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综述

大肠杆菌SeqA蛋白质结构与功能

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摘要 大肠杆菌SeqA蛋白质是染色体复制起始负调控因子。在体内, SeqA主要以四聚体或多聚体形式存在, 有N-端多聚化结构域和C-端DNA结合结构域。大肠杆菌复制原点(the origin of replication of the *Escherichia coli* chromosome, *oriC*)有11个GATC位点, 新复制的*oriC*处于半甲基化状态, 其中相邻的两个半甲基化GATC是SeqA特异性结合靶位点。SeqA通过结合新复制的半甲基化*oriC*来抑制复制起重新发生, 从而使*oriC*隔绝(sequestration)。由SeqA介导的*oriC*隔绝是抑制同一个细胞周期中复制起始重新发生的机制之一。SeqA不仅是复制起始调控因子, 也是一个转录因子, 抑制或激活一些基因的表达。该文就SeqA蛋白质的结构与功能域特点, 对DNA复制起始和基因表达调控机制以及细胞分裂中的作用作一综述。

关键词 SeqA蛋白质; 结构与功能域; DNA复制起始; 隔绝

Structure and Function of the *Escherichia coli* SeqA Protein

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Abstract The SeqA protein is a negative factor for initiation of chromosomal replication in *Escherichia coli*. *In vivo* the SeqA molecules are found in a form of tetramer or multimers. The SeqA protein has the N-terminal aggregation and the C-terminal DNA-binding domains. The *E. coli* chromosomal origin for replication (*oriC*) contains 11 GATC sites and newly replicated *oriC* is hemimethylated. The site of two neighboured hemimethylated GATC is the best target of SeqA for binding. SeqA binds to hemimthylated GATC to prevent re-initiation of replication, namely the sequestration of *oriC*. Sequestration is one of the mechanisms to make sure that each origin is initiated only once per cell cycle. The SeqA protein is not only a regulatory factor for replication initiation but also a transcription factor, inhibiting or activating expression of some genes. In the paper, we reviewed the character of structural and functional domain of SeqA, and its roles in control for initiation of DNA replication, gene expression and cell division.

Keywords the SeqA protein; structural and functional domains; DNA replication initiation; sequestration

大肠杆菌环状染色体的复制由唯一一个复制起始原点*oriC*(the origin of replication of the *Escherichia coli* chromosome)所引发, 并且在每个细胞周期中只发生一次(once-per-cell-cycle)[1-4]。大肠杆菌*oriC*(245 bp)有五个复制起始蛋白质DnaA结合位点(DnaA-box)[5-6], DnaA与*oriC*中DnaA-box的相互作用使*oriC* DNA双链扭曲, 所产生的力在IHF(integration host factor)、HU(heat unstable)等蛋白质的作用下解链*oriC*上游的AT富含区[7-8], 形成复制起始开放复合物(open complex)[9]。在DnaC协助下, DNA解旋酶

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DnaB以六聚体形式组装在复制起始开放复合物上, 形成前引物复合体(prepriming complex)[10]。之后, 引物合成酶DnaG和DNA聚合酶III组装到前引物复合体, 开始双向复制[11]。

染色体复制是个严格调控的细胞过程, 在每个细胞周期中只发生一次[3-4,12]。主要有三个机制抑制复制起始的再次发生: (1)RIDA(regulatory inactivation of DnaA)能够把具复制起始活性的ATP-DnaA水解为没有活性的ADP-DnaA, 由此降低*oriC*的复制起始频率, 且RIDA只有在复制过程中才发挥作用, 复制终止后便失去活性[13]; (2)DnaA对一个高亲和性序列*datA*的滴定(DnaA titration)[14-15]以及*datA*依赖的ATP-DnaA的水解(DDAH, *datA*-dependent DnaA-ATP hydrolysis)[16]可抑制复制起始的发生; (3)复制起始位点*oriC*的隔绝(sequestration of *oriC*)[4,17]。*oriC*区域有11个GATC位点, 复制起始前两条链的腺嘌呤N^6位点均被甲基化, 复制起始后新合成的腺嘌呤位点尚未甲基化, 与被甲基化的模板形成半甲基化的*oriC*[18-19]。相邻的半甲基化GATC位点是SeqA蛋白质的靶位点, SeqA以高亲和性结合半甲基化的GATC位点, 对全甲基化(两条链都被甲基化)的GATC位点也有一定的亲和性, 但亲和性远不如前者[18]。SeqA对半甲基化的*oriC* GATC位点的结合可抑制DnaA对*oriC*的结合以及由此引起的额外复制起始, 此现象为*oriC*的隔绝[18,20]。

