Transformation

- The transformation, the uptake of DNA by a bacterial cell.
- **Transformation** has been important in genetic analysis of some species and more recently (and to a much greater extent) because of its key role in gene cloning.
- Transformation contributes extensively to the antigenic variation observed in the gonococcus (Neisseria gonorrhoeae) through the transfer of ***pil*** genes coding for the major protein subunit of the surface appendages (pili) by which the bacteria attach to epithelial cells.
- Although the number of species in which natural transformation has been demonstrated is still quite limited, it is likely that it occurs, albeit at a low level, in many other bacteria.
- The details of the process vary between species, but some generalizations are possible.
- Competence generally occurs at a specific stage of growth, most commonly in the late log phase, just as the cells enter the stationary phase. This may be a response to cell density rather than (or as well as) growth phase.
- For example, in Bacillus subtilis, some of the genes involved in the development of competence are also involved in the early stages of sporulation.
- The development of competence at this stage is associated with the accumulation of specific secreted products (competence factors) which act via a two-component regulatory system to stimulate the expression of other genes required for competence.
- Since the level of these competence factors is dependent on cell concentration, competence will only develop at high cell density. This is a form of quorum sensing, in which the response of an individual cell is governed by the concentration of bacteria in the surrounding medium.
- Following the development of competence, double-stranded DNA fragments bind to receptors on the cell surface, but only one of the strands enters the cell. In some species, the process is selective for DNA from the same species, through a requirement for short species-specific sequences.
- For example, the uptake of DNA by the meningococcus (Neisseria meningitidis) is dependent on the presence of a specific 10-bp uptake sequence.

- The genome of N. meningitidis contains nearly 2000 copies of this sequence, which will only occur infrequently and by chance, in other genomes.
- Similarly, the transformation of Haemophilus influenzae is facilitated by the presence of a 29-bp uptake sequence, which occurs approximately 1500 times in the genome of H. influenzae. These organisms will therefore only be transformed efficiently with DNA from the same species.
- As here fragments of chromosomal DNA (rather than plasmids) are being considered, replication of the DNA will only happen if the incoming DNA is recombined with the host chromosome.
- This requires homology between the transforming DNA and the recipient chromosome. This does not constitute an absolute barrier to transformation with DNA from other species. Provided there is enough similarity in some regions of the chromosome, those segments of DNA can still undergo recombination with the recipient chromosome. The closer the taxonomic relationship, the more likely it is that they will be sufficiently similar.
- One example of this, with considerable practical significance, is the development of resistance to penicillin in ***Str. pneumoniae***. This appears to have occurred by the replacement of part of the genes coding for the penicillin target enzymes with corresponding DNA from naturally-resistant oral streptococci.
- Natural transformation is of limited usefulness for artificial genetic modification of bacteria, mainly because it works best with linear DNA fragments rather than the circular plasmid DNA that is used in genetic modification.
- For introducing foreign genes into a bacterial host, various techniques are used to induce an artificial state of competence.
- Alternatively, a mixture of cells and DNA may be briefly subjected to a high voltage, which enables the DNA to enter the cell (a process known as electroporation).
- Although the mechanisms involved are quite different, they all share the characteristic feature of the uptake of ‘naked’ DNA by the cells and are therefore referred to as transformation.

Transformation by plasmids

Plasmids are small, autonomously replicating, circular DNA molecules separate from the chromosome in a bacterial cell. Since they often contain genes for antibiotic resistance (e.g., ampicillin), their incorporation into a sensitive cell

renders the cell resistant to the antibiotic. Only these bacteria can grow in a culture medium containing the antibiotic (selective medium) Fig 1.

