Bacterial Transformation

INTRODUCTION

- Genetic recombination is the process by which genetic elements from two separate sources are brought together in a single unit.
- At molecular level, recombination can be thought of as the movement of genetic information from one molecule of nucleic acid to another.
- The genetic exchange occurring between homologous DNA sequences from two different sources is termed as general recombination. For this to happen, identical sequences on the two recombining molecules are required.
- The process of genetic exchange which occurs in eukaryotes during sexual reproduction (meiosis) is an example of this type of genetic recombination.

- Exchange of genetic material occurs between homologous chromosomes leading to the formation of recombinant DNA structures.
- The means by which DNA fragments are introduced into the recipient are: transformation, transduction and conjugation.

Three main processes of genetic recombination in prokaryotes fragments of homologous DNA from a donor chromosome are transferred to a recipient cell

(1) Transformation, which involves donor DNA free in the environment

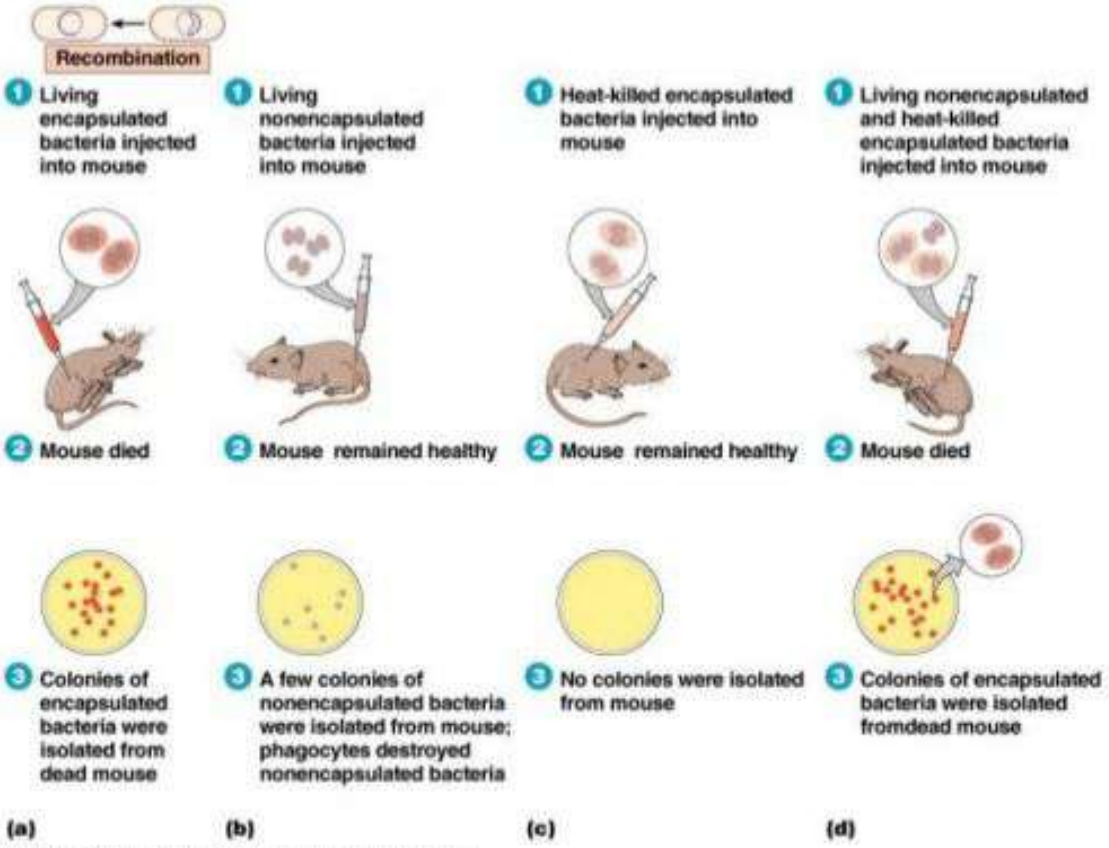
(2) Transduction, in which the donor DNA transfer is mediated by a virus

(3) Conjugation, in which the transfer involves cell-to-cell contact and a *conjugative plasmid* in the donor cell

Griffith's experiment:

- Griffith called the genetic information which could be passed from one bacterium to another the transforming principle.
- After 16 yrs., i.e, in 1944 **Oswald Avery, Collin MacLeod and Maclyn** revisited griffiths experiment and proved that the transforming principle was DNA and not protein or carbohydrate.

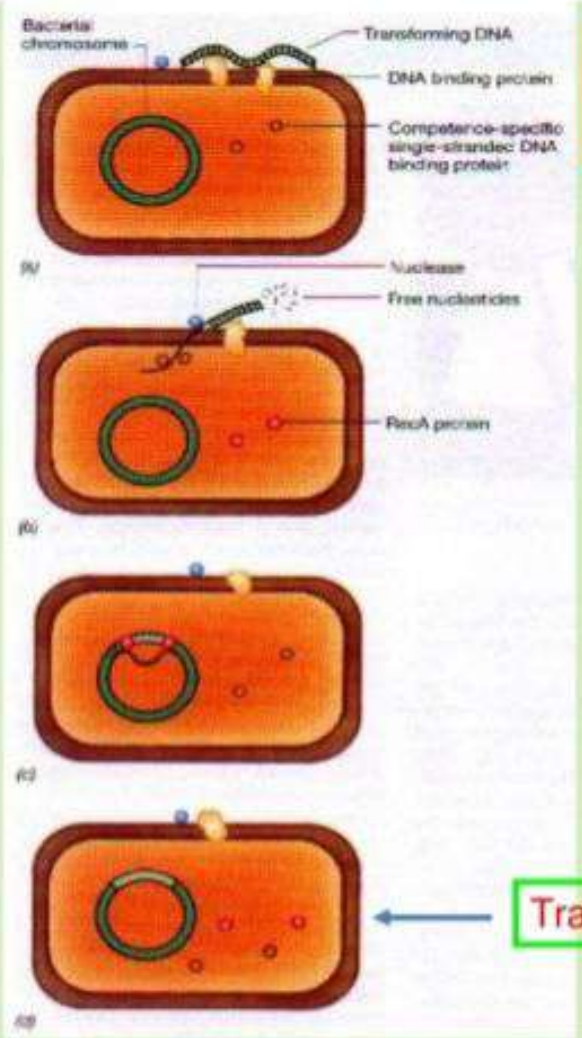
Fig : Griffith experiment



- **Transformation** is a process where certain 'competent' bacteria are able to take up free DNA released by other bacteria.
- The DNA is taken up only in relatively small amount and can be acquired only in a single event.
- Only certain strains are competent and this ability seems to be an inherited property of the organism.
- **Competence** in certain bacteria is governed by certain proteins which include membrane-associated DNA-binding protein, cell wall autolysin, and various nucleases.

- In bacterial transformation, a nonreciprocal type of recombination takes place.
- A piece of genetic material is inserted into the chromosome through the incorporation of a single strand to form a stretch of heteroduplex DNA.
- Another type of recombination, important in the integration of virus genomes into bacterial chromosomes, is site-specific recombination.
- Transfection is a process where bacteria can be transformed with DNA extracted from a bacterial virus rather than from another bacterial cell.

- Transfection has become a useful tool for studying the mechanism of transformation and recombination because, the small size of the phage genome allows the isolation of a nearly homogeneous population of DNA molecules.



