CHAPTER 20

COHEN/BOYER/BERG: THE FIRST GENETICALLY ENGINEERED ORGANISM

In 1973, Stanley Cohen, Herbert Boyer, and Paul Berg created the first genetically engineered organisms by moving ribosomal RNA genes from the African clawed toad Xenopus *into bacterial cells.*

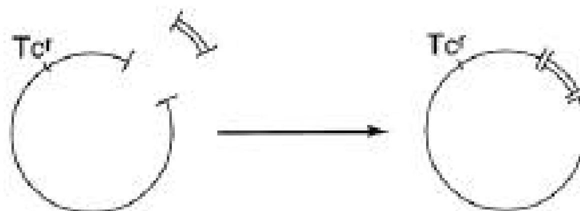
CONSTRUCTING CHIMERIC PLASMIDS

With a ready means of splicing gene fragments together, adding a restriction fragment to a plasmid vehicle is straightforward. Early efforts centered upon a group of plasmids called *resistance transfer factors*, or *R factors*. These *E. coli* plasmids carried genes whose products blocked the action of one or more antibiotics, and also carried the genes necessary for self-replication. Because antibiotic resistance had become advantageous to bacteria, R factors were selectively favored and are now common. Unfortunately, they are also contributing to the world-wide decline in efficacy of antibiotics.

R factors are usually transferred infectiously among bacteria via conjugation. Naked plasmid DNA is taken up *whole* by *E. coli*, however, if the cell membranes are first made artificially permeable by exposure to calcium chloride. While few bacterial cells actually make up R factor, they can be quickly detected and isolated by addition to the culture of the antibiotic to which they confer resistance, because then all the other (non-R factor) cells die.

The R factor is an ideal vehicle for restriction fragment propagation. Not only can it replicate itself, it carries resistance genes that permit selection for successful incorporation into bacteria. R factors are big, though, and that does pose a problem: the enzyme that produced the restriction fragment may attack the R factor at many sites, chopping it into useless bits. What is desired is a derivative plasmid, a small piece of the original R factor that still carries the replication genes and a gene for antibiotic resistance, but little or nothing else. Such derivatives can be made by shearing the R factor DNA, or by cleaving it with restriction enzymes and reannealing some of the pieces. One such derivative of an R factor, pSC101, has only 9000 base pairs (about 8 percent of the original DNA), but can still replicate itself and still carries one antibiotic-resistance gene (*Tetracycline*^{R}). All but one of the original 13 *Eco*RI restriction sites are missing. When pSC101 is cleaved by the restriction endonuclease *Eco*RI, only the single remaining site is cleaved. Thus *Eco*RI does not further fragment the derivative plasmid pSC101. It just opens the circular DNA, forming a linear duplex with sticky ends.

pSC101 was the first successful plasmid vehicle. A foreign *Eco*RI restriction fragment mixed with an *Eco*RI-cleaved pSC101 plasmid can produce a composite plasmid by two simple splicing steps:



Any gene fragment generated by *Eco*RI digestion may be added to pSC101 in this fashion.

COHEN AND BOYER'S EXPERIMENT

In 1973 Stanley Cohen, Herbert Boyer, and Paul Berg did precisely this (figure 20.1). They inserted an amphibian (*Xenopus laevis*, the African clawed toad) gene encoding rRNA into the pSC101 plasmid. The plasmid got its name by being the 101st plasmid isolated by Stanley Cohen (plasmid Stanley Cohen 101, or pSC101). This plasmid, as previously described, contained a single site that could be cleaved by the restriction enzyme *Eco*RI, as well as a gene for tetracycline resistance (Tc^r gene). The rRNA-encoding region was inserted into the pSC101 at the cleavage site by cleaving the rRNA region with *Eco*RI and allowing the complementary sequences to pair. This was the dawn of genetic engineering.

