Gene Exchange/Gene Mapping Techniques in Bacteria

Transformation & Conjugation

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Reading List

Microbial genetics. S.R.Maloy, J.E. Cronan & Freifelder

Genetics – a molecular approach. T.A. Brown

Bacterial Transformation – uptake of DNA

This technique was used to first demonstrate that DNA was the genetic material and not protein.

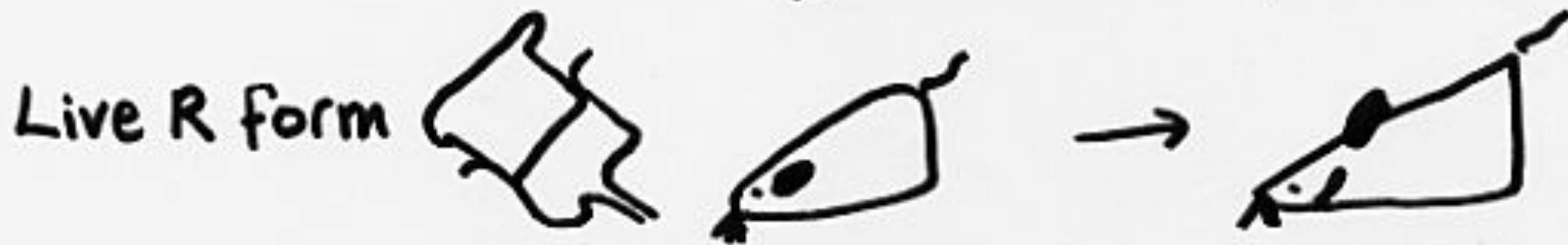
Griffith in 1928, demonstrated the ‘transformation principle’

Avery, Macleod & McCarty (1944) later demonstrated ‘transformation’ material was nucleic acid.

Work was done with ***S. pneumoniae,*** 2 forms:

1. normal or ‘S’ form (smooth shiny colonies), when injected into mice, it kills them
2. mutant or ‘R’ form (rough colonies), it does NOT kill mice when injected into them.

Griffith's Experiment



Heat killed S form TRANSFORMED Live R form

Transformation

Begins with the uptake of DNA chromosomal fragments from the surrounding media into cells competent for uptake of DNA.

The donor DNA then undergoes a physical exchange (recombination) and is incorporated into the host cell (e.g. as can be demonstrated by new antibiotic resistance).

Most bacteria are proficient at recombination but very few are competent for uptake of DNA naturally.

Even those that are naturally competent, only a small fraction are competent, but this can be enhanced by culturing under certain conditions.

Natural competence

This results from changes in the bacterial cell wall at a particular stage in the cell cycle (late log phase) and lasts just for a few minutes.