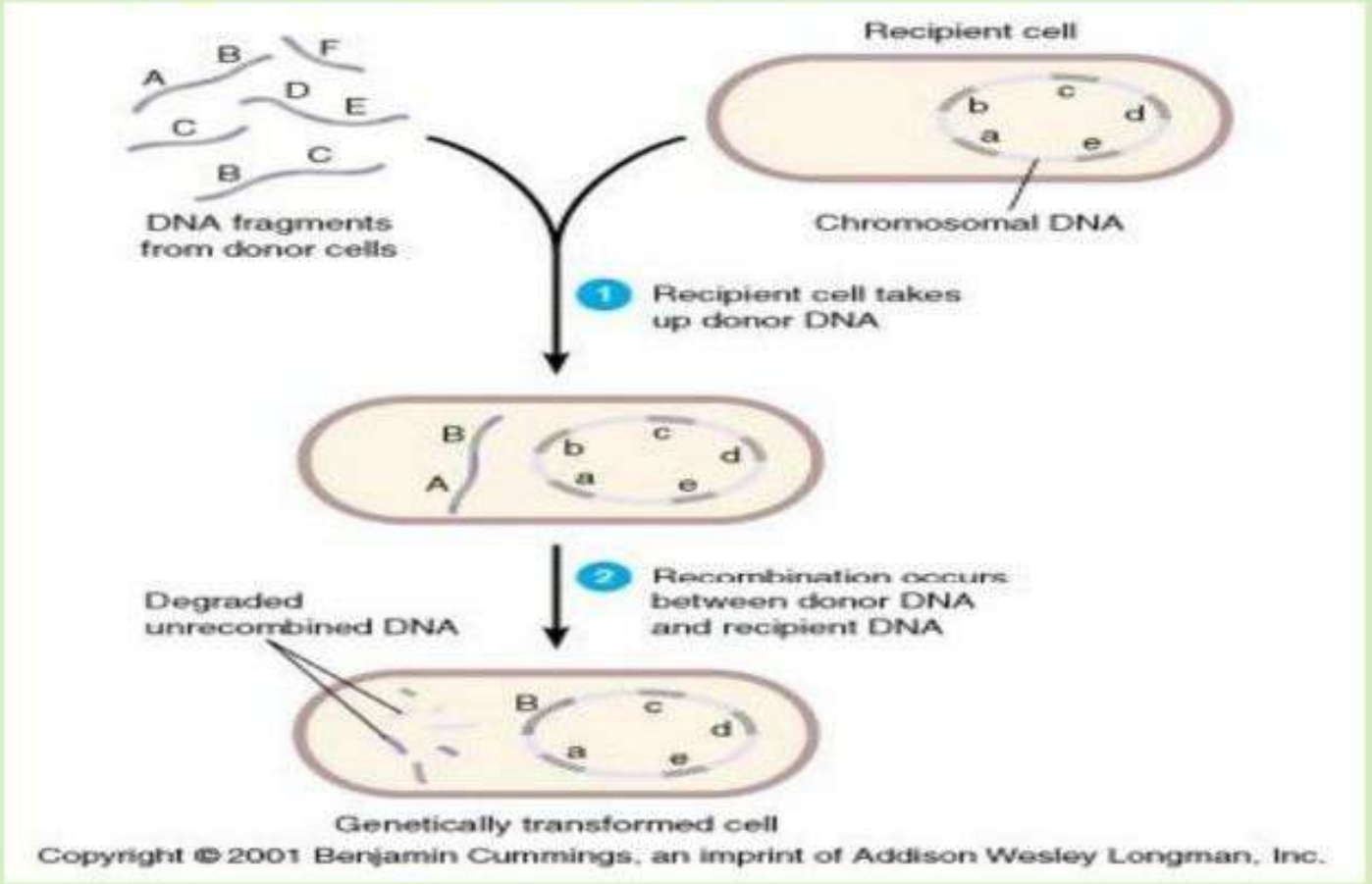
The introduction of DNA into cells by mixing the DNA and the cell

(a) Binding of free DNA by a membrane-bound DNA binding protein.

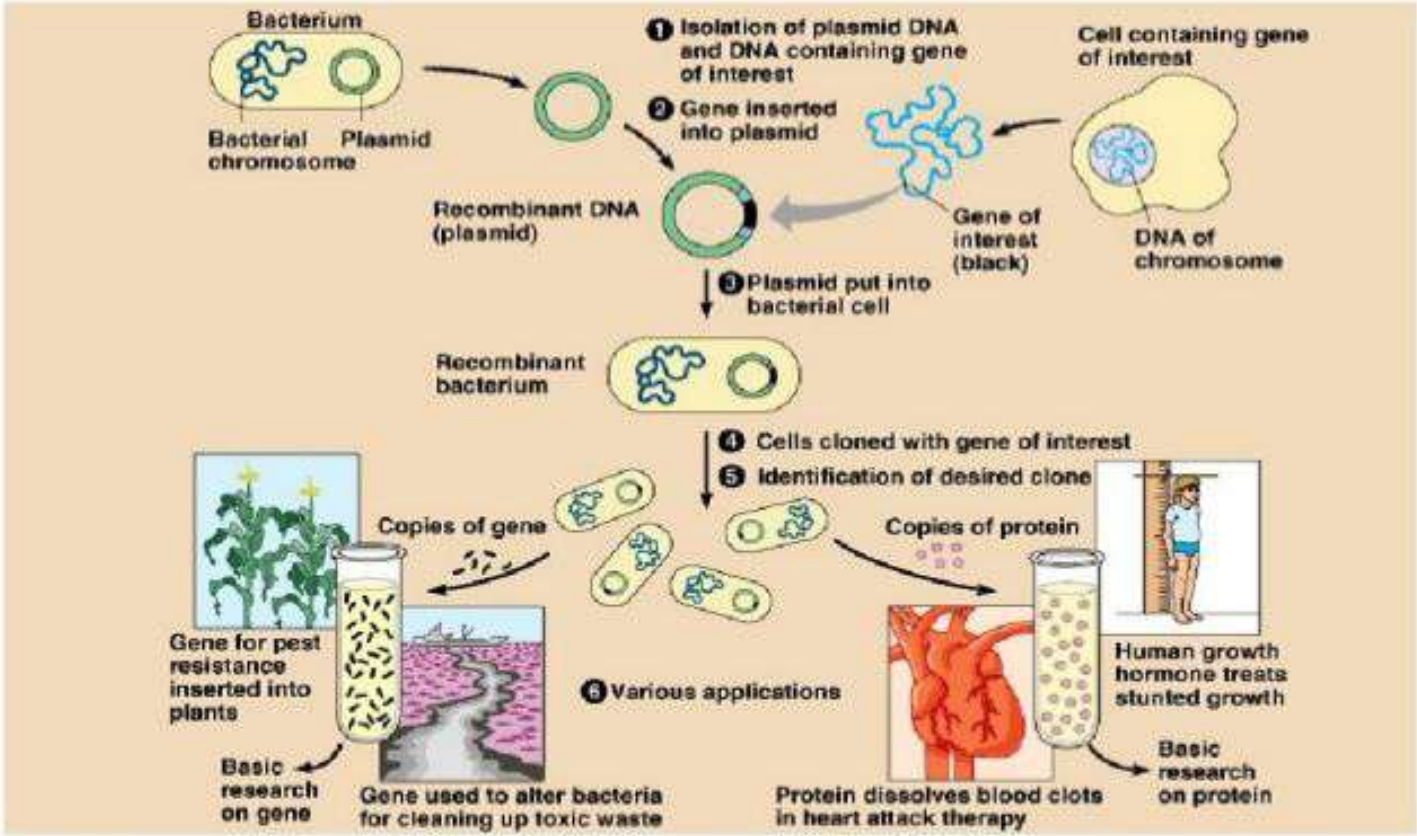
(b) Passage of one of the two strands into the cell while nuclease activity degrades the other strand.

(c) The single strand in the cell is bound by specific proteins, and recombination with homologous regions of the bacterial chromosome mediated by RecA protein occurs.

The mechanism of bacterial transformation



Transformation Applications



Competence

As mentioned above, the term "competence" refers to the state that some bacteria can enter, in which they can take up naked DNA from their environment. This capability is genetically programmed, and the process of DNA uptake is often called "natural transformation," to distinguish it from transformation induced by electroporation, heat shock, Ca^{2+} treatment of cells, or protoplast uptake of DNA. The genetic programming of competence is widespread but not universal. Generally, more than a dozen genes are involved, encoding both regulatory and structural components of the transformation process.

The general steps that occur in natural transformation differ somewhat depending on whether the bacteria are

gram negative or gram positive. If they are gram negative, the steps are (i) binding of double-stranded DNA to the outer cell surface of the bacterium, (ii) movement of the DNA across the cell wall and outer membrane, (iii) degradation of one of the DNA strands, and (iv) translocation of the remaining single strand of DNA into the cytoplasm of the cell across the inner membrane. Once in the cell, the single-stranded transforming DNA might synthesize the complementary strand and reestablish itself as a plasmid, stably integrate into the chromosome by homologous recombination of the translocated single strand into the chromosome or other recipient DNA, or be degraded. In a gram-positive organism that lacks an outer membrane, the process is similar except that movement through the outer membrane can be dispensed with, and it is necessary only to transport the DNA through the cell wall and one membrane. The uptake of DNA in both gram-positive and gram-negative bacteria is discussed in more detail below. While the DNA uptake systems of gram-positive and gram-negative bacteria have features in common, they do seem to differ in certain important respects; therefore, they are discussed separately.

COMPETENCE IN GRAM-POSITIVE BACTERIA

Two gram-positive species that have been particularly well studied are *B. subtilis* and *S. pneumoniae*. The proteins involved in transformation in these bacteria were discovered on the basis of isolation of mutants that are completely lacking in the ability to take up DNA. The genes affected in the mutants were named *com* (for *com*petence defective). In *B. subtilis*, the *com* genes are organized into several operons. The products of several of these, including the *comA* and *comK* operons, are involved in regulation of competence (see below). Others, including the products of genes in the *comE*, *comF*, and *comG* operons, become part of the competence machinery in the membrane that takes DNA up into the bacterium. The genes in these operons are given two letters, the first for the operon and the second for the position of the gene in the operon. For example, *comFA* is the first gene of the *comF* operon, while *comEC* is the third gene of the *comE* operon. The corresponding protein products of the genes have the same name with the first letter capitalized, e.g., ComFA and ComEC, respectively.

