3.1 ppGpp 与抗生素耐药性

抗生素耐药性是指细菌在高浓度抗生素存在时仍保持增长繁殖的能力(图 4A)，通过最小抑制浓度(minimal inhibitory concentration, MIC)来量化。MIC 表示抑制细菌生长的最低抗生素浓度，只有当抗生素的浓度高于该值时，才会对细菌产生杀害作用[26]。细菌对抗生素产生耐药性的分子机制主要包括：(1) 产生抗生素水解酶或修饰酶，进而破坏抗生素；(2) 降低外膜通透能力，限制抗生素进入细胞；(3) 提高药物外排泵的表达，将进入到细胞内的抗生素外排；(4) 改变或修饰抗生素作用靶点，从而减少对靶标的抑制[27-28]。

已有的研究表明，ppGpp 的积累有利于细菌对 β-内酰胺类、氨基糖苷类以及多肽类等多种抗生素产生耐药性(表 1)[29-40]。与正常的细菌相比，无法合成 ppGpp 的细菌(通常记为 ppGpp^0)对这些抗生素的耐药性显著降低，其具体原因取决于菌株类型和抗生素的种类，但都与上述耐药机制相关(图 4B)。其中，ppGpp 介导 β-内酰胺类抗生素耐药性主要通过改变抗生素作用靶点实现。例如，耐甲氧西林金黄色葡萄球菌(methicillin-resistant *Staphylococcus aureus*, MRSA)的基因组中携带 *mecA* 基因，其能够编码对 β-内酰胺类亲和力低的青霉素结合蛋白(PBP2A)，从而保证该类抗生素存在时 PBP2A 能正常合成肽聚糖。研究发现，临床菌株中 *relA*

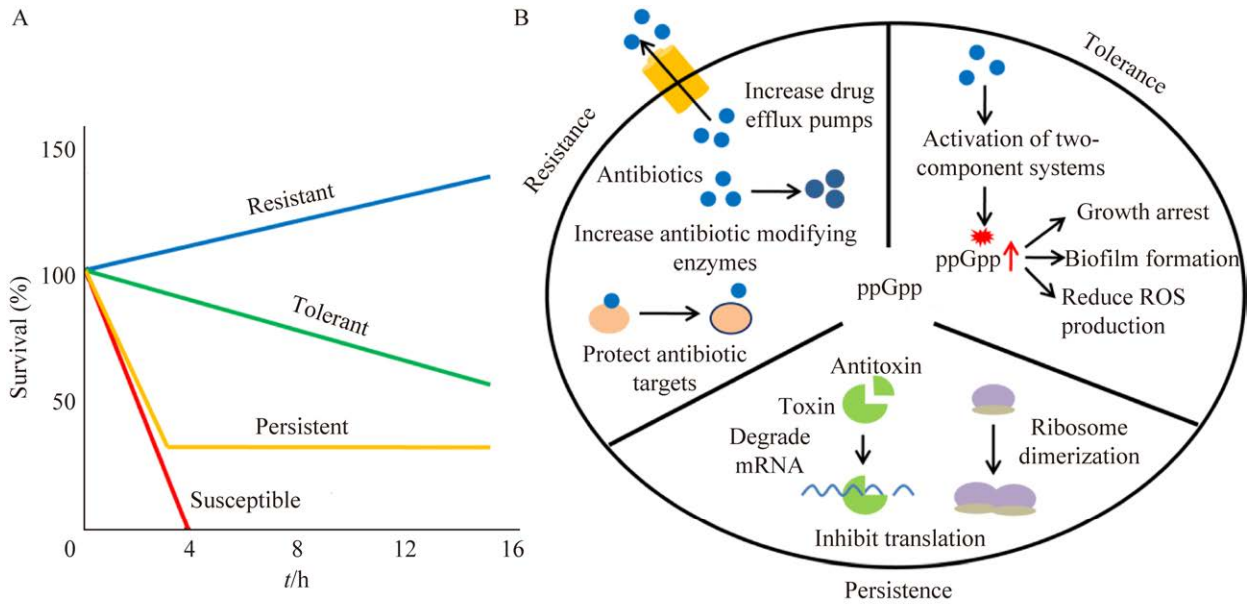

图 4 ppGpp 介导的抗生素耐药性、耐受性和持留性

Figure 4 ppGpp-mediated antibiotic resistance, tolerance and persistence. A: Graphical depiction of the definitions of susceptible, resistant, tolerant, and persistent in response to antibiotics. A hypothetical time-killing experiment is displayed. B: Role of ppGpp in antibiotic resistance, tolerance, and persistence.