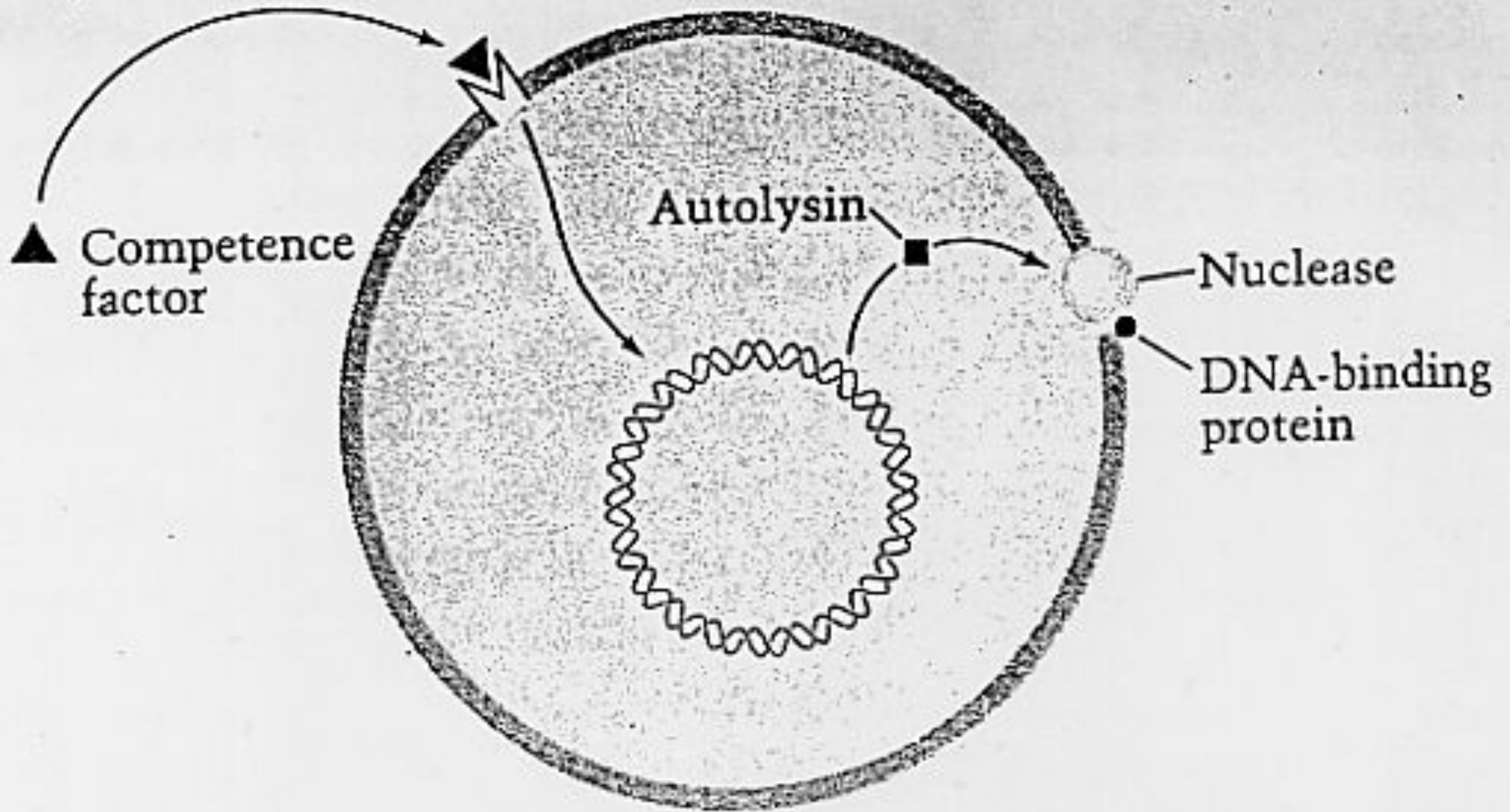
Receptors (between 4-80) on the cell wall are activated by a competence factor.

Competence factors are produced by a limited number of cells, but these then convert the rest of the culture to be competent by expressing 8 to 10 key proteins.

Competent specific proteins are expressed, e.g. autolysin.

Autolysin activates a DNA binding protein and nuclease in the cell wall.

Natural competence



Transformation cont.

