CONSTRUCTION OF *Escherichia coli* ARGinine REPRESSOR
FUSION PROTEIN AND ANALYSIS OF ITS FUNCTION
IN XER SITE-SPECIFIC RECOMBINATION

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Construction of *Escherichia coli* Arginine Repressor Fusion Protein and Analysis of Its Function in Xer Site-specific Recombination

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Declaration:

No portion of the work referred to in this dissertation, unless otherwise stated, has been submitted in support of an application for any other degree of this or any other university or institution of higher learning.

Kholis Abdurachim Audah
29 August 2000
This thesis is dedicated to "Those Who Possess Intelligence"

In the creation of the heavens and the earth, and the alteration of night and day, there are signs for those who possess intelligence.

They remember ALLAH while standing, sitting, and on their sides, and they reflect upon the creation of the heavens and the earth: Our “RABB”, You did not create all this in vain. Be You glorified. Save us from the contribution of Hell.


This thesis is specially dedicated to my parents, Umi and Abah and to my wife,

Rifia Amalia
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"Hasbunallaah wa ni’mal wakiil ni’mal maulaa wa ni’man nashiir".

Kuala Lumpur, Friday, 14 July 2000

Kholis Abdurachim Audah
CONSTRUCTION OF *Escherichia coli* ARGinine REPRESSOR FUSION PROTEIN AND ANALYSIS OF ITS FUNCTION IN XER SITE-SPECIFIC RECOMBINATION

ABSTRACT

In addition to its role in L-arginine biosynthesis in *Escherichia coli*, arginine repressor (ArgR), the product of the *argR* gene, also plays an essential role as an obligate accessory protein in Xer site-specific recombination system. A structure-function relationship study of ArgR was performed to understand more about its role in Xer site-specific recombination.

Fusion proteins between ArgRWT (wild-type ArgR) and a biotinylated peptide as well as between ArgRNV (a mutant ArgR) and a biotinylated peptide were constructed. The biotinylated peptide was fused in frame to the amino-terminus of ArgRWT and ArgRNV, respectively.

Xer recombination assays showed that the ArgRWT-biotinylated peptide fusion protein poorly supports *cer*-mediated recombination *in vivo*, whereas the ArgRNV-biotinylated peptide fusion protein proficiently supports *cer*-mediated recombination *in vivo*. A 30 kDa protein which is the expected size for ArgRWT and ArgRNV-biotinylated peptide fusion protein was succesfully expressed. ArgRNV-biotinylated peptide fusion protein was partially purified.
PEMBINAAN PROTEIN CANTUMAN DALAM *Escherichia coli* 
DAN ANALISIS FUNGSIANYA DALAM REKOMBINASI 
TAPAK KHUSUS XER

**ABSTRAK**

Tambahan daripada peranan dalam biosintesis L-arginine dalam *Escherichia coli*, protein repressor arginine (ArgR), iaitu hasilan ekspresi gene argr, juga mempunyai peranan penting sebagai protein aksessori mustahak dalam sistem rekombinasi tapak khusus Xer. Satu kajian berkaitan struktur fungsi ArgR telah dijalankan bagi memahami dengan lebih mendalam peranannya dalam sistem rekombinasi tapak khusus Xer.

Protein-protein cantuman diantara ArgRWT (ArgR jenis liar) dan peptida yang dibiotinilasikan dan juga antara ArgRNV (ArgR mutan) telah dibina. Peptida yang dibiotinilasikan telah dicantumkan dengan sempurna secara berasingan kepada kedua-dua hujung amino ArgRWT dan ArgRNV.

CONTENTS

ACKNOWLEDGEMENTS i
ABSTRACT ii
ABSTRAK iii
CONTENTS iv
ABBREVIATIONS viii

CHAPTER 1: General Introduction
1.1 Site-specific recombination 1
1.2 Xer-site specific recombination system 3
1.3 Arginine repressor of Escherichia coli K-12 (ArgR) 4
1.4 ArgR homologues 8
1.5 ArgR mutants 8
1.6 Fusion protein system 10
1.7 Objectives of study 10

CHAPTER 2: Materials and Methods
2.1 Bacterial strains and plasmids 12
2.2 Chemicals and reagents 12
2.3 Bacterial growth media 12
2.4 Growth and maintenance of bacterial culture 13
2.5 Amino acids, antibiotics, lac inducer, indicator, and vitamin 13
2.6 Isolation and purification of covalently closed circular plasmid DNA 14
2.6.1 Small-scale plasmid DNA preparation 14
2.6.2 Midi-scale plasmid DNA preparation 15
2.6.3 Large-scale plasmid DNA preparation 16
2.6.4 Purification of plasmid DNA with Cesium chloride/Ethidium bromide (CsCl-EtBr) density gradient centrifugation 17