The role played by some of the Com proteins in the competence machinery of *B. subtilis* is diagrammed in Figure 6.2A. The first gene of the *comE* operon, *comEA*, encodes the protein that directly binds extracellular double-stranded DNA. The *comF* genes encode proteins that translocate the DNA into the cell. For example, ComFA is an ATPase that may provide the energy for translocation of DNA through the membrane (not shown). It has been proposed that ComEA, ComEC, and ComFA form a sort of ATP-binding cassette (ABC) transporter, which transports DNA into the cell. A large number of different ABC transporters are known that transport molecules into and out of cells. The genes in the *comG* operon encode proteins that might form a "pseudopilus," which helps move DNA through the ComEC channel. They might bind to extracellular DNA, perhaps acting through the ComEA DNA-binding protein, and then retract, drawing the DNA into the cell. Such speculation is inspired by their similarity to type IV pilin proteins in other systems (see below).

The *comE*, *comF*, and *comG* operons are all under the transcriptional control of ComK, a transcription factor that is itself regulated by ComA, as discussed below.

Some of the genes involved in the transformation process are not designated *com*, because such genes were first discovered on the basis of their involvement in another process. For example, one of the nuclease activities which makes double-strand breaks in extracellular DNA is the *nucA* gene product (see Provedi et al., Suggested Reading). These free DNA ends become the substrates for the competence proteins. Other examples of proteins with multiple roles include single-stranded-DNA-binding protein (SSB), and RecA, which functions in the recombination of transforming DNA with the chromosome as well as generally in recombination (see chapter 10).

The lengths of single-stranded DNA incorporated into the recipient chromosome are about 8.5 to 12 kb, as shown by cotransformation of genetic markers; the incorporation takes only a few minutes to be completed.

Transformation in *S. pneumoniae* utilizes similar proteins and mechanisms to those of transformation in *B. subtilis*, although the names of the *com* genes are often different (see Berge et al., Suggested Reading).

COMPETENCE IN GRAM-NEGATIVE BACTERIA

As mentioned above, a variety of gram-negative bacteria are also capable of acquiring natural competence. Some examples are the bacterium *Acinetobacter calcoaceticus* as well as the pathogens *H. pylori, Neisseria* spp., and *Haemophilus* spp. In the last two, specific uptake sequences are required for the binding of DNA, so that these species usually take up DNA only of the same species (see below). This differs from the gram-positive bacteria and also from many other gram-negative bacteria, which do not have specific uptake sequences (discussed below).

Transformation Systems Based on Type II Secretion

Gram-negative bacteria utilize one of two fundamentally different types of DNA uptake systems. Most of them

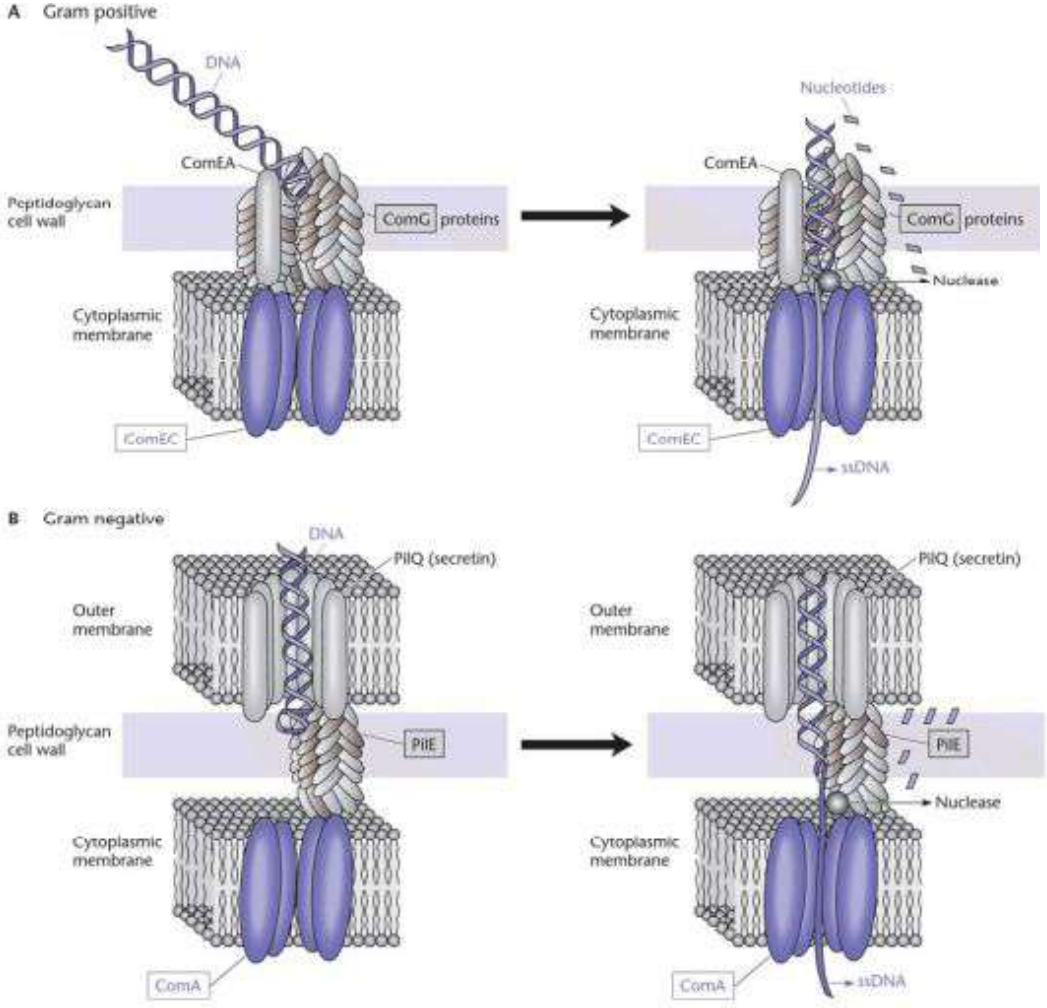


Figure 6.2 Structure of DNA uptake competence systems in gram-positive (A) and gram-negative (B) bacteria. Shown are the some of the proteins involved and the channels they form. The nomenclature in panel A is based on *Bacillus subtilis*, and that in panel B is based on *Neisseria gonorrhoeae*. Some of the *B. subtilis* ComG proteins are analogous to the *Neisseria* PilE protein (shaded boxes). The *B. subtilis* ComEC protein is an ortholog of the *Neisseria* ComA protein (unshaded boxes). ss, single stranded. The DNA is shown running through the cell wall alongside the pseudopilus (ComG proteins in *B. subtilis*; PilE in *Neisseria*), although the actual mechanism is unknown. Both gram-positive and gram-negative organisms' competence systems are related to type II protein secretion systems, which are discussed in chapter 14.

use a system related to type II secretion systems, which are used to assemble type IV pili on the cell surface and are similar to the secretion system used in the gram-positive bacteria discussed above (Figure 6.2B). The major difference between the gram-negative bacterial competence systems and the gram-positive systems is necessitated by the presence of an outer membrane in the gram-negative bacteria. In gram-negative bacteria, the water-soluble (hydrophilic) DNA must first pass through this hydrophobic outer membrane before it can pass through the cytoplasmic membrane into the cytoplasm of the cell. To facilitate DNA transfer through the outer membrane, the competence systems of gram-negative bacteria also have a pore through the outer membrane, made up of 12 to 14 copies of a secretin protein (called PilQ in *Neisseria*). This pore has a hydrophilic aqueous channel through which the double-stranded DNA can pass. One strand of the DNA is then degraded as it passes through a channel in the inner membrane; this channel is formed by a protein called ComA in *Neisseria*, which has sequences in common with the ComEC protein that forms a similar channel in *B. subtilis* (Figure 6.2B).

While these competence systems are very similar to type II secretion systems that assemble type IV pili, to the extent of often elaborating type IV pili on the cell surface, the role of pili in this process is somewhat obscure. Type IV pili are long, thin hairlike appendages that stick out from the cell and are used to attach cells to solid surfaces such as the surfaces of eukaryotic cells, they are often involved in pathogencity. They are also required for a type of cell movement called "twitching" motility in some types of bacteria such as *Myxococcus xanthus*. In these bacteria, the pili are located on the leading edge of the cell and move the cell by reaching out and attaching to the solid surface and then shortening or retracting, pulling the cell toward themselves, much like a mountain climber would climb a cliff by inserting a piton ahead of herself and pulling herself toward it. Speculation is that type IV pili systems involved in DNA transformation might work by a similar mechanism, binding to DNA on the cell surface and then retracting, pulling the DNA into the cell. However, the actual mechanism does not seem to be quite this simple. While it is true that bacteria exhibiting type IV pili on their cell surface seem to need this system for transformation, and most bacteria that are competent for transformation do seem to require systems related to type II secretion systems, not all bacteria capable of natural transformation actually exhibit type IV pili on their cell surface. Furthermore, while competence requires the protein that makes up most of the pilus, i.e., the major pilin protein (called PilE in *Neisseria* [Figure 6.2B]), some other minor pilin proteins are required for competence but not for pilus formation.

