One system bypasses DNA damaged by UV irradiation when repair has failed. It directs replication to proceed across a region badly damaged by the formation of thymine dimers. This **error-prone replication** is responsible for the mutations induced by UV light.

Some repair processes result in mutation

GENETIC EXCHANGE

Mutation and selection are important factors in bacterial evolution, but evolution proceeds far faster than it could by these processes alone. For instance, the probability that the process of random mutation alone can produce a cell that, let us say, requires five mutations for optimal growth in a new environment is extremely low. It is in fact the product of the individual mutation frequencies (eg, $10^{-6} \times 10^{-6} \times 10^{-6} \times 10^{-6} \times 10^{-6} = 10^{-30}$), and that essentially precludes a natural population from ever acquiring the new property in this manner. However, such alterations occur because organisms exchange genetic material, thereby permitting combinations of mutations to be collected in individual cells.

Evolution is speeded by exchange of genetic material

Despite the fact that bacteria reproduce exclusively asexually, the sharing of genetic information within and between related species is now recognized to be quite common and to occur in at least three fundamentally different ways. All three processes involve a one-way transfer of DNA from a **donor cell** to a **recipient cell.** The molecule of DNA introduced into the recipient is called the **exogenote** to distinguish it from the cell's own original chromosome, called the **endogenote.**

One-way passage of DNA from a donor to a recipient adds an exogenote to the recipient endogenote

One process of DNA transfer, called **transformation,** involves the release of DNA into the environment by the lysis of some cells, followed by the direct uptake of that DNA by the recipient cells. By another means of transfer, called **transduction,** the DNA is introduced into the recipient cell by a nonlethal virus that has grown on the donor cell. The third process, called **conjugation,** involves actual contact between donor and recipient cell during which DNA is transferred as part of a plasmid (an autonomously replicating, extrachromosomal molecule of circular double-stranded DNA); in conjugation, donor and recipient cells are referred to as male and female, respectively. The three means of gene transfer are summarized in Figure 4–2.

Transformation, transduction, and conjugation are the major processes of DNA transfer

Species of bacteria differ in their ability to transfer DNA, but all three mechanisms are distributed among both Gram-positive and Gram-negative species; however, only transformation is governed by bacterial chromosomal genes. Transduction is totally mediated by virus genes, and conjugation, by plasmid genes.

Transformation, transduction, and conjugation are mediated by chromosomal, viral, and plasmid genes, respectively

Transformation

Transformation was first demonstrated in 1928 by F. Griffith, a British public health officer, who showed that virulent, encapsulated *Streptococcus pneumoniae* (pneumococci) that had been killed by heat could confer on living, avirulent, nonencapsulated pneumococci the ability to make the polysaccharide capsule of the killed organisms and thus become virulent for mice. Subsequent work in 1944 by O. T. Avery, C. M. MacLeod, and M. McCarty at the Rockefeller Institute revealed that the "transforming factor" from the dead pneumococci was nothing other than DNA. This discovery had enormous impact on biology, because it was the first rigorous demonstration that DNA is the macromolecule in which genetic information is encoded. It opened the door to modern molecular genetics.

Studies on pneumococcal transformation led to identification of genetic material

The ability to take up DNA from the environment is called **competence,** and in many species of bacteria, it is encoded by chromosomal genes that become active under certain environmental conditions. In such species, transformation can occur readily and is said to be natural. Other species cannot enter the competent state but can be made permeable to DNA by treatment with agents that damage the cell envelope making an **artificial transformation** possible.

Genes encoding competence enable uptake of DNA; species lacking them must be made permeable

Natural transformation must be important in nature, judged by the variety of mechanisms that different bacteria have evolved to accomplish it. Two of the best-studied systems are those of the Gram-positive pneumococcus and a Gram-negative rod, *Haemophilus influenzae*. Pneumococcal cells secrete a protein **competence factor** that induces many of the cells of a culture to synthesize special proteins necessary for transformation, including an autolysin that exposes a cell membrane DNA-binding protein. Any DNA present in the medium is bound indiscriminately; even salmon sperm DNA can be bound and taken up as