的突变致使 ppGpp 含量增加，随后诱导 *mecA* 基因表达和提高 PBP2A 含量，引起对苯唑西林等β-内酰胺类的耐药性[29-30]。ppGpp 介导的肽类抗生素(microcin J25, MccJ25)耐药性也与抗生素靶点有关。MccJ25 是大肠杆菌产生的由 21 个氨基酸组成的抗菌肽，对沙门氏菌(*Salmonella*)和志贺氏菌(*Shigella*)等革兰氏阴性致病菌具有很好的作用效果，其主要的作用机制是 MccJ25 与 RNAP 结合，进而抑制基因转录。研究发现，细胞内 ppGpp 的积累与 MccJ25 的耐药性呈正相关，主要的原因是 ppGpp/DksA 复合体与 MccJ25 竞争性结合 RNAP，从而减少 MccJ25 对 RNAP 的抑制作用[41]。

ppGpp 还能通过提高抗生素修饰酶以及药物外排泵的表达介导抗生素耐药性。例如，肠道沙门氏菌(*S. enterica*)中 ppGpp 能够正调控氨基糖苷腺苷转移酶基因(*aadA*)的表达，从而提高对链霉素和壮观霉素等氨基糖苷类抗生素的耐药性[37]。与野生型的鲍曼不动杆菌(*Acinetobacter baumannii*)相比，无法合成 ppGpp 的菌株中药物外排泵相关基因的表达显著降低，致使对庆大霉素、四环素、红霉素和甲氧苄啶等多种抗生素的敏感性增强[36]。最新的研究表明，枯草芽孢杆菌(*Bacillus subtilis*)中 ppGpp 介导的信号转导可以增强抗生素耐药因子 VmlR 的表达，进而提高对特定抗生素(包括 pleuromutilin、lincosamides 和 type A streptogramin virginiamycin M1)的耐药性[38]。

3.2 ppGpp 与抗生素耐受性

根据抗生素对细菌作用效果的不同，可以将抗生素分为杀菌性(bacteriocidal)和抑菌性(bacteriostatic)两种类型。其中，杀菌性抗生素包括 β-内酰胺类、氨基糖苷类以及喹诺酮类，它们通过阻断特定的生物合成过程以及产生活性氧(reactive oxygen species, ROS)等多种方式杀死细菌[42]。越来越多的研究表明，部分细菌暴露于致死浓度杀菌性抗生素时虽不能生长但仍

表 1 ppGpp 介导的抗生素耐药性

Table 1 ppGpp-mediated antibiotic resistance

Organism	Method of induction	ppGpp level	Resistance phenotype
Staphylococcus aureus	Upregulation of *mecA* expression during mupirocin treatment (SR induction)	High	Increased resistance to β-lactam antibiotics[29-31]
	Δ*relQ*	Low	Decreased resistance to β-lactam antibiotics[32]
	Increased ppGpp synthase activity of RelQ/decreased ppGpp hydrolase activity of Rel due to point mutations	High	Increased resistance to oxacillin[30]
	Blocking of ppGpp with MIP-NP	Low	Increased efficiency rates to kanamycin and tetracycline[33]
Streptococcus pneumoniae	Δrel_{Spn}	Low	Decreased resistance to mupirocin[34]
Enterococcus faecalis	Δ*relQ* or Δ*relA*Δ*relQ*	Low	Decreased resistance to vancomycin[35]
Acinetobacter baumannii	$ppGpp^0$	Low	Decreased resistance to tetracycline, erythromycin and gentamicin[36]
Salmonella enterica	Small colony variants	High	Increased resistance to tetracycline and spectinomycin[37]
Bacillus subtilis	$ppGpp^0$	Low	Decreased resistance to tiamulin and iboxamycin[38]
	$ppGpp^0$	Low	Decreased resistance to tetracycline and chloramphenicol[39]
Escherichia coli	Overexpression of *relA*	High	Increased resistance to penicillin[40]