This suggests that when the pilin protein is associated with these minor pilus proteins, it is in a different state from when it is in a type IV pilus. One way out of this dilemma is to propose that competence systems and type II secretion systems elaborate a modified pilus, sometimes called a pseudopilus, to play these other roles. The pseudopilus is composed of the major pilin protein, but now it is associated with different minor pilus proteins so that it does not actually extrude from the cell but, rather, remains closely associated with the cell surface. In type II secretion systems, this pseudopilus may grow and push proteins out of the cell through the secretin channel in the outer membrane. In the case of transformation, the pseudopilus may do the reverse; it may bind DNA and retract, pulling the DNA into the cell through the secretin channel in the outer membrane. There is no evidence that any of the pilus proteins actually bind DNA, although they could of course work through other DNA-binding proteins, analogous to ComEA in *B. subtilis* (see above).

Competence Systems Based on Type IV Secretion Systems

As mentioned above, most competent bacteria have competence systems based on type II secretion systems. To date, the only known exception is *H. pylori*, which has a system based on type IV secretion-conjugation systems, discussed in chapter 5. *H. pylori* is an opportunistic pathogen involved in gastrointestinal diseases. In fact, two Australian scientists were awarded the Nobel Prize in physiology or medicine in 2005 for showing that it is the major cause of gastric ulcers, which were long thought to be due to stress. The similarity between the competence system of *H. pylori* and type IV secretion-conjugation was discovered because of the similarity between the proteins in this system and the VirB conjugation proteins in *Agrobacterium tumefaciens* that transfer T-DNA from the Ti plasmid into plants (see Boxes 5.1 and 5.2). The Com proteins of *H. pylori*, a human pathogen, were therefore given letters and numbers corresponding to their orthologs (see Box 2.7 for definitions) in the T-DNA transfer system of the Ti plasmid in *A. tumefaciens*, a plant pathogen. Table 6.1 lists these Com proteins and their orthologs in the *Agrobacterium* Ti plasmid (see Karnholz et al., Suggested Reading). Apparently, type IV secretion pathway systems can function as two-way DNA transfer systems, capable of moving DNA both into and out of the cell. Interestingly, in addition to its transformation system, *H. pylori* has a bona fide type IV secretion system that secretes proteins directly into eukaryotic cells (see Box 5.2). However, even though these two systems are related, they function independently of each other and have no proteins in common.

TABLE 6.1 Orthologous Com proteins of *H. pylori* and Vir proteins in *A. tumefaciens*

Helicobacter protein	Function	*Agrobacterium* ortholog
ComB2	Pseudopilus?	VirB2
ComB3	Unknown	VirB3
ComB4	ATPase	VirB4
ComB6	DNA binding?	VirB6
ComB7	Channel	VirB7
ComB8	Channel	VirB8
ComB9	Channel	VirB9
ComB10	Channel	VirB10

The nomenclature of competence is admittedly very confusing. To reiterate, type IV pili and type IV secretion systems are not related to each other. Type IV secretion systems actually have type II pili, and type IV pili are assembled on the cell surface by systems related to type II secretion systems! Some conjugation systems have type IV pili in addition to their type II pili. The type IV pili help to hold the cells together during DNA transfer. There is also evidence that a type IV secretion system in *N. gonorrhoeae* is involved in releasing DNA into the environment, where it can be taken up by a transformation system related to type II secretion systems (see above). When the secretion systems, transformation systems, and pili were being named, no one could have predicted their relationships to each other; this confusion is the result.

Regulation of Competence in *B. subtilis*

The regulation of competence in *B. subtilis* is achieved through a **two-component regulatory system** analogous to those used to regulate many other systems in bacteria (see chapter 13). First, information that the cell is running out of nutrients and the population is reaching a high density is registered by ComP, a **sensor protein** in the membrane (Figure 6.3). The high cell density causes this sensor-kinase protein to transfer a phosphate from ATP to itself, in other words to phosphorylate itself. The phosphate is then transferred from ComP to ComA, a **response regulator protein.** In the phosphorylated state, the ComA protein is a transcriptional activator (see chapter 2) for several genes, including some required for competence. Eventually another transcriptional activator, ComK, is made; this activator is directly responsible for activating the transcription of other *com* genes, including those that form the transformation machinery illustrated in Figure 6.2A.

COMPETENCE PHEROMONES

How does the cell know that other *B. subtilis* cells are nearby and that it should induce competence? High cell density is signaled through small peptides called **competence pheromones** that are excreted by the bacteria as they multiply (see Lazazzera et al., Suggested Reading). Cells become competent only in the presence of high concentrations of these peptides, and the concentration of these peptides in the medium is high only when the concentration of cells giving them off is high. The requirement for competence pheromones ensures that cells are able to take up DNA only when other *B. subtilis* cells are nearby and giving off DNA to be taken up. This is one example of a phenomenon called quorum sensing, by which small molecules given off by cells send signals to other cells in the population that the cell concentration is high. Many such small molecules are known, including homoserine lactones that signal cell density in some gram-negative bacteria. Other examples of quorum sensing are discussed in later chapters.

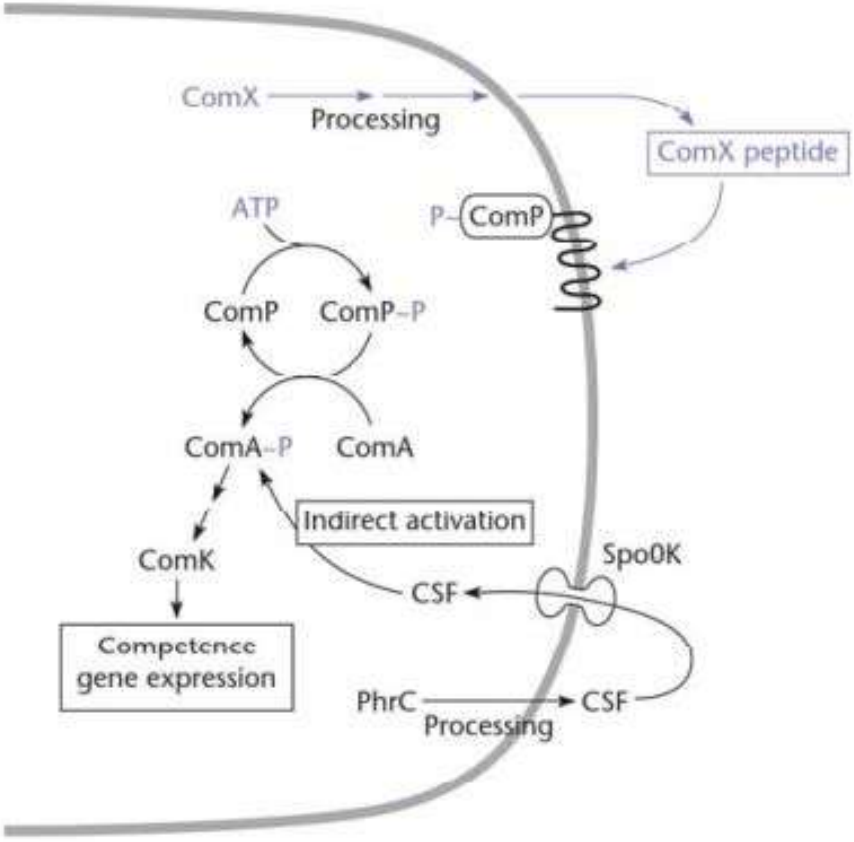


Figure 6.3 Regulation of competence development in *Bacillus subtilis* by quorum sensing. The ComP protein in the membrane senses a high concentration of the ComX peptide (shown in purple) and phosphorylates itself by transferring a phosphate from ATP. The phosphate is then transferred to ComA, which allows the transcription of many genes including ComK, the activator of the *com* genes. In a separate pathway, a peptide sometimes called CSF (competence-stimulating factor), processed from the signal sequence of another protein (PhrC), is imported into the cell by the Spo0K oligopeptide permease and indirectly activates ComA–P by inactivating another protein, RapC (not shown). There is at least one other such pathway; these pathways' purpose may be to coordinate competence with sporulation and other functions when the cell runs out of nutrients and enters stationary phase.

A second question is that of how the cell knows that the competence pheromone peptide came from other cells and was not produced internally. It does this by cutting the peptide out of a larger protein as the larger protein passes through the membrane, as shown in Figure 6.3. Once outside the cell, the peptide is diluted by the surrounding medium, and it can achieve high enough concentrations to induce competence only if the cell density is high and many surrounding cells are also giving off the peptide. In *B. subtilis*, the major competence pheromone peptide is called ComX and is cut out of a longer polypeptide, the product of the *comX* gene. Another gene, *comQ*, which is immediately upstream of *comX*, is also required for synthesis of the competence pheromone because its product is the protease enzyme that cuts the competence pheromone out of the longer polypeptide. Once the peptide has been cut out of the longer molecule, it can bind to the ComP protein in the membrane and trigger its autophosphorylation, although the mechanism remains unknown.

At best, only about 10% of *B. subtilis* cells ever become competent, no matter how favorable the conditions or how high the cell density. The advantages of this to the bacteria are obvious: if all the cells were competent, which cells would be giving off DNA to be taken up by the competent cells? This has been called a bistable state and seems to be determined somehow by autoregulation of the ComK activator protein. Bistable states are common in biological phenomena, and competence in *B. subtilis* has been used as an experimental model for such phenomena (see Maamer and Dubnau, Suggested Reading).

