

Genomic Islands and Horizontal Gene Transfer Among Bacteria

Chromosomes are usually thought to be stable molecules, which have to be copied carefully for each of the new daughter cells. Except for a few copying mistakes (“mutations”), not much is happening to the chromosomal DNA. Or is it? Bacterial chromosomes are now known to harbor what is called “genomic islands”, regions which can cut themselves out of the chromosome, in some cases travel to other bacterial cells and reinsert into the recipient’s chromosome. Their function? Very often, they provide the recipient bacteria with auxiliary capabilities for infecting eukaryotic hosts or for degrading environmental pollutants.

Almost ten years ago, we started to investigate the process of horizontal gene transfer in bacteria (see glossary). Our aim was to estimate how frequent certain types of genes are transmitted between different bacteria in the natural environment. As a model system for our studies, we chose the bacterium *Pseudomonas* sp. strain B13, which was isolated from sewage sludge and which may use 3-chlorobenzoate as sole carbon and energy source (Fig. 1). When this strain was first described in 1974, it was one of few bacterial strains capable of degrading chlorinated compounds. This had attracted considerable attention, since chlorinated aromatics are often polluting substances in the environment. Strain B13 became yet more appealing because of another spectacular feature that we discovered: these bacteria are able to transfer their genes for the 3-chlorobenzoate metabolism spontaneously to other bacterial species, and this even in wastewater treatment reactor microcosms [1]. One of the curious findings, though, was that the rate of hori-

zontal gene transfer seemed to increase in the presence of 3-chlorobenzoate. At that time, we interpreted these results such that 3-chlorobenzoate was favoring the growth of bacteria which had received the genes for 3-chlorobenzoate degradation. Furthermore, we did not have too much idea on how these genes were actually distributed from B13 to other strains.

Genes for 3-Chlorobenzoate Degradation Combined on a Genomic Island

Therefore, we looked at the mechanism of gene transfer in more detail. Roald Ravatn, who did his PhD thesis on this topic, discovered that the “recipient” bacteria had actually received a large DNA fragment of more than 100 000 basepairs from strain B13. This fragment was called the *c/c* element (Fig. 2A) and contains the genes for 3-chlorobenzoate degradation [2]. It had become inserted into one or two very specific sites of the recipient chromosome. Strain B13 itself carries two copies of the

c/c element in its chromosome, which didn’t seem to be lost after transfer to a new bacterium (Fig. 2B).

Roald Ravatn also identified the factor responsible for cutting the *c/c* element out of the chromosome and for subsequent reintegration. It is an enzyme called “integrase”. Comparison of the biochemical composition of the integrase from strain B13 with other proteins showed that it was related to integrases from bacterial viruses (bacteriophages), which place their genomes into the chromosomes of the infected cells, and to integrases from so-called genomic islands (see glossary) [3]. The gene for the B13 integrase is situated at the right end of the *c/c* element (Fig. 2A).

Since a few years, the discovery of genomic islands has accelerated enormously, mainly because of genome sequencing projects. Large sequencing laboratories determined the complete nucleotide sequence of currently around 100 bacterial genomes. With the complete nucleotide sequence at hand, it could be shown that many bacteria carry genomic islands and even have multiple different copies. The genomic islands are characterized by the presence of a gene for an integrase and a specific site on the chromosome where they have inserted (Fig. 2B). Taking together all available information, we concluded that the *c/c* element is a genomic island.

When Do Genomic Islands Move?

