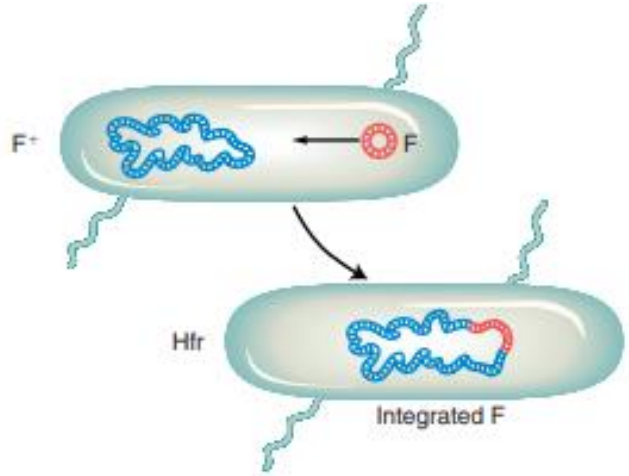


Figure 5-8 **Formation of an Hfr.** Occasionally, the independent F factor combines with the *E. coli* chromosome, creating an Hfr strain.

Hfr strains An important breakthrough came when Luca Cavalli Sforza discovered a derivative of an F+ strain with two unusual properties:

1. On crossing with F- strains this new strain produced 1000 times as many recombinants as a normal F+ strain. Cavalli-Sforza designated this derivative an Hfr strain to symbolize its ability to promote a **high frequency of recombination.**

2. In Hfr x F- crosses, virtually none of the F- parents were converted into F+ or into Hfr

Some special features of the recombination event in bacteria.

Recombination does not take place between two whole genomes, as it does in eukaryotes.

In contrast, it takes place between one *complete genome, from the F -, called the* **endogenote, and an *incomplete one, derived from the Hfr*** donor and called the **exogenote.**

The cell at this stage has two copies of one segment of DNA—one copy is the exogenote and one copy is part of the endogenote.

Thus at this stage the cell is a *partial diploid, called a* **merozygote.**

Does an Hfr cell die after donating its chromosomal material to an F- cell?
The answer is no.
Just like the F+ plasmid, during conjugation the Hfr chromosome replicates and transfers a single strand to the F- cell.

The replication of the chromosome ensures a complete chromosome for the donor cell after mating.

The transferred strand is converted into a double helix in the recipient cell, and donor genes may become incorporated in the recipient's chromosome through crossovers, creating a recombinant cell