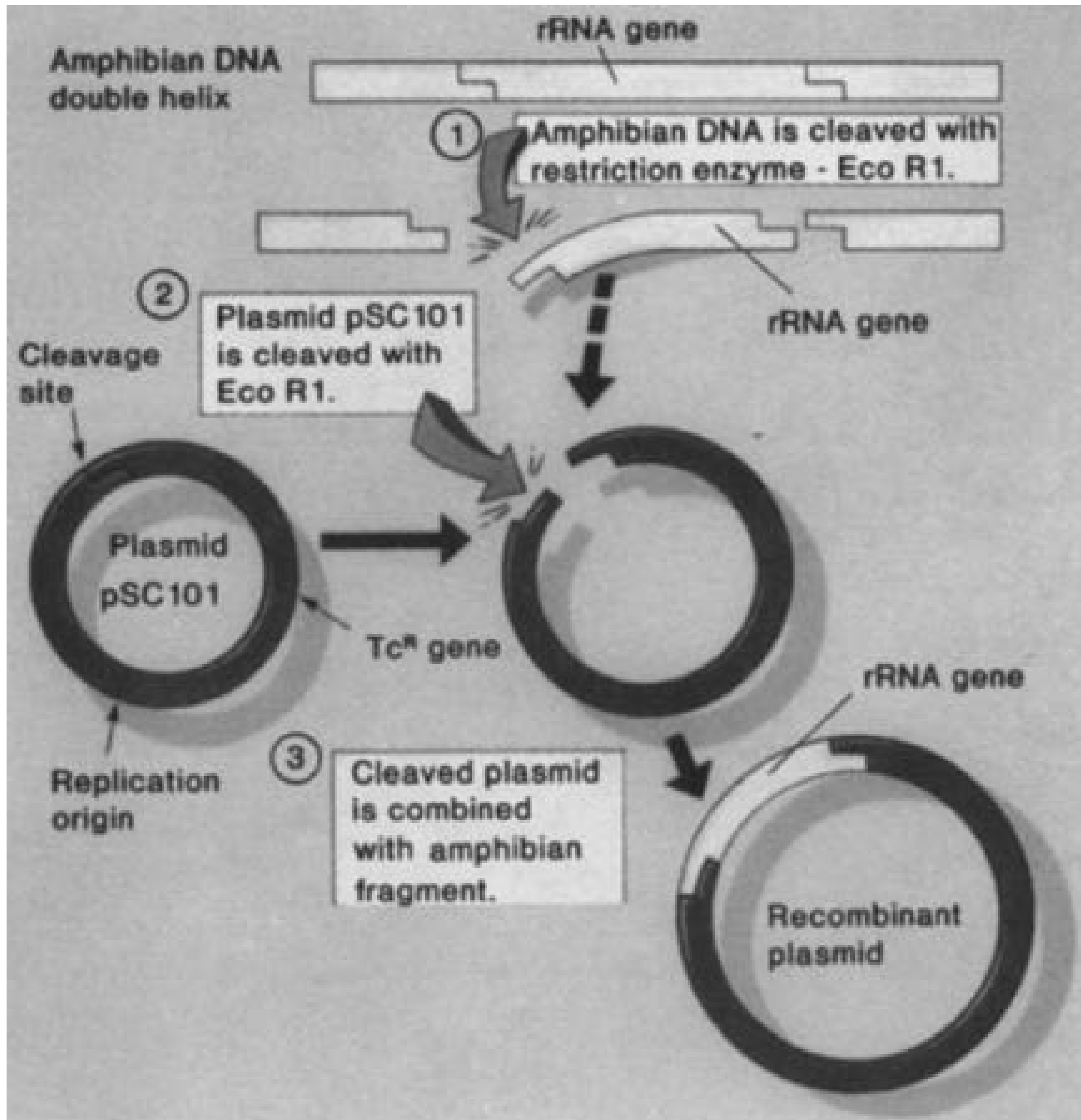


Figure 20.1
The Cohen-Boyer-Berg experiment.