微生物学报
Acta Microbiologica Sinica
2016, 56(8): 1211-1221
http://journals.im.ac.cn/actamicrocn
DOI: 10.13343/j.cnki.wsxb.20150484

Mini-Review 小型综述

细菌锌离子调控体系与感染

李博，李晶，沈立新*

西北大学生命科学学院，西部资源与现代生物技术教育部重点实验室，陕西 西安 710069

摘要：锌(Zn)是生命体不可或缺的微量元素，对细菌和宿主同等重要。细菌体内锌离子稳态的维持依赖锌离子转运和调控体系。宿主通过限制锌离子或高锌离子中毒来控制细菌感染。为了在宿主体内生存，细菌必须表达高亲和力的锌离子转运系统，如ZnuABC，以获取足够的锌离子。由于锌与细菌大量的代谢和毒性通路密切相关，在细菌建立感染的过程中尤为重要，因此通过抑制锌离子转运系统来影响锌离子的稳态，将成为一个非常有发展前途的新型抗菌策略。

关键词：锌，锌离子调控，感染，抗菌治疗

细菌的生存和繁殖严重依赖过渡金属元素的吸收，如铁、锰、铜、锌等，因为它们可以稳定金属蛋白的折叠构象同时参与重要的生化反应，有研究表明，这类金属蛋白占到总蛋白的三分之一[1]。其中锌结合蛋白参与多种重要的生化反应，包括DNA复制、转录、翻译、细胞分裂、糖酵解、pH调节等。锌之所以能够广泛存在于各种类型的蛋白质中有两方面原因。其一，锌能作为蛋白质的结构组成，如锌可以螯合在氨基酸链中形成锌指结构；其二，锌可作为催化辅因子，扮演路易斯酸的角色，作为电子受体催化生化反应[2]。为了对抗入侵细菌的生存和繁殖，宿主进化出很多机制来限制可被利用的锌离子浓度。与此同时，细菌必须产生高亲和力的锌离子转运系统(如ZnuABC)获取足够的锌以维持生存。因此，了解锌离子稳态的调节机制及其与感染的关系对控制细菌感染十分重要。

1 锌在细菌中的重要作用

锌广泛存在于各种类型的蛋白质中，锌离子的转运和调控对细菌的生存和繁殖至关重要。生物信息学研究显示大约5%的细菌蛋白包含可识别的锌结合位点[3]。在大肠杆菌(*Escherichia coli*)蛋白质组中约有1/6的蛋白可以与锌结合[4]。锌可作为结构组成或催化辅因子参与细菌多种酶和蛋白质的功能。首先，锌与一些基础代谢的酶相关，包括RNA和DNA聚合酶[5]、乙醇脱氢酶[6]，异构酶[7]等。如大肠杆菌中，锌对异戊二烯焦磷酸异

基金项目：国家自然科学基金(81171620，1210063)；西北大学研究生自主创新资助项目(YZZ14074)

*通信作者。Tel/Fax：+86-29-88302132；E-mail：shenlx@nwu.edu.cn

收稿日期：2015-10-20；**修回日期：**2015-12-01；**网络出版日期：**2015-12-16

构酶(IPP isomerase)的功能不可或缺，该酶在甲羟戊酸途径(Mevalonate pathway)中将异戊二烯焦磷酸(IPP)催化为二甲烯丙基焦磷酸(DMAPP)，DMAPP是类固醇、类萜等生物分子的合成前体[7]。其次，锌与一些关键的机制相关，包括DNA修复[8]，毒性相关蛋白的产生[9]，抗生素抗性[10]以及对氧化压力的应答[11]。如金属β-内酰胺酶，是一种有利于细菌入侵和定殖的锌结合蛋白，这类酶中的碳青霉烯酶能够钝化β-内酰胺类抗生素，使细菌对该类抗生素产生耐药[12]。锌是铜锌超氧化物歧化酶(CuZn SOD)的必需组分，而该酶是细菌抵御活性氧自由基(reactive oxygen species，ROS)的第一道防线[13]。此外，锌结合蛋白还包括核糖体蛋白[14]、主要抗原[15]和外毒素[16]等。由此可见，锌对细菌蛋白质组有多效性的影响。

尽管锌在细菌中扮演着重要的角色，但高浓度的锌会对细菌产生毒害作用。首先，过量的锌离子会竞争结合其他金属离子的蛋白结合位点，使该蛋白失活；其次，锌还可形成羟基自由基，对DNA、蛋白质和脂类造成伤害；此外，高浓度的锌离子会直接抑制电子传递链中的电子传递，影响呼吸作用，对生物体造成损伤[17]。因此细菌体内锌离子必须受到严格调控。

2 细菌中锌离子的转运和调控

在侵染宿主的过程中，为了保持锌离子的稳态，挫败宿主的防御体系，同时建立感染，细菌进化出了有效的锌转运和调节体系调控锌离子摄入和排出，使细胞内锌离子浓度处于最佳水平。

2.1 细菌锌离子的转运

细菌中存在非特异性以及特异性锌离子转运系统，当细菌生长在锌离子充足的环境中时，主要通过一些低亲和力的非特异性离子通道摄取锌。同时，处于抑制状态的特异性高亲和力锌离子转运系统也能转运部分锌离子[18]。如在大肠杆菌等革兰氏阴性菌中，通过组成型表达的转运子ZupT (属于ZIP家族)吸收锌离子[19]。ZIP家族蛋白是最早在真核生物中发现的转运子，能够转运锌、铁、锰、镉等多种金属离子[20]。大肠杆菌ZupT转运子是第一个在细菌中鉴定出来的ZIP家族蛋白[19]。大肠杆菌ZupT转运子的作用底物广泛，能够转运锌离子、亚铁离子、锰离子、镉离子和钴离子，但对锌离子比其他二价金属离子有明显的偏好[21]。研究表明，ZIP家族蛋白具有相同的拓扑结构，即该类蛋白都有八个跨膜结构域，且氨基和羧基末端都位于细胞膜外侧。在第三和第四跨膜结构域之间有一段富含组氨酸的高变区，如在ZIP1中这段高变区的序列为HAGHVHIHTHASHGHTH。该区域被证实具有潜在的结合金属离子的能力，表明该区域很可能与锌离子结合、调控和转运功能相关[22]，但ZIP转运锌离子的机制目前还不是很清楚。通过对ZupT结构研究发现，其结构与阳离子协助扩散家族(cation diffusion facilitator，CDF家族)蛋白结构类似，而CDF家族转运驱动力来自质子动力势，由此推测ZIP家族转运驱动力也可能来自质子动力势[23–24]。