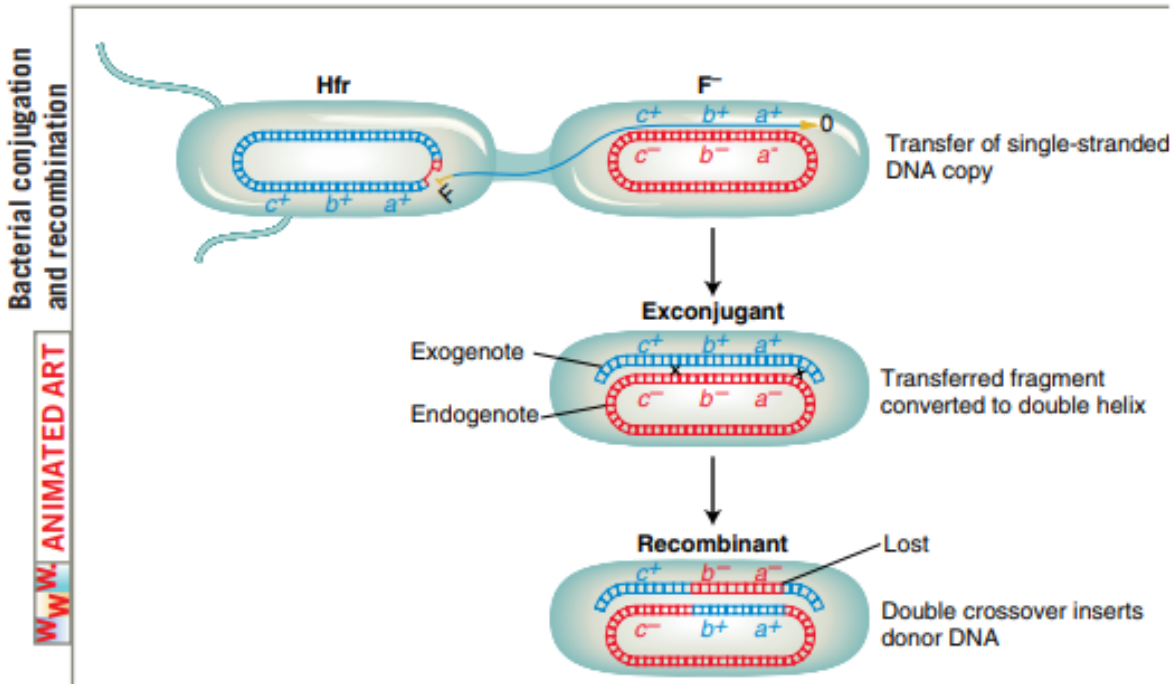
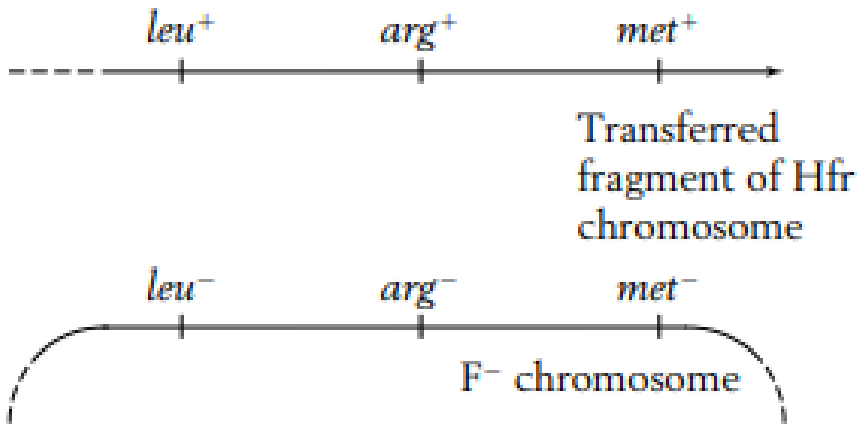
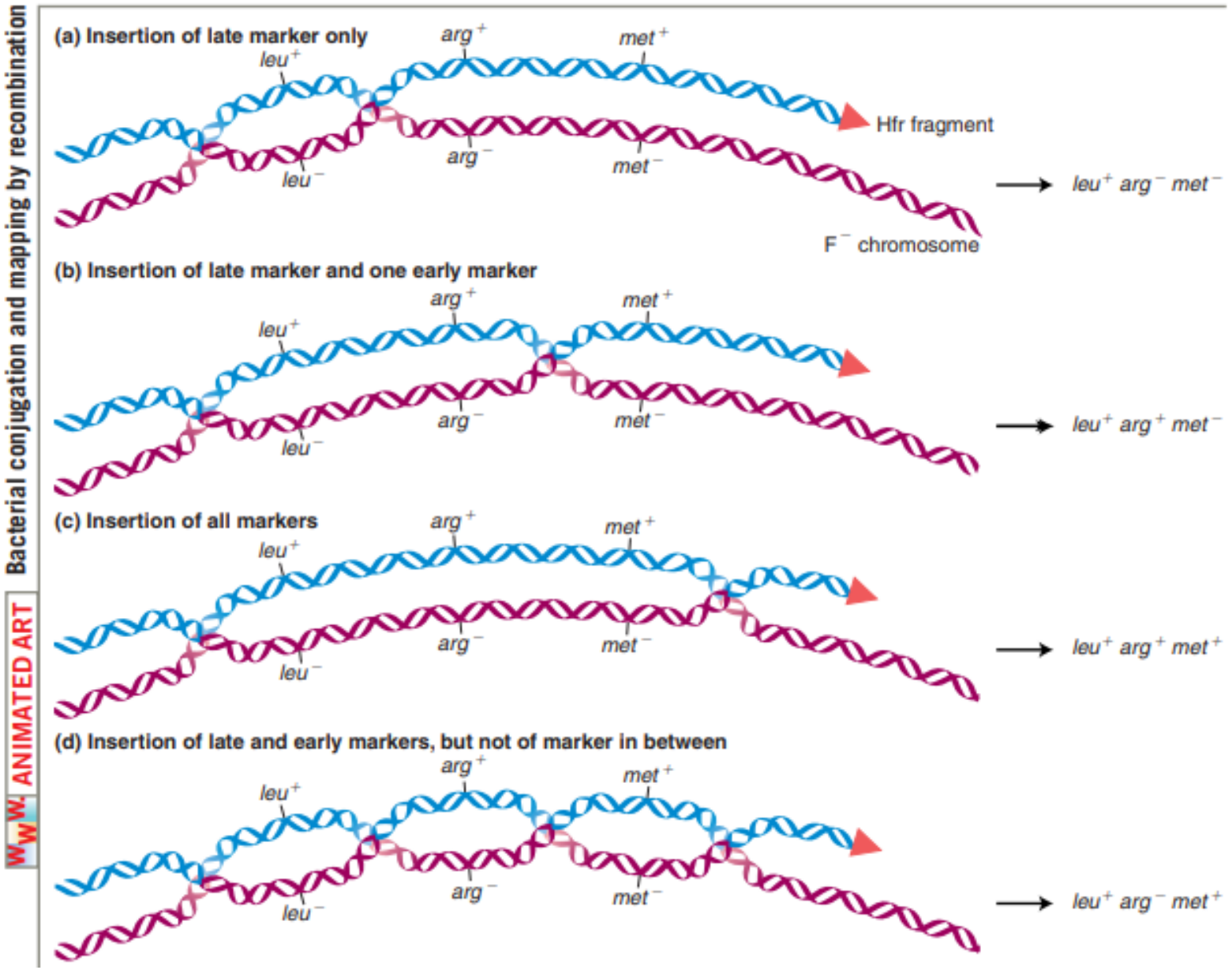


