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
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KHOLIS ABDURACHIM

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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.
Holy Qur'an (Ali 'Imran: 190-191).

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

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Kholis Abdurachim Audah

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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 FUNGSINYA 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.

Esei rekombinasi Xer telah menunjukkan bahawa protein cantuman peptida-ArgRWT tidak menyokong sepenuhnya rekombinasi berantarakan *cer in vivo*, sementara protein cantuman peptida-ArgRNV menyokong dengan sempurna rekombinasi berantarakan *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 ditulenkan.

CONTENTS

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	phenylmethanesulfonylfluoride
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
^{o}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^+	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^r	resistance to X
X^s	sensitivity to X