当环境中缺乏锌离子时，由于ZupT为组成型表达，通过该非特异性转运子转运的锌离子有限，细菌会启用高亲和力的锌离子转运系统来保证锌离子的摄入。其中最普遍存在的是一个操纵子编码的转运系统ZnuABC，它是ABC超家族的一个高亲和力的锌离子转运系统[25-26]，如图1所示。

高亲和力的锌离子转运系统ZnuABC存在于大部分细菌中，如肠道沙门菌(*Salmonella enterica*)[27]、流产布鲁菌(*Brucella abortus*)[28]、鲁氏耶尔森菌(*Yersinia ruckeri*)[29]、铜绿假单胞菌(*Pseudomonas aeruginosa*)[30]、空肠弯曲杆菌(*Campylobacter jejuni*)[31]等。该体系由3个组分构成：ZnuA，ZnuB和ZnuC。ZnuB是通透酶组分；ZnuC是ATP酶组分，为离子通过内膜提供能量；ZnuA是可溶性周质蛋白，可以高效的从细胞周质

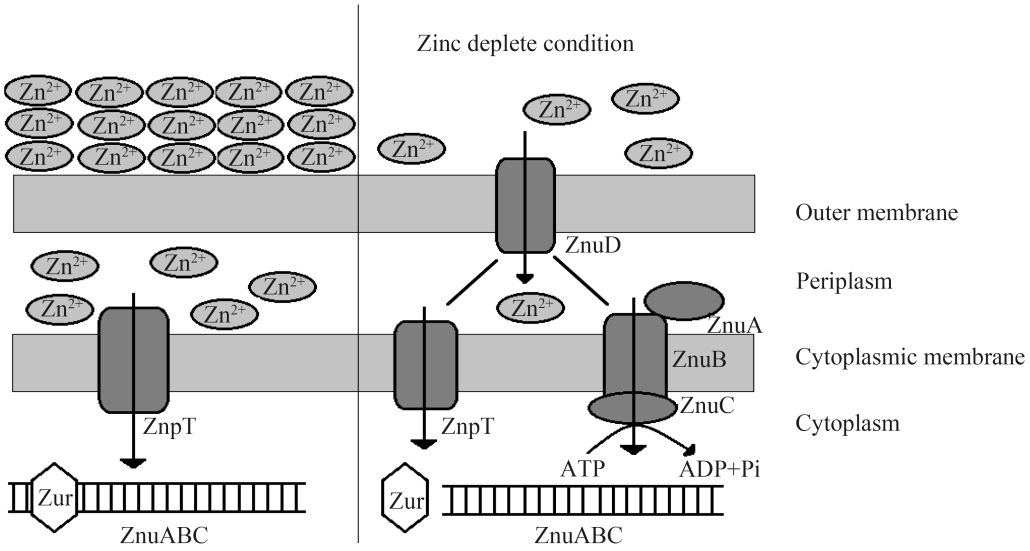

图 1. 细菌锌离子转运和调控体系[42–43]

Figure 1. Zinc transportation and regulation system in bacteria[42–43]. Under zinc replete conditions (left), the metal is imported through low affinity import systems, such as ZupT, and Zur inhibits the expression of the importer ZnuABC. Under conditions of zinc shortage (right), Zur is unable to bind DNA and the high affinity zinc importer ZnuABC is expressed. *Neisseria meningitidis* expresses a TonB-dependent outer membrane protein, ZnuD, involved in zinc uptake.

中捕获锌离子然后传递给ZnuB[32]。作者对铜绿假单胞菌(*Pseudomonas aeruginosa*, PAO1)锌离子转运系统的研究发现，在该菌中同样存在高亲和力的锌离子转运系统ZnuABC，分别由铜绿假单胞菌中PA4598，PA4501，PA4500三个基因编码。作者通过ClustalX软件对铜绿假单胞菌、鼠疫耶尔森菌(*Yersinia pestis C092*)、空肠弯曲杆菌(*Campylobacter jejuni 11168*)、大肠杆菌(*E. coli K12*)中ZnuA氨基酸序列进行多序列比对，发现这些菌的ZnuA蛋白中都有3个保守的组氨酸和1个富含组氨酸和酸性氨基酸的环状结构。已知这3个保守的组氨酸与ZnuA结合锌离子能力密切相关，而环状结构与感知细胞周质中高锌离子相关[33]。细菌中两大锌离子转运子ZupT与ZnuABC的区别如表1所示。

在一些细菌如大肠杆菌和肠道沙门菌中，ZnuABC转运子有一个附属组分ZinT，ZinT在锌存在的条件下可与ZnuA形成复合物，并能提高ZnuA结合锌的能力[27,34–35]。值得指出的是，有些细菌可以表达多个高亲和力的锌离子吸收系统，如单核细胞增多性李斯特菌(*Listeria monocytogenes*)可以表达2个ABC型锌转运系统(ZinABC和ZurLAM)[36]。在含有0.3 μmol/L Zn^{2+}的合成培养基中，ZinABC和ZurLAM任何一个敲除都不会影响单增李斯特菌的生长，但同时敲除这2个锌转运系统该菌将无法生长，这表明ZinABC和ZurLAM的功能至少有部分冗余[36]。

革兰氏阴性菌的外膜允许低分子量的分子被动扩散。细菌外膜能透过小于600道尔顿的亲水性溶质[37]，因此，仅依靠被动扩散难以保证在较差的环境中吸收足够的元素。近年来，在脑膜炎奈瑟菌(*Neisseria meningitidis*)中发现1个外膜上的依赖TonB的蛋白受体参与锌离子吸收[38]。该蛋白被命名为ZnuD，具有介导锌或血红素吸收的功能，同时受Zur或Fur的调控[39–40]。目前为止在肠道菌或其他革兰氏阴性菌中还没有鉴定出外膜上的锌

