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Escherichia coli and Recombinant DNA Technology

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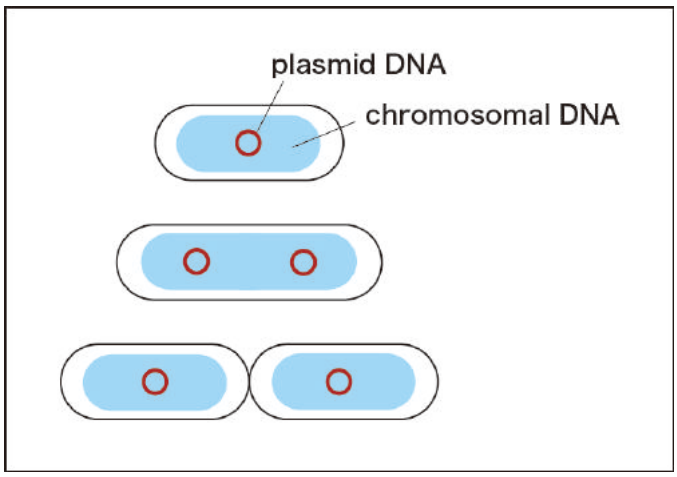
Plasmids and recombinant DNA technology

The bacterium *Escherichia coli* (*E. coli*) lives in the large intestine of the warm-blooded organisms including human. Many *E. coli* strains were isolated from feces. Most of them are harmless and work in symbiotic ways with their hosts. Virulent strains including O157

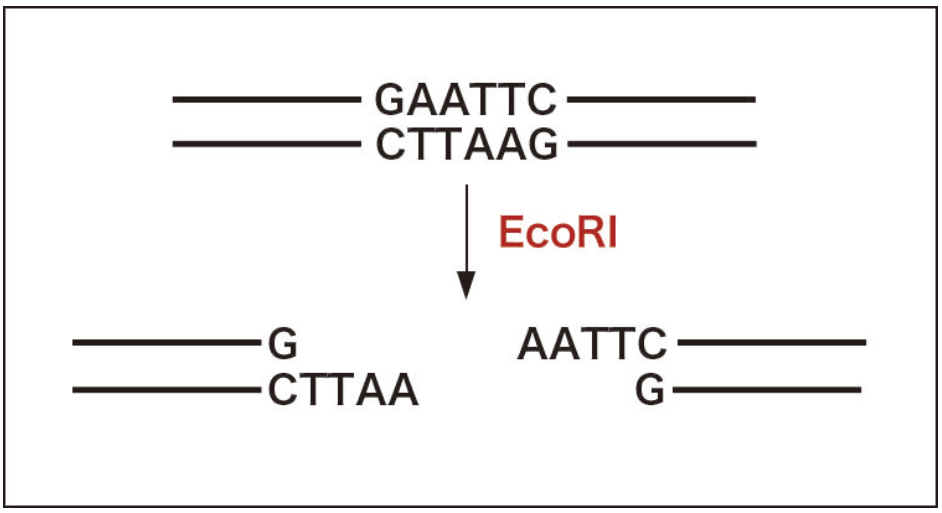
are exceptional and they cause serious illness or death. Several decades ago, a scientist made a proposal that researchers in the world would unravel mechanisms of life phenomenon common to all organisms by using *E. coli* strain K12 as a model. Consequence of the proposal was fruitful and made a tremendous progress in uncovering the molecular and genetic mechanisms basic to living organisms. A number of the results among them deserved Nobel Prize and have become classic knowledge today. When *E. coli* cells are incubated in nutrient medium at 37°C similar to human body temperature, cells grow and divide at 25-minute intervals resulting in several billions times in number within a whole day and night. Thus, *E. coli* is proved to be a very useful model organism in research. *E. coli* cell has a shape like a sausage of 2-micrometer long (1 micrometer is 1/1000 of 1 millimeter), and when grown to 4 micrometers, the center of the cell becomes narrowed to separate into two cells. There is a circular form of chromosomal DNA in the cell that governs genetic information networks. This DNA has a length about 500 times of the cell length and fits within the cell as a densely folded form. Currently, whole nucleotide sequence of chromosomal DNA has been determined to reveal that it potentially codes for about 4000 kinds of proteins. Thus, even the bacterium that seems to be simple at a glance could have a complex life mechanism.