1 SeqA蛋白质的发现

对快速生长的大肠杆菌来说, 新一轮的复制起始在前一轮复制尚未结束前就开始, 但所有*oriC*的复制起始是同步的[21], 且每一个细胞周期中复制起始只发生一次[3-4]。1987年, Russel和Zinder[22]首先提出了猜想, 即大肠杆菌体内存在着抑制"多余"复制起始的机制。他们发现, 未甲基化的微染色体(由*oriC*复制的质粒)转化*dam*(编码Dam甲基化酶的基因)删除突变体的效率高, 而全甲基化的微染色体转化效率明显低于前者[22]。*oriC*的11个GATC位点是Dam甲基化酶的靶位点[23], 正常的染色体复制中, 新合成的链尚未被甲基化, 而*oriC*是半甲基化的。因此, 他们猜测有个特殊过程抑制半甲基化*oriC*上"多余"复制起始的发生[22]。这种抑制"多余"复制起始的猜想不久就被证实, 研究人员发现, 大肠杆菌细胞中有特异性抑制半甲基化*oriC*的调控因子, 这个调控因子有可能参与隔绝半甲基化*oriC*的过程[18,24]。

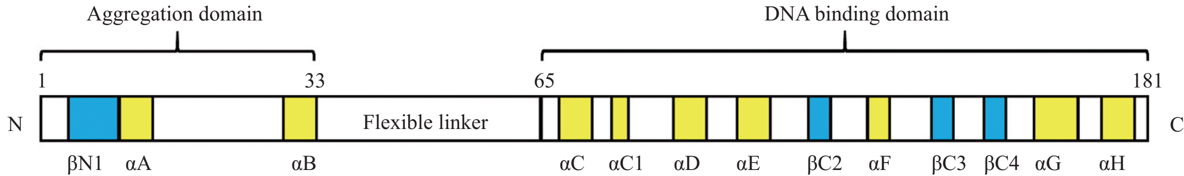
1994年, Lu等[18]报道, SeqA是*oriC*隔绝的一个成员。通过遗传图谱分析和测序比对, Lu等鉴定出*seqA*基因, 并发现*seqA*基因删除突变体失去同步的复制起始(synchronous initiation of replication), 即失去*oriC*的隔绝, 进而证明SeqA是参与*oriC*隔绝(途径)的一个成员。不久, 其他研究者用全基因组筛选方式证实了SeqA和Dam蛋白质直接参与*oriC*隔绝[25]。

2 SeqA蛋白质有N-端多聚化结构域和C-端DNA结合结构域

SeqA由181个氨基酸组成, 单体大小约为21 kDa[18,26]。SeqA有两个功能域: N-端(SeqA-NTD, 由1~33氨基酸残基构成)的多聚化结构域和C-末端(SeqA-CTD, 由65~181氨基酸残基构成)的DNA结合结构域(图1)[26-27]。两个结构域由一个柔性连接链(flexible linker)来连接, 这个连接链由中间的30个氨基酸(34~64)组成[26]。N-端的33个氨基酸残基折叠成两个α螺旋(αA和αB)和一个β折叠(βN1), 与其他SeqA分子的N-端形成多聚体[26]。βN1由氨基酸残基2~7构成, 氨基酸残基8~16、24~33分别构成了αA和αB螺旋, αA和αB之间的七个氨基酸残基(17~23)形成了αA-αB环(αA-αB loop)[28]。SeqA-NTD主链由Met1、Ile4、Val6、Leu10、Ile14、Ala15、Ile21、Ala25、Ile28、Leu29、Met32和Leu33等疏水性氨基酸残基组成, 并且不暴露在SeqA分子的表面[29]。C-末端118个氨基酸残基折叠成七个α螺旋(αC、αC1、αD、αE、αF、αG和αH)和三个反平行的β折叠(βC2、βC3和βC4)[26](图1)。αC、αC1和αD形成了α螺旋发夹结构(helical hairpin), 三个β折叠穿插在由αE、αF、αG和αH构成的另一个α螺旋发夹束中间[28]。构成连接链的30个氨基酸残基中, 有18个氨基酸是疏水性的Ala、Ile、Phe、Pro或Val, 因此, 这个连接链的表面是疏水的。Lys34是连接点, 连接N-端和C-端并介导DNA结合功能域的180º旋转[29]。

