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 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.