# All Kinds of Genetics Transformation

## Transformation:

Transformation is one of three basic mechanisms for genetic exchange in bacteria. Transformation may be either a natural process—that is, one that has evolved in certain bacteria—or it may be an artificial process whereby the recipient cells are forced to take up DNA by a physical, chemical, or enzymatic treatment. In both cases, exogenous DNA (DNA that is outside the host cell), is taken into a recipient cell where it is incorporated into the recipient genome, changing the genetic makeup of the bacterium.

In molecular biology, transformation is the genetic alteration of a cell resulting from the direct uptake and incorporation of exogenous genetic material from its surroundings through the cell membrane(s). For transformation to take place, the recipient bacteria must be in a state of competence, which might occur in nature as a time-limited response to environmental conditions such as starvation and cell density, and may also be induced in a laboratory.

Transformation is one of three processes for horizontal gene transfer, in which exogenous genetic material passes from bacterium to another, the other two being conjugation (transfer of genetic material between two bacterial cells in direct contact) and transduction (injection of foreign DNA by a bacteriophage virus into the host bacterium). In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

As of 2014 about 80 species of bacteria were known to be capable of transformation, about evenly divided between Gram-positive and Gram-negative bacteria; the number might be an overestimate since several of the reports are supported by single papers.

"Transformation" may also be used to describe the insertion of new genetic material into nonbacterial cells, including animal and plant cells; however, because "transformation" has a special meaning in relation to animal cells, indicating progression to a cancerous state, the process is usually called "transfection".

## History:

Transformation in bacteria was first demonstrated in 1928 by British bacteriologist Frederick Griffith. Griffith discovered that a strain of Streptococcus pneumoniae could be made virulent after being exposed to heat-killed virulent strains. Griffith hypothesized that some "transforming principle" from the heat-killed strain was responsible for making the harmless strain virulent. In 1944 this "transforming principle" was identified as being genetic by Oswald Avery, Colin MacLeod, and Maclyn McCarty. They isolated DNA from a virulent strain of S. pneumoniae and using just this DNA were able to make a harmless strain virulent. They called this uptake and incorporation of DNA by bacteria "transformation" (See Avery-MacLeod-McCarty experiment). The results of Avery et al.'s experiments were at first skeptically received by the scientific community and it was not until the development of genetic markers and the discovery of other

methods of genetic transfer (conjugation in 1947 and transduction in 1953) by Joshua Lederberg that Avery's experiments were accepted.

It was originally thought that Escherichia coli, a commonly used laboratory organism, was refractory to transformation. However, in 1970, Morton Mandel and Akiko Higa showed that E. coli may be induced to take up DNA from bacteriophage λ without the use of helper phage after treatment with calcium chloride solution. Two years later in 1972, Stanley Norman Cohen, Annie Chang and Leslie Hsu showed that $CaCl_2$ treatment is also effective for transformation of plasmid DNA. The method of transformation by Mandel and Higa was later improved upon by Douglas Hanahan. The discovery of artificially induced competence in E. coli created an efficient and convenient procedure for transforming bacteria which allows for simpler molecular cloning methods in biotechnology and research, and it is now a routinely used laboratory procedure.

## Definition:

Transformation is one of three forms of horizontal gene transfer that occur in nature among bacteria, in which DNA encoding for a trait passes from one bacterium to another and is integrated into the recipient genome by homologous recombination; the other two are transduction, carried out by means of a bacteriophage, and conjugation, in which a gene is passed through direct contact between bacteria. In transformation, the genetic material passes through the intervening medium, and uptake is completely dependent on the recipient bacterium.

## Natural transformation:

Natural transformation is a bacterial adaptation for DNA transfer that depends on the expression of numerous bacterial genes whose products appear to be responsible for this process. In general, transformation is a complex, energy-requiring developmental process. In order for a bacterium to bind, take up and recombine exogenous DNA into its chromosome, it must become competent, that is, enter a special physiological state. Competence development in Bacillus subtilis requires expression of about 40 genes. The DNA integrated into the host chromosome is usually (but with rare exceptions) derived from another bacterium of the same species, and is thus homologous to the resident chromosome.