3 SeqA蛋白质存在形式

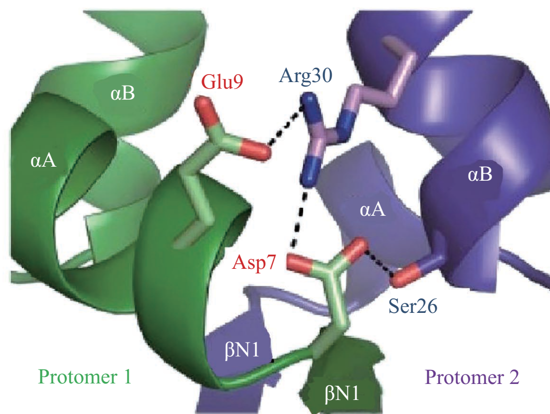
在体外, SeqA浓度较低时以四聚体形式存在, 通常情况下为多聚体[30]。单体分子量为21 kDa的SeqA, 含有1 mol/L盐的溶液中分子量大于300 kDa, 这是由于N-末端多聚化导致形成SeqA的多聚体, 从



SeqA由N-端的多聚化结构域(氨基酸残基1~33)和C-端的DNA结合结构域(氨基酸残基65~181)组成, 二者由中间的柔性的连接链连接(氨基酸残基34~64)。N-端的结构域折叠成两个α螺旋和一个β折叠, C-端的结构域折叠成七个α螺旋和三个反平行的β折叠。蓝色表示α螺旋, 黄色表示β折叠。
SeqA is organized into two domains, an N-terminal aggregation domain (residues 1-33) and a C-terminal DNA-binding domain (residues 65-181) are joined by a flexible linker (residues 34-64). The N-terminal domain folds into two α-helices and one β-strand and is required for multimerization, whereas the C-terminal DNA binding domain folds into seven α-helices and a small three-stranded antiparallel β-sheet. Yellow boxes represent α-helices, blue boxes represent β-sheet.

图1 大肠杆菌SeqA蛋白质结构(根据参考文献[26-27,29]修改)

Fig.1 Structure of the *E.coli* SeqA protein (modified from references [26-27,29])



Asp7、Glu9和Arg30之间的氢键网络。Asp7、Glu9、Ser26和Arg30等重要氨基酸已标注。绿色代表第一个SeqA分子, 紫色代表第二个SeqA分子。
Network of hydrogen bonds between Asp7, Glu9 and Arg30. Amino acids Asp7, Glu9, Ser26 and Arg30 are indicated. Green and purple are representative of SeqA protomer 1 and SeqA protomer 2, respectively.

图2 SeqA蛋白质的多聚化表面(根据参考文献[29]修改)

Fig.2 The aggregation surfaces in SeqA (modified from reference [29])

而分子量远远大于单体的分子量[26]。研究者将SeqA的N-端($SeqA_{1-59}$)和C-端($SeqA_{71-181}$)分别表达纯化后进行凝胶过滤层析, 结果发现, 单体大小为6.8 kDa的$SeqA_{1-59}$实际分子量约80 kDa, 相当于十二聚体的大小, 而$SeqA_{71-181}$的实际分子量为13 kDa, 与其单体的分子量大小一致[27]。研究还发现, 在细菌细胞内有SeqA四聚体的存在[30], $SeqA_{1-59}$的十二聚体可能由三个四聚体再次多聚化而形成[27,30]。细菌双杂交实验验证了SeqA蛋白N-端介导的SeqA-SeqA的相互作用[30], 两个SeqA的N-端β折叠(βN1)以氢键相互配对形成二聚体[28], 两个二聚体又形成四聚体或多聚体。氢键主要在第一个SeqA主链的Asp7、Glu9和第二个SeqA侧链的Ser26、Arg30间互补连接, 从而稳定了二聚体结构。Asp7一方面锚定α螺旋(αA), 另一方面与第二个SeqA侧链Arg30连接, Glu9也与第二个SeqA侧链Arg30连接(图2)。SeqA Asp7和Glu9的缺失可导致二聚体减少, 从而与DNA的结合也减少[29,31]。