表1. 细菌锌离子转运子对比

Table 1. Comparison of bacteria zinc transporters

Characteristics	ZIP family	ABC family
Gene	*zupT*	*znuABC*
Structure	Single component: eight transmembrane domains. There is a histidine-rich region between transmembrane domains 3 and 4.	Three components: periplasmic protein, ATPase, permease. ZnuA contains three conserved histidine residues and a loop, rich in histidines and acidic residues.
Specificity	Broad metal specificity, including Zn^{2+}, Fe^{2+}, Mn^{2+}, Cd^{2+}, but it displays a significant preference for Zn^{2+}	Zn^{2+} specificity
Function	A low affinity Zn^{2+} transporter, works in metal replete conditions	A high affinity zinc importer, activated in metal deplete conditions
Energy	Proton motive force	ATP

离子受体[41]。然而，有研究发现，apo-ZinT能够被挤压出细胞，这表明它可能能够从环境中获取锌[34]。

2.2 细菌体内锌离子转运的调控

细菌可以通过调节自身细胞内锌离子的含量来应对不同浓度锌离子环境[44]。锌离子的转运受转录调节子Zur的严格调控[2]。Zur可以结合两个或两个以上的锌离子[45−46]，其中一个锌离子作为结构组成稳定蛋白质构象，而其他锌离子则帮助Zur与基因启动子区结合，从而抑制编码锌离子转运系统基因的表达[47]。当细胞内锌含量减少时，缺乏锌的Zur不再能与DNA稳定结合，因此不再抑制这些基因的转录。解抑制的基因可表达高亲和力的锌离子转运系统，从低锌环境中获取锌离子。Zur对细胞内游离存在的飞摩尔水平的锌具有敏感性[47]。我们发现在铜绿假单胞菌PAO1中，Zur由PA4599编码，与正向基因*znuBC*位于同一操纵子，与反向基因*znuA*只间隔68bp。通过对LUX发光报道子(pKD-*znuA*)的检测发现，*znuA*基因的表达在Zur突变体中明显升高，这也表明Zur在铜绿假单胞菌中作为抑制子能够抑制*znuA*基因的表达。由*zur*，*znuBC*和*znuA*在铜绿假单胞菌基因组中的位置关系我们推测，Zur蛋白可结合到操纵子*zur-znuBC*和基因*znuA*中间，同时调控二者的表达[33]。

Zur除了调节锌离子的转运外，还调节其他一些基因的转录。比如，在大肠杆菌、结核分枝杆菌(*Mycobacterium tuberculosis*)、鼠疫耶尔森菌(*Yersinia pestis*)中，Zur解抑制可以改变核糖体蛋白的表达[48]。在结核分枝杆菌中，Zur可调节早期分泌抗原6(ESAT-6)的表达，该抗原可以与巨噬细胞toll样受体2 (TLR2)相互作用，增加巨噬细胞吞噬功能[49]。在脑膜炎奈瑟菌中，Zur可以改变参与tRNA修饰的蛋白的表达[40]。综上所述，在锌离子限制性条件下，Zur除了能增加锌的摄入外，还能调节许多新陈代谢过程以适应低锌环境。

近期研究发现，完整的Zur-ZnuABC系统与致病菌毒力因子表达的调控密切相关[31,50−51]。首先ZnuA本身就是一种重要的毒力因子，如：流产布鲁杆菌的*znuA*突变体感染小鼠BALB/c细胞的毒性降低[52]；红色耶尔森菌的*znuABC*突变体在虹鳟鱼肾感染中不能与野生型竞争且很难存活[29]；肠炎沙门菌的*znuA*突变体对小鼠腹腔或口咽感染的致病性显著降低[50]。其次*znuA*基因突变还会引起细菌运动性，粘附能力，过氧化压力耐受性的变化。如大肠杆菌*E. coli* CFT073中*znuB*突变体的swimming运动能力明显降低[53]；*E. coli* O157：H7中缺失*znuA*会降低其对上皮细胞的粘附能力[34]。此外，Zur除了能调控锌离子转运系统外，还能调控一些毒性相关基因的表达。如在鼠伤寒沙门菌

中，Zur的突变使小鼠在腹膜腔接种时半致死量下降10倍左右，致病力下降的原因还未清楚，可能是由于Zur的突变导致与致病性相关基因*flitZ*的表达量下降[51]。在野油菜黄单胞菌(*Xanthomonas campestris*)中，Zur正调节致病性转录调节子*hrpX*的表达。而HrpX调节毒性因子*hrpA*和*hrpF*的表达[54]。在铜绿假单胞菌中，*zur*突变体在小鼠和线虫感染模型中毒性显著降低可能是因为喹诺酮信号分子PQS产量降低，PQS进一步影响细菌毒性[55]。尽管Zur在这些细菌中与毒性有关，但在金黄色葡萄球菌和结核分枝杆菌中，*zur*突变并未影响其毒性[56]。Anna Maciag等认为出现这一表型可能是因为Zur是抑制子，被抑制的基因在Zur突变体中能够组成型表达，其中有与毒性和应激反应相关的基因，所以突变体中没有出现预期的表型。

3 细菌锌离子调控与感染的关系

细菌建立感染的过程中必需准确感知宿主体内锌离子浓度的变化，并及时做出反应。细菌通过调节自身细胞内锌离子的含量来应对宿主体内随时变化的锌离子浓度。因此细菌锌离子的调控与其感染能力密切相关。

有研究指出，对入侵细菌来说，宿主体内可被利用的锌离子浓度非常低。将鼠伤寒沙门菌(*Salmonlla typhimurium*)培养在锌离子浓度低至1 μmol/L的合成培养基中时，*znuABC*的表达仍被抑制。相比之下，将该菌培养在上皮细胞或巨噬细胞中时，*znuABC*的表达被强烈诱导[50]。由此可知，宿主体内锌离子浓度远远低于1 μmol/L。宿主为了控制细菌的感染，通过转运、结合、储藏等手段限制可被利用的锌离子浓度，而细菌必需通过高表达ZnuABC快速获取“游离状态”的锌。