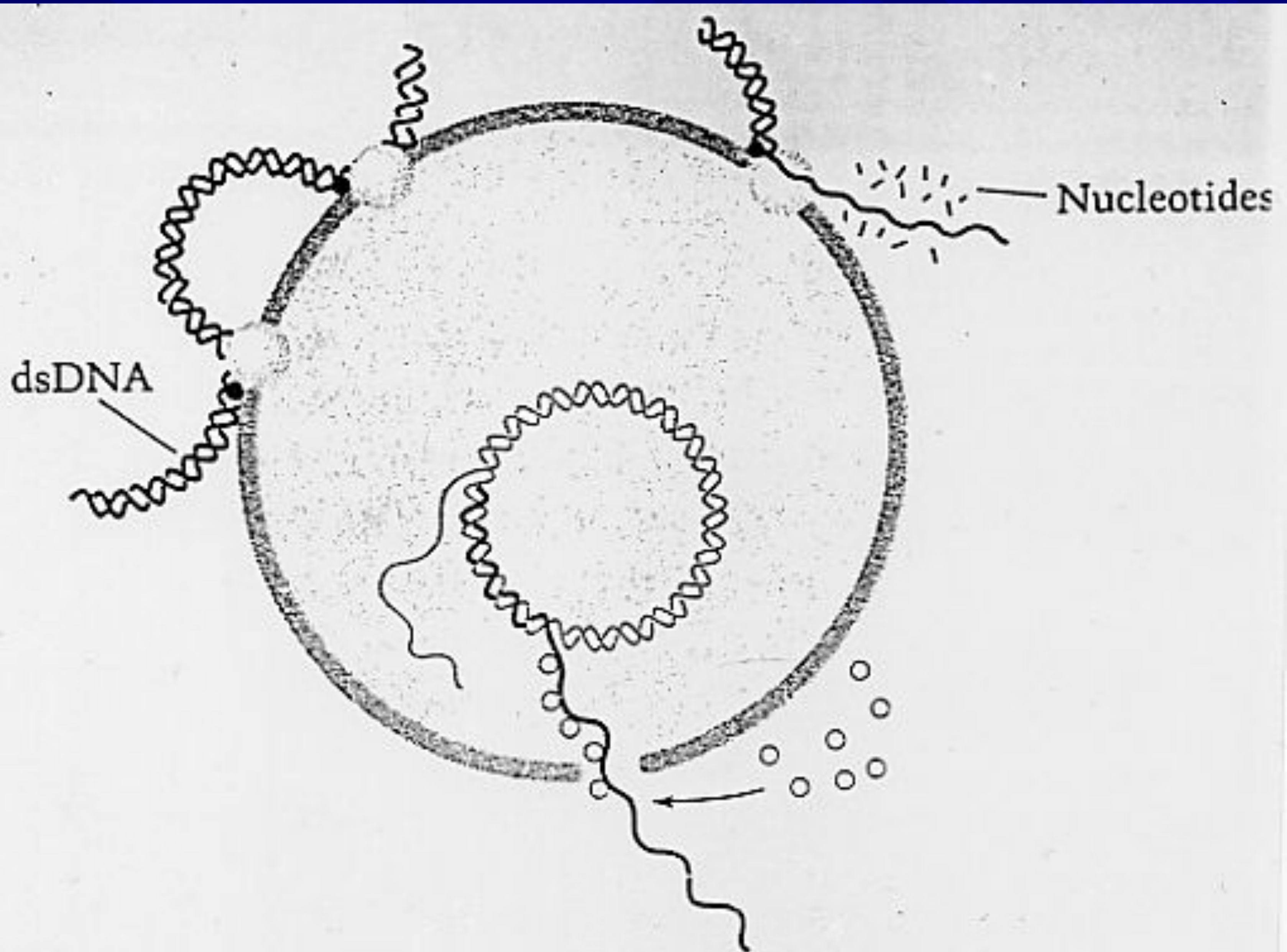
Double-stranded DNA (dsDNA) released by bacteria (e.g. during starvation) bind to the ‘competent’ cell surface.

The dsDNA is nicked and one strand is degraded.

The remaining DNA strand then binds to a protein and enters the cell.

The ssDNA if homologous can then recombine into the host genome.

Transformation

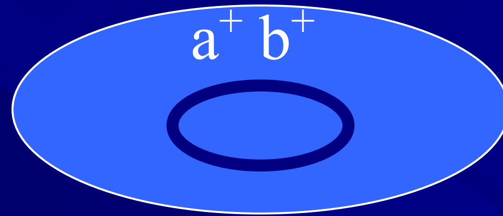


Competence ‘for uptake of DNA’

Relatively few bacteria are naturally competent, best examples are *Bacillus subtilus*, *Streptomyces pneumoniae* and *Haemophilus influenzae*.

However a wide range of prokaryotic and eukaryotic cells can be made competent.

Genetic Mapping by Transformation



DNA released & degraded

a^+ $a^+ b^+$ b^+

Transformation of an $a^- b^-$ recipient

If $a^+ b^+$ are closely linked (physically close), will see $a^+ b^+$, $a^+ b^-$ and $a^- b^+$.