4 SeqA蛋白质与DNA相互作用

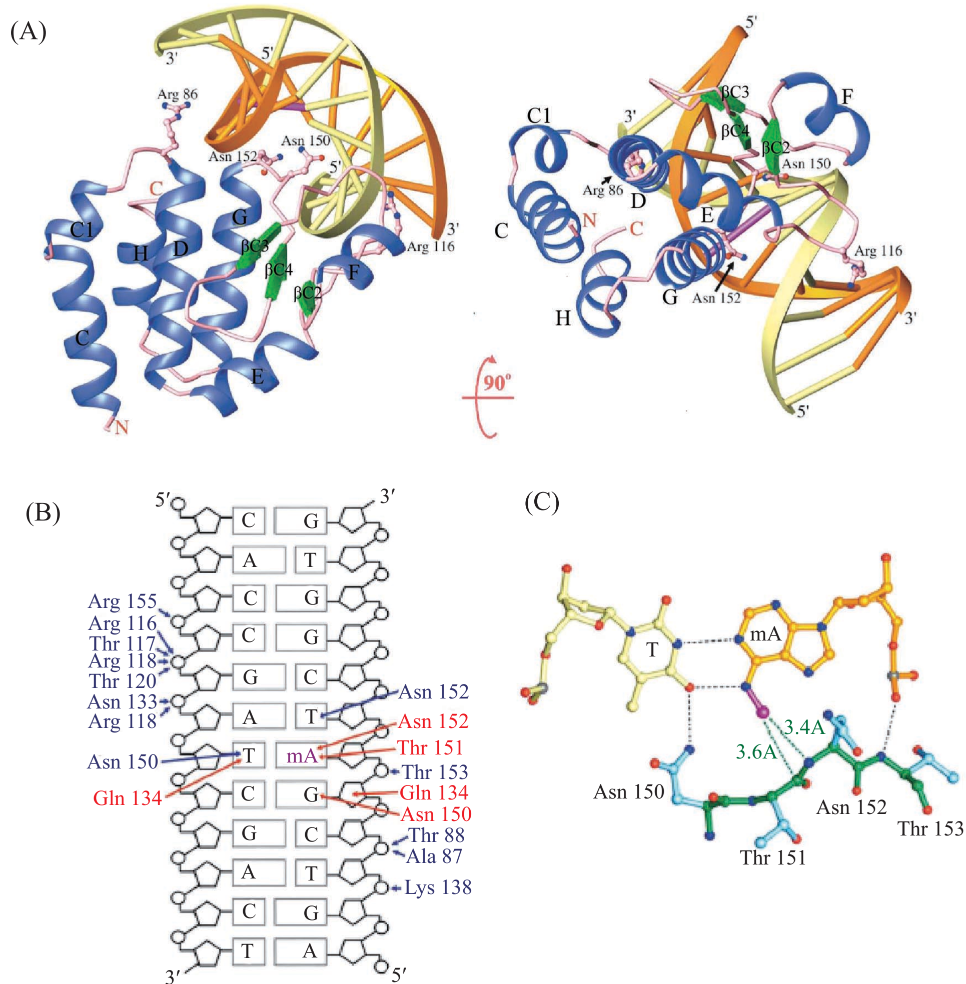
4.1 SeqA与DNA结合

GATC位点的半甲基化是SeqA结合的前提, 由Dam甲基化酶完成。依据目标识别结构域(target recognition domain, TRD)不同, 甲基化酶分为α、β、γ、ζ、δ和ε这六类, 大肠杆菌Dam(EcoDam)甲基化酶属于α类型, 在GATC的腺嘌呤N^6位点进行甲基化[32-34]。EcoDam甲基化酶在体内大约有130个分子, 参与众多细胞过程, 如DNA复制起始、基因表达调控、碱基错配修复等[35-37]。大肠杆菌染色体基因组大约有20 000个GATC位点, 其中大概130个甲基化的GATC位点(由EcoDam甲基化酶完成)高度配合多种细胞过程, 保证基因组的稳定性[35,38]。在大肠杆菌*dam*基因删除突变体中, 除了复制起始非同步以外, RNA和蛋白水平均有较大的变化, 进一步体现了甲基化的重要作用[39]。有趣的是, 越来越多的研究发现, 抑制细菌病原体毒素因子的甲基化成为开发新型抗生素