2.7 In vitro DNA manipulation 18
2.7.1 Restriction endonuclease digestion of DNA 18
2.7.2 Dephosphorylation of DNA restriction fragment 19
2.7.3 Phenol extraction and ethanol precipitation of DNA 19
2.7.4 Ligation of DNA fragment 19

2.8 Bacterial transformation 20
(a) Preparation of competent cells 20
(b) Transformation 20

2.9 Rapid screening of plasmid DNA 20
(a) Phenol-chloroform-isopropanol method 20
(b) SCFSB method 21

2.10 DNA sequencing 21

2.11 Gel electrophoresis of DNA 22

2.11.1 Staining of DNA gel 22
2.11.2 Extraction of DNA from agarose gel 22

2.12 Protein expression and detection 23
2.12.1 Cell growth and induction 23
2.12.2 Cell lysis 24

2.13 SDS-PAGE analysis 24
2.14 Western blotting 25
2.14.1 Preparation of the gel for protein transfer 26
2.14.2 Preparation of the transfer membrane 26
2.14.3 Assembling of the transfer stack 26
2.14.4 Protein transfer 26
2.14.5 Visualization of the proteins 27
2.14.6 Drying of the blotted membrane 27
2.14.7 Detection of biotinylated protein 28
2.14.7.1 Blocking of the blotted membrane 28
2.14.7.2 Chromogenic substrate incubation 29
2.15 In vivo Xer-site specific recombination assay 29
2.16 Protein purification 30
2.17 Photography 31

CHAPTER 3: Construction of ArgRWT and ArgRNV-Biotinylated peptide fusion protein

3.1 Introduction 32
3.2 Preparation of cloning vector and DNA insert 33
3.2.1 Isolation of plasmid DNA 33
3.2.2 Purification of plasmid DNA 34
3.2.3 Digestion of plasmid DNA 35
3.3 Construction of ArgRWT-biotinylated peptide fusion protein 36
3.3.1 Subcloning of argRWT gene into PinPoint™ Xa-3 cloning vector 36
3.3.2 Analysis of argRWT-biotinylated peptide fusion recombinant DNA 36
3.4 Construction of ArgRNV-biotinylated peptide fusion protein 37
3.4.1 Subcloning of argRNV gene into PinPoint™ Xa-3 cloning vector 38
3.4.2 Analysis of argRNV-biotinylated peptide fusion recombinant DNA 38
3.5 Characterization of recombinant plasmids 39
3.5.1 Restriction endonucleases analysis for argRWT fusion derivative 39
3.5.2 Restriction endonuclease analysis for argRNV fusion derivative 40
3.5.3 DNA sequence analysis 41
CHAPTER 4: Determination of the Xer phenotype of ArgRWT- and ArgRNV-biotinylated peptide fusion protein

4.1 Introduction 42
4.2 \textit{In vivo cer}-mediated recombination using pCS202 as reporter plasmid 43
4.3 \textit{In vivo cer}-mediated recombination using pSH10 as reporter plasmid 44

CHAPTER 5: Protein expression and analysis

5.1 Introduction 46
5.2 Small-scale expression of ArgRWT-biotinylated peptide fusion protein 46
5.3 Small-scale expression of ArgRNV-biotinylated peptide fusion protein 47
5.4 Partial purification of fusion proteins 48
5.4.1 Expression conditions 48
5.4.2 Fractionation of cellular proteins 48
5.4.2.1 Purification by Batch capture method 49
5.4.2.2 Purification by Column capture method 49

CHAPTER 6: Discussion

6.1 Introduction 51
6.2 Effects of additional amino acids residues to ArgR structure and activity 53
6.3 Expression of \textit{argR} fusion genes and the related properties of the fusion products 55
6.3.1 Protein degradation systems 56
6.3.2 Potential toxicity of the protein 58
6.4 Use of ArgR fusion protein 58
6.5 Possible experiments to be carried out 60
6.6 Conclusions and suggestions 60

