

**P039** Transcriptional Regulation and Physiological Studies  
of a Hybrid Cluster Protein

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Hybrid Cluster Protein (HCP) 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 *E. coli hcp* gene is located in a two-gene operon with *hcr*, which encodes an NADH-dependent HCP reductase. *E. coli* HCP is only 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, a *hcp::lacZ* fusion was constructed and transformed into *fnr*, *arcA* and *norR* 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 response proteins NarL and NarP. Gel retardation assays were used to show that FNR and NarL form a complex with the *hcp* promoter. The *hcp* mutation had no effect on anaerobic, nitrate-dependent growth of strains that express only the periplasmic or the membrane-associated nitrate reductase.

It was shown that transcription of the *hcp-hcr* operon initiates at thymine nucleotide located 31 bp upstream of the translation-initiation codon. Further work includes overexpression experiments and physiological studies of HCP.