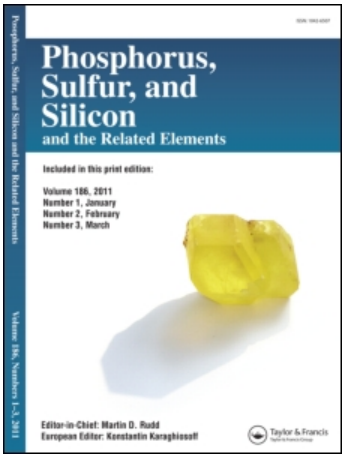


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# FACTORS CONTROLLING SYNTHESIS OF A BACTERIAL SELENOPROTEIN

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# FACTORS CONTROLLING SYNTHESIS OF A BACTERIAL SELENOPROTEIN

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Abstract Organisms from bacteria to mammals contain a highly discriminatory system for insertion of selenium into polypeptides. Elucidation of the mechanisms governing this system could illuminate various aspects of gene expression and metabolism. The bacterium Escherichia coli produces two selenium-dependent formate dehydrogenase enzymes under anaerobic conditions. They are expressed alternatively in the presence ($FDH_N$) or absence ($FDH_H$) of nitrate. Factors controlling the synthesis of $FDH_H$ were studied using fusions of the $FDH_H$ gene with the beta-galactosidase structural gene. Unlike other anaerobically-expressed proteins, inhibitors of DNA supercoiling increase synthesis of the gene product. An in vitro system for coupled transcription and translation of the fusion gene has been analyzed to determine components essential for selenium incorporation into polypeptides.



## INTRODUCTION

Mammals, birds and several species of bacteria incorporate selenium as selenocysteine at specific sites of a few proteins.[1] In all known cases, the messenger RNA codon for selenocysteine is UGA, which normally is a stop codon terminating synthesis of the polypeptide chain. The mechanism by which selenocysteine is incorporated into protein remains a mystery.

The bacterium Escherichia coli produces a selenocysteine-containing enzyme, hydrogenase-linked formate dehydrogenase ($FDH_H$), under anaerobic conditions in the absence of nitrate.[2] The gene encoding $FDH_H$ has been cloned, isolated and its sequence determined

in the laboratory of August Bock.[3] Fusions of the gene to the β-galactosidase structural gene have provided a facile assay for factors affecting $FDH_H$ expression.

We have found that in vivo expression of formate dehydrogenase is increased under conditions where the DNA is less supercoiled. This is in direct contrast to the results found for other anaerobically-induced genes. This difference may be due to differences in expression regulation. An in vitro system for transcription and translation of the formate dehydrogenase gene is being constructed. This will provide a system for identification of the biochemical machinery necessary for selenocysteine incorporation.

## ANAEROBIC GENE EXPRESSION

Facultative anaerobes such as E. coli are able to change the expression of the enzymes of oxidative metabolism due to changes in the available electron acceptor.[4] Thus, the efficiency of metabolism is maximized by expressing metabolic enzymes which use the available electron acceptor with the highest redox potential.

Two regulatory genes control the expression of anaerobically-induced enzymes. The fnr gene is required for expression of anaerobic respiration enzymes, while the oxrC gene regulates synthesis of anaerobic fermentation enzymes.[5] The mechanism by which these genes control other genes is not known at present.

Recent reports[6-8] have suggested that anaerobically-induced genes require the activity of DNA gyrase for expression. DNA gyrase is an enzyme which adds negative supercoils to DNA.[9] A model has been proposed whereby anaerobiosis causes the DNA of facultative anaerobes to become more supercoiled, and this induces the expression of enzymes required for anaerobic metabolism.

## FORMATE DEHYDROGENASES

In the absence of oxygen, E. coli oxidizes formate with either of two enzymes.[4] If nitrate is available, it is the preferred electron acceptor, and electrons are shuttled to it via the nitrate reductase-linked formate dehydrogenase ($FDH_N$). In the absence of nitrate, formate is oxidized by the formate-hydrogen lyase complex, which includes a distinct formate dehydrogenase ($FDH_H$).