RELATIONSHIP BETWEEN COMPETENCE, SPORULATION, AND OTHER CELLULAR STATES

At about the same time as *B. subtilis* reaches the stationary phase, some cells acquire competence and some cells sporulate (see chapter 14). Sporulation, a developmental process common to many bacteria, allows a bacterium to enter a dormant state and survive adverse conditions such as starvation, irradiation, and heat. During sporulation, the bacterial chromosome is packaged into a resistant spore, where it remains viable until conditions improve and the spore can germinate into an actively growing bacterium. To coordinate sporulation and competence, *B. subtilis* cells may produce other competence peptides (see Bongiorni et al., Suggested Reading). There are at least two such peptides that regulate ComA indirectly by inhibiting proteins called Rap proteins, which bind to the C-terminal DNA-binding domain of phosphorylated ComA and prevent it from binding to DNA and activating transcription. These peptides are processed from the signal sequences of longer polypeptides, the

products of the *phr* genes, and are transported into the cell by the oligopeptide permease, Spo0K (see below and Figure 6.3).

The *spo0K* gene is an example of a regulatory gene that is required for sporulation and also for the development of competence. This gene was first discovered because of its role in sporulation. A *spo0K* mutant is blocked in the first stage, the "0" stage, of sporulation. The *K* means that it was the 11th gene (as K is the 11th letter in the alphabet) involved in sporulation to be discovered in that collection.

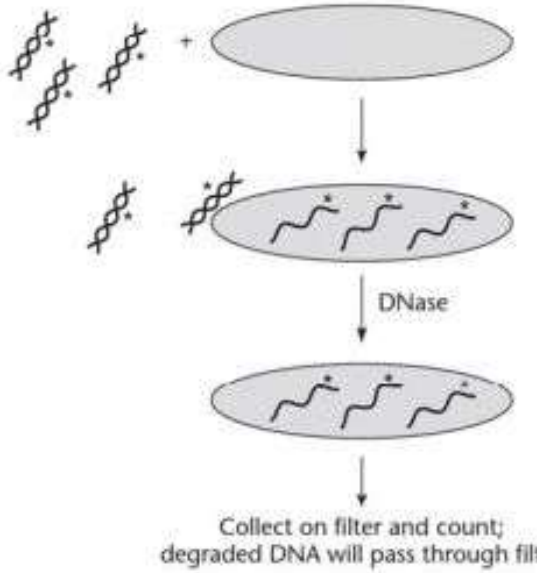
Experimental Evidence for Models of Natural Transformation

The above models for natural transformation are based on experiments with a number of different systems. Experiments directed toward an understanding of DNA uptake during natural transformation have sought to answer three obvious questions. (i) How efficient is DNA uptake? (ii) Can only DNA of the same species enter a given cell? (iii) Are both of the complementary DNA (cDNA) strands taken up and incorporated into the cellular DNA?

EFFICIENCY OF DNA UPTAKE

The efficiency of uptake is fairly easy to measure biochemically. Figure 6.4 shows an experiment based on the fact that transport of free DNA into the cell makes the DNA insensitive to DNases, which cannot enter the cell because competent cells permit only DNA, not proteins,

Figure 6.4 Determining the efficiency of DNA uptake during transformation. DNA in the cell is insensitive to DNase. Degraded DNA passes through a filter. The asterisk refers to radioactively labeled DNA.



to enter. In the experiment, donor DNA is radioactively labeled by growing the cells in medium in which the phosphorus has been substituted with phosphorus-32, the radioisotope of phosphorus. The radioactive DNA is then extracted and mixed with competent cells, and the mixture is treated with DNase at various times. Any DNA that is not degraded and survives intact must have been taken up by the cells, where it is protected from the DNase. Undegraded DNA can be distinguished from degraded DNA because the former DNA can be precipitated with acid and collected on a filter whereas degraded DNA does not precipitate and passes through the filter. Therefore, if the medium containing the cells is precipitated with acid and collected on a filter, the radioactivity on the filter is due to undegraded DNA that must have been taken up by the cells. If the radioactivity on the filter is counted and compared with the total radioactivity of the DNA that was added to the cells, the percentage of DNA that is taken up, or the efficiency of DNA uptake, can be calculated. Experiments such as these have shown that some competent bacteria take up DNA very efficiently.

SPECIFICITY OF DNA UPTAKE

The second question, i.e., whether DNA from only the same species is taken up, is also fairly easy to answer. By using the same assay of resistance to DNases, it has been determined that some types of bacteria take up DNA from only their own species whereas others can take up DNA from any source. The first group includes *N. gonorrhoeae* and *Haemophilus influenzae*.

Bacteria that preferentially take up the DNA of their own species do so because their DNA contains specific **uptake sequences.** Figure 6.5 shows the minimal uptake sequences for *H. influenzae* and *N. gonorrhoeae*. Uptake sequences are long enough that they almost never occur by chance in other DNAs. In contrast, bacteria such as *B. subtilis* seem to take up any DNA. Possible reasons why some bacteria should preferentially take up DNA from their own species while others take up any DNA are subjects of speculation and are discussed later.

FATE OF DNA TAKEN UP IN *S. PNEUMONIAE*

Although the genetic requirements for transformation are best known for *B. subtilis*, the more efficient uptake of DNA by some other naturally transformable bacteria has allowed investigators to perform biochemical experiments on the uptake of DNA by these species, which has led to the models described above. The general pathway for DNA uptake was first worked out for *S. pneumoniae* but is probably similar in most other naturally transformable bacteria.

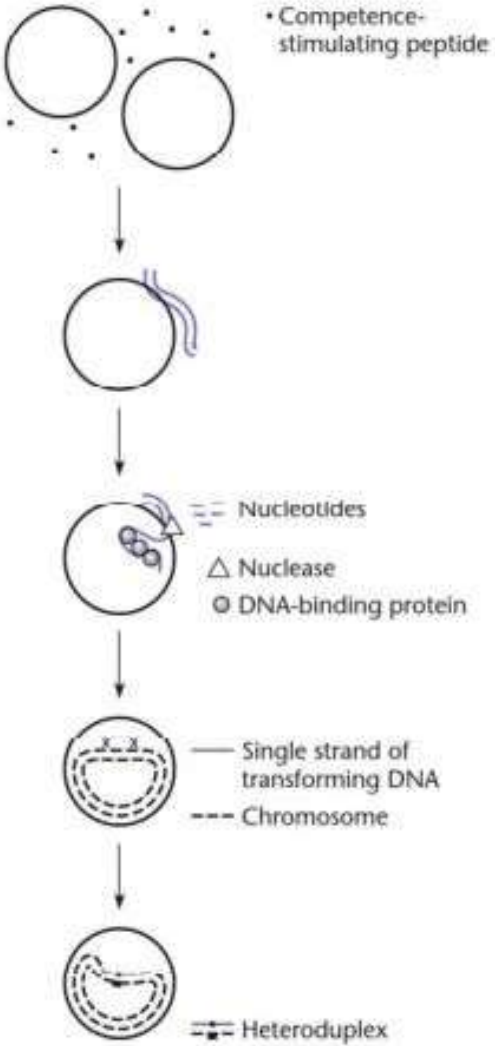
Figure 6.5 The uptake sequences on DNA for some types of bacteria. Only DNA with these sequences is taken up by the bacteria indicated. Only one strand of the DNA is shown.

Haemophilus influenzae	5′ AAGTGCGGT 3′
Neisseria gonorrhoeae	5′ GCCGTCTGAA 3′

Figure 6.6 shows a general scheme for DNA uptake during the transformation of *S. pneumoniae*. In the first step, double-stranded DNA released by lysis of the donor bacteria is bound to specific receptors on the cell surface of the recipient bacterium. The bound DNA is then broken into smaller pieces by endonucleases; one of the two complementary strands is degraded by an exonuclease; and the remaining strand is transported into the

Figure 6.6 Transformation in *Streptococcus pneumoniae*. Competence-stimulating peptide accumulates as the cells reach a high density. Double-stranded DNA binds to the cell, and one strand is degraded. The remaining single strand protected by a DNA-binding protein replaces the strand of the same sequence in the chromosome, creating a "heteroduplex" in which one strand comes from the donor and one comes from the recipient.