Among the many *E. coli* strains isolated from large intestine, some strains have small circular double-stranded DNA besides the chromosomal DNA. Such DNA is called 'plasmid'. Plasmid can do self-multiplication. Many kinds of plasmids were isolated. In some kinds, one or two plasmid molecules exist per cell, while in other kinds several tens molecules exist per cell. Some plasmids called R plasmid are also known that carry genes making the cells resistant to antibiotics. When an *E. coli* cell of male strain having F plasmid (sexual plasmid) conjugates to *E. coli* cell (female strain) harboring no F plasmid, F plasmid is transferred to the female cells. In this conjugation, chromosomal DNA may also be transferred, resulting in crossing of genes between both strains.

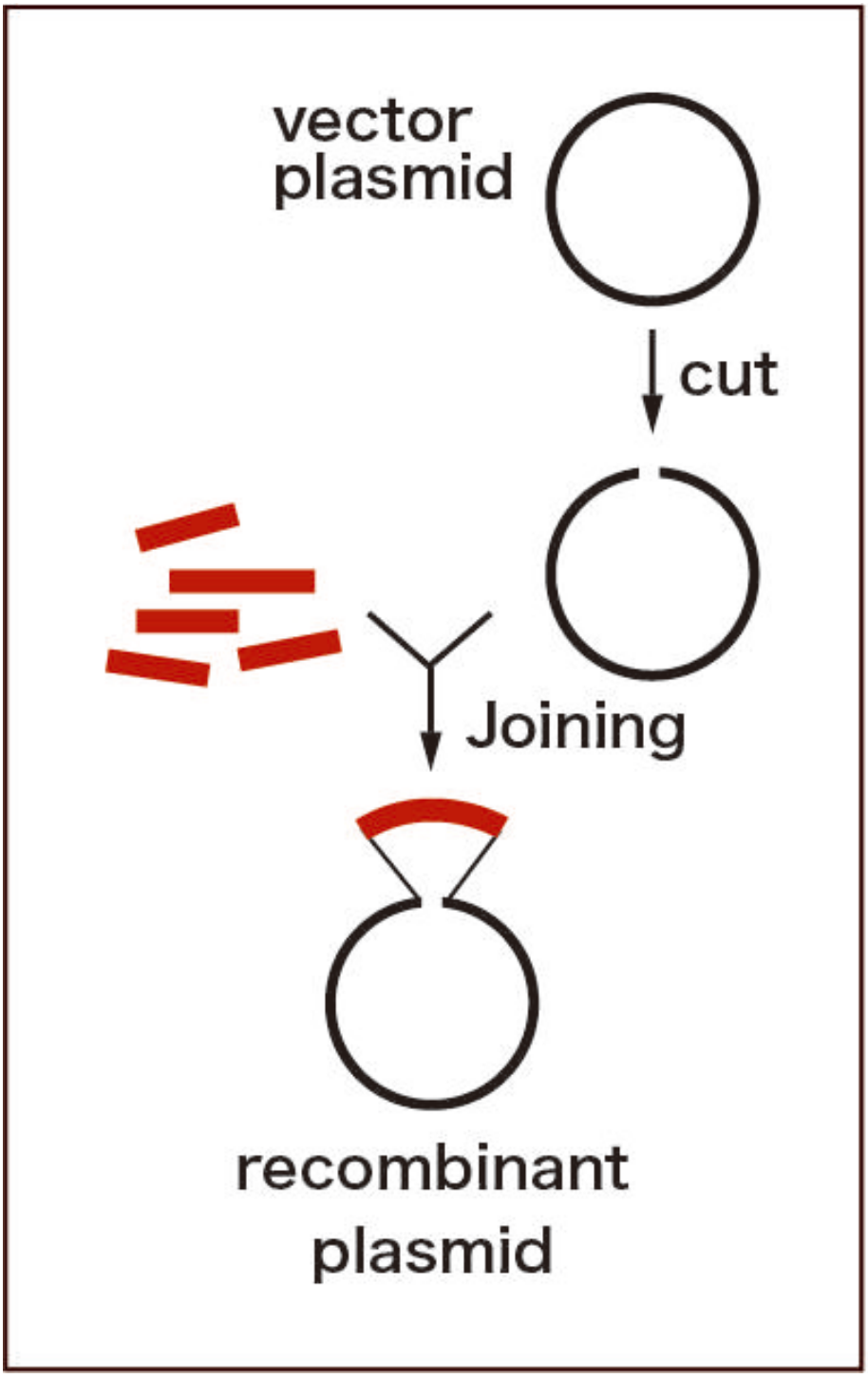


Plasmid in *E. coli*

Plasmids also play an important role in "recombinant DNA technology". This technology made a rapid progress in concert with the discovery of restriction enzymes. The technique is now wildly used, for example, in "DNA profiling", "production of genetically modified crops", "preparation of transgenic mouse as an animal model of human disease", "iPS cell preparation", "biological evolution research", *etc*. A restriction enzyme to be used is an enzyme that cleaves DNA into fragments at or near specific recognition base sequence and cleaves a special base sequence on DNA. Various kinds of restriction enzymes are currently used. For example, the restriction enzyme *Eco*RI recognizes and cleaves the GAATTC base sequence as shown in the figure below.