药物的一种战略[40]。

为了鉴定SeqA的DNA结合结构域, Fujikawa等[27]分别检测了SeqA的N-端($SeqA_{1-59}$)和C-端($SeqA_{71-181}$)与全甲基化、半甲基化和未甲基化DNA的结合能力。他们发现, $SeqA_{71-181}$与半甲基化的DNA形成稳定的复合物, 而N-末端$SeqA_{1-59}$不能与任何形式的DNA(全甲基化、半甲基化或未甲基化)形成复合物。因此, 他们认为, SeqA的C-端$SeqA_{71--181}$特异性识别半甲基化的GATC, 不识别未甲基化的GATC, 以低亲和力结合全甲基化的GATC[27]。

SeqA主要作用于DNA双螺旋大沟的GATC位点, 其C-端的α螺旋αD(氨基酸残基86~88)和αG(氨基酸残基152~155)以及αE-βC2环(氨基酸残基116~120)和αF-βC3环(氨基酸残基131~136)夹住半甲基化或全甲基化的DNA链, 推向DNA大沟中[26,28](图3A)。侧链的Arg86和Arg116伸向周围的小沟将DNA包起来, SeqA-DNA复合体900A^{2}的分子表面埋在里面(图3A)。连接α螺旋发夹束和αE-βC2(和αF-βC3)环的氨基酸残基148~152, 深入地插入到大沟中, 稳定SeqA与DNA的结合(图3A和图3B)。SeqA



A: SeqA-C与12 bp半甲基化DNA结合的两个结构图。蓝色表示α螺旋(αC、αC1、αD、αE、αF、αG和αH); 绿色表示β折叠(βC2、βC3和βC4)。Arg86和Arg116与小沟周围的GATC结合, Asn150和Asn152与中心的A-T结合。B: 半甲基化的GATC与SeqA-C的识别。Asn150和Asn152间形成的氢键与甲基化的A-T碱基对连接, Thr151和Asn152间的范德华力与GATC位点N6-甲基化的腺嘌呤(mA)连接, 形成SeqA-DNA复合物。C: 半甲基化的A-T碱基对。氢键和范德华力分别用蓝色和绿色的虚线表示。

A: ribbon diagram of a SeqA-C bound to the 12 bp hemimethylated DNA in two orthogonal views. α-helices (αC, αC1, αD, αE, αF, αG and αH) are blue; β-sheets (βC2, βC3 and βC4) are green. The unmethylated strand is yellow, and the methylated one is orange. Arg86 and Arg116 interact with the minor groove surrounding the GATC site, and Asn150 and Asn152 interact with the central A-T base pairs. B: recognition of hemimethylated GATC by SeqA-C. Asn150 and Asn152 stabilized by a hydeogen bonds and contects with methylated A-T base pairs. Thr151 and Asn152 bounds by close van der Waals and contects with the N6-methyl group of adenine (mA), and forms the SeqA-DNA complex. C: hemimethylated A-T base pairs. Hydrogen bounds and close van der Waals contacts are indicated by blue and green dashed lines.

图3 SeqA蛋白质与DNA的结合(根据参考文献[26]修改)

Fig.3 Interaction of SeqA protein and DNA (modified from reference [26])

的Asn150和Asn152由氢键稳定, 并形成短的氢键与A-T碱基对连接。Thr151和Asn152间形成范德华力与GATC位点N^6-甲基化的腺嘌呤(^mA)连接(图3C), 当G-C位点替换成A-T时SeqA不再与其结合, 说明G-C碱基在SeqA结合GATC位点过程中有重要作用[26]。

