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Transcriptional control of RfaH on polysialic and colanic acid synthesis by *Escherichia coli* K92

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ABSTRACT

The transcriptional antiterminator RfaH promotes transcription of long operons encoding surface cell components important for the virulence of *Escherichia coli* pathogens. In this paper, we show that RfaH enhanced *kps* expression for the synthesis of group 2 polysialic acid capsule in *E. coli* K92. In addition, we demonstrate for the first time that RfaH promotes *cps* expression for the synthesis of colanic acid, a cell wall component with apparently no role on pathogenicity. Finally, we show a novel RfaH requirement for growth at low temperatures.

1. Introduction

Bacterial capsules are structures surrounding the cell surface which exhibit extraordinary diversity and confer important advantages upon micro-organisms. Nearly eighty capsular polysaccharides have been described in *Escherichia coli* alone [1]. *E. coli* K92 synthesizes two different capsular polysaccharides in a temperature-dependent manner [2]. When inside the host (37 °C), this bacterial strain synthesizes a Group 2 capsule known as polysialic acid (PA), a polysaccharide responsible for bacterial virulence [3,4]. The chromosomal loci responsible for PA biosynthesis is designated *kps* operon (Fig. 1A), has a conserved organization consisting of three regions [5] and its transcription is driven by two convergent temperature-regulated promoters located upstream of regions 1 and 3 [6,7]. Transcription in region 1 is driven by the region 1 promoter, whereas regions 2 and 3 are organized into one transcriptional unit under the control of the region 3 promoter. Unlike what happens in region 1, efficient transcription in regions 2 and 3 requires RfaH [8]. This acts as a transcriptional anti-terminator for large operons and its loss promotes transcriptional polarity without affecting initiation from the promoters [8]. RfaH-dependent operons share a short element termed *ops* (operon polarity suppressor) that is essential for rfaH function [8–10]. The *ops* sequence recruits RfaH and other factors to the RNA polymerase complex, increasing its processivity by reducing pausing and termination and allowing transcription to proceed over long distances [11].

The PA capsule in *E. coli* K92 is co-expressed with colanic acid (CA), an exopolysaccharide predominantly synthesized at low temperatures (20 °C), which provides protection against stressful conditions outside the mammalian host [12]. However, CA does not play a directly role in pathogenesis [13].

The *cps* CA operon (Fig. 1B) comprises one large transcription unit encoding proteins involved in colanic acid biosynthesis [14]. In addition, the *ugd* gene is located outside the *cps* CA operon but is also involved in colanic acid synthesis [5,14].

Previous findings suggest that the expression of the *cps* CA operon is mediated by RfaH [8]. Thus, this transcriptional anti-terminator is often required for the expression of long operons encoding bacterial capsules [8]. Furthermore, the *cps* CA operon in *E. coli* K-12 is preceded by *ops* elements [15]. RfaH also modulates the expression of *cps* encoding Group 1 K30 capsule with genetic organization and gene content is similar to *cps* CA [16]. However, despite the fact that the expressions of both *cps* K30 and *cps* CA operons share many regulatory characteristics, their regulatory mechanisms are quite different. Most notable are the facts that

Abbreviations: CA, colanic acid; Glc-Pro, glucose-proline; MM, minimal media; PA, polysialic acid; Xyl-Asn, xylose-asparagine

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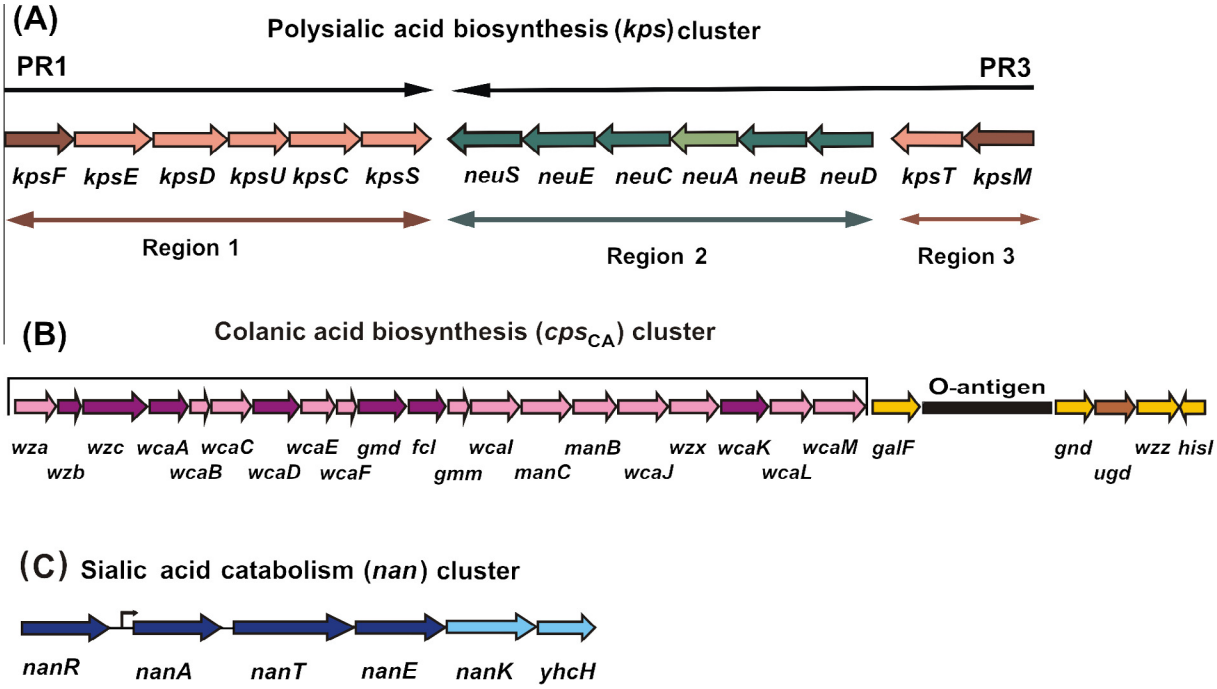