For example, cloning of a human gene, human DNA is cleaved with a restriction enzyme, whereas vector plasmid, which has only one site of the nucleotide sequence for the restriction enzyme, is cleaved at the nucleotide sequence by the restriction enzyme. Both DNA samples are mixed and an enzyme joining DNA is added and incubated. Plasmids inserted human DNA fragments can be generated. The DNA solution is taken into *E. coli* cells and the bacteria are plated on agar medium. Some colonies of *E. coli* having plasmids containing human DNA fragments are detected.

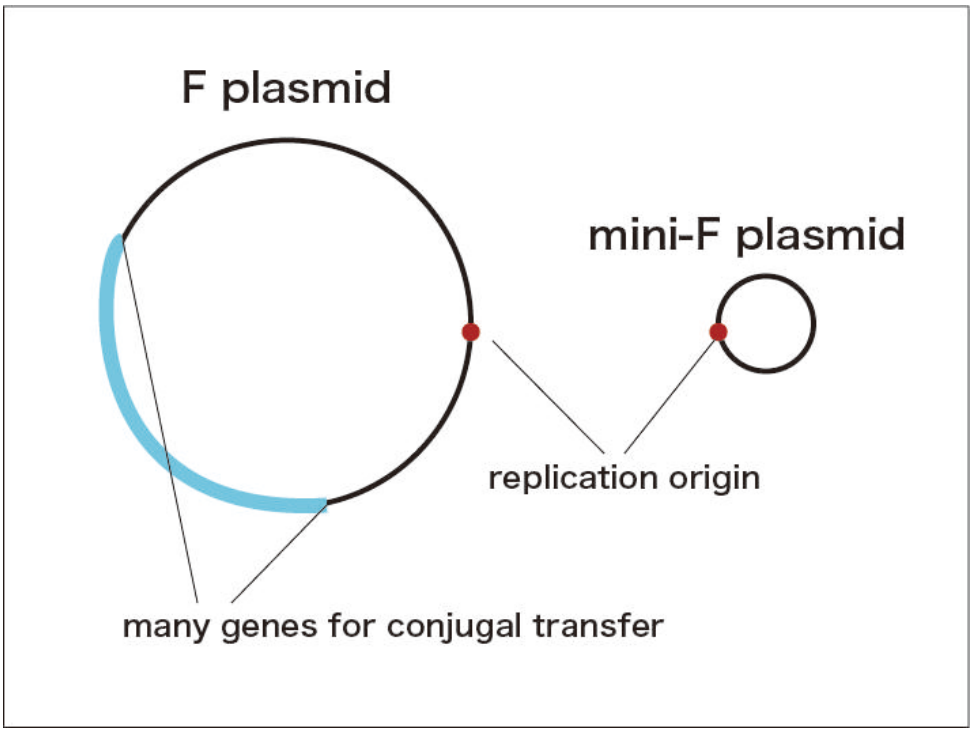


Cloning of human DNA

E. coli cell having a plasmid containing human insulin gene or growth hormone gene is thus obtained so that insulin or growth hormone can be produced in a large amount. In the past when the recombinant DNA technique was yet invented, growth hormone was extracted and purified from pituitary glands of cadavers. Pituitary glands from several bodies were necessary for treating one patient whose height would not sufficiently have grown otherwise during adolescence.

