Controlled Expression and Functional Analysis of the Iron-Sulfur Cluster (Isc) Biosynthetic Machinery in *Azotobacter vinelandii*

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(ABSTRACT)

A system was developed for the controlled expression of genes in *Azotobacter vinelandii* by using genomic fusions to the sucrose catabolic regulon. This system was used for the functional analysis of the *A. vinelandii* *isc* genes, whose products are involved in the maturation of [Fe-S] proteins. For this analysis the *scrX* gene, contained within the sucrose catabolic regulon, was replaced by the *A. vinelandii* *iscS, iscU, iscA, hscB, hscA, fdx, iscX* gene cluster, resulting in duplicate genomic copies of these genes, one whose expression is directed by the normal *isc* regulatory elements (P*isc*) and the other whose expression is directed by the *scrX* promoter (P*scrX*). Functional analysis of [Fe-S] protein maturation components was achieved by placing a mutation within a particular P*isc*-controlled gene with subsequent repression of the corresponding P*scrX*-controlled component by growth on glucose as the carbon source.

This experimental strategy was used to show that IscS, IscU, HscBA and Fdx are essential in *A. vinelandii* and that their depletion results in a deficiency in the maturation of aconitase, an enzyme that requires a [4Fe-4S] cluster for its catalytic activity. Depletion of IscA results in null growth only when cells are cultured under conditions of elevated oxygen, marking the first null phenotype associated with the loss of a bacterial IscA-type protein. Furthermore, the null growth phenotype of cells depleted for HscBA could be partially reversed by culturing cells under conditions of low oxygen. These results are interpreted to indicate that HscBA and IscA could have functions related to the protection or repair of the primary IscS/IscU machinery when grown under aerobic conditions. Conserved amino acid residues within IscS, IscU, and IscA that are essential for their respective functions and/or display a partial or complete dominant-negative growth phenotype were also identified using this system. Inactivation of the IscR repressor protein resulted in a slow growth phenotype that could be specifically attributed to the elevated expression of an intact [Fe-S] cluster biosynthetic system.
This system was also used to investigate the extent to which the two [Fe-S] biosynthetic systems in *A. vinelandii*, Nif and Isc, can perform overlapping functions. Under normal laboratory growth conditions, no cross-talk between the two systems could be detected. However, elevated expression of Isc components as a consequence of inactivation of the IscR repressor protein results in a modest ability of the Isc [Fe-S] protein maturation components to replace the function of Nif-specific [Fe-S] protein maturation components. Similarly, when expressed at very high levels the Nif-specific [Fe-S] protein maturation components could functionally replace the Isc components. Oxygen levels were also found to affect the ability of the Nif and Isc systems to perform common functions. Nevertheless, the lack of significant reciprocal cross-talk between the Nif and Isc systems when they are produced only at levels necessary to satisfy their respective physiological functions, indicates a high level of target specificity with respect to [Fe-S] protein maturation.
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# TABLE OF CONTENTS

ABSTRACT ....................................................................................................................ii  
ACKNOWLEDGEMENTS ...................................................................................................iv 
TABLE OF CONTENTS ...............................................................................................v 
LIST OF FIGURES ............................................................................................................vii  
LIST OF TABLES .............................................................................................................x

## CHAPTER 1: Introduction to [Fe-S] Cluster Biosynthesis:
The requirement of a controlled expression system in *A. vinelandii* .............................1

## CHAPTER 2: Literature Review: Structure, Function, and Formation of Biological Iron-Sulfur Clusters

2.1 Introduction and Perspectives .................................................................................6  
2.2 Functions of Biological [Fe-S] Clusters .................................................................6  
2.3 Structures and Properties of Biological [Fe-S] Clusters .......................................11  
2.4 Formation of Biological [Fe-S] Clusters .................................................................12  
2.5 Nitrogenase Maturation – The First Paradigm for Biological [Fe-S] Cluster Assembly ..................................................................................................................12  
2.6 Systems Involved in Generalized [Fe-S] Protein Maturation ...............................21  
2.7 Biochemical Features of Proteins Involved in [Fe-S] Protein Maturation ...........28  
2.8 IscS has a General Role in Intracellular Sulfur Trafficking .................................39  
2.9 [Fe-S] Protein Maturation in Eukaryotes ..............................................................41  
2.10 Summary and Outlook .........................................................................................43

## CHAPTER 3: Controlled Expression and Functional Analysis of Iron-Sulfur Cluster Biosynthetic Components within *Azotobacter vinelandii*

3.1 Introduction .............................................................................................................46  
3.2 Materials and Methods .........................................................................................49  
3.3 Results ....................................................................................................................59  
3.4 Discussion ...............................................................................................................78
CHAPTER 4: Cross-Talk Studies: Analyzing Possible Overlapping Functions between the Isc and Nif Systems

Part 1: NifU and NifS are Required for the Maturation of Nitrogenase and Cannot Replace the Function of isc-Gene Products