If not closely linked will only see $a^+ b^-$ or a-b^+

Bacterial conjugation

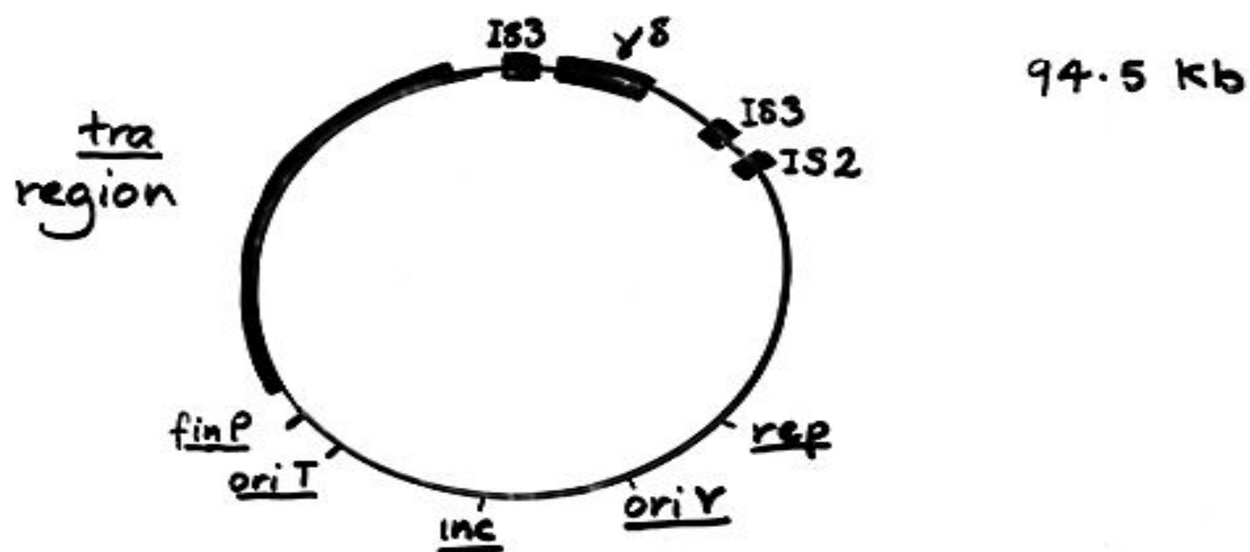
Is based on cell to cell contact due to sex-pili encoded by the F (fertility) plasmid.

Occurs by crossing an F^- (female) strain with

1. an HFr (High Frequency of recombination) due to an F plasmid integrated into the donor chromosome.

2. an F' (F-prime) strain.

THE F PLASMID



94.5 Kb

ori V : ORIGIN FOR VEGETATIVE REPLICATION

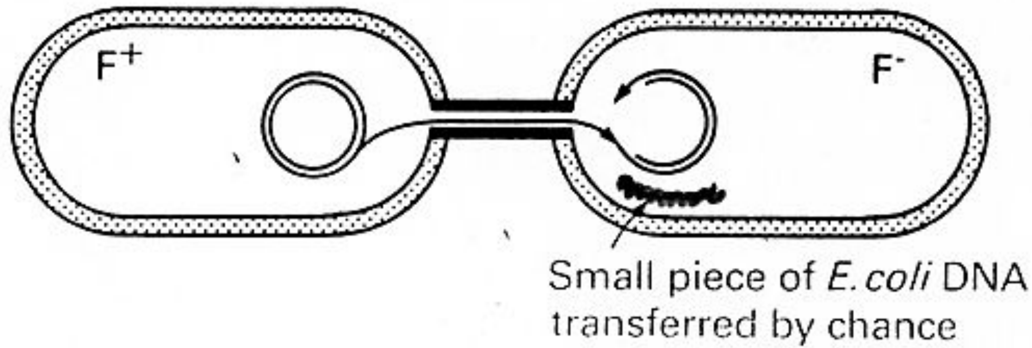
inc, rep : PROMOTE REPLICATION FROM ori V

