This is the day appointed for the combat
and ready are the appellant and the defendant

William Shakespeare, Henry VI

Chapter 23
Plasmids and resistances

One type of plasmid has already been introduced, the F-plasmid. Joshua Lederberg (1925–2008) and others noticed in their work on bacterial conjugation that the information for this process was not localized on the bacterial chromosome but on the so-called F-factor, which was then designated "plasmid." In 1961 it was shown by Stanley Falkow and colleagues (Stanford, USA) that plasmids are small, circular DNA molecules that are present in bacterial cells in addition to the chromosome. These plasmids can be made visible as shown in Figure 46.

Why did nature come up with the idea of storing genetic information outside of the chromosomes?

Let's first look briefly at an analogy with personal computers or laptops on one side and CD-ROMs on the other. CD-ROMs have the advantage of storing information without taking up space on the hard drive. As long as this information is not needed, it can be stored as CDs on a shelf. If the situation changes, the information or data are quickly available. The situation with plasmids is similar. Happily growing bacteria usually do not need them. However, when a stress situation comes up, cells that more or less by chance still contain a useful plasmid are in demand. The whole arsenal of DNA uptake (conjugation or transformation) mechanisms is then activated because the availability of plasmid-encoded genetic information may decide over the life or death of these bacteria, over growing happily or wasting away.

This sounds a bit theoretical. What kind of stress situations do you have in mind?

Let's take a situation in which bacteria can only survive, for instance, by becoming resistant to heavy metals. The principle was described by Hans-Guenter Schlegel in Chapter 19. In this case, those plasmids conferring resistance are armed with information required to synthesize efflux pumps and to bring them into position. These pumps are rapidly synthesized and installed into the cytoplasmic membrane so that the intercellular heavy-metal concentration can be kept low. We learned about another resistance mechanism in Chapter 20. Bacteria produce plasmid-encoded beta-lactamases in order to cleave and thereby inactivate beta-lactam

Discover the World of Microbes: Bacteria, Archaea, and Viruses, First Edition. Gerhard Gottschalk.

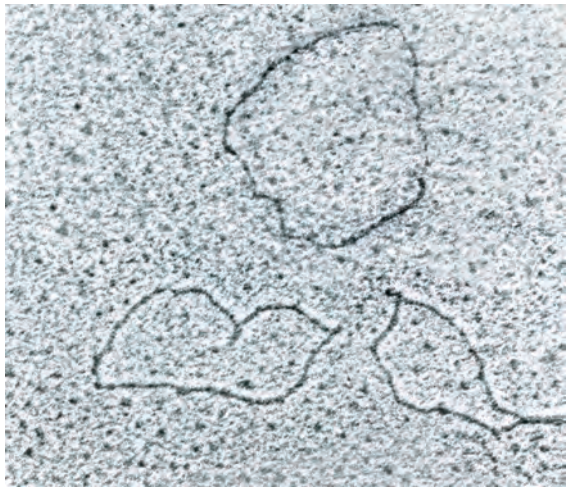

Figure 46 Electron micrograph of a plasmid (pBR322). The diameter of the upper ring is approximately 0.25 µm. (Photograph: Michael Hoppert, Goettingen, Germany.)

antibiotics. It was also discussed how beta-lactamases altered by mutation and selection are able to inactivate new types of beta-lactam antibiotics that have come onto the market to treat infections. There are also additional mechanisms conferring resistance to certain antibiotics, which in some cases are inactivated by phosphorylation or acetylation as soon as they arrive inside the cells. The protein synthesis factories, the ribosomes, are the targets of a number of antibiotics, including streptomycin. Bacteria have gained resistance to these antibiotics by modifying the target. The protein that normally interacts with streptomycin, for example, is so modified that the antibiotic is no longer able to bind, resulting in the development of resistance.