4.1 Introduction

4.2 The isc and suf Systems also have [Fe-S] Cluster Biosynthetic Functions

4.3 Controlled Expression of isc and nif Genes

4.4 NifU and NifS Cannot Functionally Replace IscU and IscS

Part 2: ‘Cross-talk’ revisited: Conditions under which the Nif and Isc [Fe-S] Protein Maturation Systems Exhibit Full or Partial Functional Equivalence

4.5 Introduction

4.6 Materials and Methods

4.7 Results and Discussion

CHAPTER 5: Summary and Future Directions

REFERENCES

APPENDIX I: Evidence of the accumulation of apo-forms of the [2Fe-2S] enzyme, Benzoate Dioxygenase, in cells depleted for IscU

APPENDIX II: Plasmids constructed during this project

APPENDIX III: Strains constructed during this project

VITA
LIST OF FIGURES

CHAPTER 2

Figure 1  Structures, core oxidation states and spin states of crystallographically defined Fe-S clusters………………………………………………………9
Figure 2  Salient mechanistic feature of the NifS/IscS class of cysteine desulfurases……………………………………………………………………………15
Figure 3  Organization of genes from various organisms whose products are known or suspected to be involved in [Fe-S] protein maturation……………………………………………………………………………18
Figure 4  Comparison of primary sequences of representative members of theNifU/IscU/SufU family of proteins………………………………………………………19
Figure 5  Comparison of primary sequences of members of the IscA/SufA family of proteins from Azotobacter vinelandii and Escherichia coli……………………………………………………………………………23

CHAPTER 3

Figure 1  Schematic representation of the relevant genetic organization of key strains used in this work………………………………………………………61
Figure 2  Effect of depletion of Isc components in A. vinelandii……………………………………………………………………………………………………63
Figure 3  Depletion of Isc components in A. vinelandii has a detrimental effect on aconitase activity……………………………………………………………66
Figure 4  Primary sequence comparisons for IscU from A. vinelandii (Av) and E. coli (Ec), and SufU from Clostridium acetobutylicum (Ca) and Thermotoga maritima (Tm)……………………………………………………………68
Figure 5  Growth phenotypes exhibited by strains having selected residues of the IscR-regulated copy of IscU substituted by alanine……………………………69
Figure 6  Comparison of the primary amino acid sequences of three IscA homologs encoded within A. vinelandii………………………………………………71
CHAPTER 4

Figure 1 Organization of the isc gene cluster and nifUS genes in A. vinelandii strains used in this work…………………………...88
Figure 2 Growth of A. vinelandii strains cultured under different conditions………………………………………………………..91
Figure 3 Key features of plasmids, pDB1551 and pDB1562, which respectively contain the A. vinelandii nifUS and iscSUAhscBAfxiscX genes under control of the strong ara transcriptional and translational elements from E.coli…………………….98
Figure 4 Expression of the Nif system under low oxygen concentrations rescues the null growth phenotype of a strain with an in-frame deletion in iscU…………………………………………………………...102
Figure 5 Arabinose-dependent and abundant expression of NifU and NifS in A. vinelandii strain DJ1626………………………………104
Figure 6 Arabinose-induced, elevated expression of NifU and NifS provides permissive growth conditions for a strain with an in-frame deletion in iscU……………………………………………………………105
Figure 7 Elevated expression of NifU and NifS does not provide permissive growth conditions for a strain with an in-frame deletion in \textit{iscU} under oxidative stress conditions..........................107

Figure 8 Elevated expression of the Isc system, resulting from inactivation of \textit{iscR}, improves the growth rate of a strain deleted for \textit{nifU}.............................................................................108

Figure 9 Arabinose-induced, elevated expression of the \textit{isc} operon provides permissive growth conditions for a strain with an in-frame deletion in \textit{nifU}.................................................................110

APPENDIX I

Figure 1 The complete degradation of benzoate by aerobic bacteria is initiated by the conversion of benzoate to benzoate diol by a multicomponent benzoate 1,2-dioxygenase (XylXYZ)..........................154

Figure 2 Transcriptional organization of the \textit{xyl} genes in \textit{Azotobacter vinelandii} whose products catalyze the initial reactions of the benzoate catabolic pathway..........................155

Figure 3 Q-sepharose elution profiles of the crude extracts of WT cells grown in the presence and absence of 25 mM sodium benzoate..........156

Figure 4 Q-sepharose elution profiles of the crude extracts of WT cells compared to cells depleted for IscU in glucose media supplemented with 25 mM sodium benzoate.................................158
LIST OF TABLES

CHAPTER 2

Table 1 Functions of some biological [Fe-S] clusters………………………………7

CHAPTER 3

Table 1 Key parent plasmids and relevant derivatives of these plasmids
used for the construction of *A. vinelandii* mutant strains………………..51
Table 2 Mutant strains constructed and/or used in this study…………………56
Table 3 β-galactosidase activity of *A. vinelandii* strains with mutations
in *iscR*……………………………………………………………………..76

CHAPTER 4

Table 1 Mutant strains used in this study…………………………………………97