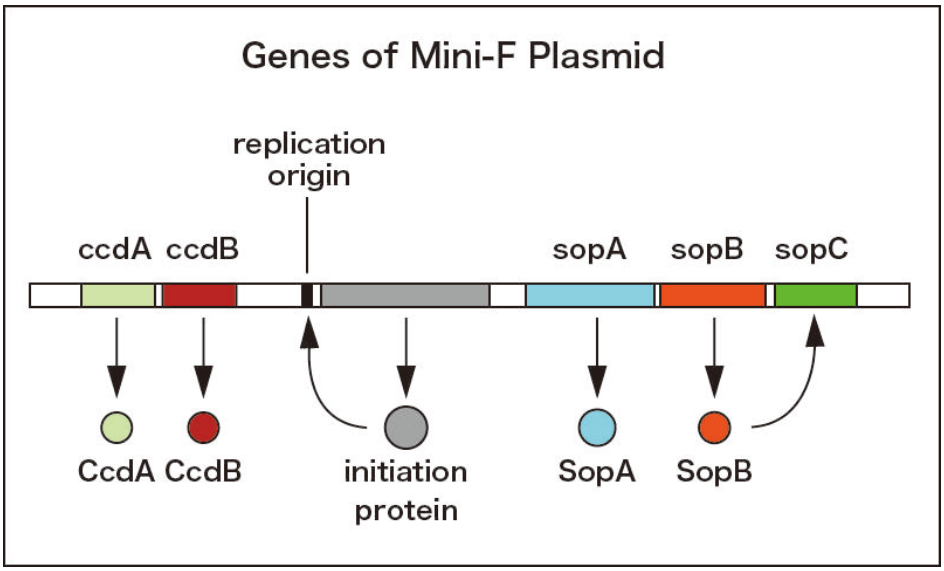
Discovery of partition mechanism of mini F plasmid

We chose F plasmid (sexual plasmid) as one of the research themes. F plasmid has a lot of genes that work to conjugal transfer. We actually used a small "mini-F plasmid", which lost the genes of conjugal transfer, for research.



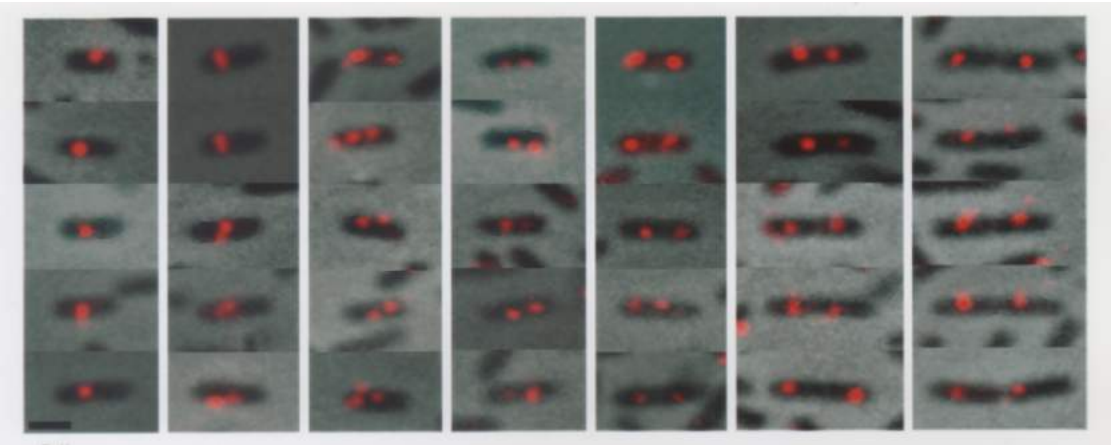
Mini-F plasmid is replicated by the role of initiation protein
that is specific for replication initiation of the F plasmid.

As in the case of F plasmids, only one or two mini-F plasmid molecules are present per cell, and the plasmids are inherited to daughter cells one by one upon cell division. Thus "partition" works normally as in the case of F plasmids. The mini-F plasmid has a replication origin and a gene for replication initiation protein for autonomous proliferation. When the DNA region in the right side of the gene for replication initiation protein was deleted (see the figure below), the deleted mini-F plasmid became unstable in terms of partition and daughter cells that lost mini-F plasmid appeared more frequently. From this observation, genes necessary for normal partition could be present in the deleted DNA region. Further analysis of this area in detail revealed that SopA and SopB proteins corded by *sopA* and *sopB* genes (abbreviation of stability of plasmid) and a specific nucleotide sequence of *sopC* are involved in controlling plasmid partition. The purified SopA and SopB proteins react with each other, and the SopB protein has the property of binding strongly to the *sopC* DNA region.



Mini-F plasmid is replicated by the role of initiation protein that is specific for replication initiation of the F plasmid.

We developed fluorescence phase-contrast microscopic technology to investigate location of plasmids and distribution of the proteins in *E. coli* cells. A mini-F plasmid molecule was initially present in the center of the cell, but then it became two molecules by replication and each of them moves to be located at positions of 1/4 and 3/4 of the cell length along elongation of the growing cell as shown in the photos below.