4.2 SeqA蛋白质影响DNA拓扑结构

快速生长的大肠杆菌染色体DNA复制和细胞分裂没有明显的间隔时间, 可以重叠进行, 这意味着DNA复制后立即浓缩成螺旋状态。一些类核相关蛋白(nucleoid associated proteins, NAPs)有助于DNA浓缩[41-43], 这类蛋白质包括HU、Fis(factor for inversion stimulation)和H-NS(histone-like nucleoid protein)等[44], 功能上与真核细胞的组蛋白类似。SeqA虽然没有归类为NAPs, 但是与*oriC*的半甲基化GATC位点相互作用, 并以SeqA高级结构(high order structure)形式辅助组织复制中的复制叉[45]和复制后DNA分子。SeqA与DNA的相互作用并不局限于*oriC*, 因为大肠杆菌染色体上的约20 000个GATC位点中2%的位点在对数生长的细胞中处于半甲基化状态, 这些位点中任意相邻两个半甲基GATC位点均可与SeqA相互作用[46]。SeqA二聚体先结合一个半甲基化GATC, 随后再结合第二个半甲基化GATC位点, 使DNA链弯曲, 从而稳定SeqA-DNA结构[47]。两个半甲基化的GATC位点相距31 bp时SeqA与DNA的结合较牢固[48]。无论在快速生长还是慢速生长的细胞中SeqA结构(SeqA structure)紧随着复制叉结合新合成的半甲基化GATC, 分别在引导链和滞后链上的两个SeqA结构之间保持30 nm左右的距离, SeqA蛋白结构与复制叉保持200~300 nm的距离[49]。在体外多聚化的SeqA抑制DNA负超螺旋, 而过表达SeqA可引起质粒DNA的正超螺旋[50], 体内SeqA的缺失导致负超螺旋的增加[50], 说明SeqA可通过自身多聚化影响DNA的构象[50-51]。

5 SeqA与ParC蛋白相互作用

染色体DNA的超螺旋结构由拓扑酶I(Topo I)、DNA解旋酶(DNA gyrase)和拓扑酶IV(Topo IV)调控, 其中DNA解旋酶可引入负超螺旋, 拓扑酶I解开负超螺旋, 拓扑酶IV则负责解开正超螺旋和负超螺旋[52]。通过细菌双杂交实验, Kang等[53]发现, SeqA与拓扑酶IV的ParC亚基[54]C-末端结构域相互作用, SeqA-T18和大肠杆菌基因组文库构建的T25-融合蛋白共同转化DHP1细胞, 得到两个与SeqA-T18相互作用的蛋白质, 一个SeqA-T25, 另一个为T25-ParC-CTD。SeqA-T18与SeqA-T25相互作用是SeqA的多聚化, 而体外实验表明, SeqA-T18与T25-ParC-CTD的相互作用可促进拓扑酶IV解开超螺旋的活性[53], 进一步解释了SeqA缺失突变体中DNA负超螺旋增加[51]是由SeqA与ParC相互作用所介导的[53]。

6 SeqA蛋白的生物学功能

6.1 *oriC*隔绝中的作用

SeqA主要生物学功能是在DNA复制起始后使*oriC*隔绝, 并且持续细胞周期1/3时间[55], 这种暂时的*oriC*隔绝能够定时DNA复制起始, 并确保在同一细胞周期中复制起始只发生一次[3-4]。*oriC*以外的GATC位点也会发生隔绝, 但是持续的时间较*oriC*区域要短[17-18], 而且发生隔绝的区域随着细胞分裂进入子细胞中[36,56]。*seqA*基因删除突变体会失去*oriC*隔绝, 其复制起始非同步[18]。同样, *dam*基因删除也会引起复制起始的非同步[57], 而过表达Dam甲基化酶使*oriC*隔绝时间缩短。在Δ*seqA*Δ*dam*双突变体中, *oriC*隔绝时间明显比野生型细胞短, 甚至不发生[18,58], 说明*oriC*隔绝是由SeqA和Dam甲基化酶共同控制的[18,59]。支持这个看法的是, 过表达SeqA可以延长*oriC*隔绝时间, 甚至可能持续整个细胞周期, 从而抑制DNA复制起始[60]。体外实验证实, Dam甲基化酶与*oriC*结合的亲和性较SeqA低, 因此, Dam也不能从半甲基化的*oriC*解离SeqA[59]。此外, 除了SeqA, DnaA似乎通过抑制*oriC* GATC位点重新甲基化的方式也参与*oriC*的隔绝[61]。

6.2 基因转录中的调控作用

SeqA也是一个转录因子, 可以参与基因表达调控。全基因组转录芯片分析发现, *seqA*删除突变体中一些基因的转录水平与野生型不同[62]。在野生型细胞中转录水平较高的基因在*seqA*删除突变体中其转录较低, 而野生型细胞中转录水平较低的基因在*seqA*删除突变体中却较高。染色体上约20 000个GATC位点的分布较广, 间隔4~4 000 bp不同。生物信息学分析发现, 相隔10、19、70和1 100 bp的两个GATC序列“簇”经常出现在离*oriC*较远的蛋白质读码框中[38], 但是没有发现这类基因的转录与SeqA有关。