Pneumoccal competence involves a nonspecific DNA-binding protein

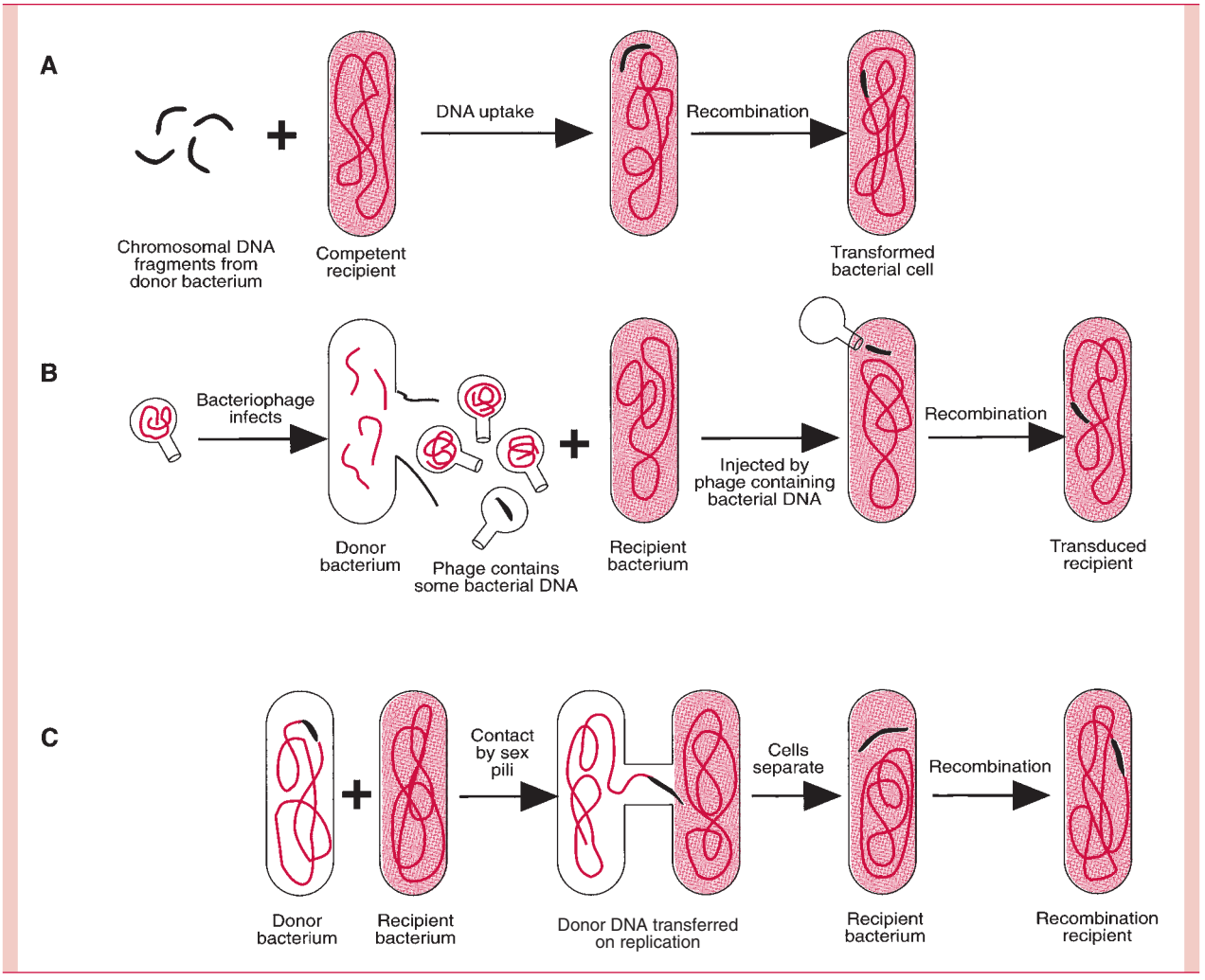

FIGURE 4–2
Chromosomal gene transfer mechanisms in bacteria. **A.** Transformation. **B.** Transduction. **C.** Conjugation.

All DNA is taken up, but heterologous DNA is degraded

readily as DNA from another pneumococcal cell. The surface-bound double-stranded DNA is cleaved into fragments of about 6 to 8 kilobases (kb). One strand is degraded by a nuclease, while the complementary strand of each fragment is taken up by a process that seems to be driven by the proton-motive force of the cell membrane (see Chapter 3). The fate of the internalized DNA fragment then depends on whether it shares homology (the same or similar in base sequence) with a portion of the recipient cell's DNA. If so, recombination can occur by a process described later, but heterologous DNA (no similarity to the endogenote) is degraded and causes no heritable change in the recipient.

