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A method for the construction of in frame substitutions in operons: Deletion of the essential *Escherichia coli holB* gene coding for a subunit of the DNA polymerase III holoenzyme

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Abstract

To investigate the putative five-gene operon at 24.9 min on the *Escherichia coli* genome, which comprises the genes *pabC*, *yceG*, *tmk*, *holB* and *ycfH*, a method for the construction of an in frame deletion strain of the essential *E. coli holB* gene was developed. HolB, also referred to as delta prime or δ′, is a subunit of the DNA polymerase III (Pol III) holoenzyme. The *holB* gene was replaced by the kanamycin resistance gene *kka1*, coding for amino glycoside 3′-phosphotransferase kanamycin kinase. The kanamycin resistance gene was expressed under the control of the promoter(s) of the putative five-gene operon. The *holB* gene is essential for bacterial growth and the deletion of *holB* exhibits no polar effects on the adjacent genes *tmk* or *ycfH* in terms of cell viability. The method of the *holB* null construction presented in this work allows for a simplified studying of interactions between the different subunits of DNA polymerase III.

Keywords: Co-transduction; DNA polymerase III; Gene substitution in operons; *holB*; In frame deletion

1. Introduction

The *E. coli holB* gene product designated HolB, delta prime or δ′, is a subunit of the *E. coli* DNA polymerase III. The DNA polymerase III (Pol III) holoenzyme is essential for bacterial growth and is highly processive. It is responsible for the major part of DNA synthesis during the replication of the *E. coli* chromosome and is composed of at least 10 subunits (α, ε, θ, τ, γ, δ, δ′, χ, ψ, and β) (Kornberg and Baker, 1991; McHenry, 2003). HolB is thought to stimulate the DNA-dependent ATPase activity in the so-called clamp loading complex (Carter et al., 1993; O'Donnell et al., 2001; Onrust and O'Donnell, 1993). However, in vitro studies showed that replication activity can be reconstructed without HolB. HolB is supposed to stimulate the assembly of the subunits of the DNA polymerase and to increase the ATPase activity of the γ complex (Onrust et al., 1991). Recent results indi-

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cate an essential role of HolB in DNA synthesis (Song et al., 2001).

The *holB* gene is located downstream of the *tmk* gene, coding for thymidylate kinase at 24.9 min on the *E. coli* chromosome. The *holB* gene was first identified using a reverse genetic approach (Carter et al., 1993) and found to be 1002 nucleotides long. Sequencing data showed a ribosomal binding site (RBS), a putative promoter site, and overlap of the *holB* structural gene and the *tmk* gene. The *holB* gene codes for a 36.9 kDa protein and its mRNA shows a rare codon usage, indicating a low HolB overexpression level (Konigsberg and Godson, 1983). Two HolB variants of similar protein mass appear on SDS-PAGE when detected with antibodies against the holoenzyme of DNA polymerase III (Dong et al., 1993). HolB has sequence similarity to other prokaryotic HolB proteins, to the *dnaX* gene products of *E. coli* (Dong et al., 1993; Flower and McHenry, 1986), to the replication factor C of HeLa cells (Chen et al., 1992), and to the gene *44* product of bacteriophage T4 (Spicer et al., 1984). Although the homology to the last two proteins is rather low, they are all involved in DNA replication.

In this work, a method for the construction of a *holB* deletion strain was established in which the *holB* gene has been replaced by the kanamycin resistance gene *kka1*. Co-transduction experiments indicate that *holB* is essential for growth of *E. coli*. No polarity effects on viability due to the deletion construct were observed under the conditions tested. The *holB* deletion construct presented in this report allows a simplified studying of interactions of the components of the clamp loading complex, or of *holB* homologues from other organisms. This article describes a method of gene substitutions in operons in the presence of a plasmid-encoded gene, facilitating the introduction of selectable markers in essential genes for complementation experiments.

2. Materials and methods

2.1. Bacterial strains, plasmids and phages, media and culture conditions

The bacterial strains, plasmids, and phages used and constructed during the course of this study are listed in Table 1. LB medium containing 10 g of tryptone, 5 g of yeast extract and 5 g of NaCl per liter, pH 7.0, was used for bacterial growth. For LB agar plates, 10 g of agar was added per liter of medium. For selection of antibiotic resistance in liquid and solid medium, 100 μg/ml ampicillin, 25 μg/ml kanamycin, 10 μg/ml chloramphenicol and 10 μg/ml tetracycline were used. Cells were grown at 37 °C with the exception of strains harboring the insertion *yceG*::miniTn*10* (Kan^r) or when otherwise indicated.

2.2. PCR, oligonucleotides and plasmid construction

Polymerase chain reactions were performed with *Pfu* DNA polymerase according to the manufacturer's protocol (Promega, Madison, WI, USA). The plasmid pSR1613 (Table 1) served as template in the PCR reactions and for the amplification of the kanamycin resistance gene *kka1*, the plasmid pACYC177 was used. The PCR fragment used in linear DNA transformation was obtained using plasmid pDC10 as template.

DNA sequences of PCR primers used in this work: Primer 1 (N-*tmk* 5′→3′): 5′-GGAGGAATTCACCATGCGCAGTAAGTATATCGT-3′, primer 2 (C-*tmk* 3′→5′): 5′-ACGCGCATGCTCATGCGTCCAACTCCTTC-3′, primer 3 (N-*holB* 5′→3′): 5′-GGAGGAATTCACCATGAGATGGTATCCATGGTTA-3′, primer 4 (C-*holB* 3′→5′): 5′-ACGCGCATGCTTAAAGATGAGGAACCG GTA-3′, primer 5 (5′ of the operon): 5′-GTAGTGGCGGGCGAGG-3′, primer 6 (N-*tmk* 3′→5′): 5′-ATCTGCATGCTTCCAGCCCCTCAATG-3′, primer 7 (C-*tmk* 5′→3′): 5′-ATCTGCATGCTGGGTGAAGGAGTTGG-3′, primer 8 (*SalI ycfH* 3′→5′): 5′-GCCAGAACGTCATCCACGTC-3′, primer 9 (N-*holB* 3′→5′): 5′-ATCTGCATGCTCTCATGCGTCCAACTC-3′, primer 10 (C-*holB* 5′ →3′): 5′-ATCTGCATGCCTTTAAGAGAGACATCATGTTTT-3′, primer 11 (universal primer): 5′-TAATACGCTCACTATAGGG-3′, primer 12 (N-Kan 5′→3′): 5′-ATCTGCATGCTAAGTTATGAGCCATATTCAAC-3′, primer 13 (C-Kan 3′→5′): 5′-ATCTGCATGCCATTTAGAAAAACTCATCGAGCA-3′, primer 14 (C-*yceG* 5′→3′): 5′-ATCTGCATGCCCAGGTGCGATAGCGA-3′. Lyophilized primers synthesized by Invitrogen Ltd. (Paisley, UK) were dissolved in H_2O and stored at −20 °C.