哺乳动物体内有24种锌离子转运子，被分为2个家族。其中14种属于SLC39家族蛋白，分别命名为ZIP1到ZIP14，负责将锌从细胞外转运到细胞质中。其余10种蛋白属于SLC30家族，分别为ZnT1到ZnT10，作用相反，是将细胞质中的锌转运到细胞外。血浆中存在高水平的锌，且70%与蛋白松散结合，这些锌极易被入侵细菌利用[57]。但在急性感染期，巨噬细胞分泌的白细胞介素6(IL-6)等细胞因子诱导肝细胞膜上转运子ZIP14表达上调，将锌从血浆转运到肝细胞内，并与金属硫蛋白(metallothioneins, MTs)结合积累在肝脏中[58]。细菌的入侵除了会使血浆中锌离子浓度急剧降低外，还会导致巨噬细胞吞噬体内锌离子浓度显著降低。同时伴随着细胞质中游离的锌离子减少，其中一部分被转移到高尔基体内，另一部分与MTs结合积累在细胞质中。细胞质中游离锌离子的减少会激活NADPH氧化酶(Nox)，从而生成大量活性氧(ROS)清除病原菌，如图2所示[59]。巨噬细胞由于积累了大量锌饱和的MTs对ROS的抗性明显增强，而吞噬体内的细菌极度缺乏锌离子，使得其抗氧化防御体系被瓦解，对ROS更敏感[13]。

从细菌的角度出发，建立感染的过程是对抗宿主体内低锌离子环境的过程，比如，巨噬细胞可被利用的锌离子只有皮摩尔浓度[60]。为了挫败宿主的防御体系，同时建立感染，细菌必须感知和应答低锌离子环境。Zur负责感知低锌环境，对*znuABC*基因解抑制，使得高亲和力锌离子转运系统ZnuABC高表达，以至于细菌才有可能在宿主体内存活并建立感染。ZnuABC突变体与野生型相比在感染过程中的存活率会显著降低，一方面是由于锌离子缺乏而直接导致细菌新陈代谢受阻，另一方面是由于锌缺乏使得细菌对抗氧化压力的酶(如CuZn SOD)活性和敏感性丧失[61]。

近期的研究表明，在肠道感染中ZnuABC对于抵抗宿主分泌的锌结合蛋白有一定的作用[62]。这些锌结合蛋白包括S100家族的钙网蛋白(calprotectin)，牛皮癣素(psoriasin)等。钙网蛋白

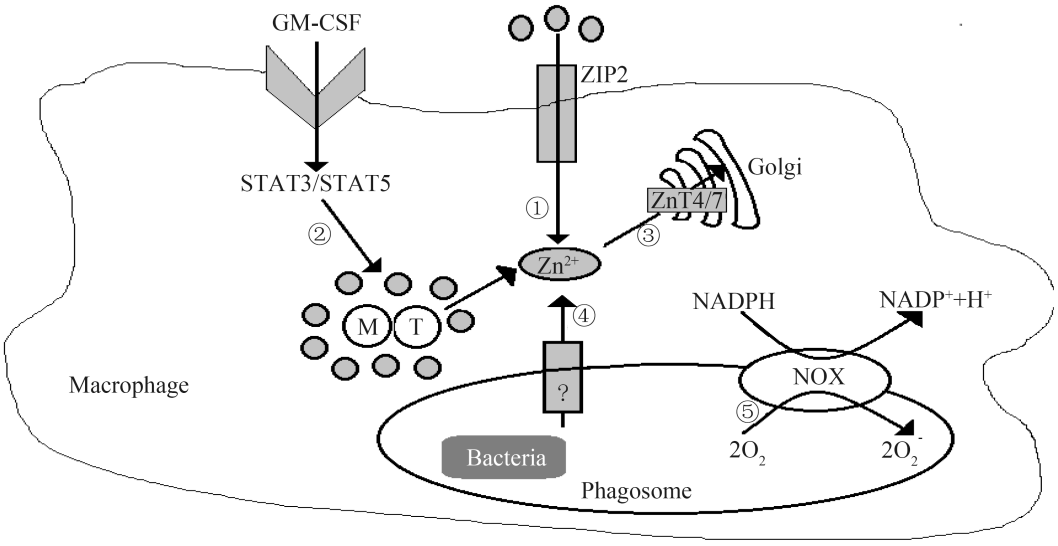

图 2. 巨噬细胞内锌离子的调控[61]

Figure 2. Zinc regulation in macrophages[61]. ①A dual stimulus involving GM-CSF and bacteria infection potently induces zinc influx by ZIP2. ②GM-CSF binds to the GM-CSF receptor on macrophages, activates STAT3 and STAT5 signaling, and triggers the production of MTs that constrict the labile zinc by binding the metal. ③Zinc is mobilized into the Golgi apparatus, associated with increased expression of Golgi membrane transporters ZNT4 and ZNT7.④Speculated zinc deprivation by influx into the cytosol by a potential ZIP. ⑤The zinc-deprived environment activating NADPH oxidase (Nox) and generating large amounts of ROS which effectively eliminated pathogen.

是1种中性钙结合蛋白，它被充分释放到感染部位通过结合锌和锰来控制病原菌的增殖[63]。牛皮癣素(S100A7)能通过增加细菌细胞膜的渗透性阻止其生存[64]。鼠伤寒沙门菌之所以能够逃脱S100家族锌结合蛋白制造的低锌环境，是因为与其他共生的肠道菌相比，该菌能够表达高亲和力的锌离子转运系统ZnuABC[50]。这个发现进一步证实了锌的摄取对细菌的重要性。同样的，对于金黄色葡萄球菌(*Staphylococcus aureus*)、大肠杆菌、白色念珠菌(*Monilia albican*)等细菌来说表达ZnuABC能够抵抗宿主锌结合蛋白的作用，有利于微生物在发炎的肠道中成长[62]。

我们针对铜绿假单胞菌感染宿主能力的研究发现，基因*znuA*突变体与野生型相比粘附性和毒性均明显降低。粘附是细菌在宿主中定殖的第一步，我们的研究发现*znuA*突变体对聚苯乙烯平板和人结肠癌细胞(Caco-2)的粘附能力均明显降低。除此之外，我们利用果蝇感染模型检测了*znuA*突变体致病性，结果显示感染*znuA*突变体果蝇的死亡率明显低于野生型，而感染*znuA*互补体果蝇的死亡率能恢复至野生型水平[33]。这都表明，ZnuABC转运系统在细菌感染宿主的过程中至关重要[33]。