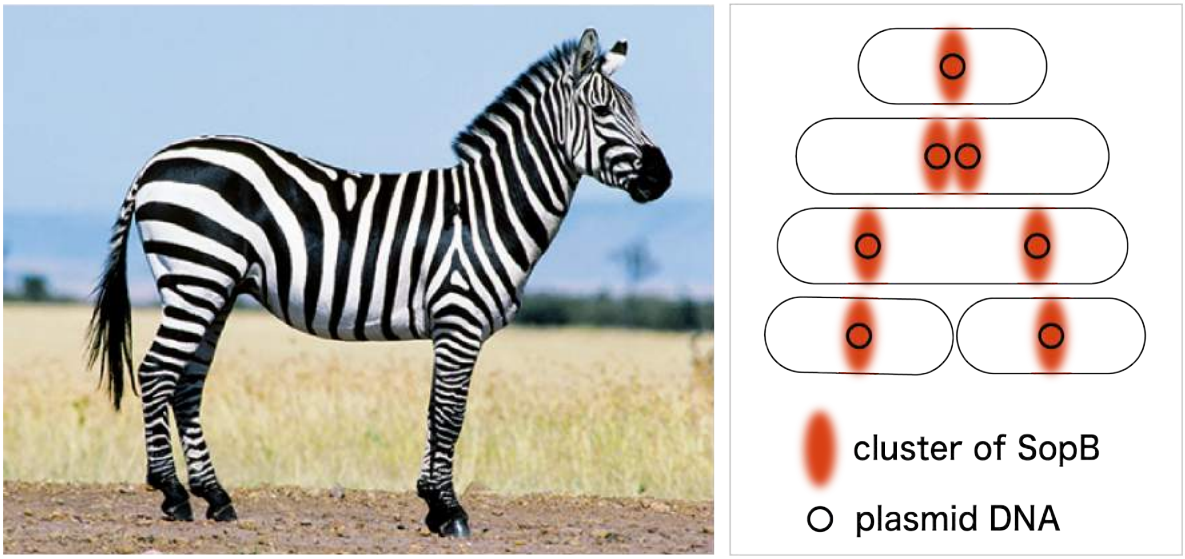
Fluorescence phase-contrast microscopic images of *E. coli* cells in which mini-F plasmid molecules were stained by *in situ* hybridization with mini-F plasmid DNA fragments binding with red fluorescence compound.

On the other hand, mini-F plasmid molecules lacking the *sopA-sopB-sopC* region were localized at random in cell poles where were no chromosomal DNA. Therefore there were

cells in which two molecules were localized in a cell pole, so that plasmid-free daughter cells appeared frequently upon cell division. These results indicated that the *sopA-sopB-sopC* system determined subcellular position of plasmid molecules.

We developed a new technique to observe subcellular localization of proteins in *E. coli* cells. Using this technique we found that SopA and SopB proteins were distributed in striped patterns in the cell, and mini-F plasmid DNA molecules were located at highly concentrated regions of the SopB protein. Based on this experimental data, we speculated that this phenomenon could be explained by "reaction-diffusion model". This model was proposed by the genius mathematician Alan Turing, who discovered the principle of computer, to explain the mechanism of striped hair pattern formation in animals such as tigers and zebras (1952). In brief, it is a hypothesis that when two proteins having different diffusion rates react with each other, the coupled proteins form striped patterns. This can happen not only in the fetus of animals but also in single cells of bacteria.

Regarding the plasmid partition in *E. coli* cells, the striped patterns are formed by the action of SopA protein and SopB protein which react with each other, and since the DNA of *sopC* region of plasmid DNA binds to SopB protein, plasmid molecules can be located at highly concentrated clusters of SopB protein. Computer simulation matched well the observed results. We published this conclusion in 2006 when 23 years had passed since our discovery of the partition control mechanism of the mini-F plasmid (1983).