These are just a few examples of how antibiotic resistances develop. There has been an additional fatal development. Not only is there a permanent race between the application of new antibiotics and the appearance of new resistances, but also bacteria are able to collect resistances on their plasmids, like trophies. Due to the rapid propagation of bacteria and the conjugative events resulting in plasmid transfer, the accumulated resistances may spread like an avalanche. Therefore, bacteria containing such plasmids can be isolated everywhere. Just one example: the research group of Michael Teuber from the Swiss Federal Institute of Technology/ETH Zurich (Switzerland) isolated a strain of *Enterococcus faecalis* from raw sausage with plasmid-encoded resistances against tetracycline, lincomycine, chloramphenicol, and erythromycin. Harmless microbial populations that are normally common in man and animals are transformed more and more into enemy legions that no longer can be controlled effectively. One example, the plasmid PSMS-130 of a pathogenic strain of *Escherichia. coli,* is depicted in Figure 47. The resistance genes against eight antibiotics are united on this plasmid. In

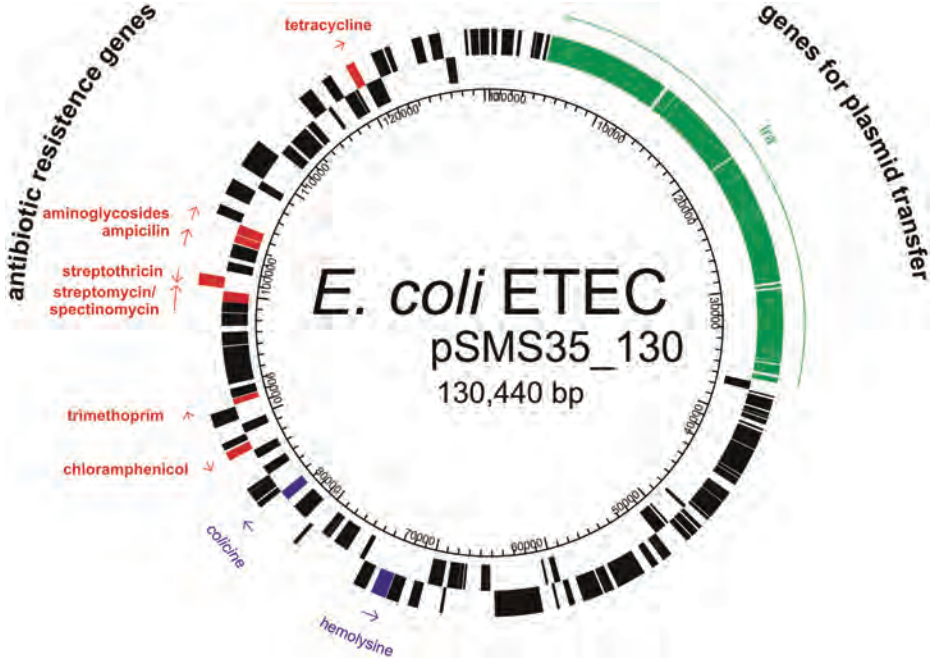

Figure 47 A plasmid of enterotoxinogenic *E. coli* strain, an ETEC strain carrying various resistance genes. The double ring depicted consists of 130440 base pairs. The tra region (in green) contains the genes for plasmid transfer by conjugation. Genes in red confer resistance against eight different antibiotics. Also present are genes encoding a toxin (colicin) and a virulence factor (hemolysin). (Diagram: Elzbieta Brzuszkiewicz, Goettingen, Germany.)

addition, genes for a toxin (colicine) and a hemolysin (destroys erythrocytes) are present. What a potential threat is present on just this one type of resistance plasmid!

What about MRSA? There was something about it in the newspaper yesterday.

MRSA is the abbreviation for methicillin-resistant *Staphylococcus aureus*. Since methicillin is no longer commonly used as an antibiotic, MRSA now also refers to multiresistant *Staphylococcus aureus*. The danger associated with MRSA is twofold: First, the mechanism of resistance rests on the fact that a certain protein on the bacterial cell surface is so modified that antibiotics such as penicillin, methicillin, or oxacillin are no longer bound. As a result, cell-wall synthesis cannot be prevented. Secondly, the information for this modified binding protein is not encoded on a plasmid but on the bacterial chromosome and is therefore stable. This makes MRSA a real threat inside and outside of hospitals. Thousands of people die each year because such infections cannot be treated effectively with antibiotics.

Is there anything good about plasmids?