Both of these formate dehydrogenases contain a polypeptide subunit in which selenium is specifically incorporated as selenocysteine. $FDH_H$ contains a 80 kDa selenopolypeptide, whereas that of $FDH_N$ is 110 kDa.

The gene for the 80 kDa selenopolypeptide of $FDH_H$ has been cloned and isolated in the laboratory of August Bock.[3] Fusions have been made which link portions of this gene and its promoter with the structural gene for β-galactosidase.[10] Using these fusions Bock's group has found that selenium is co-translationally inserted into the growing polypeptide chain.

We have used these fusion genes to analyze factors which affect $FDH_H$ expression. Unlike other anaerobic-specific enzymes, the expression of the $FDH_H$ selenopolypeptide does not require the activity of DNA gyrase. Rather, inhibition of gyrase activity enhances the expression of this $FDH_H$ subunit. This was found by observing fusion protein synthesis when gyrase activity was inhibited with any of three chemical inhibitors or a temperature-sensitive mutant.

The chemical gyrase inhibitors were added to E. coli cells upon switching to anaerobic conditions. The inhibitor coumermycin gave the greatest enhancement of expression, with fusion protein levels reaching 3.5 times that of controls at 25 μg/ml coumermycin. The gyrase inhibitors novobiocin and nalidixic acid also induced significant increases in fusion protein synthesis.

The fusion gene was introduced into an E. coli strain (a gift of Dr. M. Gellert) with a temperature-sensitive mutation in the

gyrase protein. At 42°C this strain loses greater than 99% of the activity of gyrase.[11] Fusion protein synthesis by this strain is enhanced about 30% at 42 C as compared to 30°C.

Addition of coumermycin partially overcame repression of fusion protein synthesis due to nitrate, but not oxygen. This suggests oxygen conveys a stronger repressive signal as has been shown previously.[12]

Gyrase inhibition also enhances expression of the intact $FDH_H$ selenopolypeptide in addition to the fusion protein. This was shown by labeling cells with $^{75}Se$ under anaerobic conditions in the presence and absence of coumermycin. Gel electrophoresis of the labeled proteins revealed an enhancement of $FDH_H$ selenopolypeptide synthesis due to gyrase inhibition. When nitrate was made available to the cells, a decrease of $FDH_N$ selenopolypeptide synthesis was observed upon addition of a gyrase inhibitor. Thus, $FDH_N$ can be added to the list of anaerobic-specific enzymes whose expression requires gyrase activity, although $FDH_H$ is a striking exception to that rule.

The reason that $FDH_H$ expression differs from that of other anaerobic gene products is not clear at present. All of the anaerobic enzymes whose expression require gyrase activity are involved in respiration, and thus their expression is regulated by the *fnr* gene. However, $FDH_H$ is a fermentative enzyme primarily under the control of the *oxrC* gene. We propose that *fnr*-regulated enzymes require gyrase activity for expression, while oxrC-regulated genes do not. The gyrase effect could be due either to alterations in the synthesis of the regulatory gene products, or to altered binding of regulatory proteins to the control regions of the genes.

## IN VITRO TRANSLATION

An *in vitro* system for synthesis of formate dehydrogenase would allow dissection of the essential biochemical components for selenium utilization. Such a system is presently being developed

in our laboratory. The fusion gene has been synthesized *in vitro* using a cell-free extract from wild-type *E. coli* cells. This occurs whether or not the selenocysteine-encoding TGA codon is present.

A mutant of *E. coli* has been isolated which is unable to incorporate selenium into protein, although it will incorporate selenium into tRNA.[10] We plan to construct the *in vitro* transcription-translation system using extracts from these mutant cells. Such a system will be unable to synthesize the fusion protein from genes containing the selenocysteine codon. Addition of extracts of wild-type cells will restore the ability to incorporate selenium and thus synthesis of the fusion protein. This provides an assay for isolation and identification of biochemical components essential for selenium incorporation.



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