Figure 5-10 Bacterial conjugation and recombination. Transfer of single-stranded fragment of donor chromosome and recombination with recipient chromosome.


leu+
arg+
met+
Transferred
fragment of Hfr
chromosome
leu−
arg−
met−
F− chromosome


Bacterial conjugation and mapping by recombination
WWW ANIMATED ART
(a) Insertion of late marker only
leu+
arg+
met+
leu−
arg−
met−
Hfr fragment
F− chromosome
leu+ arg− met−
(b) Insertion of late marker and one early marker
leu+ arg+ met−
(c) Insertion of all markers
leu+ arg+ met+
(d) Insertion of late and early markers, but not of marker in between
leu+ arg− met+

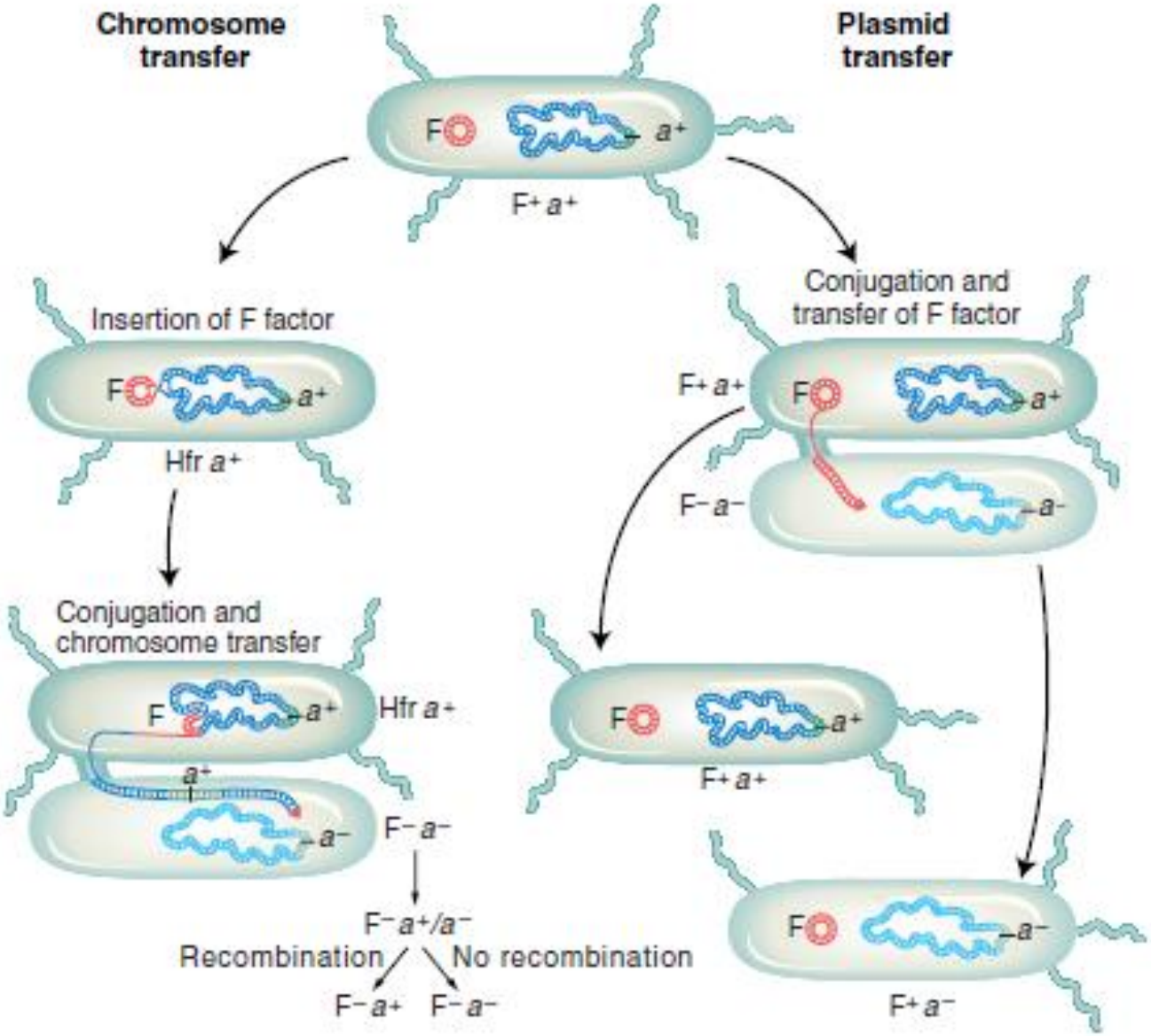
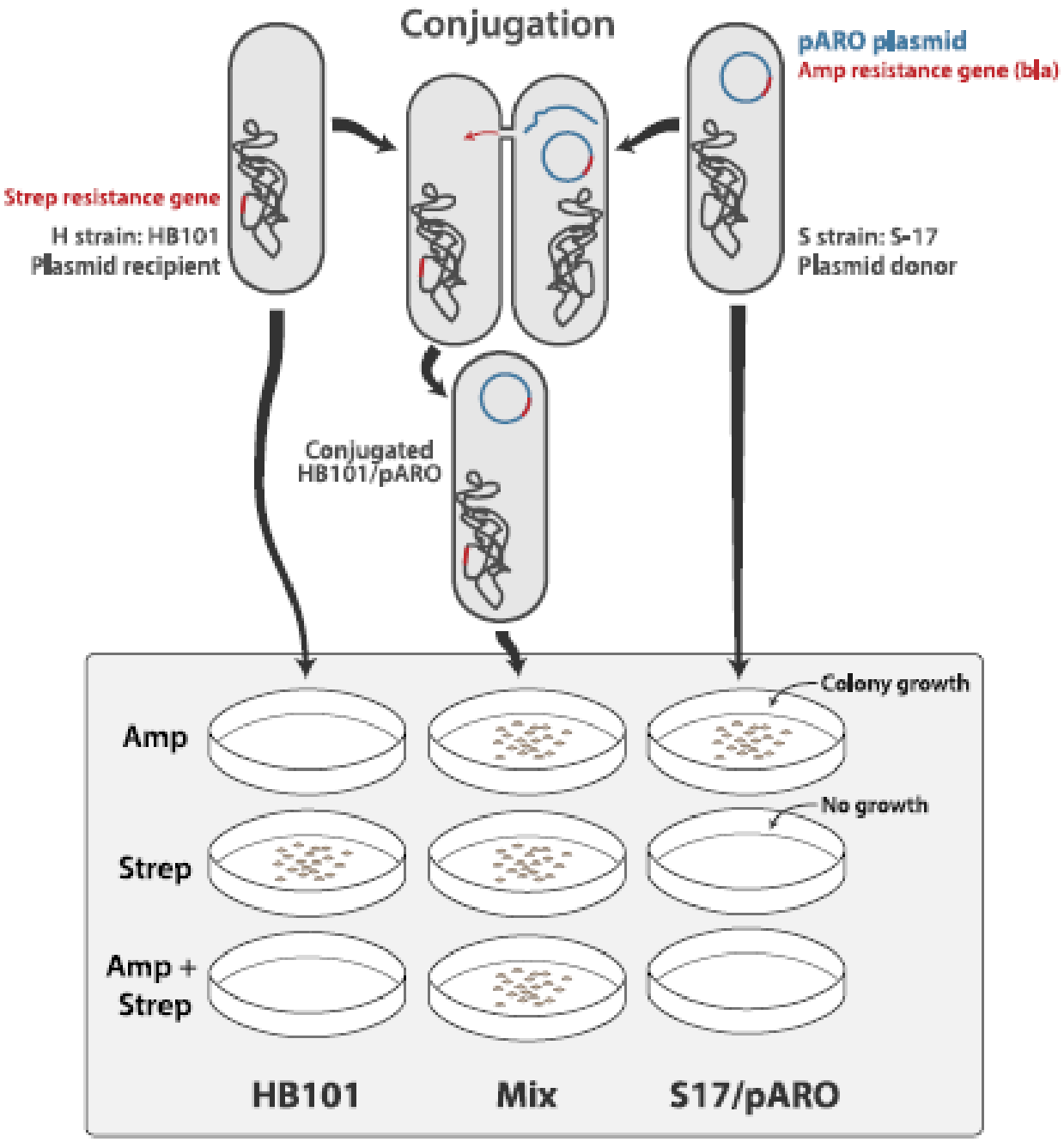