Fig. 1. Genetic organization of *E. coli* polysialic acid and colanic acid metabolism clusters: (A) Polysialic acid synthesis (*kps*), (B) colanic acid synthesis (*cps* CA) and (C) polysialic acid catabolism (*nan*). Dark arrows indicates the genes used in this study. PR1 and PR3: promoters located upstream of regions 1 and 3 in the *kps* cluster.

the virulent K30 antigen is expressed at 37 °C, whereas CA is mainly synthesized at low temperatures and that the Rcs phosphorelay controls *cps* CA gene expression [17] in contrast to *cps* K30 expression [16].

This report investigates the function of RfaH in *E. coli* K92 in controlling *kps* and *cps* gene expression for PA and CA biosynthesis, respectively, an RfaH-deficient mutant having been used for this purpose.

2. Materials and methods

2.1. Strains, culture media, and growth conditions

The strains and plasmids used in this study are shown in Table 1. Bacterial cultures were grown in Luria–Bertani (LB) complex medium, LA (LB supplemented with 2% w/v agar) and Xylose-Asparagine (Xyl-Asn) or Glucose-Proline (Glc-Pro) minimal media (MM) for *E. coli* K92. We chose Xyl-Asn or Glc-Pro MM because they induce maximal PA [19] and CA production in *E. coli* K92, respectively [18]. When indicated, Glc-Pro MM was supplemented with agar 2% (w/v). During the allelic exchange experiments, LA medium (LB supplemented with 2% (w/w) agar), with the addition of with 5% (wt/vol) sucrose, and without NaCl, was used to select plasmid excision from the chromosome [20]. When required, the following supplements were added to the culture media: rifampicin (25 and 10 μg/ml for liquid and solid media, respectively), ampicillin (100 μg/ml), and chloramphenicol (30 μg/ml).

2.2. DNA manipulations, RNA isolation and qRT-PCR

Routine molecular biology techniques, including the use of restriction enzymes, plasmid DNA and RNA isolation, mobilization of plasmids between *E. coli* strains, DNase treatment, reverse transcription and qPCR (qRT-PCR), were performed as previously

Table 1
Strains, plasmids and constructions used in this work.

	Description	Reference or source
E. coli strains		
DH5α′	F$^-$ *Δlac*U169 □80d*lac*Z1M15 *hsd*R17 *rec*A1 *end*A1 *gyr*A96 *thy*-1 λ$^-$ *rel*A1 *sup*E44 *deo*R	[35]
S17λpir	λ*pir recA thi pro hsdR* M$^+$, RP4:2-Tc::Mu::km Tn7 Tp^r Sm^r	[36]
K92	Wild type	ATCC 35860
K92*ΔrfaH*	K92*ΔrfaH::cat*; constructed using pDS132-UD	This work
K92*ΔrfaHprfaH*	*E. coli* K92 harboring plasmid pMCrfaHxp	This work
Plasmids and constructions		
pGEM-TEasy	Ap^r *ori*ColE1 *lacZ*α$^+$ SP6 T7 *lac* promoter, direct cloning of PCR products	PROMEGA
pDS132	R6K ori mobRP4 cat sacB	[37]
pGEM-U	*rfaH* upstream sequences PCR amplified with primers rfaHup5′ and rfaHup3 cloned into pGEMT-easy; Ap^r	This work
pGEM-D	rfaH downstream sequences PCR amplified with primers rfaHdown5′ and rfaHdown3 cloned into pGEMT-easy; Ap^r	This work
pGEM-UD	*ΔrfaH*; *rfaH* upstream sequence from pGEM-U removed with EcoRI and ligated with *rfaH* downstream sequence from pGEM-D removed with EcoRI; Ap^r	This work
pDS132-YZ	ΔrfaH sequences from pGEM-UD removed with SacI and SphI and inserted into pDS132 digested with the same enzymes; Cat^r	This work
pGEM*rfaHxp*	*rfaH* sequences PCR amplified with primers rfaHxpup and rfaHxpdown cloned into pGEMT-easy; Ap^r	This work
pBBR1MCS-3	Broad host range-cloning vector, Tc^R	[21]
pMC*rfaHxp*	*E. coli* K92*ΔrfaH* cloned into pBBR1MCS-3	This work