ori T : ORIGIN FOR TRANSFER REPLICATION

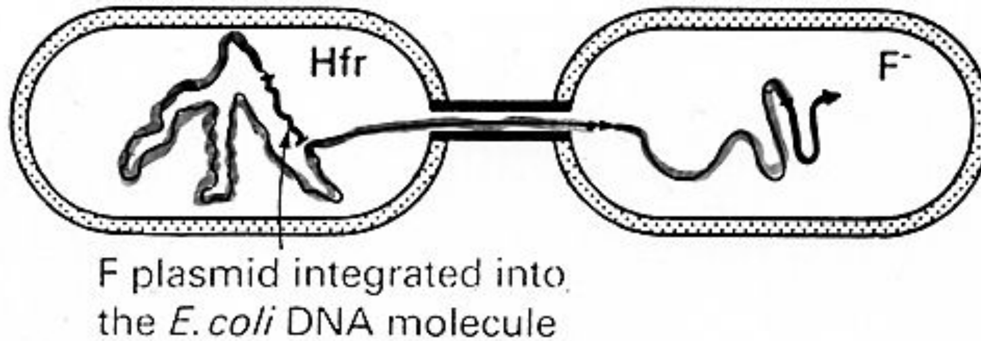
tra : OPERON → TRANSFER REPLICATION

IS3, γδ, IS2 : INSERTION SEQUENCES

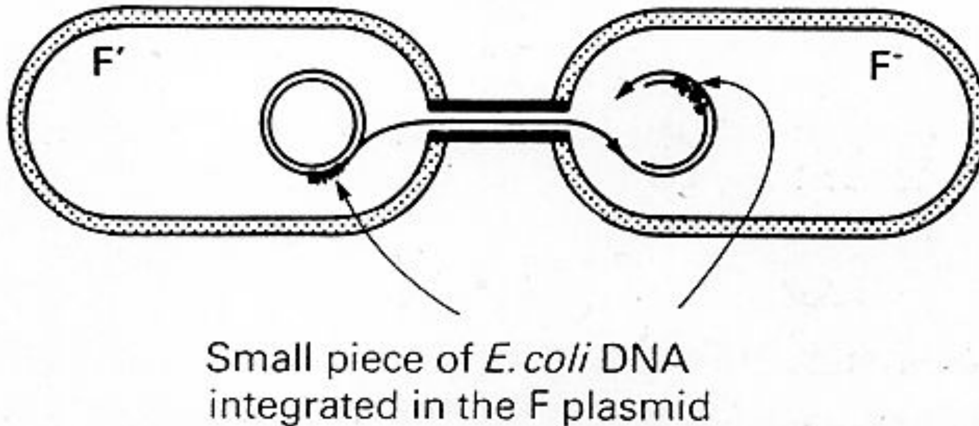
(a) Transfer during F⁺ to F⁻ conjugation



(b) Hfr to F⁻ conjugation



(c) Transfer by F′ to F⁻ conjugation



(from Genetics by Brown)