能保持存活状态，这种现象称之为抗生素耐受性(图 4A)。抗生素耐受性是细菌从敏感性向耐药性进化的一种中间状态，也是抗生素耐药性形成的基础[3-4,43]。

细菌对抗生素产生耐受性的机制复杂多样，ppGpp 的积累是其中最重要的机制之一[44]。大量的研究表明，ppGpp 与抗生素耐受性密切相关，ppGpp 的积累能够提高细菌对众多抗生素的耐受性，而 ppGpp 的缺失则会降低细菌在这些抗生素中的存活能力(表 2)[45-60]。ppGpp 介导抗生素耐受性形成最主要的原因是影响细菌生长速度(图 4B)，细胞中 ppGpp 的含量与细菌生长速度之间呈负相关，而生长速度与 β-内酰胺类等多种杀菌性抗生素的杀菌效率呈正相关[61]。ppGpp 抑制细菌生长与其对细胞整体生理代谢的调控密切相关。当 ppGpp 积累时，DNA 复制、转录和翻译等生命过程以及细胞壁、细胞膜和核糖体等细胞结构的生物合成受到明显抑制，导致细胞生长变慢；与此同时，ppGpp 积累的细胞尺寸变小[17,62]。

SASs 类型 ppGpp 合成酶对抗生素的耐受性起着重要作用(图 4B)。例如，革兰氏阳性细菌面临细胞壁靶向抗生素胁迫时，通过双组分系统 WalKR (枯草芽孢杆菌)或 VraRS (金黄色葡萄球菌)诱导 *relP* 和 *relQ* 的表达，致使 ppGpp 积累从而保护细菌免受抗生素的杀伤作用[15,63]。金黄色葡萄球菌中 *relP* 和 *relQ* 的诱导表达还能促进生物被膜的形成，进一步增强细菌对抗生素的耐受性[48]。笔者实验室研究发现，革兰氏阴性细菌希瓦氏菌对 β-内酰胺类、万古霉素和 D-环丝氨

表 2　ppGpp 介导的抗生素耐受性

Table 2　ppGpp-mediated antibiotic tolerance

Organism	Method of induction	ppGpp level	Tolerance phenotype
Staphylococcus aureus	Point mutation in *rel*	High	Increased tolerance to daptomycin[45]
	Δ*rsgA*	High	Increased tolerance to β-lactams and vancomycin[46]
	Δ*relP*Δ*relQ* or ppGpp^0	Low	Decreased tolerance to vancomycin and ampicillin[15]
	Point mutation in *rel*	High	Increased tolerance to ciprofloxacin[47]
	ppGpp^0	Low	Decreased biofilm-related antibiotic tolerance[48]
	Mupirocin treatment	High	Increased tolerance to vancomycin[49]
Enterococcus faecalis	Δ*rel*Δ*relQ* or Δ*relQ*	Low	Decreased tolerance to vancomycin[35]
	Point mutation in *rel*	High	Increased tolerance to vancomycin, linezolid, and daptomycin[50]
Bacillus subtilis	ppGpp^{0}after chloramphenicol treatment	Low	Decreased tolerance to vancomycin[39]
Escherichia coli	Isoleucine deprivation/Lysine deprivation	High	Increased tolerance to ampicillin, penicillins, cephalosporins and carbapenems[51-52]
	Amino acid starvation	High	Increased antibiotic tolerance in biofilms[53]
	Antibiotic pretreatment	High	Increased tolerance to ampicillin[54]
	Overexpression of *hipA*	High	Increased tolerance to carbenicillin, ampicillin, ciprofloxacin, norfloxacin, cefotaxime, ofloxacin, and mitomycin C[55-56]
Vibrio cholerae	Δ*dksA* or ppGpp^0	Low	Decreased tolerance to tetracycline, erythromycin, and chloramphenicol in stationary phase[57]
Shewanella oneidensis	ppGpp^0	Low	Decreased tolerance to cell wall-acting antibiotics[17]
Pseudomonas aeruginosa	Δ*relA*Δ*spoT* (ΔSR)	Low	Decreased tolerance to ofloxacin, gentamicin, and meropenem[58-59]
	Δ*spoT*	High	Increased tolerance to quinolones[60]

酸等靶向细胞壁生物合成的抗生素具有耐受性，这些抗生素能够激活该菌中的双组分系统PghKR，进而诱导 ppGpp 合成酶基因 *relV* 的表达[64]。当 *relV* 过表达后，细菌生长变慢并且对细胞壁靶向抗生素的耐受性显著提高；而当PghKR 缺失后耐受性显著降低[17,64-65]。

除细胞壁靶向抗生素外，ppGpp 还能介导细菌对其他多种类型抗生素产生耐受性。霍乱弧菌中 ppGpp 的积累显著提高了细菌对四环素、红霉素和氯霉素的耐受性，该过程与 TCA 循环和有氧呼吸强度减弱有关，进一步减缓了抗生素引起的氧化压力，最终阻止细胞死亡(图 4B)[57]。粪肠杆菌中 ppGpp 能够保护细菌在诺氟沙星胁迫时的存活能力[66]。金黄色葡萄球菌中 ppGpp 合成酶 Rel 的突变致使细菌对达托霉素和诺氟沙星产生耐受性[45-46]。一项最新的研究表明，枯草芽孢杆菌和粪肠球菌中氯霉素的处理诱导 ppGpp 的合成，进而保护细菌免受氯霉素杀死；当 ppGpp 缺失后，氯霉素对这些细菌的作用从抑菌转变为杀菌[39]。