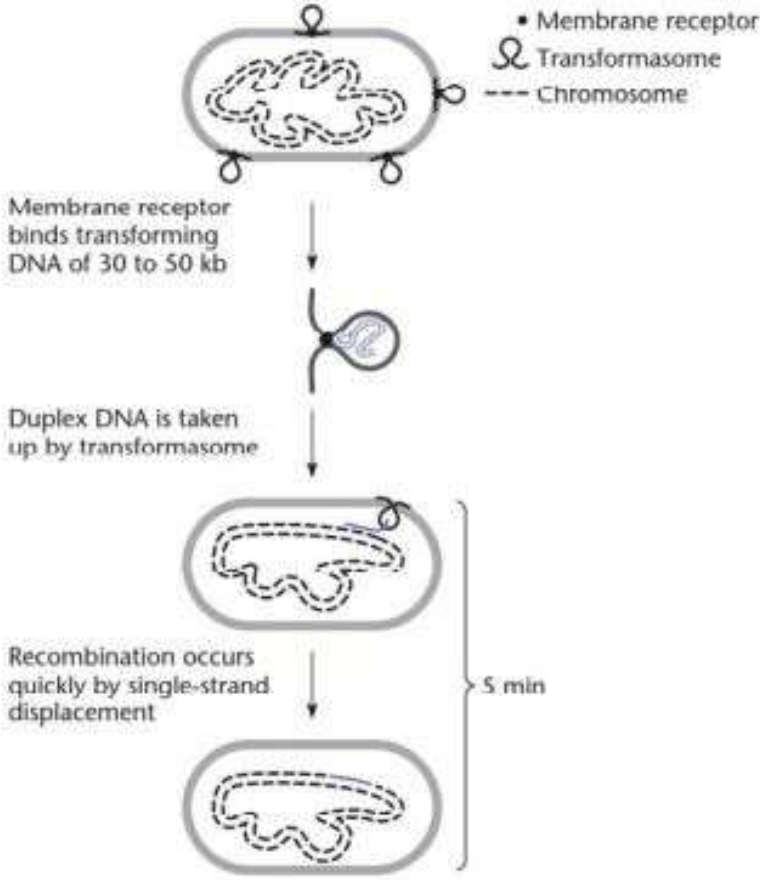
cell, being protected by a DNA-binding protein. The transforming DNA integrates into the cellular DNA in a homologous region by means of strand displacement, a mechanism in which the new strand invades the double helix and displaces an old strand with the same sequence. The old strand is then degraded. If the donor DNA and recipient DNA sequences differ slightly in this region, recombinant types can appear. Evidence for this model comes from several different experiments, some of which are discussed below. Also, the gene for a membrane-bound DNase that may be involved in degrading one of the two strands of the incoming DNA has been found in *S. pneumoniae*.



TRANSFORMASOMES

The basic scheme described above probably differs among different types of naturally competent bacteria. For example, *H. influenzae* may first take up double-stranded DNA in subcellular compartments called **transformasomes** (Figure 6.7). The new DNA may not become single stranded until it enters the cytoplasm. However, the basic process of all natural transformation is the same. Only one strand of the DNA enters the interior of the cell and integrates with the cellular DNA to produce recombinant types.

Figure 6.7 Transformation in *Haemophilus influenzae*. Double-stranded DNA is first taken up in transformasomes. One strand is degraded, and the other strand invades the chromosome, displacing one chromosome strand.



GENETIC EVIDENCE FOR SINGLE-STRANDED DNA UPTAKE

Genetic experiments taking advantage of the molecular requirements for transformation can be used to study the molecular basis for transformation; in other words, transformation can be used to study itself. Evidence that DNA has transformed cells is usually based on the appearance of recombinant types after transformation. A recombinant type can form only if the donor and recipient bacteria differ in their genotypes and if the incoming DNA from the donor bacterium changes the genetic composition of the recipient bacterium. The chromosome of a recombinant type has the DNA sequence of the donor bacterium in the region of the transforming DNA.

Experiments have shown that only double-stranded DNA can bind to the specific receptors on the cell surface, so that double-stranded but not single-stranded DNA can transform cells and yield recombinant types. However, we can also conclude from these experiments that the cells actually take up only *single*-stranded DNA, because the DNA enters an "eclipse" phase in which it cannot transform. For example, in the experiment shown in Figure 6.8, an Arg^- mutant requiring arginine for growth is used as the recipient strain and the corresponding Arg^+ prototroph is the source of donor DNA. At various times after the donor DNA has been mixed with the recipient cells, the recipients are treated with DNase, which cannot enter cells but destroys any DNA remaining in the medium. The surviving DNA in the recipient cells is then extracted and used for retransformation of more auxotrophic recipients, and Arg^+ transformants are selected on agar plates without the growth supplement arginine. Any Arg^+ transformants must have been due to double-stranded donor DNA in the recipient cells.

Whether transformants were observed depends on the time the DNA was extracted from the cells. When the DNA is extracted at time 1 in Figure 6.8, while it is still outside the cells and accessible to the DNase, no Arg^+ transformants are observed because the Arg^+ donor DNA is all destroyed by the DNase. At time 2, some of the DNA is now inside the cells, where it cannot be degraded by the DNase, but this DNA is single stranded. It has not yet recombined with the chromosome, and so Arg^+ transformants are still not observed in step 4. Only at time 3, when some of the DNA has recombined with the chromosomal DNA and so is again double stranded, do Arg^+ transformants appear in step 4. Thus, the transforming DNA enters the eclipse period for a short time after it is added to competent cells, as expected if it enters the cell in a single-stranded state.

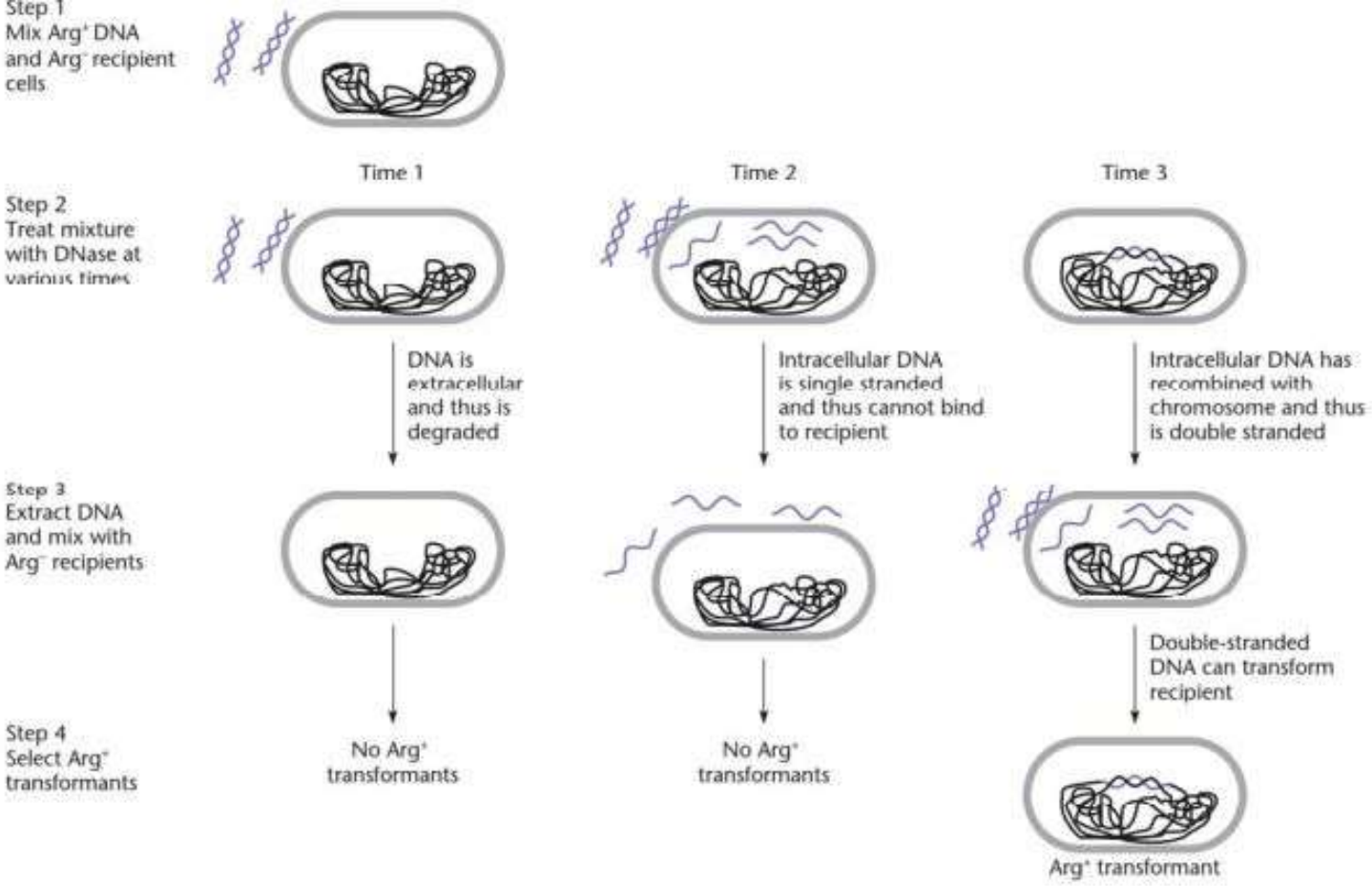


Figure 6.8 Genetic assay for the state of DNA during transformation. Only double-stranded DNA binds to the cell to initiate transformation. The appearance of transformants in step 4 indicates that the transforming DNA was double stranded at the time of DNase treatment.