Now that we knew that the genes for the 3-chlorobenzoate degradation lie on a genomic island, we turned back to our earlier observation indicating an increased transfer of the *c/c* element when 3-chlorobenzoate is present. At this point, Vladimir Sentschilo started his PhD thesis in 1999 considering the question of which environmental or cellular factors regulate the transfer of the *c/c* element. Because the transfer of the *c/c* element is always preceded by the activation of the integrase gene, our assumption was that we could take the activation of the

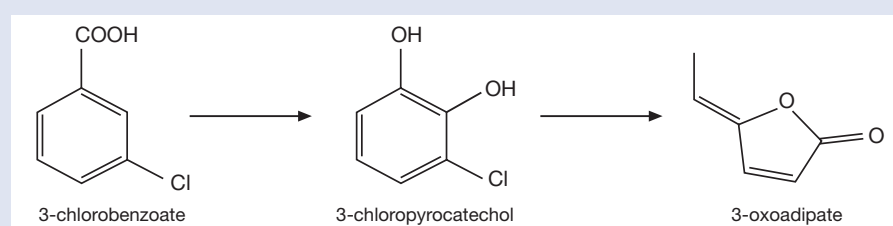


Fig. 1: Specific degradation pathway of the chlorinated aromatic 3-chlorobenzoate. The product 3-oxoadipate will be further degraded in the general cellular metabolism.

Glossary

Genomic Islands

Unstable regions on the chromosomes of bacteria, which sometimes transfer themselves from one bacterium directly into the genome of another one. They increase bacterial fitness and can be divided into several subtypes: e.g., “ecological islands” in environmental bacteria and “pathogenicity islands” in pathogenic bacteria with auxiliary functions in infection, toxin synthesis or adhesion [4].

Green Fluorescent Protein or GFP

Reporter protein; those cells in which GFP is synthesized are fluorescent and can be observed under the epifluorescence microscope.

Horizontal Gene Transfer

DNA exchange between bacteria; in contrast to vertical gene transfer signifying the inheritance of a gene from a progenitor. Bacterial reproduction is usually described as asexual, because bacteria have no equivalent of the genetic fusion of two different cells that is characteristic of sexual reproduction in eukaryotes. Nonetheless, bacteria do have the ability to exchange segments of DNA with other bacteria. Because these segments can become fixed in a bacterium's genome and confer new traits, gene exchange among bacteria could be considered to be a form of bacterial sex.

Promoter

Regulating region of a gene in front of the coding region. Activation of the promoter will lead to transcription of the coding region and to subsequent synthesis of the respective protein.

integrase gene as indicator for the subsequent excision and transfer of the *cIc* element. Therefore, Vladimir Sentchilo focused on the gene for the integrase and constructed specific reporter bacteria (similar to the arsenic biosensor, see p. 12). These reporter bacteria carried a molecular switch consisting of the integrase gene promoter (see glossary) coupled to the reporter gene for the Green Fluorescent Protein (= GFP, see glossary). Presence of GFP in the cell would thus signify that the promoter of the integrase gene had been activated and that the transfer process will subsequently proceed.

To our astonishment, we observed that only very few cells became fluorescent in a culture of the transgenic strain B13 (Fig. 3), implying that the transfer mechanism was only activated in a small subset of the pop-

ulation. Mainly, however, cells became fluorescent when they were no longer actively growing (i.e., starvation conditions). Strangely enough, though, when cells had been grown in the presence of 3-chloro-

