

R  
r

PERPUSTAKAAN UNIVERSITI MALAYA

ACM-3638  
INVC 3/11/2009

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

**KHOLIS ABDURACHIM**

**INSTITUTE OF BIOLOGICAL SCIENCES  
FACULTY OF SCIENCE  
UNIVERSITY OF MALAYA  
KUALA LUMPUR  
2000**

Perpustakaan Universiti Malaya



A510033251

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

**KHOLIS ABDURACHIM**

**A dissertation submitted to the Faculty of Science  
University of Malaya  
for the Degree of Master of Science**



**Institute of Biological Sciences  
Faculty of Science  
University of Malaya  
Kuala Lumpur  
2000**

**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.

A handwritten signature in black ink, appearing to read 'Kholis', written in a cursive style.

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.

Holy Qur’an (Ali ‘Imran: 190-191).

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

## ACKNOWLEDGEMENTS

In the name of Allah, Most Gracious, Most Merciful. Praise be to Allah, “Rabb” of the universe and peace be upon the Prophet Muhammad S. A. W., his family, and his followers.

I am greatly indebted to my supervisor, Dr Amir Feisal Merican bin Aljunid Merican for his invaluable guidance, encouragement, support and help throughout this project.

I would also like to thank the staffs of Institute of Biological Sciences, for their cooperation especially to Ms Chan Lee Choo, Mr Harun Kedol, Mr Abdul Kudus Abdullah, Mr Raizan, Ms Anusooya, Shina, and Hazu. Thanks to the National Biotechnology Directorate (NBD), Ministry of Science, Technology and Environment, Malaysia for the research grants provided for this project, and the research assistantship granted to me during this project.

To all my lab mates, Faizul, Arul, Rowyna, Syantia, Talha, and Zali, for their support and help. Thanks to Yasota (Assoc. Prof. Dr. Rohana Yusuf’s Laboratory, Biochemistry Department, Faculty of Medicine) for helping out with Western blotting technique. A special thanks to my friends Mr. Meriksa Sembiring and family, Mr. Mahmud Zaki Fuad and family, Mr. Jamiludin Hidayat and family, and Ms. Lindayani for their support, help and advice. A very special thanks to Dr. Mohamad Dahlan Darip and family, Mr. Muhammad Audah and family, and Mrs. Jamilah Ali and family, for their help, encouragement and advice. Thanks to everyone who has directly and indirectly supported me throughout my study.

My gratitude also goes to my beloved wife, Rifia Amalia, my wonderful parents Umi and Abah, and family members for their love, faith, and continuous prayers throughout these hard years. I wouldn’t be able to make it without your support.

**“Hasbunallaah wa ni’mal wakiil ni’mal maulaa wa ni’mannashiir”.**

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 successfully expressed. ArgRNV-biotinylated peptide fusion protein was partially purified.

**PEMBINAAN PROTEIN CANTUMAN DALAM *Escherichia coli*  
DAN ANALISIS FUNGSINYA DALAM REKOMBINASI  
TAPAK KHUSUS XER**

**ABSTRAK**

Tambahan daripada peranan dalam biosintesis L-arginine dalam *Escherichia coli*, protein repressor arginine (ArgR), iaitu hasil ekspresi gene *argR*, juga mempunyai peranan penting sebagai protein aksesori 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.

Esei rekombinasi Xer telah menunjukkan bahawa protein cantuman peptida-ArgRWT tidak menyokong sepenuhnya rekombinasi berantaraan *cer in vivo*, sementara protein cantuman peptida-ArgRNV menyokong dengan sempurna rekombinasi berantaraan *cer in vivo*. Suatu protein bersaiz 30 kDa iaitu saiz yang dijangkakan untuk protein cantuman peptida-ArgRWT dan ArgRNV telah berjaya diekspresikan. Protein cantuman peptida-ArgRNV telah berjaya dituliskan.

## CONTENTS

	<b>Page</b>
<b>ACKNOWLEDGEMENTS</b>	<b>i</b>
<b>ABSTRACT</b>	<b>ii</b>
<b>ABSTRAK</b>	<b>iii</b>
<b>CONTENTS</b>	<b>iv</b>
<b>ABBREVIATIONS</b>	<b>viii</b>
<b>CHAPTER 1:      General Introduction</b>	
1.1               Site-specific recombination	1
1.2               Xer-site specific recombination system	3
1.3               Arginine repressor of <i>Escherichia coli</i> 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
<b>CHAPTER 2:      Materials and Methods</b>	
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, <i>lac</i> 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	<i>In vitro</i> 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	<i>In vivo</i> Xer-site specific recombination assay	29
2.16	Protein purification	30
2.17	Photography	31
<b>CHAPTER 3:</b>	<b>Construction of ArgRWT and ArgRNV- Biotinylated peptide fusion protein</b>	
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 <i>argRWT</i> gene into PinPoint™ Xa-3 cloning vector	36
3.3.2	Analysis of <i>argRWT</i> -biotinylated peptide fusion recombinant DNA	36
3.4	Construction of ArgRNV-biotinylated peptide fusion protein	37
3.4.1	Subcloning of <i>argRNV</i> gene into PinPoint™ Xa-3 cloning vector	38
3.4.2	Analysis of <i>argRNV</i> -biotinylated peptide fusion recombinant DNA	38
3.5	Characterization of recombinant plasmids	39
3.5.1	Restriction endonucleases analysis for <i>argRWT</i> fusion derivative	39
3.5.2	Restriction endonuclease analysis for <i>argRNV</i> fusion derivative	40
3.5.3	DNA sequence analysis	41

<b>CHAPTER 4:</b>	<b>Determination of the Xer phenotype of ArgRWT- and ArgRNV-biotinylated peptide fusion protein</b>	
4.1	Introduction	42
4.2	<i>In vivo cer</i> -mediated recombination using pCS202 as reporter plasmid	43
4.3	<i>In vivo cer</i> -mediated recombination using pSH10 as reporter plasmid	44
<b>CHAPTER 5:</b>	<b>Protein expression and analysis</b>	
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
<b>CHAPTER 6:</b>	<b>Discussion</b>	
6.1	Introduction	51
6.2	Effects of additional amino acids residues to ArgR structure and activity	53
6.3	Expression of <i>argR</i> 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

## 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- $\beta$ -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- $\beta$ -D galactoside

### (b) Antibiotics

Ap	ampicillin
Cm	chloramphenicol
Km	kanamycin
Tc	tetracycline

### (c) Units

bp	base pair
$^{\circ}$ C	degree Celsius
Da	dalton
g	gram
hr	hour
kb	kilobase pairs ( $10^3$ bp)
kDa	kilodalton

l	litre
M	molar
mA	milliampere
min	minutes
mg	milligram
µg	microgram
ml	millilitre
µl	microlitre
mol	moles
nm	nanometer
rpm	revolutions per minute
sec	seconds
V	volts

#### (d) Amino acids and genetic code

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

#### (e) Genotype and phenotype

Xer <sup>+</sup>	strain proficient in Xer site-specific recombination
Xer <sup>-</sup>	strain deficient in Xer site-specific recombination
<i>argR</i> <sup>-</sup>	<i>argR</i> null mutant

**(f) Miscellaneous**

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