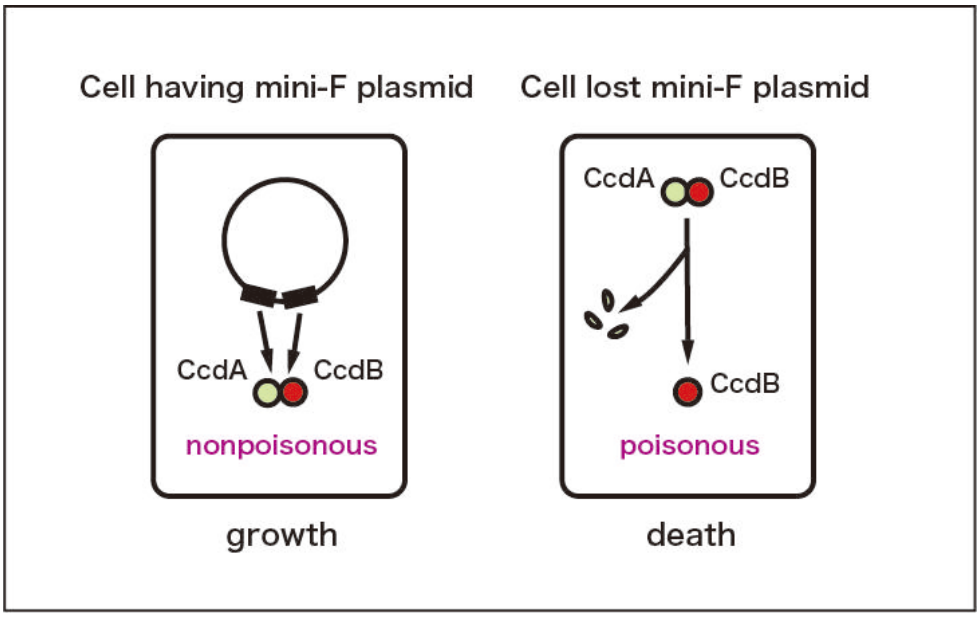
The “reaction-diffusion mode

Our experimental results on this partition mechanism are now applied to a vector plasmid used for recombinant DNA technology. The vector plasmid carrying *sopA*, *sopB,*

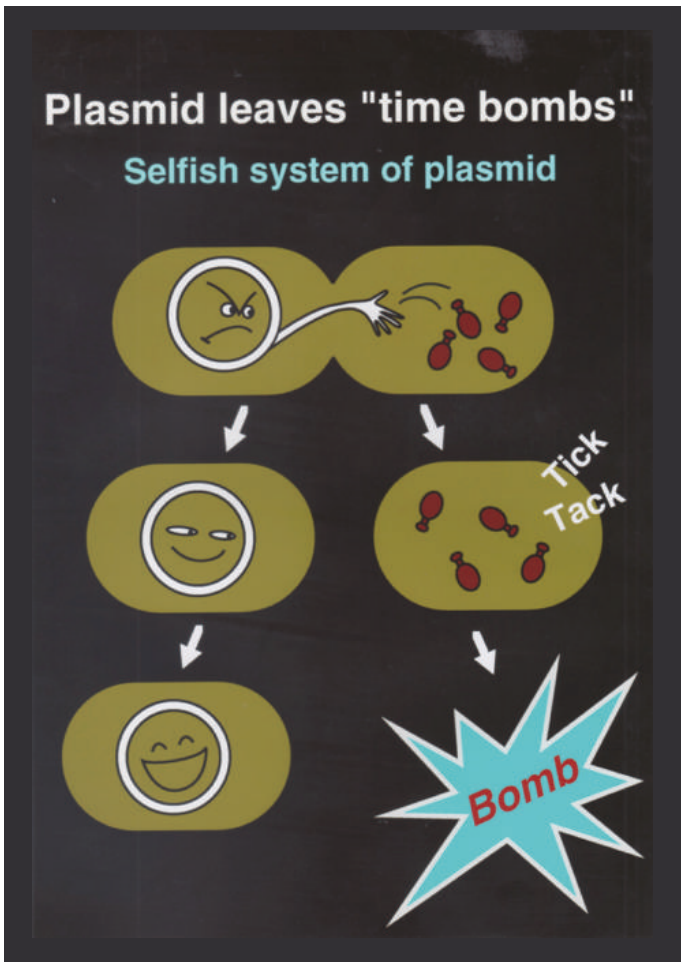
and *sopC* genes has been sold by an overseas drug company. As this vector plasmids are stably maintained in the cells, the efficiency to yield the desired human protein from the cloned gene is improved. Since *E. coli* cells can be cultured in a large quantity, use of the vector plasmids is proved enormously beneficial.

