35. Prediction of Heat-Induced Replication Sites in Replication Origins from Escherichia Coli and Other Plasmidic Replicons

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Keywords: replication origin, stress-induced destabilization sites (SIDD), thermodynamic, chromosome, plasmids.

We present the first in-silico approximation for the prediction of destabilization sites in origins of replication of plasmids: F, R1, P1 and ColE1 and oriC from E.coli using the WebSIDD program from Craig J. Benham in order to predict sites where Heat-Induced Replication described in E.coli could take place.

Local DNA strand separation is a necessary step in the initiation of chromosome replication. For this reason, the locations of strand separation needed for replication initiation must be stringently controlled in vivo. The replication in Escherichia coli starts in a fixed site in the chromosome, the origin of replication oriC. Replication origins have common features in their sequences and in their structure. In general, the local thermodynamic stability of DNA at these sites is very low. In the case of the E. coli chromosome, the replication is a highly controlled process. Indeed, only once replication takes place in each cell cycle. However, it has been previously described by our group that a temperature up-shift of 10ºC or more degrees in the growth temperature of an E coli culture causes induction of extra rounds of chromosome replication. This heat-induced replication (HIR) initiates at oriC. A thermodynamic alteration of the oriC region was proposed.

In order to investigate if HIR was a common phenomenon or only restricted at oriC, we tested its presence in other replicons. We choose E.coli – plasmid replicons because of their phylogenetic relationship to start with this approach. In this work, we present the first approximation in silico for the prediction of desestabilization sites in the origins of replication of plasmid: F, R1, P1 and ColE1 type from E.coli. Firstly, we compared the primary structure between the origins of these plasmids and oriC using sequence alignments. Phylogenetic distances resulted to be very narrow, as we can predict. The second objetive was to test the presence of stress-induced duplex desestabilization sites in these replicons. We used the WebSIDD program from Craig J. Benham that is accessible at the web address: http://genome.bme.ucdavis.edu/sidd/. The input of this program is a DNA sequence and the output is the transition probability profile, p(x), and destabilization energy profile, G(x), for each location of nucleotide bases in the molecule. The interface controls parameters as type of DNA molecules, type of energetics, temperature and salt concentration, the superhelix density, open region size and torsional stiffness. Our results agree with the primary structure analysis. We found that the destabilization energy is lower in oriC than in other replicons. The second would be oriR, the origin of replication from plasmid P1 that is the nearest in the phylogenic tree. OriR1, the origins from plasmid R1, is a bit more stable and oriS, the origin of replication from miniF, has only one predicted site and a more stable structure. Considering the case of ColE1-type plasmids, we couldn't find destabilization sites in their origins and the free energy needed to destabilize this structure resulted to be higher.
These in silico data validate our in vivo experiments; we found heat-induced replications in R1 and P1; however, we couldn't find it in miniF. Currently, we are testing its presence in ColE1 plasmid. This is a case where in silico results can be directly compared with experimental data.