REFERENCES vii
ABBREVIATIONS

(a) Buffers/Chemicals/Enzymes/Reagents

APS  ammonium persulphate
ATP  adenosine triphosphate
BSA  bovine serum albumin
DNA  deoxyribonucleic acid
DMF  dimethylformamide
DNase I  deoxyribonuclease I
dNTP  deoxynucleoside triphosphate
DTT  dithiothreitol
EDTA  ethylenediaminetetraacetic acid (disodium salt)
EtBr  ethidium bromide
EtOH  ethanol
FSB  final sample buffer
IPTG  isopropyl-β-D-thiogalactopyranoside
NaCl  sodium chloride
PMSF  phenylmethanesulfonyl fluoride
RNA  ribonucleic acid
RNase A  ribonuclease A
SCFSB  single colony final sample buffer
SDS  sodium dodecyl sulphate
TAE  Tris-acetate-EDTA buffer
TBE  Tris-borate-EDTA buffer
TE  Tris-EDTA buffer
TEMED  NNN’N’- tetramethylethylenediamine
TM  Tris-magnesium buffer
Tris  tris (hydroxymethyl) amino ethane
X-gal  5-bromo-4-chloro-3-indolyl-β-D galactoside

(b) Antibiotics

Ap  ampicillin
Cm  chloramphenicol
Km  kanamycin
Tc  tetracycline

(c) Units

bp  base pair
°C  degree Celsius
Da  dalton
g  gram
hr  hour
kb  kilobase pairs (10^3 bp)
kDa  kilodalton
<table>
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<td>l</td>
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<td></td>
</tr>
<tr>
<td>M</td>
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</tr>
<tr>
<td>mA</td>
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<td></td>
</tr>
<tr>
<td>min</td>
<td>minutes</td>
<td></td>
</tr>
<tr>
<td>mg</td>
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</tr>
<tr>
<td>µg</td>
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<tr>
<td>ml</td>
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<tr>
<td>µl</td>
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</tr>
<tr>
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<tr>
<td>nm</td>
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</tr>
<tr>
<td>rpm</td>
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(d) **Amino acids and genetic code**

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<th>Genetic Code</th>
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<tr>
<td>A</td>
<td>Ala alanine</td>
<td>GCT, GCC, GCA, GCG</td>
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<tr>
<td>C</td>
<td>Cys cysteine</td>
<td>TGT, TGC</td>
</tr>
<tr>
<td>D</td>
<td>Asp aspartic acid</td>
<td>GAT, GAC</td>
</tr>
<tr>
<td>E</td>
<td>Glu glutamic acid</td>
<td>GAA, GAG</td>
</tr>
<tr>
<td>F</td>
<td>Phe phenylalanine</td>
<td>TTT, TTC</td>
</tr>
<tr>
<td>G</td>
<td>Gly glycine</td>
<td>GGT, GGC, GGA, GGG</td>
</tr>
<tr>
<td>H</td>
<td>His histidine</td>
<td>CAT, CAC</td>
</tr>
<tr>
<td>I</td>
<td>Ile isoleucine</td>
<td>ATT, ATC, ATA</td>
</tr>
<tr>
<td>K</td>
<td>Lys lysine</td>
<td>AAA, AAG</td>
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<tr>
<td>L</td>
<td>Leu leucine</td>
<td>TTG, TTA, CTT, CTC, CTA, CTG</td>
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<tr>
<td>M</td>
<td>Met methionine</td>
<td>ATG</td>
</tr>
<tr>
<td>N</td>
<td>Asn asparagine</td>
<td>AAT, AAC</td>
</tr>
<tr>
<td>P</td>
<td>Pro proline</td>
<td>CCT, CCC, CCA, CCG</td>
</tr>
<tr>
<td>Q</td>
<td>Gln glutamine</td>
<td>CAA, CAG</td>
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<tr>
<td>R</td>
<td>Arg arginine</td>
<td>CGT, CGC, CGA, CGG, AGA, AGG</td>
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<tr>
<td>S</td>
<td>Ser serine</td>
<td>TCT, TCC, TCA, TCG, AGT, AGC</td>
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<tr>
<td>T</td>
<td>Thr threonine</td>
<td>ACT, ACC, ACA, ACG</td>
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<tr>
<td>V</td>
<td>Val valine</td>
<td>GTT, GTC, GTA, GTG</td>
</tr>
<tr>
<td>W</td>
<td>Trp tryptophan</td>
<td>TGG</td>
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<tr>
<td>Y</td>
<td>Tyr tyrosine</td>
<td>TAT, TAC</td>
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(e) **Genotype and phenotype**

- **Xer**
  - strain proficient in Xer site-specific recombination
- **Xer**
  - strain deficient in Xer site-specific recombination
- **argR**
  - *argR* null mutant
(f) Miscellaneous

~ approximately
i.e. (Latin *id est*) that is to say, in other words
LB Luria-Bertani
MW molecular weight
OD$_x$ optical density at x nm
ori origin of replication
ORF open reading frame
% percentage
PAGE polyacrylamide gel electrophoresis
$Tn$ transposon
UV ultra violet
(v/v) volume to volume ratio
WT wild-type
(w/v) weight to volume ratio
$X'$ resistance to X
$X^s$ sensitivity to X