Plasmid Transformation and Phage Transfection of Naturally Competent Bacteria

Chromosomal DNA can efficiently transform any bacterial cells from the same species that are naturally competent. However, neither plasmids nor phage DNAs can be efficiently introduced into naturally competent cells for two reasons. First, they must be double stranded to replicate. Natural transformation requires breakage of the double-stranded DNA and degradation of one of the two strands so that a linear single strand can enter the cell. Second, they must recyclize. However, pieces of plasmid or phage DNAs cannot recyclize if there are no repeated or complementary sequences at their ends.

Transformation of naturally competent bacteria with plasmid or phage DNA usually occurs only with DNAs that are **dimerized** or **multimerized** into long **concatemers** (see chapters 4 and 7). A dimerized or multimerized DNA is one in which two or more copies of the molecule are linked head to tail, as illustrated in Figure 6.9. If a dimerized plasmid or phage DNA is cut only once, it still has complementary sequences at its ends that can recombine to recyclize the plasmid, as illustrated in the figure. Such dimers and higher multimers often form naturally while plasmid or phage DNA is replicating, so that most preparations of plasmid or phage DNAs contain some dimers. The fact that only dimerized plasmid or phage DNAs can transform naturally competent bacteria supports the model of uptake of single-stranded DNA during transformation described earlier in this chapter.

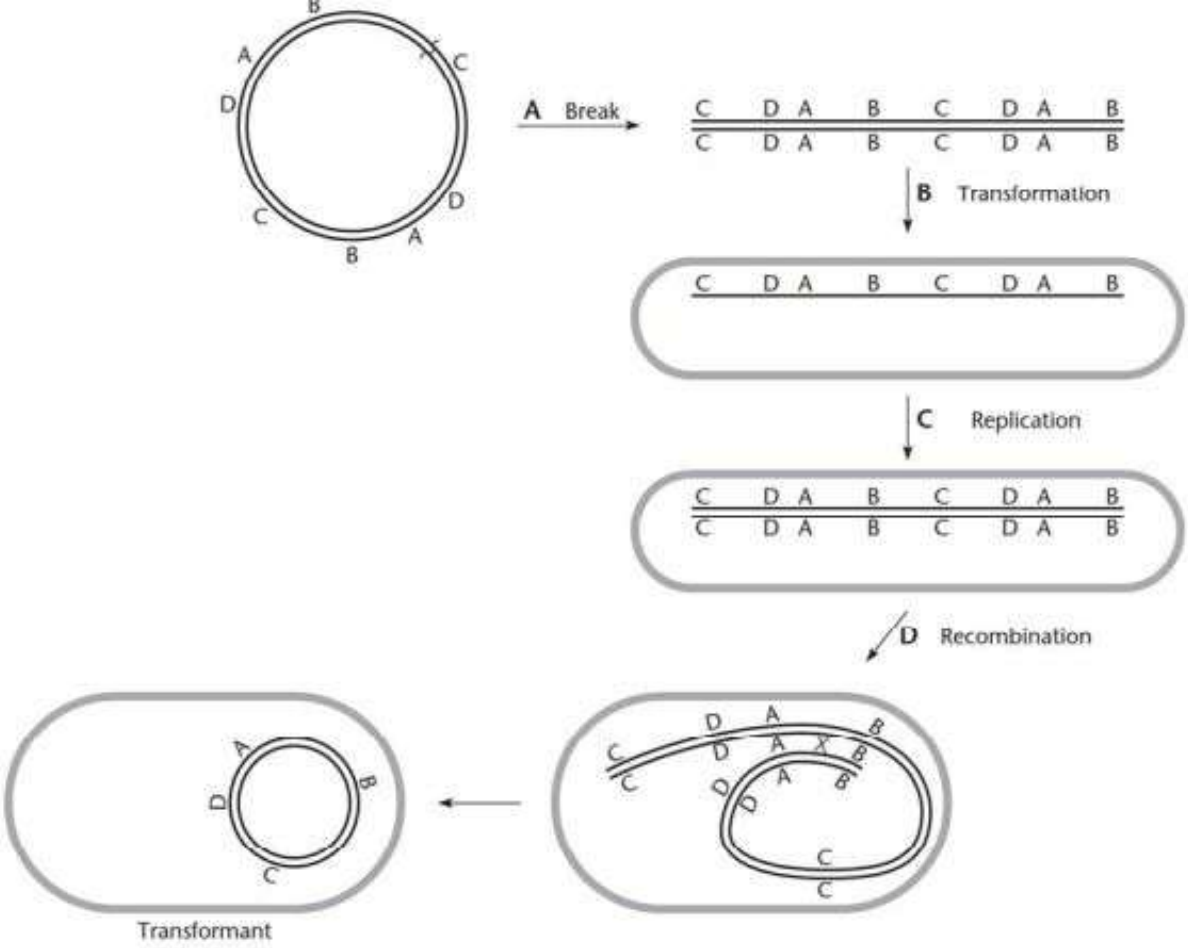


Figure 6.9 Transformation by dimeric plasmids. After the single-stranded dimeric plasmid DNA is taken up, it can serve as a template to make the double-stranded DNA. The repeated ends can recombine with each other to re-form the circular plasmid.

Role of Natural Transformation

The fact that so many gene products play a direct role in competence indicates that the ability to take up DNA from the environment is advantageous. Below, we discuss three possible advantages and the arguments for and against them.

NUTRITION

Organisms may take up DNA for use as a carbon and nitrogen source (see Redfield, Suggested Reading). One argument against this hypothesis is that taking up whole DNA strands for degradation inside the cell may be more difficult than degrading the DNA outside the cell and then taking up the nucleotides. In fact, *B. subtilis* excretes a deoxyribonuclease (DNase) which degrades DNA so that it can be taken up more easily. The major argument against this hypothesis as a general explanation for transformation in all bacteria is that some bacteria take up only DNA of their own species, since DNA from other organisms should offer the same nutritional benefits. Moreover, the fact that competence develops only in a minority of the population, at least in *B. subtilis*, argues against the nutrition hypothesis, since all the bacteria in the population would presumably need the nutrients.

These arguments are attractive but do not disprove the nutrition hypothesis. The bacteria may consume DNA of only their own species because of the danger inherent in taking up foreign DNAs, which might contain prophages, transposons, or other elements that could become parasites of the organism. Furthermore, consumption of DNA from the same species may be a normal part of colony development; cell death and cannibalism are thought to be part of some prokaryotic developmental processes. These processes would require that only some of the cells in the population become DNA consumers while the others become the "sacrificial lambs." The existence of specific cell-killing mechanisms that kill some cells in the population as *B. subtilis* enters the stationary phase lends support to such interpretations (see Box 4.3 in chapter 4).

REPAIR

Cells may take up DNA from other cells to repair damage to their own DNA (see Mongold, Suggested Reading). Figure 6.10 illustrates this hypothesis, in which a population of cells is exposed to UV irradiation. The radiation damages the DNA, causing pyrimidine dimers and other lesions to form (see chapter 11). DNA leaks out of some of the dead cells and enters other bacteria. Because the damage to the DNA has not occurred at exactly the same places, undamaged incoming DNA sequences can replace the damaged regions in the recipient, allowing at least some of the bacteria to survive. This scenario explains why some bacteria take up DNA of only the same species, since, in general, this is the only DNA that can recombine and thereby participate in the repair.

If natural transformation helps in DNA repair, we might expect that repair genes would be induced in response to developing competence and that competence would develop in response to UV irradiation or other types of DNA damage. In fact, in some bacteria, including *B. subtilis* and *S. pneumoniae*, the *recA* gene required for recombination repair is induced in response to the development of competence (see Haijema et al. and Raymond-Denise and Guillen, Suggested Reading). However, in other bacteria, such as *H. influenzae*, the *recA* gene is not induced in response to competence. There is also no evidence that competence genes are induced in response to DNA damage. Nevertheless, the need for DNA repair is an attractive explanation for why at least some types of bacteria develop competence.

RECOMBINATION

The possibility that transformation allows recombination between individual members of the species is also an attactive hypothesis but is difficult to prove. According to this hypothesis, transformation serves the same function that sex serves in higher organisms: it allows the assembly of new combinations of genes and thereby increases diversity and speeds up evolution. Bacteria do not have an obligatory sexual cycle; therefore, without some means of genetic exchange, any genetic changes that a bacterium accumulates during its lifetime are not necessarily exchanged with other members of the species.

The gene exchange function of transformation is supported by the fact that cells of some naturally transformable bacteria leak DNA as they grow. It is hard to imagine what function this leakage could perform unless the leaked DNA is supposed to be taken up by other bacteria.

In several *Neisseria* species, including *N. gonorrhoeae*, transformation may enhance antigenic variability, allowing the organism to avoid the host immune system (Box 6.1). In mixed laboratory cultures, transformation does contribute substantially to the antigenic diversity in this species. However, under natural conditions, it is debatable whether most of this antigenic diversity results from recombination between DNAs brought together by transformation or simply from recombination between sequences within the chromosomal DNA of the bacterium itself.

We still do not know why some types of bacteria are naturally transformable and others are not. It seems possible that most types of bacteria are naturally transformable at low levels. Transformation may serve different purposes in different organisms. Perhaps transformation is used for DNA repair in soil bacteria such as *B. subtilis* but is used to increase genetic variability in obligate parasites such as *N. gonorrhoeae*.