总之，现有的研究表明，ppGpp 介导的抗生素耐受性是细菌应答抗生素的一种普遍方式，但具体机制还有待进一步研究。

3.3 ppGpp 与抗生素持留性

当使用高浓度杀菌性抗生素处理敏感细菌群体时，绝大多数细菌都被快速杀死，但仍有小部分细菌群体存活下来，这些存活的亚群被称为持留菌(persister)，对抗生素具有持留性(图 4A)[67-68]。与耐受性不同的是，持留性只存在于细菌细胞的一个亚群中而且是不稳定的，当抗生素压力去除后，细菌会恢复到抗生素敏感的状态。持留菌实际上是微生物种群中进入休眠或缓慢生长状态、抗生素高度耐受的表型变异细胞[69-70]。

研究表明，细胞中 ppGpp 的积累诱导持留菌的形成。大肠杆菌和铜绿假单胞菌等革兰氏阴性菌中 *relA* 和 *spoT* 缺失菌株不再形成持留菌，表明 ppGpp 在持留菌形成过程中起着重要作用[71]。同样，在粪肠球菌和金黄色葡萄球菌等革兰氏阳性细菌中，ppGpp 也被证实与持留菌的形成密切相关[13]。ppGpp 促进持留菌形成的机制依赖于毒素-抗毒素(toxin-antitoxin, TA)系统(图 4B)[12,72]。TA 系统由两个基因组成，分别编码毒素蛋白和抗毒素蛋白；其中，毒素蛋白抑制细菌的生长；而抗毒素蛋白则与毒素蛋白结合，解除其抑制作用；大肠杆菌中含有约 30 个 TA 系统，其中大多数都受 ppGpp 调控；在 HipBA TA 系统中，HipA 为毒素蛋白，而 HipB 为抗毒素蛋白，受 Lon 蛋白酶降解[72]。ppGpp 竞争性抑制多聚磷酸盐水解酶 PPX 活性进而积累多聚磷酸盐(inorganic polyphosphate, polyP)，而后 polyP 激活 Lon 蛋白酶使其降解 HipB，毒素蛋白 HipA 发挥 mRNA 核酸内切酶作用，从而在全局水平抑制翻译，诱导持留菌的形成[72]。

近年来，通过对大肠杆菌的研究，Song 和 Wood 提出一种不依赖于 TA 系统的新机制：ppGpp 促进细菌中的核糖体由 70S 二聚化为 100S，核糖体二聚化后活性丧失，但保护核糖体蛋白和 rRNA 免受降解。在此过程中，蛋白质的翻译被抑制，导致细胞休眠或生长缓慢，进而形成持留菌。当胁迫消失后，二聚化核糖体解聚为正常的核糖体[73]。

4 总结与展望

ppGpp 是细菌面对不利环境时合成的“报警”信号分子，能够在全局水平调控基因表达，对核糖体和代谢类蛋白等资源进行优化配置，从而渡过不利的环境。抗生素是细菌生长过程中常见的胁迫因素之一，细菌进化出耐药性、耐受性以及持留性等多种策略应答抗生素胁迫，而这些

应答策略都与 ppGpp 密切相关。ppGpp 介导的抗生素胁迫应答机制复杂多样，目前已经取得了许多研究进展，但仍有一些问题有待进一步研究。例如，细菌细胞通常含有多个与 ppGpp 合成和水解相关的酶，它们如何分工协作进而将 ppGpp 浓度维持在合理的水平？ppGpp 介导的不同抗生素胁迫应答机制其根本的原因是什么？抗生素耐受性和持留性形成过程中 ppGpp 发挥何种作用？这些问题的阐明将有助于理解细菌在复杂环境中的生存机制，尤其是细菌在抗生素胁迫时的响应机制。

抗生素耐药性、耐受性和持留性是造成抗菌药物在临床治疗中失败的重要原因。与此同时，新型抗生素研发停滞不前，迫切需求新的策略应对由细菌引起的感染性疾病。由于 ppGpp 与抗生素耐药性、耐受性和持留性密切相关，ppGpp 合成酶可以作为有效靶点开发抗生素联合治疗药物，从而增强现有抗生素的临床疗效。

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