限制锌离子浓度被宿主广泛用于控制细菌的感染，最近揭示了另外一种完全相反的抗菌策略，即通过积累大量锌使细菌锌中毒死亡。如巨噬细胞吞噬大肠杆菌、结核分枝杆菌形成的吞噬体内锌离子浓度会大幅提升[34]。分枝杆菌通过诱导P型ATP酶CtpC的高表达阻止细胞内的锌离子的积累。结核分枝杆菌*ctpC*缺失突变体在宿主体内的存活率显著降低[65]。同样，在包括大肠杆菌(ZntA)链球菌(CzcD)在内的大量细菌中都已鉴定出了结构同源的锌离子外排泵[66]。大肠杆菌ZntA缺失突变与野生型相比更容易被宿主清除。这些结果显示细菌锌离子外排泵对抵抗高锌离子环境中巨噬细胞的清除有显著作用。

4 锌离子转运系统可作为抗菌治疗的靶点

细菌的繁殖和毒性依赖于锌离子稳态，因此可将锌转运系统作为抗菌治疗的靶点。一方面可以生产抑制该系统的化合物对抗细菌感染；另一方面可以基于该系统研发减毒疫苗。

大多数细菌只有一个高亲和力的锌转运系统ZnuABC，该系统中各结构域非常保守，且哺乳动物体内没有ABC转运系统[67]。因此，以ZnuABC为靶点筛选抗菌药物将成为一种非常有前途的抗菌策略。开发抑制该系统的新型抗菌剂主要依赖对其蛋白晶体结构和分子作用机制的研究。获得候选药物的方法主要有两种，一是通过高通量药物筛选(High throughput screening，HTS)技术对化合物库进行筛选；二是基于分子片段的药物筛选[68]。Simm等通过对2000种小分子化合物的筛选，在白色念珠菌中鉴定出了几个分子能阻止锌吸收[69]。从最初开发利用ABC转运系统到现在已有35年的时间，但是这一技术到目前为止仍不成熟。可能由于活性蛋白体外表达量、蛋白晶体的获得以及药物筛选技术的限制使得这种开发抗菌剂的方法仍不能被普遍利用，但毋庸置疑这一方法有着巨大的潜能。

细菌中ZnuABC突变株有被开发为活的减毒疫苗的潜能。已有研究表明，鼠伤寒沙门菌的ZnuABC突变株能够短时间感染小鼠模型，而这一短时间的感染可以诱导一个稳固且持久的对该菌毒性菌株的免疫保护[70−71]。同样的在豚鼠感染模型中ZnuABC突变株也表现出低毒性同时诱导对毒性菌株的免疫保护[72]。与之类似，接种布鲁菌ZnuA突变株可以保护老鼠免受该菌的感染[73]。近期研究发现脑膜炎奈瑟菌中的外膜蛋白ZnuD高度保守，可作为疫苗研发的潜在靶点。用包裹ZnuD的囊泡感染小鼠和豚鼠能够使其产生抗体，同时激活补体介导的细胞毒作用杀死脑膜炎奈瑟菌[74]。这些证据表明，锌离子转运系统可作为有效靶点用于对新型抗菌药物或疫苗的研发[68]。

5 小结和展望

锌是生命代谢中重要的微量元素，广泛参与糖酵解、核酸和蛋白质代谢等生理活动。锌离子缺乏和过剩都会影响细菌的生存与感染的建立。因此，通过影响细菌体内锌离子稳态就能够在一定程度上控制感染。目前为止，虽然人们对于锌离子在细菌和宿主锌竞争过程中的重要作用进行了广泛的研究，但仍有许多问题需要深入研究。如巨噬细胞如何决定何时选择锌缺乏的方式或锌中毒的方式杀死细菌；以及锌离子转运、贮存、调节等代谢活动的详细机制等问题还有待进一步研究。相信对于锌离子稳态调控机制的深入研究，将有助于预防和治疗细菌感染。

参考文献

[1] Banerjee S, Wei BX, Bhattacharyya-Pakrasi M, Pakrasi HB, Smith TJ. Structural determinants of metal specificity in the zinc transport protein ZnuA from *Synechocystis* 6803. *Journal of Molecular Biology*, 2003, 333(5): 1061–1069.

[2] Patzer SI, Hantke K. The ZnuABC high-affinity zinc uptake system and its regulator Zur in *Escherichia coli*. *Molecular Microbiology*, 1998, 28(6): 1199–1210.

[3] Andreini C, Banci L, Bertini I, Rosato A. Zinc through the three domains of life. *Journal of Proteome Research*, 2006, 5(11): 3173–3178.

[4] Andreini C, Bertini I, Cavallaro G, Holliday GL, Thornton JM. Metal ions in biological catalysis: from enzyme databases to general principles. *Journal of Biological Inorganic Chemistry*, 2008, 13(8): 1205–1218.

[5] Scrutton MC, Wu CW, Goldthwait DA. The presence and possible role of zinc in RNA polymerase obtained from *Escherichia coli*. *Proceedings of the National Academy of Sciences of the United States of America*, 1971, 68(10): 2497–2501.

[6] Potter AJ, Kidd SP, McEwan AG, Paton JC. The MerR/NmlR

family transcription factor of *Streptococcus pneumoniae* responds to carbonyl stress and modulates hydrogen peroxide production. *Journal of Bacteriology*, 2010, 192(15): 4063–4066.

[7] Carrigan CN, Poulter CD. Zinc is an essential cofactor for type I isopentenyl diphosphate: dimethylallyl diphosphate isomerase. *Journal of the American Chemical Society*, 2003, 125(30): 9008–9009.

[8] Kropachev KY, Zharkov DO, Grollman AP. Catalytic mechanism of *Escherichia coli* endonuclease Ⅷ: roles of the intercalation loop and the zinc finger. *Biochemistry*, 2006, 45(39): 12039–12049.

[9] Ammendola S, Pasquali P, Pacello F, Rotilio G, Castor M, Libby SJ, Figueroa-Bossi N, Bossi L, Fang FC, Battistoni A. Regulatory and structural differences in the Cu, Zn-superoxide dismutases of *Salmonella enterica* and their significance for virulence. *Journal of Biological Chemistry*, 2008, 283(20): 13688–13699.