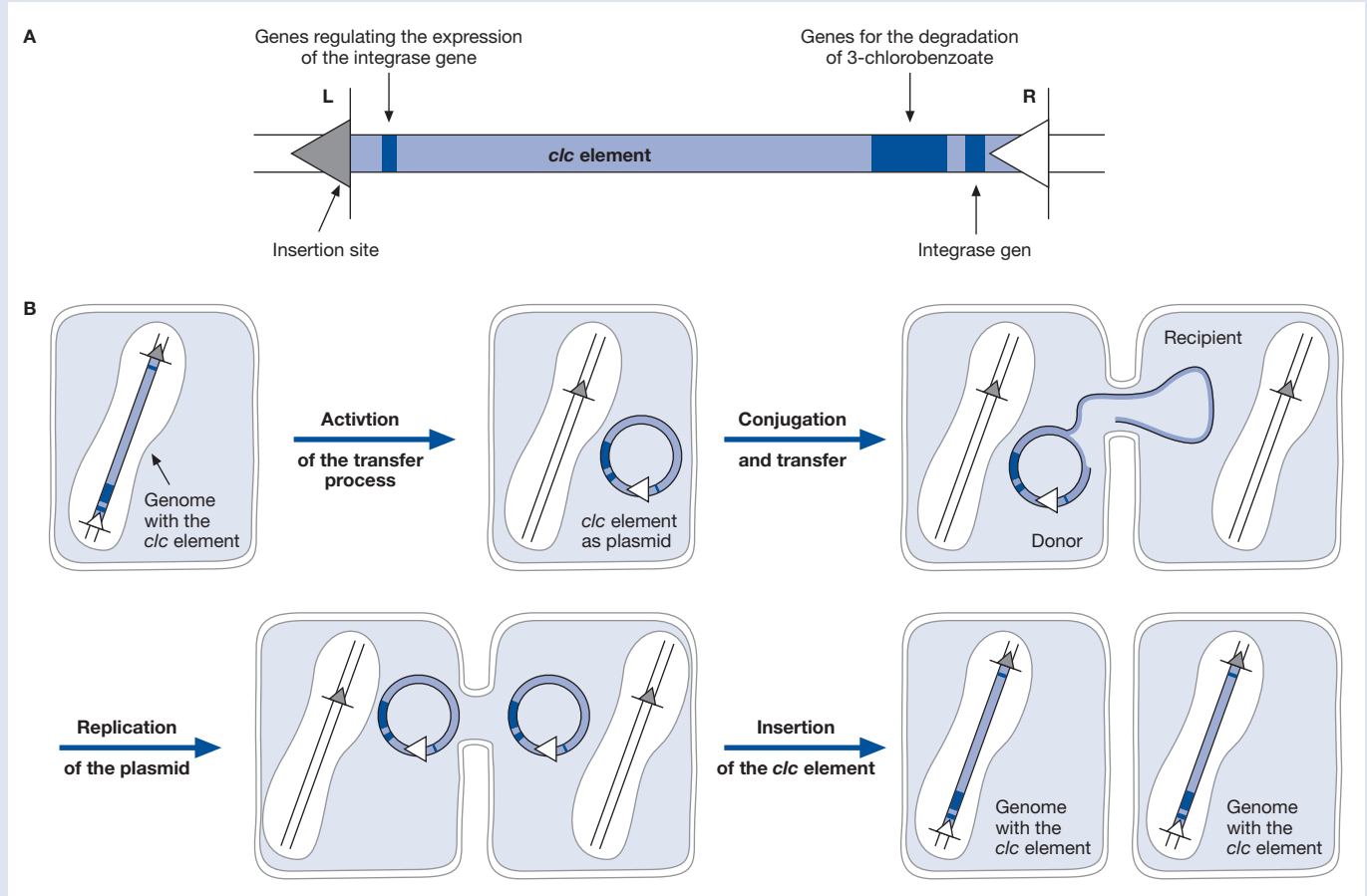


Fig. 2: The *cIc* element (A) and its hypothetical life on its own (B). After activation of the transfer process, the *cIc* element is cut out of the genome by the integrase and forms a circular molecule (= plasmid) in the bacterial cell. In case this cell comes into contact with another bacterium lacking the *cIc* element, the *cIc* element will be transferred as single-stranded molecule to the second cell. After replication, the *cIc* element integrates at predetermined insertion sites into the genomes of both cells, a process during which the integrase also plays an important role.