Discovery of the CCD mechanism of mini F plasmid

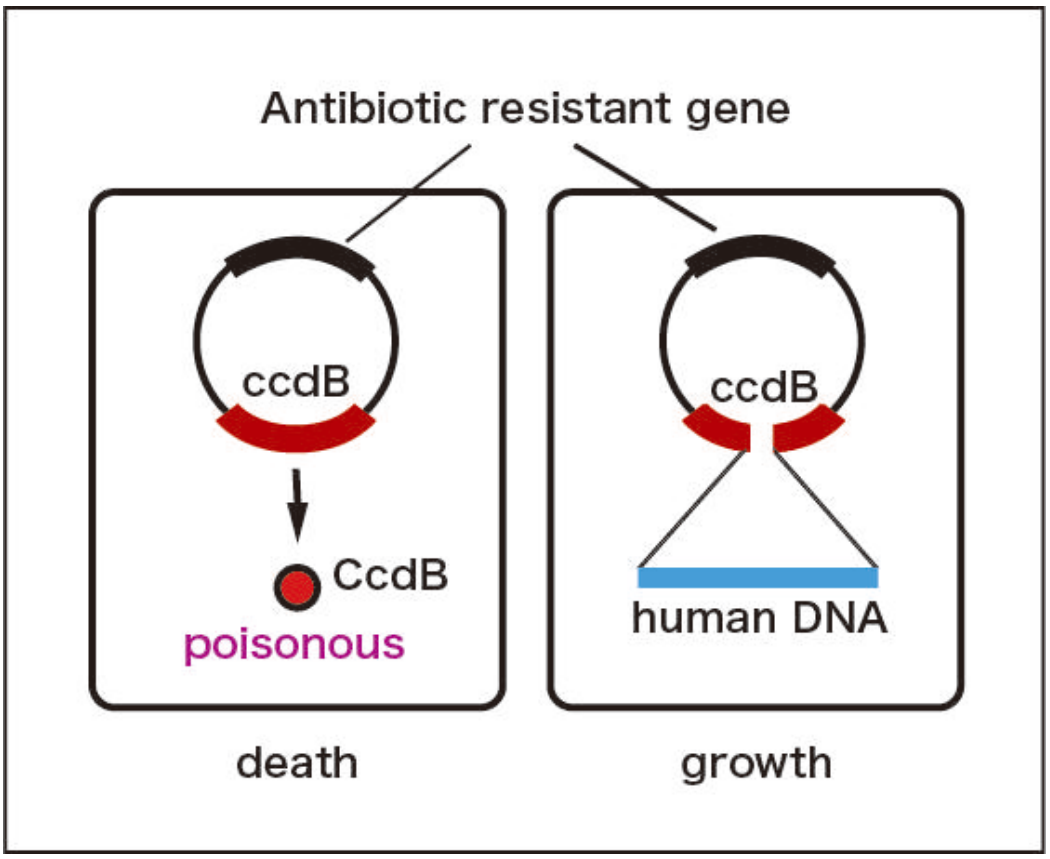
Furthermore, we discovered a new mechanism for stable maintenance of mini-F plasmid in cells and named it "CCD mechanism" (CCD stands for control of cell death). This mechanism is carried out by the action of two proteins (CcdA protein and CcdB protein) coded by the genes named *ccdA* and *ccdB*, respectively (see the figure at page 6). Results of detailed experiments revealed that CcdB protein has a function of killing cells, and CcdA protein has the ability to erase the toxicity of CcdB protein. Therefore, when both proteins coexist, the cell does not die and grow. However, when a daughter cell that has lost mini-F plasmid appears upon cell division as a result of some impairment, CcdA protein that remains in the mini-F plasmid-less daughter cell is fragile in nature and disappears in due course, while CcdB protein is stable. Therefore the cell is killed by the toxicity of CcdB protein. Cells carrying mini-F plasmid are able to grow predominantly in the environment than cells which lost mini-F plasmid (1985), therefore the CCD mechanism is a 'selfish mechanism' of mini-F plasmid.



CCD mechanism



The discovery of the CCD mechanism has acquired a reputation and spread among plasmid researchers. An overseas drug company has made and sold the vector plasmid carrying the *ccdB* gene for using in recombinant DNA technology. To isolate a certain gene of humankind using this vector plasmid, a particular nucleotide sequence in the *ccdB* gene is cleaved with a restriction enzyme in a test tube and mixed with humankind genomic DNA fragments treated by cleaving by the same restriction enzyme. The cleaved humankind DNA fragments are mixed with the cleaved plasmids and incubated with the enzyme joining DNA fragments. The DNA mixture is introduced into *E. coli* cells and the cells are spread on the agar medium with antibiotic. This plasmid carries an antibiotic resistant gene. *E. coli* cells that did not receive the plasmid are killed by the antibiotic. Only *E. coli* cells carrying the vector plasmid in which the humankind DNA fragment is inserted into the *ccdB* gene can grow to form colonies (a cell group of bacteria grown from a single cell) on the agar medium, but *E. coli* cells receiving the vector plasmid without humankind DNA fragment die entirely by production of CcdB protein. The vector plasmid that is very effective in gene cloning is currently widely used in the world. The commercially available vector plasmid is due to our fundamental research on mini-F plasmid.



