REPLICATION OF PLASMIDS

Summary

One of the characteristics that differentiates plasmids from other extrachromosomal genetic elements, transposons and viruses, is the controlled replication that allows stable inheritance in dividing cells. At the same time, the mechanism of replication of some plasmids is very similar to that of some viruses, and indeed some plasmids might have viral origin. In the case of other plasmids, their replication mechanism is similar to that used by the host. On the one hand, therefore, the study of plasmid replication may give hints as to the origin of a particular group of these extrachromosomal elements. On the other, the research on replication of plasmids helped to solve basic questions pertaining to this basic molecular process in general. The first basic problem of any process of DNA replication is that of priming, since none of the replicative polymerases can start DNA synthesis using just free nucleotides. Plasmids use three strategies to solve this problem. Some linear plasmids code for a replication protein which provides an OH group to which the first nucleotide of the copy molecule can be attached by a plasmid-coded DNA polymerase. Another strategy is to bring the host primase to the origin of replication. This enzyme synthesizes a short RNA molecule the 3´OH end of which can be elongated by the DNA polymerase of the host. The mechanism of replication of such plasmids shows many similarities to that of the host chromosome. Some plasmids, it may be added, code for their own primase. Finally, at the origin of replication of other plasmids, for instance those replicating using the rolling-cycle mechanism, one of the parent DNA strands becomes cleaved to provide a free 3'OH group. Research on plasmid replication provided many answers not only to basic questions on replication in general: it was also very important for understanding the mechanisms of gene regulation. Plasmid replication needs to be controlled so that the metabolic burden on the host is not excessive. There are two known mechanisms used to control the replication of bacterial plasmids. In one, the role of a regulator is played, actually, by the plasmid DNA sequences in the origin region. This is the mechanism of plasmid handcuffing, named after the appearance of two plasmid molecules brought together when such regions from two plasmids interact indirectly, the mechanism by which replication is repressed. As the interaction is reversible, when plasmid concentration in the growing and dividing cell drops, the molecules can separate and replicate. In a different mechanism of replication regulation, the role of the regulator is played by small molecules (RNA, and sometimes also a protein) the concentration of which depends on plasmid copy number and which inhibit the synthesis of another molecule coded for a plasmid (usually a protein, sometimes RNA) that is important for replication initiation. Indeed, it was thanks to the research on regulation of plasmid replication that the mechanism of regulation by small RNA molecules (antisense RNA) has been first described in detail. The reason for such a historical importance of research on plasmid replication is that it is easier to achieve the genetic modification of plasmids than modification of the host genome. Moreover, the effects of a modification of an element which is not necessary for survival of the cell can be observed and interpreted with less difficulty. For the same reasons of easy manipulation, introduction of artificial plasmids into the cells is the main method of genetic modification. Such molecules may not be replicative in the target host, and thus either are eliminated from the cells with time, or integrate into the host chromosomes. In the case of a majority of biotechnological applications, however, the plasmids used are replicative, and therefore it is important to be able to control their copy level in the cells. Replicative elements are being also considered for use in therapy. Thus, the research on plasmid replication remains of vital importance.