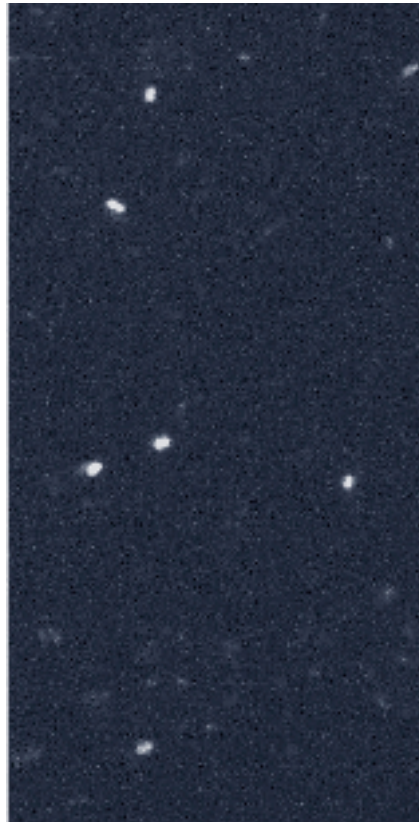
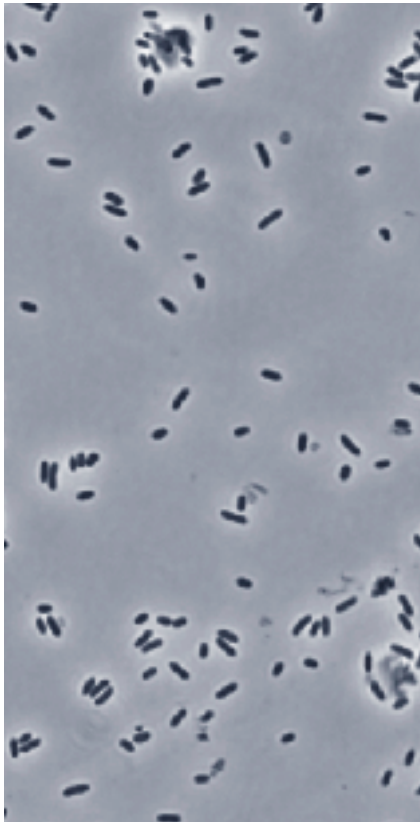


Fig. 3: The transfer process of the *clc* element ist activated only in a small number of bacterial cells of a culture of *Pseudomonas* sp. strain B13. Compare the phase contrast image (left, black on grey) with the same section showing the activated bacteria as bright cells on a black background.

benzoate, the number of fluorescent cells in starvation conditions was higher than when other carbon sources were used. This result confirmed our initial observation and showed, moreover, that 3-chlorobenzoate stimulates the transfer of the *clc* element at a very early stage, i.e., by activating the integrase gene expression. However, it is still unknown why the integrase gene is activated in some bacteria but not in others. Vladimir Sentschilo was also able to identify two proteins which seem to influence the expression of the integrase gene and may perhaps interact with cellular or environmental signals. Interestingly, these two proteins are encoded by the genomic island itself and a database comparison showed similar proteins in a number of other bacteria. In order to better understand the func-

tion of the genomic island in strain B13 and its evolutionary relationship to other genomic islands, we are now determining the complete DNA sequence. This is done with the help of the Institute Pasteur in Paris and the Genome Center in Bielefeld, Germany. With this knowledge, we hope to get a better idea of how the transfer of the B13 element and other genomic islands is regulated.

Desirable and Undesirable Implications

If it turns out that certain chemical compounds in the environment, like 3-chlorobenzoate, really act as a trigger for gene transfer, this could have profound influence of the rates of distribution of certain gene functions among bacterial communities. From the perspective of degradation of en-

vironmental pollutants, it wouldn't seem too problematic if the genes for their degradation became more widely distributed, since this would result in a faster degradation of the pollutant. However, faster distribution of pathogenicity characteristics providing other bacteria with auxiliary capabilities for infecting eukaryotic hosts might not really be an attractive perspective. It seems that even the genomes of what we usually consider to be the smallest organisms have smaller entities, e.g., the genomic islands with a peculiar life-style of their own.



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