Indeed, a number of plasmids are very useful. The genetic information for the degradation of compounds that are only available from time to time is encoded on plasmids. As examples, let's take the degradation of naphthalene, toluene, salicylic acid, or the components of crude oil. These compounds are not found everywhere in nature, so it is unnecessary for bacteria to always have the genes for degradation enzymes available. These genes are outsourced to plasmids. Among the huge populations of bacteria in soil and in bodies of water, there are always a few containing plasmids with the genetic information required in case these populations are confronted with any of the above-mentioned compounds. Tanker and oil-platform accidents are negative examples of conditions under which bacteria carrying plasmids with genetic information for degradation of oil components are real champions. But we shouldn't forget the examples of nickel-resistant bacteria in New Caledonia (Chapter 19) and MRSA. If the challenge for bacteria is an ongoing situation, the genetic defense machinery will shift from plasmids to the chromosome. The fact that bacteria have the necessary tools for such shifts makes it even more difficult to combat them during infections.

I am trying to imagine how life on Earth would be without plasmids. Of course, horizontal gene transfer would be less efficient. And what about gene transfer into certain plants? At least the spread of resistances would be greatly reduced.

Speculations of this kind are not very useful. You should view the plasmids from the standpoint of the so-called selfishness of DNA. In his famous book, *The Selfish Gene*, Richard Dawkins writes about the existence of numerous rebellious DNA fragments. We only notice them if they contain information for self-replication and if they are in an environment that provides the machinery and resources for replication. Such an environment is the microbial cell. We should consider the microbe–plasmid systems from a somewhat different point of view. Plasmids keep bacteria in order to survive and to propagate. Those plasmids encoding resistances have a better chance of survival because their bacterial hosts are not destroyed so easily.

But where did resistance genes against antibiotics come from?

We have asked Julian Davies (Vancouver, Canada):

> "This is a complex question. One of the most striking examples of the influence of human activity on the biosphere is the development of antibiotic-resistant bacteria following the introduction of therapeutic antibiotics in 1941. Millions of metric tons of potent antimicrobials have been produced and released into the environment in the last 60 years. Plasmids carrying antibiotic resistance genes were first reported in Japan in the mid-1950s and were greeted with skepticism by scientists and doctors in the

west. How could multidrug resistance possibly be infectious? Plasmid-encoded transmissible drug resistance rapidly became a major worldwide threat to infectious disease treatment and the pharmaceutical industry has been unable to keep up with new resistance mechanisms. The origins of plasmids and their resistance genes pose a question of great evolutionary importance: it is generally assumed that they are environmental in origin; in fact a bacterial penicillinase was identified in 1940, before penicillin was in clinical use! In 1974 it was shown that soil actinomycetes possess enzymes that inactivate the antibiotics that they produce. It is assumed that these are for "self-protection" of the producer, but there is no convincing proof of this supposition. Antibiotic-producing organisms have biochemical mechanisms of drug inactivation, export systems and target inactivation protection mechanisms. These are related in mechanism to those found in pathogenic organisms in the clinic. Do all resistance mechanisms arise from producing strains in the environment? Recent metagenomic studies have shown that antibiotic resistance is widespread in nature; this has been termed the "resistome". Interestingly, resistance genes have been detected in bacterial strains that do not produce antibiotics. For example, resistance to vancomycin is widespread. Thus, resistance and production do not necessarily go together. The human gut microbiome is also a reservoir of many resistance genes but their relationship with disease bacteria is not known. Are the resistance genes in hospitals the same as those found in soils and other environments that have not been exposed to the human use of antibiotics? They work in the same way but the genes differ in their DNA sequences and gene regulation signals. The connection between the resistome and the clinic is not yet complete! It would require significant genetic "tailoring" to convert an environmental streptomycin phosphotransferase gene to that found in a *Shigella* or *Staphylococcus* pathogen.

The ecology of antibiotics and their resistance genes is poorly understood, especially since it is difficult to detect the presence of antibiotics in native soils and water sources. A better understanding of the roles that these wonderful small molecules play in nature might provide information on how resistance develops and perhaps lead to better ways of finding much-needed new antibiotics!"

So, in all our discussions on resistance, it has to be taken into account that the production of antibiotics by microorganisms is intrinsically tied to resistance of the producer. From there, resistance will spread, and the genetic "tailoring" mentioned may then lead to the complex picture drawn by Julian Davies. But we must go on with the isolation and design of new antibiotics.