MOLECULAR MECHANISMS THAT AFFECT STABLE MAINTENANCE AND SPREAD OF PLASMIDS CARRYING TYPE II RESTRICTION-MODIFICATION SYSTEMS

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Plasmids are self-replicating genetic elements that are separated from the host chromosome content. They can be found in most bacteria, but also in archea and yeast. Some of them are able to transfer themselves to a new host by conjugation or mobilization. Among many features that are characteristic to plasmids lifestyle, two are the most distinctive: (i) autonomous, self-controlled replication driven by cell proteins, and (ii) persistence even without selective pressure and at an obvious metabolic burden to the host. The latter is especially challenging to analyze, as apart from general mechanisms that are involved in plasmid maintenance such as (i) random partition, (ii) active partition; and (iii) plasmid addiction systems, there are other factors that play a pivotal role here. These include: horizontal gene transfer; positive selection for plasmid encoded genes, and compensatory adaptation. All of these make plasmid persistence a complex phenomenon that needs to be approached from different angles.

The purpose of this study was to investigate the role of maintenance mechanisms and the routes of transmission of pEC156, a naturally occurring ColE1-type plasmid of *E.coli* E1585-68 that carries genes of the EcoVIII restriction-modification system. This plasmid possess determinants involved in two stability mechanisms. The first of them relies on site specific recombination depending on the Xer/*cer* molecular machinery, while the second involves a restriction-modification system. For analysis of the maintenance mechanism, we constructed a set of pEC156 derivatives that were deficient in one or more genetic determinants. Their stability was investigated in wild-type *E. coli* as well as in strains deficient in homologous recombination and the Xer/*cer* multimer resolution system. Our results indicated that three factors affected the maintenance of pEC156: (i) the presence of a *cer* site involved in resolution of plasmid multimers, (ii) a gene coding for EcoVIII endonuclease, and (iii) plasmid copy number.

To study high-copy plasmid's maintenance we propose a simple theoretical model based on one input parameter which is the copy number of plasmids present in a host cell. The Monte Carlo approach was used to analyze random fluctuations affecting plasmid replication and segregation leading to gradual reduction in the plasmid population within the host cell. Proposed model is applicable to high-copy plasmids exemplified by ColE1-type, where newly synthesized plasmid units are segregated randomly upon cell division. This model was employed to investigate maintenance of pEC156 derivatives in selected *Escherichia coli* strains (MG1655, wild type; MG1655 *pcnB*, and hyper-recombinogenic JC8679 *sbcA*). We have compared the

experimental data concerning plasmid maintenance with the simulations and found that the theoretical stability patterns exhibited an excellent agreement with those empirically tested. In our simulations, we have investigated the influence of replication fails (α parameter) and uneven partition as a consequence of multimer resolution fails (δ parameter), and the postsegregation killing factor (β parameter). All of these factors act at the same time and affect plasmid inheritance at different levels. The only input parameter was the mean plasmid copy number at the beginning of the stability experiments. The obtained results indicate that the errors associated with plasmid segregation has a stronger effect on plasmid maintenance than restriction-modification system. Uneven segregation of *cer*-deficient pEC156 derivatives suggest that multimerization is a key factor that affects even distribution of plasmid units between daughter cells at the cell division what leads to the formation of plasmid-free cells. Use of the proposed mathematical model can provide a valuable description of plasmid maintenance, as well as enable prediction of the probability of the plasmid loss.

Moreover, we have shown that pEC156 can be stably maintained in different members of the *Enterobacteriaceae* family. This prompted us to study possible mechanisms that are involved in dissemination of this plasmid among other bacteria. Analysis of the pEC156 nucleotide sequence revealed a lack of the *mob* genes, but the presence of two loci with similarity to *oriT* of plasmid F and *oriT* of plasmid R64, respectively. We have shown that this two origin of transfer are functional and pEC156 plasmid can be mobilized by two narrow host range conjugative plasmids like F (IncF) and R64 (IncI1). In our work, we performed single-species and heterospecific matings between selected members of the *Enterobacteriaceae* family by the conjugal plasmid F'ts114*lac*::Tn5 (Km^R). In some hosts we noticed a striking difference in frequency of pEC156 derivatives transfer with respect to functionality of the EcoVIII RM system. The pIB8 (EcoVIII R^+M^+) plasmid was acquired and mobilized less efficiently compared to the pIB9 plasmid deprived of the RM system. In addition, we found that bacteria that possess the EcoVIII restriction-modification system can efficiently release plasmid content to the environment. We have shown that *E. coli* cells can be naturally transformed with pEC156-derivatives, however, with low efficiency. The transformation protocol employed, neither involved chemical agents (e.g. $CaCl_2$) nor temperature shift which could induce plasmid DNA uptake.