F+ STRAINS TRANSFER F PLASMID TO F− STRAINS

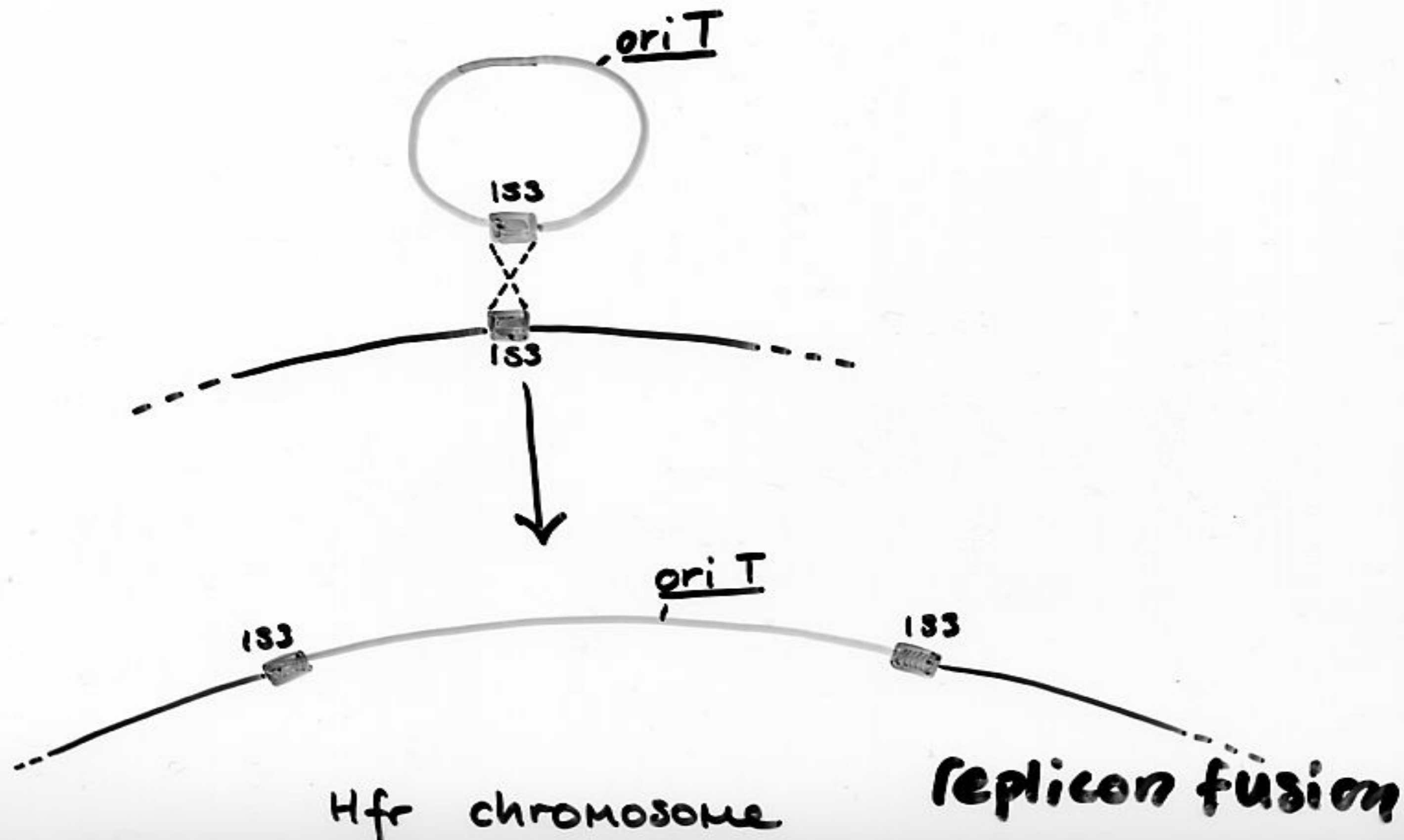
⇓

F− ⟶ F+

MINORITY OF CELLS BECOME

Hfr STRAINS

(High frequency of recombination)



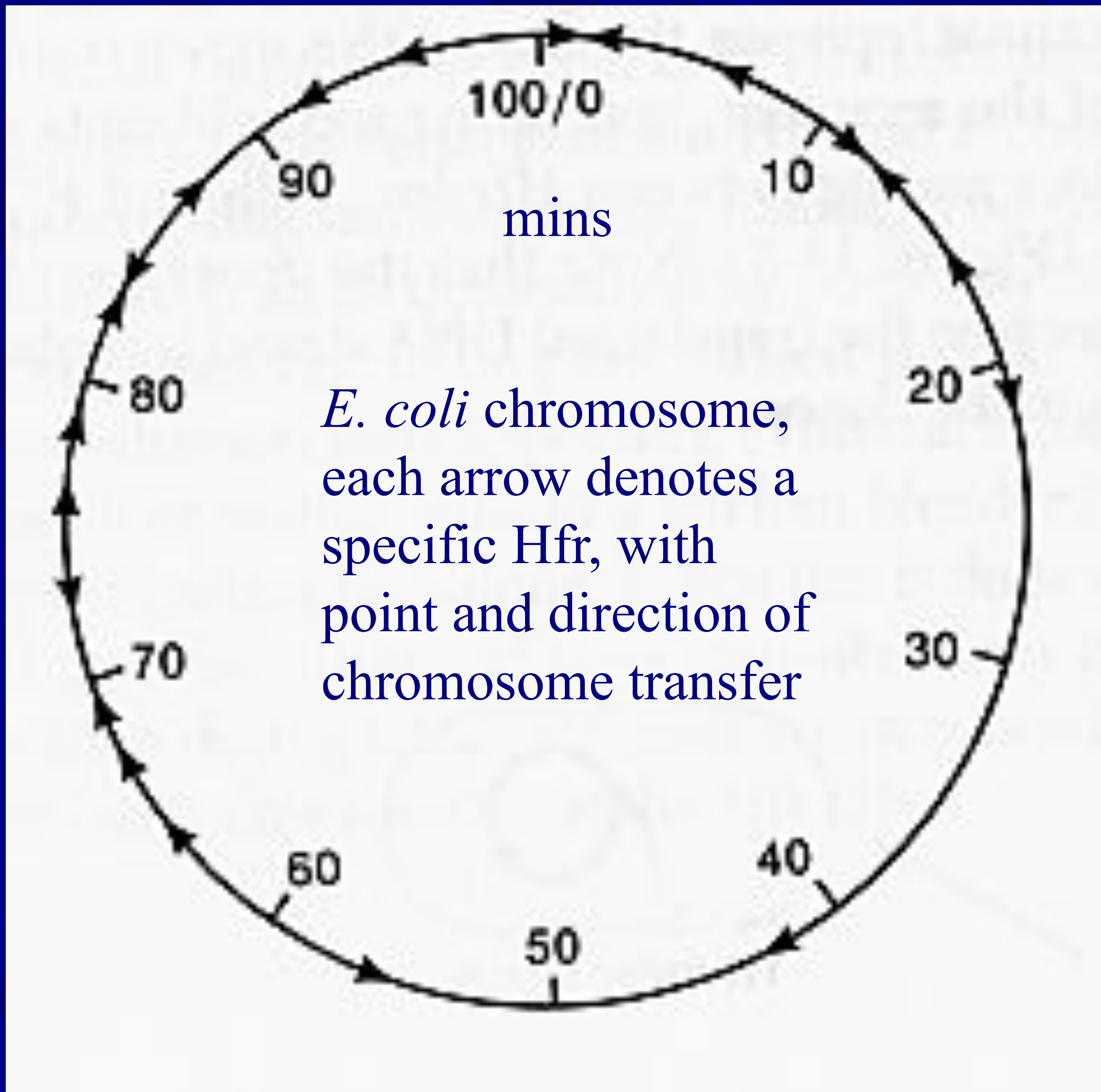
Integration of F into the chromosome produces an Hfr of defined position