有趣的是, *dnaA*基因启动子区有多个GATC位

表1 含有*seqA*基因的革兰氏阴性菌属

Table 1 The *seqA* gene was found in a subset of Gram negative bacteria

含有*seqA*基因的革兰氏阴性菌属 The *seqA* gene was found in a subset of Gram negative bacteria	相关文献 Related references
Escherichia coli(大肠杆菌)	[17-18,69]
Salmonella typhimurium(鼠伤寒沙门氏菌)	[66,69,73-75]
Yersinia pestis(鼠疫耶尔森菌)	[69]
Photorhabdus luminescens(发光杆菌)	[69]
Pasteurella multocida(多杀巴斯德杆菌)	[69]
Haemophilus influenzae(流感嗜血杆菌)	[69]
Vibrio cholerae(霍乱弧菌)	[68-69,76]
Shewanella oneidensis(沙雷菌)	[69]

点, 在对数生长期, *dnaA*基因启动子被隔绝的时间长达1/3世代, 与*oriC*隔绝时间相同。但是, 有更多GATC位点的*recB*基因(编码RecBCD解旋酶的RecB亚基)并没有隔绝那么长时间, 可能是由于DnaA结合自身的启动子区抑制基因转录, 从而帮助隔绝。位于*oriC*上游的*gidA*基因(离*oriC* 30~50 bp, 编码tRNA修饰酶的MnmG蛋白)的转录在复制起始10 min后"关闭", 是由SeqA引起的隔绝作用所致, 因为在*dam*或*seqA*删除突变体中其"转录关闭"现象消失。另外一个离*oriC*较近的*mioC*基因(编码生物素合成相关的黄素蛋白MioC)位于*oriC*下游, 其转录不被SeqA所抑制[63]。SeqA作为转录激活因子能够促进半甲基化或全甲基化的原噬菌体λP_R启动子活性[64]。*seqA*基因上游185 bp的基因间隔区没有GATC位点, 因此, SeqA不调控自身基因的转录, 而是由HU蛋白质抑制其转录[65]。在鼠伤寒沙门氏菌(*Salmonella enterica*)中SeqA与Dam甲基化酶和HdfR蛋白质(HNS-dependent flhDC regulator, HNS依赖的flhDC调节蛋白)协同调控*std*操纵子的表达[66]。

6.3 染色体分离和细胞分裂中的作用

Bach等[60]发现, 过表达SeqA, 除了延长*oriC*的隔绝时间外, 细胞分裂也推迟发生, 推测SeqA可能延迟已复制的两条染色体的分离[60]。只有在适量提高SeqA浓度时染色体分离才会有延迟现象, 说明并非SeqA特异性抑制染色体分离[60]。荧光显微镜成像定位实验发现, 少数SeqA-YFP定位在即将分离的细胞中间, 但并不与*ter*位点(染色体复制终止位点)相互共定位[67]。在霍乱弧菌(*Vibrio cholerae*)中过表达SeqA可抑制染色体复制起始, 且每个细胞中染色体DNA含量有所提高, 由此推测, SeqA可能抑制细胞分裂[68]。

7 总结与展望

通过与半甲基化的GATC位点特异性结合, SeqA不仅在复制起始调控中起重要作用, 而且参与基因表达调控。*seqA*基因在进化过程中并不保守, 只在革兰氏阴性菌中存在[17,69-70], 目前研究较多的有大肠杆菌、鼠伤寒沙门氏菌和霍乱弧菌[17,66,68](表1)。而在革兰氏阳性菌如新月柄杆菌(*Caulobacter crescentus*)和根瘤土壤杆菌(*Agrobacterium tumefaciens*)中, 由CcrM蛋白介导复制起始负调控, 但是CcrM并不与SeqA同源, 而是功能上类似于大肠杆菌Dam甲基化酶[71-72]。本文总结了SeqA的各结构域功能和生物学功能, 但是对于SeqA多聚体随着复制叉移动的机理、基因表达调控机制、染色体分离和细胞分裂中的作用机制尚不清楚。

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