H. influenzae endocytoses only homologous dsDNA, recognized by a characteristic 11-bp sequence

Transformation in *H. influenzae* is somewhat different. There is no competence factor, and cells become competent merely by growth in an environment rich in nutrients. Only homologous DNA (ie, DNA from the same or a closely related species of *Haemophilus*) is taken up, and it is taken up in double-stranded form. The selectivity is brought about by the presence of a special membrane protein that binds to an 11-base pair (bp) sequence (5'-AAGTGCGGTCA-3') that occurs frequently in *Haemophilus* DNA and infrequently in other DNAs. Following binding to molecules of this protein, the homologous DNA is internalized by a mechanism that resembles membrane invagination, resulting in the temporary residence of the exogenote in cytosolic membrane vesicles. Although the DNA taken up is double stranded, only one of the two strands participates in the subsequent recombination with the endogenote.

The common use of *E. coli* as a host cell in which to clone genes on hybrid plasmids (see Invertible DNA Segments and Recombinational Regulation of Gene Expression) depends on procedures involving treatment with salt and temperature shocks to bring about artificial transformation; this organism has no natural competence mechanism. In contrast, the pathogen, *Neisseria gonorrhoeae* regularly uses transformation to bring about changes in the antigenic nature of its pili, as described later in the section on recombination.

Transformation is common among many pathogens; artificial transformation enables use of *E. coli* for gene cloning

Transduction

Transduction is virus-mediated transfer of genetic information from donor to recipient cell. To understand transduction and its several mechanisms, it is necessary to preview the nature of bacterial viruses, a topic dealt with more extensively in Chapters 5, 6, and 7.

Viruses are capable of reproduction only inside living cells. Those that grow in bacteria are called **bacteriophages**, or simply **phages.** They are minimally composed of protein and nucleic acid, although some may have a very complex structure and composition. The individual virus particle or virion consists of a protein capsid enclosing genomic nucleic acid, which is either RNA or DNA, but never both. Virions infect sensitive cells by adsorbing to specific receptors on the cell surface and then, in the case of phages, injecting their DNA or RNA. Phages come in two functional varieties according to what happens after injection of the viral nucleic acid. **Virulent** (lytic) **phages** cause lysis of the host bacterium as a culmination of the synthesis of many new virions within the infected cell. **Temperate phages** may initiate a lytic growth process of this sort or can enter a quiescent form (called a **prophage**), in which the infected host cell is permitted to proceed about its business of growth and division but passes on to its descendants a prophage genome capable of being **induced** to produce phage in a process nearly identical to the growth of lytic phages. The bacterial cell that harbors a latent prophage is said to be a **lysogen** (capable of producing lytic phages), and its condition is referred to as **lysogeny**. Lysogens are immune to infection by virions of the type they harbor as prophage. Occasionally, lysogens are spontaneously induced and lysed by the phage and release mature virions (as many as 75 to 150 or more per cell) into the environment. When triggered by UV irradiation or certain chemicals, an entire population of lysogens are induced simultaneously to initiate reproduction of their latent virus followed by lysis of the host cells. Infection of a sensitive cell with the temperate phage can lead to either lysis or lysogeny. How this choice comes about is described in Chapter 7.

Phages are viruses of diverse structure and modes of replication

Virulent phages produce new virions in the host bacterial cell, usually lysing it

Temperate phages can either lyse a bacterial host cell or lysogenize it as a prophage

Prophage induction leads to virion production and cell lysis

The prophage of different temperate phages exists in one of two different states. In the first, the prophage DNA is physically integrated into a bacterial chromosome; in the second, it remains separate from the chromosome as an independently replicating, circularized, molecule of DNA. Prophages of this sort are in fact plasmids.

Some prophages integrate; others behave as plasmids

For the most part, transduction is mediated by temperate phage, and the two broad types of transduction result from the different physical forms of prophage and the different means by which the transducing virion is formed. These are termed **generalized transduction,** by which any bacterial gene stands an equal chance of being transduced to a recipient cell, and **specialized** or **restricted transduction,** by which only a few genes can be transduced.

Transduction, whether generalized or specialized, is mediated by temperate phage

Generalized Transduction

Some phages package DNA into their capsids in a nonspecific way, the headful mechanism, in which any DNA can be stuffed into the capsid head until it is full. (The head is the principal structure of the virion to which, in some cases, a tail is attached; see Chapter 5.) An endonuclease then trims off any projecting excess. If fragments of host cell DNA are around during the assembly of mature virions, they can become packaged in place of virus DNA, resulting in **pseudovirions.** Pseudovirions are the transducing agents. They can adsorb to sensitive cells and inject the DNA they contain as though it were viral DNA. The result is the introduction of donor DNA into the recipient cell.