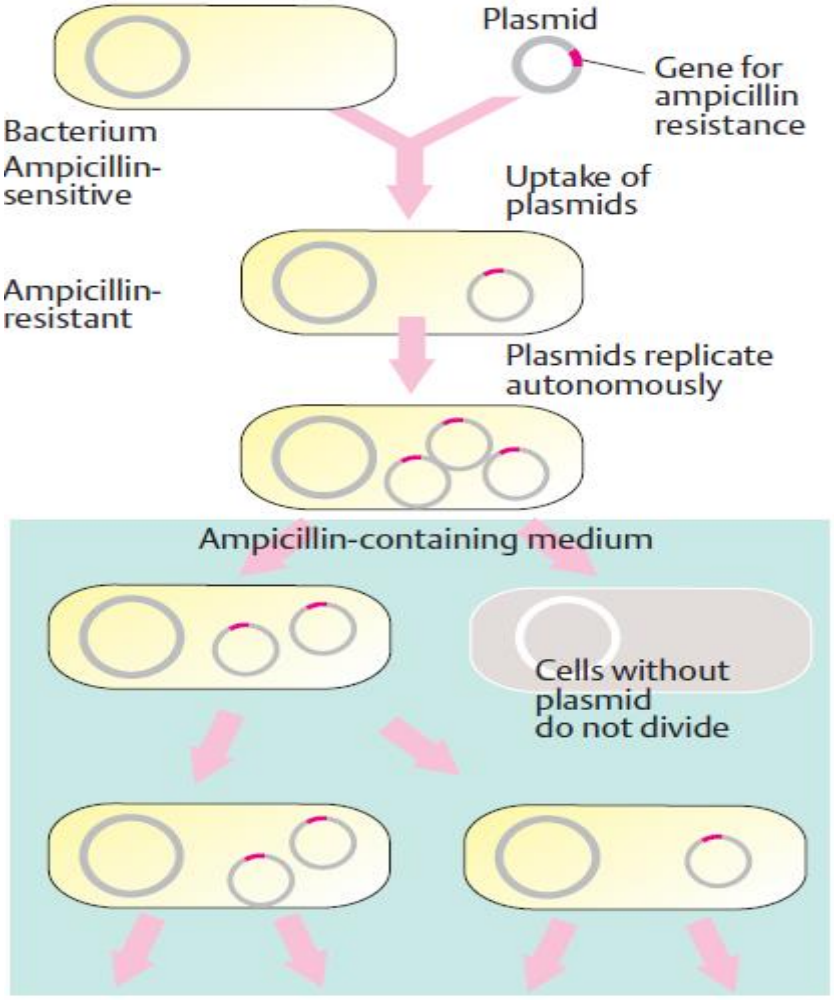

Fig 1.Transformation by plasmids

Multiplication of a DNA segment in transformed bacteria

A DNA fragment to be studied can be inserted into a vector and multiplied in rapidly dividing cells. A plasmid containing one or more genes conferring antibiotic resistance can be used for selecting bacteria that have taken up this plasmid. In an antibiotic-containing medium (selective medium) only those bacteria can grow that have incorporated a recombinant plasmid containing the DNA to be investigated Fig 2.

Transfection by DNA

•In the 1970s it was discovered that pure DNA added to a bacterial culture in the presence of a high concentration of calcium ions (Ca++) can transform the bacteria.

•This discovery allowed any piece of DNA to be introduced into any bacterial genome and the genetic consequences to be observed. DNA can also be introduced into mammalian cells by a process called transfection.