Vector plasmid carrying the *ccdB* gene

When I gave a talk about the discovery of this CCD mechanism at the Gordon Research Conference on Plasmid in New Hampshire, I predicted as follows. "Other plasmids may also have a toxic protein and a neutralizing protein in pairs similar to the case of F plasmid, but these proteins will not be identical with those of the CCD mechanism in F plasmid."

Thereafter, CCD mechanisms were found one after another in various plasmids as I had predicted at the conference and they became booming study subjects in the plasmid research field. Furthermore, Takeshi Miki and coworkers (1996) of Kyushu University discovered that the purified CcdB protein binds to DNA gyrase (one of topoisomerases that causes supercoiling of the DNA) to inactivate its function, resulting in killing cells.

Credit for fundamental research on prosperous progress in life sciences

The discovery of restriction enzymes by Werner Arbor was brought from the basic research of "restriction and modification mechanism" of *E. coli* against invading bacteriophage. "Restriction and modification mechanism" means a phenomenon in that the bacteriophage grown in K strain of *E. coli* can grow well in the K strain, but the phage can hardly grow in B strain of *E. coli*, and *vice versa*. Then, Hamilton Smith isolated and characterized other type of restriction enzymes from the bacterium *Haemophilus*

influenzae. Restriction enzymes of the latter type are more useful for laboratory work as they cleave DNA at the site of their recognition sequence and most commonly used as the molecular biology tools.

After various kinds of restriction enzymes, each having specific recognition base sequence, were discovered from various bacteria, these enzymes are widely used for DNA analysis as a vital tool, so-called "recombinant DNA technology" as described earlier.

Osamu Shimomura and collaborators studied the bioluminescent jellyfish *Aequorea victoria*, which they collected during many summers at the Friday Harbor Laboratories of the University of Washington, and discovered and developed Green Fluorescent Protein (GFP) in the study of the jellyfishes. By linking this GFP to a specific protein by recombinant DNA technology, it became possible to analyze the localization of proteins in living cells. Thus, GFP is contributing tremendously to research all over the world as an indispensable tool in life sciences. Professor Shimomura had been immersing in analyzing the nature of the protein that exhibits bright green fluorescence in response to light in the range of blue to ultraviolet range. It is needless to say that he did not work for winning fame. Can you imagine that laborious and patient collection of 850,000 jellyfishes led him to such a successful result in the fundamental research. Professor Shimomura actually received the Nobel Prize anyway.

What underlies the decline in scientific research level in Japan?

These days, one may often see articles in the news media that raise serious concern about decline in scientific research capabilities in Japan. It is pointed out that the activity levels in the field of fundamental research in particular are falling down. The scientific research environment in recent Japan is becoming in such that one research subject can‘t be continued in a small way under the long-term perspective. One of the underlying reasons for the fall could be that enormous amount of research funds are allocated to a limited number of some big research projects on practical use under a nearsighted social and bureaucratic view. For fundamental research, scientists need work environment, in which they can have research projects of long-range perspective together with long-term guaranteed modest granting and life. Despite of pointing out this serious difficulty, funding for small and steady research are being reduced in inverse proportion to the total budget for scientific research. Also, under the present circumstance, young scientists who have just completed a doctoral course of a graduate program have to remain as a postdoctoral fellow (postdoctoral researcher) and many of them are employed for a short term contractual position mostly in the above mentioned big research projects. Therefore, they have to repeat moving institution one after another under different research theme until they find a guaranteed position where they

can pursue research project of their fully creative idea for long-range project. Accordingly, it is not surprising that young people can't get passionate for research under nowadays system in Japan of poor academic freedom.

In recent years, Japanese scientists have been awarded the Nobel Prize like an *annual event*, but we should emphasize that it must be a legacy of basic researches performed 20 to 30 years ago. Since a major scientific discovery stem s from fundamental research, it is necessary to continue supporting a basic research by sustainable funding. It is also inevitable to support research projects proposed by young researchers, taking a long-range view of scientific future as well as their stable workplace

Written in March 201 9

Acknowledgment

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If you would like to know more detailed results, please see No. 6 PDF file entitled "*Escherichia coli* and my life" in "Hiraga Sota's Homepage: Sequel" https://hs12345.jimdo.com.