Figure 5-14 Conjugation summary. Summary of the various events that take place in the conjugational cycle of *E. coli*.


Conjugation
pARO plasmid
Amp resistance gene (bla)
Strep resistance gene
H strain: HB101
Plasmid recipient
S strain: S-17
Plasmid donor
Conjugated
HB101/pARO
Colony growth
No growth
Amp
Strep
Amp +
Strep
HB101
Mix
S17/pARO

You'll start with liquid cultures of with two strains, or genetic types, of *E. coli*:
***E. coli* S17 containing the plasmid pARO180.** The plasmid has a gene providing resistance to ampicillin, but the S17 strain isn't resistant to streptomycin.

***E. coli* HB101 with no plasmid.** The chromosome contains a gene providing resistance to streptomycin, but without a plasmid, this strain isn't resistant to ampicillin.

You'll mix liquid cultures of these two strains to make a new liquid culture:

"Mix." The two strains mixed together in one culture tube. Some of the cells are expected to conjugate, meaning that the plasmid pARO is copied from S17 to HB101.

The resulting conjugated HB101 cells will contain both the amp resistance gene and the strep resistance gene, so they will be able to grow on plates containing both amp and strep (they're double resistant).

Not all the cells will conjugate, so the "mix" liquid culture will actually contain both original strains plus the new strain created by conjugation. Only the conjugated cells will grow on amp + strep plates.

Transformation as another type of bacterial gene transfer

Some bacteria can take up fragments of DNA from the external medium.

The source of the DNA can be other cells of the same species or cells of other species. In some cases the DNA has been released from dead cells; in other cases the DNA has been secreted from live bacterial cells.

The DNA taken up integrates into the recipient's chromosome. If this DNA is of a different genotype from the recipient, the genotype of the recipient can become permanently changed, a process aptly termed **transformation.**

Transformation was discovered in the bacterium *Streptococcus pneumoniae in 1928 by Frederick Griffith.*

In 1944, Oswald T. Avery, Colin M. MacLeod, and Maclyn McCarty demonstrated that the "transforming principle" was DNA.

In ***conjugation,*** *DNA is transferred* from one living cell to another through close contact, whereas in ***transformation*** *isolated pieces of external* DNA are taken up by a cell through the cell wall and plasma membrane.