[10] Meini MR, González LJ, Vila AJ. Antibiotic resistance in Zn(Ⅱ)-deficient environments: metallo-β-lactamase activation in the periplasm. *Future Microbiology*, 2013, 8(8): 947–979.

[11] de Orué Lucana DO, Wedderhoff I, Groves MR. ROS-mediated signalling in bacteria: zinc-containing Cys-X-X-Cys redox centres and iron-based oxidative stress. *Journal of Signal Transduction*, 2012, 2012: 605905.

[12] Kim Y, Tesar C, Mire J, Jedrzejczak R, Binkowski A, Babnigg G, Sacchettini J, Joachimiak A. Structure of apo- and monometalated forms of NDM-1-a highly potent carbapenem-hydrolyzing metallo-β-lactamase. *PLoS One*, 2011, 6(9): e24621.

[13] Vignesh KS, Landero Figueroa JA, Porollo A, Caruso JA, Deepe GS, Jr. Granulocyte macrophage-colony stimulating factor induced Zn sequestration enhances macrophage superoxide and limits intracellular pathogen survival. *Immunity*, 2013, 39(4): 697–710.

[14] Patrich Hensley M, Tierney DL, Crowder MW. Zn(Ⅱ) binding to *Escherichia coli* 70S ribosomes. *Biochemistry*, 2011, 50(46): 9937–9939.

[15] Galeotti CL, Bove E, Pezzicoli A, Nogarotto R, Norais N, Pileri S, Lelli B, Falugi F, Balloni S, Tedde V, Chiarot E, Bombaci M, Soriani M, Bracci L, Grandi G, Grifantini R. Surface interactome in *Streptococcus pyogenes*. *Molecular & Cellular Proteomics*, 2012, 11(4): M111.015206.

[16] Baker M, Gutman DM, Papageorgiou AC, Collins CM, Ravi Acharya K. Structural features of a zinc binding site in the superantigen strepococcal pyrogenic exotoxin A (SpeA1): implications for MHC class Ⅱ recognition. *Protein Science*, 2001, 10(6): 1268–1273.

[17] Iqbal M, Kenney PB, Al-Humadi NH, Klandorf H. Relationship between mechanical properties and pentosidine in tendon: effects of age, diet restriction, and aminoguanidine in broiler breeder hens. *Poultry Science*, 2000, 79(9): 1338–1344.

[18] Hantke K. Bacterial zinc uptake and regulators. *Current Opinion in Microbiology*, 2005, 8(2): 196–202.

[19] Grass G, Wong MD, Rosen BP, Smith RL, Rensing C. ZupT is a Zn(Ⅱ) uptake system in *Escherichia coli*. *Journal of Bacteriology*, 2002, 184(3): 864–866.

[20] Guerinot ML. The ZIP family of metal transporters. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, 2000, 1465(1/2): 190–198.

[21] Grass G, Franke S, Taudte N, Nies DH, Kucharski LM, Maguire ME, Rensing C. The metal permease ZupT from *Escherichia coli* is a transporter with a broad substrate spectrum. *Journal of Bacteriology*, 2005, 187(5): 1604–1611.

[22] Alex Gaither L, Eide DJ. Functional expression of the human hZIP2 zinc transporter. *Journal of Biological Chemistry*, 2000, 275(8): 5560–5564.

[23] Karlinsey JE, Maguire ME, Becker LA, Crouch MLV, Fang FC. The phage shock protein PspA facilitates divalent metal transport and is required for virulence of *Salmonella enterica* sv. Typhimurium. *Molecular Microbiology*, 2010, 78(3): 669–685.

[24] Taudte N, Grass G. Point mutations change specificity and kinetics of metal uptake by ZupT from *Escherichia coli*. *Biometals*, 2010, 23(4): 643–656.

[25] Chandra BR, Yogavel M, Sharma A. Structural analysis of ABC-family periplasmic zinc binding protein provides new insights into mechanism of ligand uptake and release. *Journal of Molecular Biology*, 2007, 367(4): 970–982.

[26] Lim CK, Hassan KA, Penesyan A, Loper JE, Paulsen IT. The effect of zinc limitation on the transcriptome of *Pseudomonas protegens* Pf-5. *Environmental Microbiology*, 2013, 15(3): 702–715.

[27] Ilari A, Alaleona F, Tria G, Petrarca P, Battistoni A, Zamparelli C, Verzili D, Falconi M, Chiancone E. The *Salmonella enterica* ZinT structure, zinc affinity and interaction with the high-

affinity uptake protein ZnuA provide insight into the management of periplasmic zinc. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 2014, 1840(1): 535–544.

[28] Kim S, Watanabe K, Shirahata T, Watarai M. Zinc uptake system (*znuA* locus) of *Brucella abortus* is essential for intracellular survival and virulence in mice. *Journal of Veterinary Medical Science*, 2004, 66(9): 1059–1063.

[29] Dahiya I, Stevenson RMW. The ZnuABC operon is important for *Yersinia ruckeri* infections of rainbow trout, *Oncorhynchus mykiss* (Walbaum). *Journal of Fish Diseases*, 2010, 33(4): 331–340.

[30] Ellison ML, Jarrow MF Ⅲ I, Parrish W, Danell AS, Pesci EC. The transcriptional regulator *Np20* Is the zinc uptake regulator in *Pseudomonas aeruginosa*. *PLoS One*, 2013, 8(9): e75389.

[31] Davis LM, Kakuda T, DiRita VJ. A *Campylobacter jejuni znuA* orthologue is essential for growth in low-zinc environments and chick colonization. *Journal of Bacteriology*, 2009, 191(5): 1631–1640.

[32] Berducci G, Mazzetti AP, Rotilio G, Battistoni A. Periplasmic competition for zinc uptake between the metallochaperone ZnuA and Cu, Zn superoxide dismutase. *FEBS Letters*, 2004, 569(1/3): 289–292.

[33] Li B, Wang BB, Gao XL, Gao L, Liang Y, Duan KM, Shen LX. The zinc uptake system (*znuA* Locus) is important for bacterial adhesion and virulence in *Pseudomonas aeruginosa*. *Biomedical Engineering and Environmental Engineering*, 2014, 24/25: 135–142.