Hfrs contain the integrated F factor at a defined position in the bacterial chromosome.

Hfrs behave like a big F factor and transfer the chromosome from the point at which the F factor is integrated.

E. coli chromosome is mapped in minutes, these minutes are the time taken to transfer part of the chromosome. The whole chromosome can theoretically be transferred in 100 minutes.

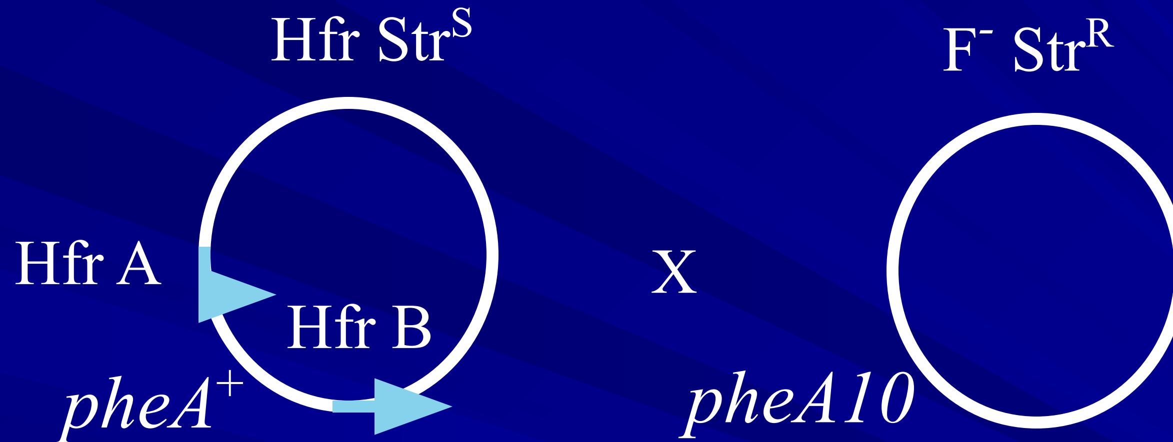
This allows gene mapping experiments.



(from Microbial Genetics Maloy *et al.*)

Genetic Mapping by Conjugation

1. Can the position of a gene by crossing an Hfr x F- and asking if gene is transferred by specific Hfrs



Add Hfr to F^- at 1:10 leave for 60 mins at 37^0C.
Plate on minimal media + streptomycin.
Only Hfr B x F- will yield cells (transconjugants) that will grow on minimal media containing streptomycin, *pheA* maps between the origin of transfer of Hfr A and Hfr B.