Natural transformation is a physiological process that is genetically encoded in a wide range of bacteria. Most bacteria must shift their physiology in order to transform DNA; that is, they must become "competent" for taking up exogenous DNA. There appear to be two basic mechanisms by which bacteria can become competent for transformation. In some bacteria, including Streptococcus pneumoniae and Bacillus subtilis, competence is externally regulated. These bacteria produce and secrete a small protein called competence factor that accumulates in the growth medium.

When the bacterial culture reaches a sufficient density, the concentration of competence factor reaches a level high enough to bind receptors on the outside of the cell. This event causes an internal signal to turn on the expression of the genes needed for transformation. Thus, competence development is controlled by cell density. There are a number of other bacterial functions that are similarly regulated, and these processes are collectively called quorum sensing mechanisms. In other bacteria, including Haemophilus influenzae and Pseudomonas stutzeri, competence development is internally regulated. When there is a shift in the growth dynamics of the bacterium, an internal signal triggers competence development.

Once competence is induced, three additional steps are required for natural transformation. After induction of competence, double-stranded DNA is bound to specific receptors on the surface of the competent cells. These receptors are lacking in noncompetent cells. The double-stranded DNA is nicked and one strand is degraded while the other strand enters the cell. This process is called DNA uptake. Finally, the recombination enzymes of the recipient cell will bind the single-strand DNA that has entered it, align it with its homologous DNA on the recipient chromosome, and recombine the new DNA into the chromosome, incorporating any genetic differences that exist on the entering DNA.

## Transformation, as an adaptation for DNA repair:

Competence is specifically induced by DNA damaging conditions. For instance, transformation is induced in Streptococcus pneumoniae by the DNA damaging agents mitomycin C (a DNA crosslinking agent) and fluoroquinolone (a topoisomerase inhibitor that causes double-strand breaks). In B. subtilis, transformation is increased by UV light, a DNA damaging agent. In Helicobacter pylori, ciprofloxacin, which interacts with DNA gyrase and introduces double-strand breaks, induces expression of competence genes, thus enhancing the frequency of transformation Using Legionella pneumophila, tested 64 toxic molecules to determine which of these induce competence. Of these only six, all DNA damaging agents, caused strong induction. These DNA damaging agents were mitomycin C (which causes DNA inter-strand crosslinks), norfloxacin, ofloxacin and nalidixic acid (inhibitors of DNA gyrase that cause double-strand breaks), bicyclomycin (causes single- and double-strand breaks), and hydroxyurea (induces DNA base oxidation). UV light also induced competence in L. pneumophila. suggested that competence for transformation probably evolved as a DNA damage response.

## Artificial Transformation:

While a wide variety of bacteria can transform naturally, many species cannot take up DNA from an outside source. In some cases DNA can be forced into these cells by chemical, physical, or enzymatic treatment. This is especially important in genetic engineering, as artificial transformation is essential for the introduction of genetically altered sequences into recipient cells. One of the two most common methods is a chemical process where cells are heat-shocked, then treated with the DNA and a high concentration of

calcium ions. The calcium ions precipitate the DNA on the surface of the cell, where the DNA is forced into the recipient.

In transformation, bacteria pick up DNA from their environment. This illustration shows Frederick Griffith's classic experiment which first demonstrated transformation. The live nonvirulent bacteria absorb DNA from the dead nonvirulent bacteria, and become virulent themselves. Adapted from Curtis and Barnes, 1994.

More recently a new method, called electroporation, has been used to introduce DNA by artificial transformation. In this process a suspension of recipient bacteria and transforming DNA is placed in a container with metal sides. A high-voltage electrical current is passed through the sample, temporarily creating small pores, or channels, in the membranes of the bacteria. The DNA enters the cells and the pores close. Thus, exogenous (outside) DNA is introduced into the recipient.

Because exogenous DNA is not enclosed within cell walls, it is susceptible to enzymes that degrade DNA, called DNases. A hallmark of transformation is that it is sensitive to DNase, while the other two processes of genetic exchange, transduction and conjugation, are DNase resistant. Transduction is DNase resistant because the DNA is protected inside a viral protein coat. Conjugation is DNase resistant because fusion occurs between donor and recipient cells, meaning the DNA is never exposed to the outside environment or to enzymes.