[34] Gabbianelli R, Scotti R, Ammendola S, Petrarca P, Nicolini L, Battistoni A. Role of ZnuABC and ZinT in *Escherichia coli* O157: H7 zinc acquisition and interaction with epithelial cells. *BMC Microbiology*, 2011, 11: 36.

[35] Petrarca P, Ammendola S, Pasquali P, Battistoni A. The Zur-regulated ZinT protein is an auxiliary component of the high-affinity ZnuABC zinc transporter that facilitates metal recruitment during severe zinc shortage. *Journal of Bacteriology*, 2010, 192(6): 1553–1564.

[36] Corbett D, Wang JH, Schuler S, Lopez-Castejon G, Glenn S, Brough D, Andrew PW, Cavet JS, Roberts IS. Two zinc uptake systems contribute to the full virulence of *Listeria monocytogenes* during growth *in vitro* and *in vivo*. *Infection and Immunity*, 2012, 80(1): 14–21.

[37] Nikaido H. Molecular basis of bacterial outer membrane permeability revisited. *Microbiology and Molecular Biology Reviews*, 2003, 67(4): 593–656.

[38] Stork M, Bos MP, Jongerius I, de Kok N, Schilders I, Weynants VE, Poolman JT, Tommassen J. An outer membrane receptor of *Neisseria meningitidis* involved in zinc acquisition with vaccine potential. *PLoS Pathogens*, 2010, 6: e1000969.

[39] Kumar P, Sannigrahi S, Tzeng YL. The *Neisseria meningitidis* ZnuD zinc receptor contributes to interactions with epithelial cells and supports heme utilization when expressed in *Escherichia coli*. *Infection and Immunity*, 2012, 80(2): 657–667.

[40] Pawlik MC, Hubert K, Joseph B, Claus H, Schoen C, Vogel U. The zinc-responsive regulon of *Neisseria meningitidis* comprises 17 genes under control of a Zur element. *Journal of Bacteriology*, 2012, 194(23): 6594–6603.

[41] Ho TD, Davis BM, Ritchie JM, Waldor MK. Type 2 secretion promotes enterohemorrhagic *Escherichia coli* adherence and intestinal colonization. *Infection and Immunity*, 2008, 76(5): 1858–1865.

[42] Cerasi M, Ammendola S, Battistoni A. Competition for zinc binding in the host-pathogen interaction. *Front Cell Infect Microbiol*, 2013, 3: 108.

[43] Hantke K. Bacterial zinc transporters and regulators. *Biometals*, 2001, 14(3/4): 239–249.

[44] Garmory HS, Titball RW. ATP-binding cassette transporters are targets for the development of antibacterial vaccines and therapies. *Infection and Immunity*, 2004, 72(12): 6757–6763.

[45] Lubelski J, Konings WN, Driessen AJM. Distribution and physiology of ABC-type transporters contributing to multidrug resistance in bacteria. *Microbiology and Molecular Biology Reviews*, 2007, 71(3): 463–476.

[46] Shin JH, Jung HJ, An YJ, Cho YB, Cha SS, Roe JH. Graded expression of zinc-responsive genes through two regulatory zinc-binding sites in Zur. *Proceedings of the National Academy of Sciences of the United States of America*, 2011, 108(12): 5045–5050.

[47] Outten CE, O'Halloran TV. Femtomolar sensitivity of metalloregulatory proteins controlling zinc homeostasis. *Science*, 2001, 292(5526): 2488–2492.

[48] Panina EM, Mironov AA, Gelfand MS. Comparative genomics of bacterial zinc regulons: enhanced ion transport, pathogenesis, and rearrangement of ribosomal proteins. *Proceedings of the National Academy of Sciences of the United States of America*, 2003, 100(17): 9912–9917.

[49] Maciag A, Dainese E, Marcela Rodriguez G, Milano A, Provvedi R, Pasca MR, Smith I, Palù G, Riccardi G, Manganelli R. Global analysis of the *Mycobacterium tuberculosis* Zur (FurB) regulon. *Journal of Bacteriology*, 2007, 189(3): 730–740.

[50] Ammendola S, Pasquali P, Pistoia C, Petrucci P, Petrarca P, Rotilio G, Battistoni A. High-affinity Zn^{2+} uptake system ZnuABC is required for bacterial zinc homeostasis in intracellular environments and contributes to the virulence of *Salmonella enterica*. *Infection and Immunity*, 2007, 75(12): 5867–5876.

[51] Campoy S, Jara M, Busquets N, Pérez de Rozas AM, Badiola I, Barbé J. Role of the high-affinity zinc uptake *znuABC* system in *Salmonella enterica* serovar Typhimurium virulence. *Infection and Immunity*, 2002, 70(8): 4721–4725.

[52] Yang XH, Becker T, Walters N, Pascual DW. Deletion of *znuA* virulence factor attenuates *Brucella abortus* and confers protection against wild-type challenge. *Infection and Immunity*, 2006, 74(7): 3874–3879.

[53] Gunasekera TS, Herre AH, Crowder MW. Absence of ZnuABC-mediated zinc uptake affects virulence-associated phenotypes of uropathogenic *Escherichia coli* CFT073 under Zn (Ⅱ)Absence of ZnuABC-mediated zinc uptake affects virulence-associated phenotypes of uropathogenic *Escherichia coli* CFT073 under Zn-depleted conditions. *FEMS Microbiology Letters*, 2009, 300(1): 36–41.

[54] Yang WF, Liu Y, Chen L, Gao TC, Hu BS, Zhang DF, Liu FQ. Zinc uptake regulator (*zur*) gene involved in zinc homeostasis and virulence of *Xanthomonas oryzae* pv. oryzae in rice. *Current Microbiology*, 2007, 54(4): 307–314.

[55] Gallagher LA, McKnight SL, Kuznetsova MS, Pesci EC, Manoil C. Functions required for extracellular quinolone signaling by *Pseudomonas aeruginosa*. *Journal of Bacteriology*, 2002, 184(23): 6472–6480.

[56] Desrosiers DC, Bearden SW, Mier I, Jr., Abney J, Paulley JT, Fetherston JD, Salazar JC, Radolf JD, Perry RD. Znu is the predominant zinc importer in *Yersinia pestis* during *in vitro* growth but is not essential for virulence. *Infection and Immunity*, 2010, 78(12): 5163–5177.