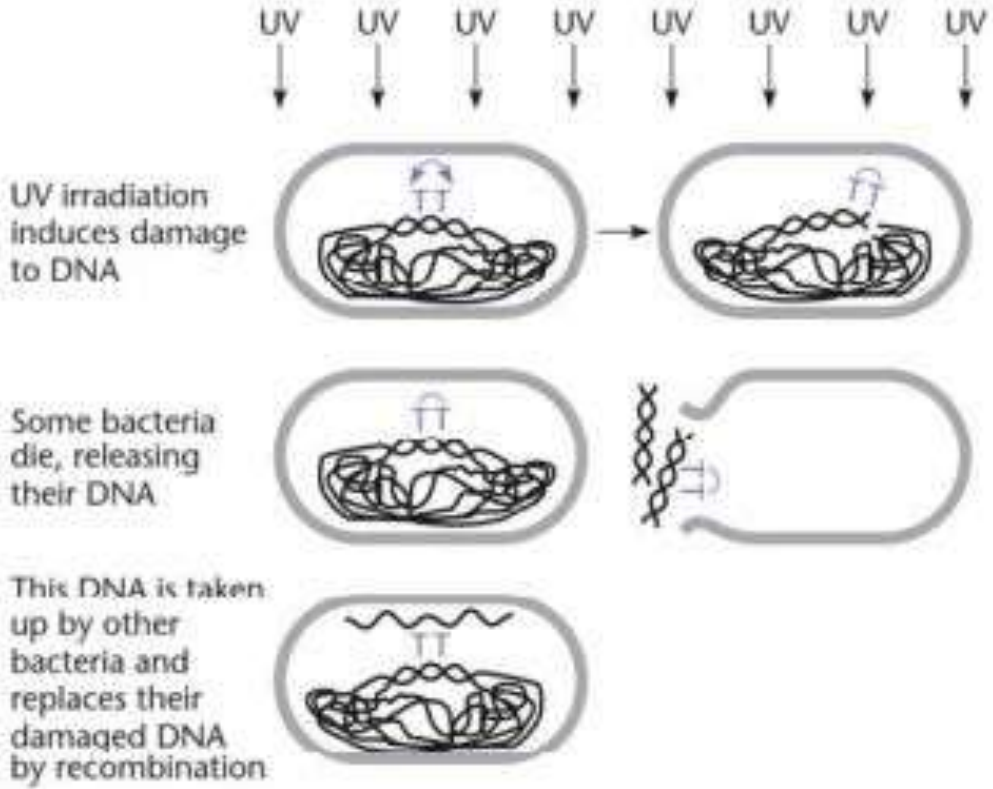


Figure 6.10 Repair of DNA damage by transforming DNA. A region containing a thymine dimer (TT) induced by UV irradation is replaced by the same, but undamaged, sequence from the DNA of a neighbor killed by the radiation. This mechanism could allow survival of the species.

Importance of Natural Transformation for Forward and Reverse Genetics

Whatever its purpose for individual bacterial species, natural transformation has many uses in molecular genetics. Transformation has been used in many bacteria to map genetic markers in chromosomes in many bacteria and to reintroduce DNA into cells after the DNA has been manipulated in the test tube, which has made them ideal model systems for molecular genetic studies. As mentioned, the interpretation of genetic data obtained by transformation is similar to the interpretation of data obtained by transduction. The interpretation of genetic data obtained by transduction and transformation is discussed in chapter 3. More recently, the presence of efficient natural transformation systems in some bacteria, for example, species of *Neisseria, Acinetobacter,* and cyanobacteria, has made them ideal subjects for functional genomics, i.e., attempts to determine the function of every gene of an organism. One example has been the use of pMUTIN in *B. subtilis* (see chapter 4). In the bacterium *Acinetobacter baylyi*, transformation is so efficient that it can be done merely by spotting DNA restriction fragments or PCR-amplified fragments on streaks of recipient bacteria on plates. This offers opportunities to construct many different types of mutations in genes, including **loss-, gain-,** or **change-of-function mutations** (see Young et al., Suggested Reading). Such manipulations are more difficult in bacteria that do not have efficient natural competence systems.

Artificially Induced Competence

Most types of bacteria are not naturally transformable, at least not at easily detectable levels. Left to their own devices, these bacteria do not take up DNA from the environment. However, even these bacteria can sometimes be made competent by certain chemical treatments, or DNA can be forced into them by a strong electric field in a process called electroporation.

Calcium Ion Induction

Treatment with calcium ions (see Cohen et al., Suggested Reading) can make some bacteria competent, including *Escherichia coli* and *Salmonella* spp. as well as some *Pseudomonas* spp., although the reason is not understood.

Chemically induced transformation is usually inefficient, and only a small percentage of the cells are ever transformed. Accordingly, the cells must be plated under conditions selective for the transformed cells. Therefore, the DNA used for the transformation should contain a selectable gene such as one encoding resistance to an antibiotic.

TRANSFORMATION BY PLASMIDS

In contrast to naturally competent cells, cells made permeable to DNA by calcium ion treatment will take up both single-stranded and double-stranded DNA. Therefore, both linear and double-stranded circular plasmid DNAs can be efficiently introduced into chemically treated cells. This fact has made calcium ion-induced competence very useful for cloning and other applications that require the introduction of plasmid and phage DNAs into cells.

TRANSFECTION BY PHAGE DNA

In addition to plasmid DNAs, viral genomic DNAs or RNAs can often be introduced into cells by transformation, thereby initiating a viral infection. This process is called **transfection** rather than transformation, although the principle is the same. To detect transfection, the potentially transfected cells are usually mixed with indicator bacteria and plated using top agar (see the introductory chapter). If the transfection is successful, a plaque forms where the transfected cells had produced phage which then infected the indicator bacteria.

Some viral infections cannot be initiated merely by transfection with the viral DNA. These viruses cannot transfect cells, because in a natural infection, proteins in the viral head are normally injected along with the DNA, and these proteins are required to initiate the infection. For example, the *E. coli* phage N4 carries a phage-specific RNA polymerase in its head that is injected with the DNA and used to transcribe the early genes (see chapter 7). Transfection with the purified phage DNA does not initiate an infection, because the early genes are not transcribed without this phage-encoded RNA polymerase. Another example of a phage in which the infection cannot be initiated by the nucleic acid alone is phage ϕ6 (see Box 7.1 in chapter 7). This phage has RNA instead of DNA in the phage head and must inject an RNA replicase to initiate the infection, so that the cells cannot be transfected by the RNA alone. Such examples of phages that inject required proteins are rather rare; for most phages, the infection can be initiated by transfection.

As an aside, many animal viruses do inject proteins required for multiplication, and these proteins cannot be made after injection of the naked DNA or RNA. For example, a retrovirus such as human immunodeficiency virus, which causes AIDS, injects a reverse transcriptase required to make a DNA copy of the incoming RNA before it can be transcribed to make viral proteins. Therefore, human cells cannot be transfected with human immunodeficiency virus RNA alone.

TRANSFORMATION OF CELLS WITH CHROMOSOMAL GENES

Transformation with linear DNA is one method used to replace endogenous genes with genes altered in vitro. However, most types of bacteria made competent by calcium ion treatment are transformed poorly by chromosomal DNA because the linear pieces of double-stranded DNA entering the cell are degraded by an enzyme called the RecBCD nuclease. This nuclease degrades DNA from the ends; therefore, it does not degrade circular plasmid and phage DNAs. However, inactivating the RecBCD nuclease by a mutation would preclude recombination between the incoming DNA and the chromosome, because the enzyme is required for normal recombination in *E. coli* and other bacteria (see chapter 10).

Nevertheless, methods have been devised to transform competent *E. coli* with linear DNA. One way is to use a mutant *E. coli* lacking the D subunit of the RecBCD nuclease. These *recD* mutants are still capable of recombination, but because they lack the nuclease activity that degrades linear double-stranded DNA, they can be transformed with linear double-stranded DNAs. Other methods, sometimes called recombineering, use the recombination systems of phages such as λ phage. Double-stranded DNA can be introduced into cells expressing the λ recombination functions because of the ability of a λ protein named γ (gamma) to inhibit RecBCD. The method can also be used with single-stranded DNAs, such as PCR primers, which are not degraded by RecBCD. Such procedures are discussed further in chapter 10.

Electroporation

Another way in which DNA can be introduced into bacterial cells is by **electroporation**. In the electroporation process, the bacteria are mixed with DNA and briefly exposed to a strong electric field. It is important that the recipient cells first be washed extensively in buffer with very low ionic strength such as distilled water. The buffer usually also contains a nonionic solute such as glycerol to prevent osmotic shock. The brief electric fields across the cellular membranes might create artificial pores of H_2O lined by phospholipid head groups. DNA can pass through these temporary hydrophilic pores (see Tieleman, Suggested Reading). Electroporation works with most types of cells, including most bacteria, unlike the methods mentioned above, which are very specific for certain species. Also, electroporation can be used to introduce linear chromosomal and circular plasmid DNAs into cells. However, electroporation requires specialized equipment.