Transcriptional regulation of a hybrid cluster (prismane) protein

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Abstract
HCP (hybrid-cluster protein) contains two Fe/S clusters, one of which is a hybrid [4Fe-2S-2O] cluster. Despite intensive study, its physiological function has not been reported. The Escherichia coli hcp gene is located in a two-gene operon with hcr, which encodes an NADH-dependent HCP reductase. E. coli HCP is detected after anaerobic growth with nitrate or nitrite: possible roles for it in hydroxylamine or nitric oxide reduction have been proposed. To study the regulation and role of HCP, an hcp: lacZ fusion was constructed and transformed into fnr, arcA and narR mutant strains of E. coli. Transcription from the hcp promoter was induced during anaerobic growth. Only the fnr mutant was defective in hcp expression. Nitrate- and nitrite-induced transcription from the hcp promoter was activated by the response regulator proteins NarL and NarP. Gel retardation assays were used to show that FNR (fumarate-nitrate regulation) and NarL form a complex with the hcp promoter. Transcription of the hcp-hcr operon initiates at a thymine nucleotide located 31 bp upstream of the translation-initiation codon. HCP has been overexpressed from a recombinant plasmid for physiological studies.

Introduction
The HCP (hybrid-cluster protein) was initially purified from strictly anaerobic sulphate-reducing bacteria of the genus Desulfovibrio: D. vulgaris (Hildenborough) [1] and D. desulfuricans [2]. This protein contains two types of Fe/S cluster: a [4Fe-4S] cubane cluster and a novel type of hybrid [4Fe-2S-2O] cluster, which can attain four redox states.

The hcp gene encoding the HCP in Escherichia coli and other facultative anaerobes occurs, in contrast with hcp genes in obligate anaerobic bacteria and archaea, in a small operon with a gene encoding a putative NADH oxidoreductase. The NADH oxido-reductase catalyses the reduction of the HCP with NADH as an electron donor [3]. Western hybridization demonstrated that, in the facultative anaerobes E. coli and Morganella morgani, HCP is detected after cultivation under anaerobic conditions in the presence of nitrate or nitrite, which suggested a role for HCP in nitrate or nitrite respiration [3].

In Salmonella enterica, hcp transcription was shown to be up-regulated in the presence of acidified nitrite [4], which is regarded as an in vitro model to mimic reactive nitrogen intermediates. These authors suggested that the hcp promoter is regulated by physiological nitrogen oxides as it was up-regulated in activated macrophage-like cells, which produce NO through the inducible nitric oxide synthase.

The aims of this work were to determine the transcription start point of the hcp/hcr operon of E. coli and to study the transcription activator FNR (fumarate-nitrate regulation) in response to anaerobiosis, and by transcription regulators NarL and NarP in response to nitrite and nitrate.

Materials and methods
Media and growth of strains
Strains used during this study were E. coli RK 4353 (ΔlacU169 araD139 rpsL gyrA) [5], JCB 302 (Δa chl+ RV) [6], JCB 3893 (JCB302 narL), JCB 3894 (JCB302 narL narP) and JCB 3875 (Δnir Δlac narP). E. coli strains were grown anaerobically at 37°C in MS medium (4.5 g/l KH2PO4, 10.5 g/l K2HPO4, 1 g/l NH4SO4, 0.05 g/l MgCl2, 1 μM ammonium molybdate, 1 μM sodium selenite and 1 ml/l of E. coli sulphur-free salts), supplemented with 2.5 g/l nutrient broth, 20 mM NaNO3 and 0.5% (w/v) glucose.

Subcloning
The promoter region of gene ybjW (hcp) was isolated by PCR amplification of genomic DNA from an E. coli K-12 strain RK 4353. Two primers were designed for amplification of the hcp promoter and its cloning into the promoter probe vector pAA182 [7] to create plasmid pNF383. The primers were 5′-aaagaatggtggcgcgag-3′ and 5′-aaaagaagtctcctgctgctgctag-3′.

Chemical assays
For β-galactosidase assays, the method described in [7] was used. The 5′-RACE System for rapid amplification of cDNA ends (Invitrogen) was used to identify the transcription start site. Gel retardation assays with purified FNR and NarL proteins were made as detailed in [8].

Key words: anaerobiosis, hybrid-cluster protein, iron-sulphur-oxygen cluster, nitrogen cycle, transcription start mapping, transcriptional regulation.

Abbreviations used: FNR, fumarate-nitrate regulation; HCP, hybrid-cluster protein.

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Results

Transcription start mapping of the hcp/hcr operon and gel retardation assays
To facilitate the in vitro work, the transcription initiation site of the hcp/hcr operon was determined. Total RNA of RK4353 expressing HCP was isolated and used as a template in a reverse transcription reaction to synthesize cDNA. The cDNA was poly-C tailed and used as a template for a PCR. One transcription initiation site of the hcp-hcr operon was identified, located at a thymine nucleotide 31 bp upstream of the translation-initiation codon (Figure 1). The binding of FNR and NarL to the hcp regulatory region was investigated by gel retardation assays, using the EcoRI–HindIII hcp fragment and purified FNR and NarL proteins. The results indicated that both FNR and NarL bind to the hcp promoter. The FNR-binding site was located at nucleotide 73 upstream from the transcription start site. Formally this promoter arrangement is similar to that of the napF (periplasmic nitrate reductase) gene, indicating that the hcp promoter is a class I FNR-dependent promoter [9].

Nitrate- and nitrite-mediated expression of the hcp is dependent on NarL and NarP
Sequence analysis of the hcp regulatory region suggested that it contains a binding site for FNR and also binding sites for nitrite- and nitrate-activated regulators, NarL and NarP (Figure 1). We investigated the possibility that NarL and NarP are the hcp gene regulators by examining in vivo expression of hcp: lacZ fusion in the narL+, narP+, narL, narP and narL narP strains. As shown in Table 1, in the narL+ narP+ parental strain nitrate- and nitrite-mediated activity of the promoter was approx. 2.5-fold higher than that during anaerobic growth in the absence of nitrite or nitrate. In the narL or narP single mutants, nitrate and nitrite induction was retained. In contrast, when genes for both regulators were deleted, nitrite- and nitrate-mediated induction was completely abolished. This demonstrated that either NarL or NarP is required to activate transcription from the hcp promoter.

Concluding remarks
The hcp gene has been shown to be regulated by FNR, NarL and NarP proteins. Its up-regulation in the presence of nitrite and nitrate suggests that HCP is involved in nitrite or nitrate reduction. Being a redox-active protein, based on the structural and spectroscopic studies [3,10,11], HCP appears to accomplish an as yet unidentified reaction in the nitrogen cycle. To investigate this possibility HCP has been overexpressed from a recombinant plasmid for physiological studies.

Table 1 | Effects of nitrate and nitrite and response regulators NarL and NarP on the hcp promoter
<table>
<thead>
<tr>
<th>Genotype</th>
<th>Anaerobic</th>
<th>Anaerobic + NO₂⁻</th>
<th>Anaerobic + NO₃⁻</th>
</tr>
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<td>14 400</td>
<td>36 500</td>
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<td>12 500</td>
<td>36 000</td>
<td>42 500</td>
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<tr>
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<tr>
<td>narL narP</td>
<td>7 300</td>
<td>11 000</td>
<td>10 100</td>
</tr>
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References

Received 25 September 2004