In generalized transduction, pseudovirions inject a random piece of host DNA into a recipient

Any given gene has an equal probability of being transduced by this process. With the temperate phage P1 of *E. coli,* this probability is approximately one transduction event per 10^5 to 10^8 virions, because nearly 1 out of every 1000 phage particles made in a P1

Genes have low but equal probability of being transduced

lytic infection are pseudovirions, and the bacterial DNA fragments packaged are 1 to 2% of the length of the chromosome. Cotransduction of two bacterial genes by a single pseudovirion occurs only if they are located close together within this small length of the chromosome, and this fact facilitates mapping the position of a newly discovered gene.

Once injected into the host cell, the transduced DNA is lost by degradation unless it can recombine with the chromosome of the recipient cell, usually by homologous recombination (see below, Invertible DNA Segments and Recombinational Regulation of Gene Expression) in which both strands of the exogenote cross into and replace the homologous segment of the recipient's chromosome. However, sometimes the exogenote can persist without degradation by assuming a stable circular configuration.

Specialized Transduction

Specialized transduction involves imprecise excision of an integrated prophage

A few genes adjacent to the prophage are transferred to the recipient and cointegrated with prophage

It has been noted that the prophage of some phages is integrated into the lysogen's chromosome. This integration does not occur haphazardly but is restricted to usually one site, called the *att* (attachment) site. When a lysogen carrying such a prophage is induced to produce virions, excision of the viral genome from the bacterial chromosome occasionally (eg, in 1 of 10^5 to 10^6 lysogens) occurs imprecisely, resulting in a pickup of genes of the bacterium adjacent to the *att* site. The resulting virion may be infectious (if no essential phage genes are missing) or defective (if one or more essential genes are missing). In either case, adsorption to a sensitive cell and injection of the DNA can occur, and integration of the aberrant phage genome into the chromosome of the new host cell results in the formation of a lysogen containing a few genes that have been transduced as hitchhikers with the phage genome. Integration of the phage genome automatically accomplishes the recombinational event needed to guarantee reproduction of the transduced genes. Only genes that border the *att* site stand a chance of being transduced by this process, which is why it is called specialized or restricted transduction.

All virions produced by lysogenic transductants carry original transduced gene

Because the original pickup event is rare, the first transducing process is termed **low-frequency transduction;** however, when a lysogenic transductant is, in turn, induced to produce phage, all of the new virions carry the originally transduced bacterial gene. The resulting mixture of lysed cells and virions now brings about **high-frequency transduction** of the attached genes.

Specialized transduction has been valuable in gene cloning and sequencing

Bacterial geneticists have learned to move genes of interest near the phage integration site and thereby construct specialized transducing phages containing these genes. Such transducing phages are valuable aids to cloning and sequencing genes and to studying their function and regulation. Obviously a temperate phage that could form a prophage by integrating randomly at any site in the bacterial chromosome would be of special use. The temperate phage Mu of *E. coli* has this property.

Transduction is common in nature, important clinically, and useful in research

Although both generalized transduction and specialized transduction can be regarded as the result of errors in phage production, transfer of genes between bacterial cells by phage is a reasonably common phenomenon. It occurs at significant frequency in nature; for example, genes conferring antimicrobic resistance in staphylococci are often transduced from strain to strain in this way. The toxins responsible for the severe clinical symptoms of diphtheria and of cholera are encoded by genes transduced into *Corynebacterium diphtheriae* and *Vibrio cholerae,* respectively. Transduction is also used extensively as a tool in molecular biology research.

Conjugation

Conjugation is plasmid-encoded and requires cell contact

Conjugation is the transfer of genetic information from donor to recipient bacterial cell in a process that requires intimate cell contact; it has been likened to mating. By themselves, bacteria cannot conjugate. Only when a bacterial cell contains a self-transmissible **plasmid** (see below for definition) does DNA transfer occurs. In most cases, conjugation involves transfer only of plasmid DNA; transfer of chromosomal DNA is a rarer event, and is mediated by only a few plasmids. Plasmids are of enormous importance to medical microbiology. They are discussed in detail later in this chapter, but to understand conjugation we should introduce some of their features at this point.