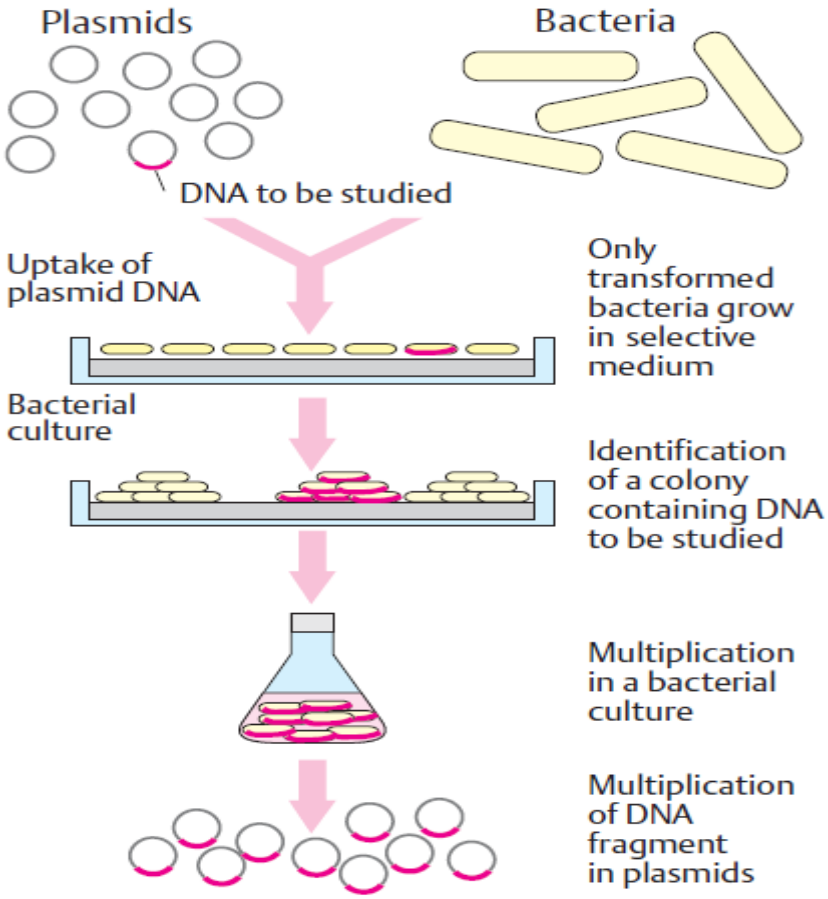

Fig 2.Multiplication of a DNA fragment in transformed bacteria

•In Fig 3 (**Transfection by DNA**) on the left, a DNA transfer experiment is shown in a culture of mouse fibroblasts; on the right, in a culture of human tumor cells .

•The mouse fibroblast culture is exposed to the chemical carcinogen methyl cholanthrene (left). DNA from these cells is precipitated with calcium phosphate, extracted, and then taken up by a normal culture (transfection).

• About 2 weeks later, cells appear that have lost contact inhibition (transformed cells). When these cells are injected into mice that lack a functional immune system (nude mice), tumors develop. DNA from cultured human tumor cells (right) can also transform normal cells after several transfer cycles. Detailed studies of cancer-causing genes (oncogenes) in eukaryotic cells were first carried out using transfection. Many eukaryotic cell lines have been generated from tumor cells. (Figures 3)

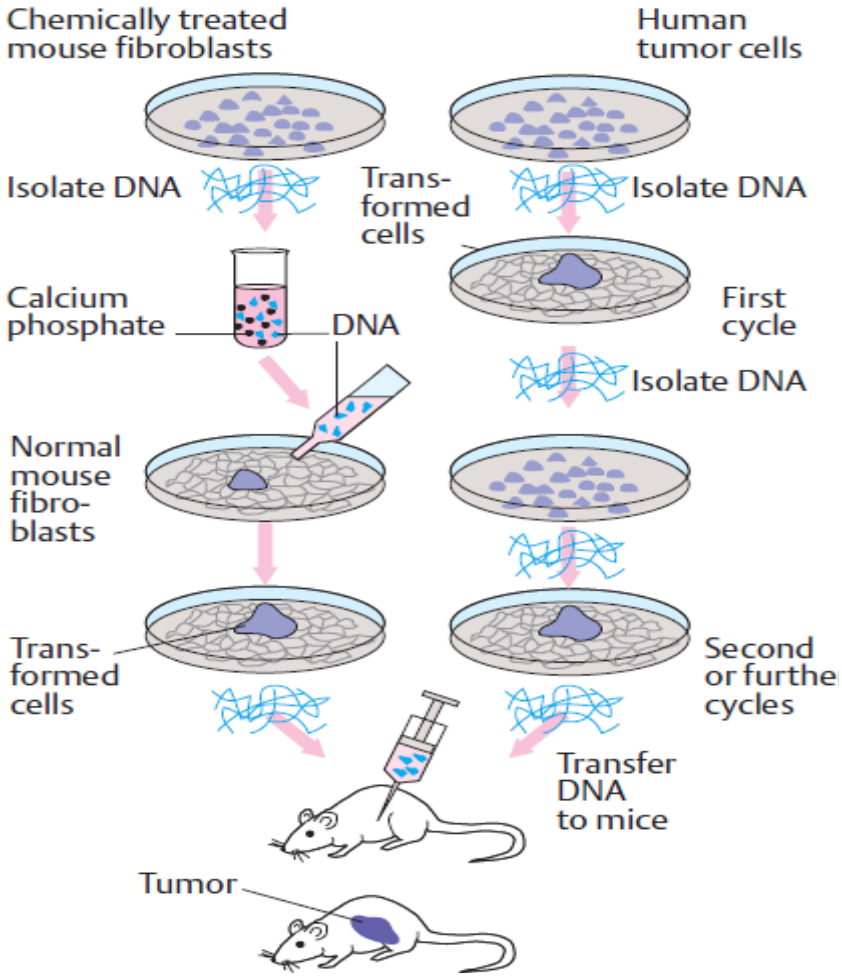

Fig 3. **Transfection by DNA**