Genetic Mapping by Conjugation

2. Time of transfer – interrupted mating (vortex by jigsaw motor)

Hfr Str^S

F^- Str^R

thrA$^+$

cysG$^+$

pheA$^+$

Hfr A

X

*thrA*13

*cysG*22

*pheA*10

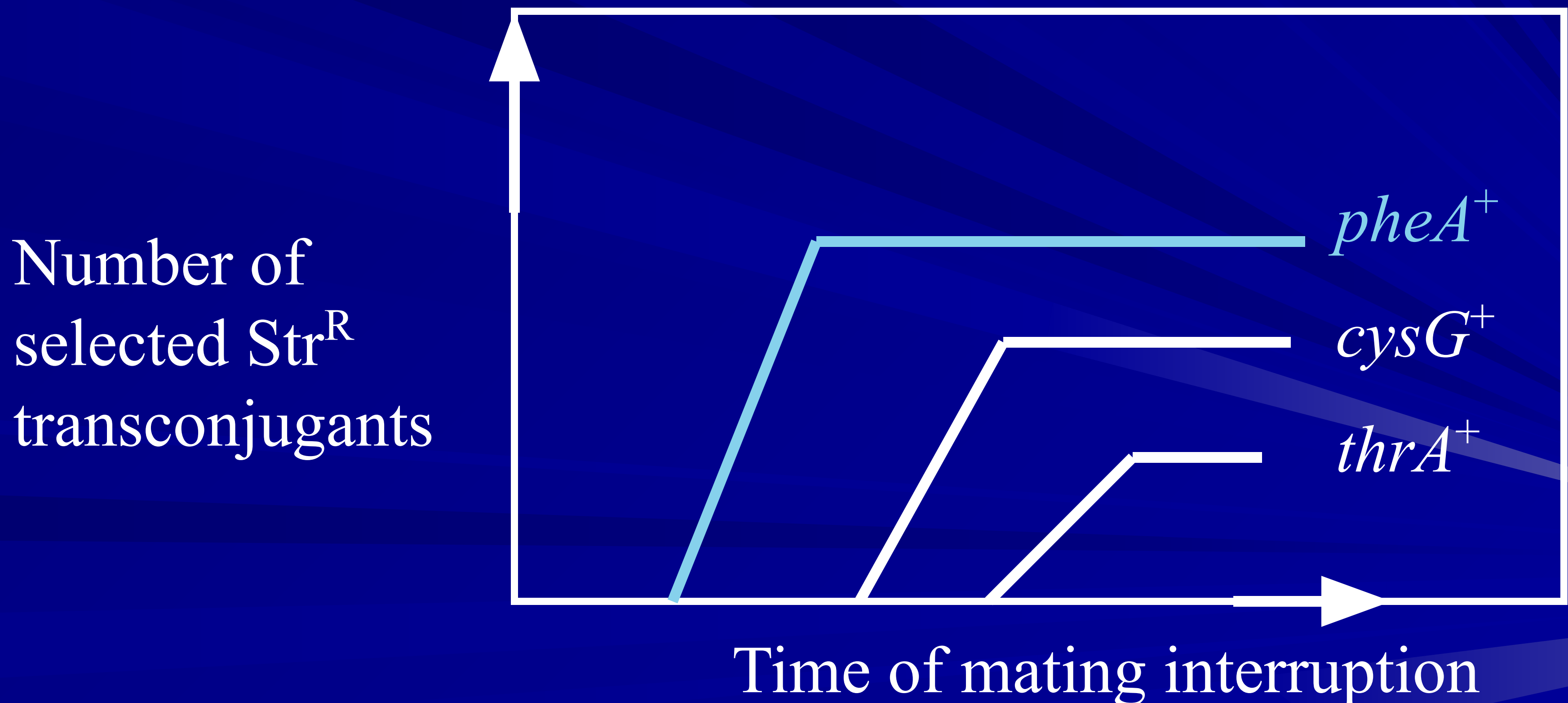
thrA$^+$ *cysG*$^+$ *pheA*$^+$

35 min 25 min 15 min

Order of transfer into F^-

Genetic Mapping by Conjugation

2. Time of transfer – interrupted mating (vortex by jigsaw motor) Mating as described previous but now plate on minimal media + streptomycin + phenyalanine OR cysteine OR threonine



Summary

Transformation is simply the uptake of external DNA by competent cells, but this is a rare process.

Conjugation is a another process by which DNA is introduced into other bacterial cells.

Both processes promote gene transfer and genes on homologous DNA are taken into the cells by recombination (most cells are proficient at this).

Once genes have been transferred and recombined into the host chromosome, it is possible to measure how physically close they are by how frequently they are co-transferred with the other gene! Applies to all mapping techniques!