Filling the gap between *hns* and *adhE* in *Escherichia coli* K12

Antoine Danchin and Evelyn Krin

Author for correspondence: Antoine Danchin. Tel: +331 45 68 84 41. Fax: +331 45 68 89 48.
e-mail: adanchin@pasteur.fr

Régulation de l'Expression
Génétique, Institut
Pasteur, 28 rue du Docteur
Roux 75724 Paris Cedex 15,
France

As a consequence of the absence of a general coordination process in the sequencing of the *Escherichia coli* K12 genome, to completely sequence the genome in a reasonable time it is important to fill in gaps between known regions. We report the sequence of the *hns*–*adh* region, at 27 min on the genome.

Keywords: *Escherichia coli* K12, *hns*, *tdk*, IS4, genome sequencing

The region including the *hns* gene is somewhat unusual in *Escherichia coli* because this gene has been identified as being involved in many different cellular functions, e.g. virulence, control of osmolarity, control of pili or curli synthesis (see references quoted by Lucht *et al.*, 1994, and Tupper *et al.*, 1994). Moreover the *hns* gene itself has been sequenced several times, and mapped at different positions on the genome. In particular the work by Gualerzi and co-workers gave a mistaken location of the gene because the sequenced fragment was presumably chimaeric (Pon *et al.*, 1988). It is therefore interesting to place the gene in context. Here we report the 3060-nucleotide sequence encompassing most of the *hns* gene as well as the upstream region bordering the *adh* gene.

The sequence was obtained by subcloning into pBR322 a fragment complementing an *hns* defect, and then screening for thymidine kinase activity. The restriction map of the cloned fragment is identical to the known restriction map of the region (see Fig. 1). The fragment was then sequenced on both strands using the method of Sanger *et al.* (1977). Table 1 lists the characteristics of the *hns*–*adhE* region.

The sequenced region also includes the *tdk* gene, encoding thymidylate kinase, and an unknown coding sequence (CDS) which encodes a putative protein displaying significant similarity to an hypothetical 442-residue protein encoded by the *E. coli* insertion element IS4 (see Fig. 2).

In addition, a small segment of the 657 bp region of the *E. coli* genome upstream of an IS5 insertion, in which Liu & Reeves (1994) proposed that a rearrangement had oc-

Fig. 1. Restriction map of the *hns*–*adhE* region. Known sequences, present in the EMBL database [accession numbers X59940 (ECMSYA), X53733 (ECTDKG), and M33504 (ECADHE)], are aligned with respect to the base positions of the sequenced fragment. Restriction sites of the Kohara map are also shown, and the gene coding sequences, arrowed, are displayed underneath the restriction map.

Table 1. The *hns*–*adhE* region

Feature	Position	Identification	Orientation
<i>hns</i> CDS	1–405	Experimental	Complement
<i>hns</i> RBS	414–417	Similarity	Complement
<i>hns</i> promoter –10	447–453	Similarity	Complement
<i>hns</i> promoter –35	471–476	Similarity	Complement
<i>tdk</i> promoter –35	937–942	Similarity	Direct
<i>tdk</i> promoter –10	959–964	Similarity	Direct
<i>tdk</i> RBS	995–998	Similarity	Direct
<i>tdk</i> CDS	1010–1624	Experimental	Direct
IS4 insert-like CDS	1912–2499	Pattern similarity	Complement
Palindrome	2809–2838	Pattern	Direct
Palindrome	2850–2875	Pattern	Direct
<i>adhE</i> CDS	2932–3060	Similarity	Complement

Abbreviations: CDS, coding sequence; RBS, ribosome-binding site.

The EMBL accession number for the nucleotide sequence reported in this paper is X67326.

IS4	124	WCGLTLLAIDGVFWRTPDTPENDAAF-PRQTHAGNPALYPQVKMVCQEMELTSHLLTAAAF
CDS	20	WHGLQLFAIDGAQFRTPDKPELREYYGSANTSTKRQNAYPVMRLVALMNLGSHILLNAV
IS4	183	GTMKNSENELAEQLIEQTGDNTLTLMKGYYSLGLLNAWSLAGEHRHWMIPLRKGAQYEE
CDS	80	APYRQSETVLAHSMLATIPDNSITLFDKLFYSEDLTLNQKGCNRHWLLPAWKNIASE
IS4	243	IRKLGKG(insert)ASPGRIPELMRDLASMGQLVKLPTRRERAFPRVVKERPWKYP
CDS	139	MIELGNT ASPGTIPKRLEHLRGALEVVFITKRPRPSRPSVKISKTRYP

Fig. 2. Comparison of amino acid sequences of IS4-encoded protein and IS4 insert-like CDS.

curred, is almost identical to part of this IS4-encoded protein. It seems therefore that the CDS identified in the *hns-adbE* region, which is similar to an IS4-encoded protein, is a scar of some insertion event, a phenomenon which is likely to be observed elsewhere on the *E. coli* genome.

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