[57] Jayaram L, Chunilal S, Pickering S, Ruffin RE, Zalewski PD. Sputum zinc concentration and clinical outcome in older asthmatics. *Respirology*, 2011, 16(3): 459–466.

[58] Lichten LA, Cousins RJ. Mammalian zinc transporters: nutritional and physiologic regulation. *Annual Review of Nutrition*, 2009, 29(1): 153–176.

[59] Youseff BH, Holbrook ED, Smolnycki KA, Rappleye CA. Extracellular superoxide dismutase protects *Histoplasma* yeast cells from host-derived oxidative stress. *PLoS Pathogens*, 2012, 8(5): e1002713.

[60] Colvin RA, Holmes WR, Fontaine CP, Maret W. Cytosolic zinc buffering and muffling: their role in intracellular zinc homeostasis. *Metallomics*, 2010, 2(5): 306–317.

[61] Haase H. An element of life: competition for zinc in host-pathogen interaction. *Immunity*, 2013, 39(4): 623–624.

[62] Liu JZ, Jellbauer S, Poe AJ, Ton V, Pesciaroli M, Kehl-Fie TE, Restrepo NA, Hosking MP, Edwards RA, Battistoni A, Pasquali P, Lane TE, Chazin WJ, Vogl T, Roth J, Skaar EP, Raffatellu M. Zinc sequestration by the neutrophil protein calprotectin enhances *Salmonella* growth in the inflamed gut. *Cell Host & Microbe*, 2012, 11(3): 227–239.

[63] Kehl-Fie TE, Skaar EP. Nutritional immunity beyond iron: a role for manganese and zinc. *Current Opinion in Chemical Biology*, 2010, 14(2): 218–224.

[64] Michalek M, Gelhaus C, Hecht O, Podschun R, Schröder JM, Leippe M, Grötzinger J. The human antimicrobial protein psoriasin acts by permeabilization of bacterial membranes. *Developmental & Comparative Immunology*, 2009, 33(6): 740–746.

[65] Padilla-Benavides T, Long JE, Raimunda D, Sassetti CM, Arguello JM. A novel P_{1B}-type Mn^{2+}-transporting ATPase is required for secreted protein metallation in *Mycobacteria*. *Journal of Biological Chemistry*, 2013, 288(16): 11334–11347.

[66] Botella H, Stadthagen G, Lugo-Villarino G, de Chastellier C, Neyrolles O. Metallobiology of host-pathogen interactions: an intoxicating new insight. *Trends in Microbiology*, 2012, 20(3): 106–112.

[67] Wouters J. Crystallography of membrane proteins, major targets in drug design. *Mini-Reviews in Medicinal Chemistry*, 2003, 3(5): 439–448.

[68] Counago RM, McDevitt CA, Ween MP, Kobe B. Prokaryotic substrate-binding proteins as targets for antimicrobial therapies. *Current Drug Targets*, 2012, 13(11): 1400–1410.

[69] Simm C, Luan CH, Weiss E, O'Halloran T. High-throughput screen for identifying small molecules that target fungal zinc homeostasis. *PLoS One*, 2011, 6(9): e25136.

[70] Pasquali P, Ammendola S, Pistoia C, Petrucci P, Tarantino M,

Valente C, Marenzoni ML, Rotilio G, Battistoni A. Attenuated *Salmonella enterica* serovar Typhimurium lacking the ZnuABC transporter confers immune-based protection against challenge infections in mice. *Vaccine*, 2008, 26(27/28): 3421–3426.

[71] Pesciaroli M, Aloisio F, Ammendola S, Pistoia C, Petrucci P, Tarantino M, Francia M, Battistoni A, Pasquali P. An attenuated *Salmonella enterica* serovar Typhimurium strain lacking the ZnuABC transporter induces protection in a mouse intestinal model of *Salmonella* infection. *Vaccine*, 2011, 29(9): 1783–1790.

[72] Gradassi M, Pesciaroli M, Martinelli N, Ruggeri J, Petrucci P, Hassan WH, Raffatellu M, Scaglione FE, Ammendola S, Battistoni A, Alborali GL, Pasquali P. Attenuated *Salmonella enterica* serovar Typhimurium lacking the ZnuABC transporter: an efficacious orally-administered mucosal vaccine against salmonellosis in pigs. *Vaccine*, 2013, 31(36): 3695–3701.

[73] Clapp B, Skyberg JA, Yang XH, Thornburg T, Walters N, Pascual DW. Protective live oral brucellosis vaccines stimulate Th1 and th17 cell responses. *Infection and Immunity*, 2011, 79(10): 4165–4174.

[74] Hubert K, Devos N, Mordhorst I, Tans C, Baudoux G, Feron C, Goraj K, Tommassen J, Vogel U, Poolman JT, Weynants V. ZnuD, a potential candidate for a simple and universal *Neisseria meningitidis* vaccine. *Infection and Immunity*, 2013, 81(6): 1915–1927.

Zinc regulation system in bacteria and its relationship with infection-A review

Bo Li, Jing Li, Lixin Shen*

Key Laboratory of Resources Biology and Biotechnology in Western China, Ministry of Education, Faculty of Life Sciences, Northwest University, Xi'an 710069, Shaanxi Province, China

Abstract: Zinc is an indispensable trace element and important for both bacteria and eukaryotes. Zn homeostasis is established by Zn^{2+} transport and regulation system. Hosts have developed mechanisms for Zn restricting or toxicosis in response to infections. In order to grow and multiply in the infected host, bacteria have progressed strict zinc transportation and regulation system, such as ZnuABC. Zinc is critically involved in a plethora of metabolic and virulence pathways and is paramount important in infection. Therefore, there could be possibilities to use zinc transporters as a very promising target for the development of novel antimicrobial strategies.

Keywords: zinc, zinc regulation, infection, antimicrobial treatment

(本文责编：张晓丽)

Supported by the National Nature Science Foundation of China (81171620, 1210063) and by the Postgraduate Innovation Project of Northwestern University (YZZ14074)

*Corresponding author. Tel/Fax: +86-29-88302132; E-mail: shenlx@nwu.edu.cn

Received: 20 October 2015; Revised: 1 December 2015; Published online: 16 December 2015