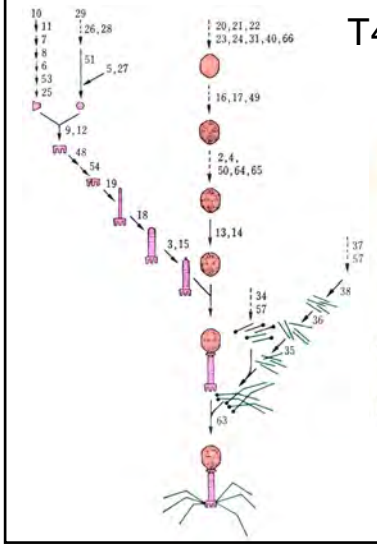
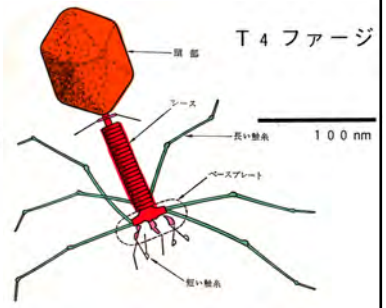
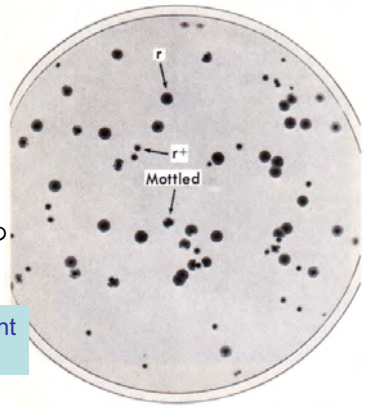
T4ファージの形成過程





11. Photograph of phage T2 plaques on a lawn of *E. coli* bacteria

T2 phage

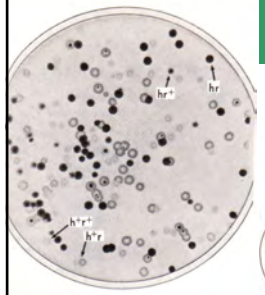


T2 mutant phage

原因は分からないが、プラークの形をかえる変異を得ることが容易にできた

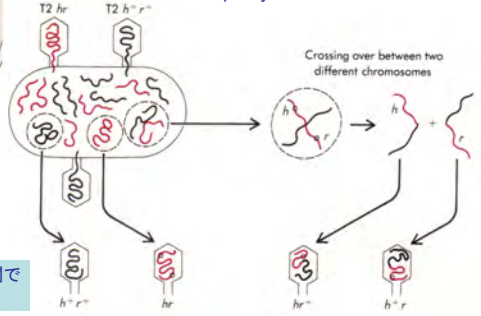
T2 r : rapid-lysis mutant
T2 r⁺:wild-type

13. Phage recombination in cells infected with two different strains of phage T2



大腸菌B株とB/2株の混合菌でプラークを作らせることで、h⁺r⁺, hr, hr⁺, h⁺rを区別

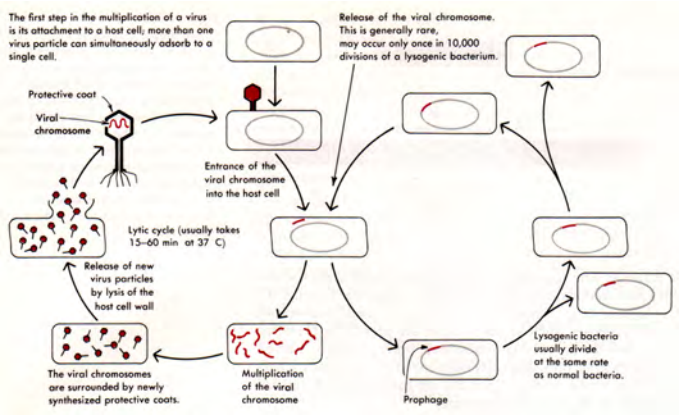
T2h:野生型T2が吸着できない大腸菌B/2に感染できる変異株
T2 r : rapid-lysis 変異株



1945年 : 変異株ファージ間での遺伝的組み換えの発見

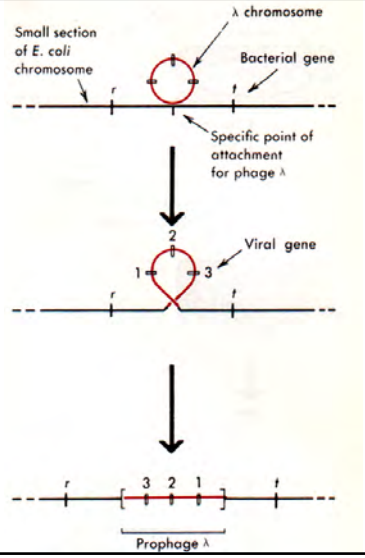
ファージ治療

29. The life cycle of a lysogenic bacterial virus

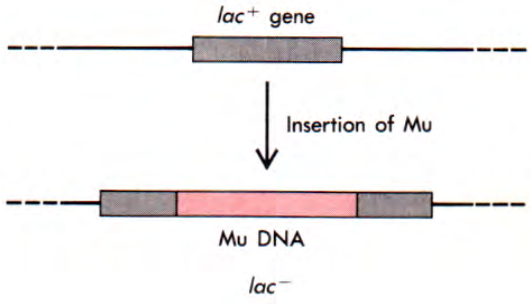


溶原化ファージ◎λファージ◎プロファージ

30. Insertion of the chromosome of phage λ into *E.coli* chromosome

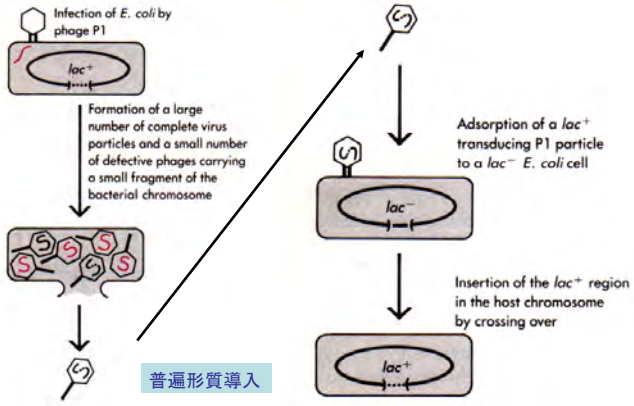


Insertion of Mu DNA



MuはλファージとIと異なり、挿入が起こる場所はランダムである

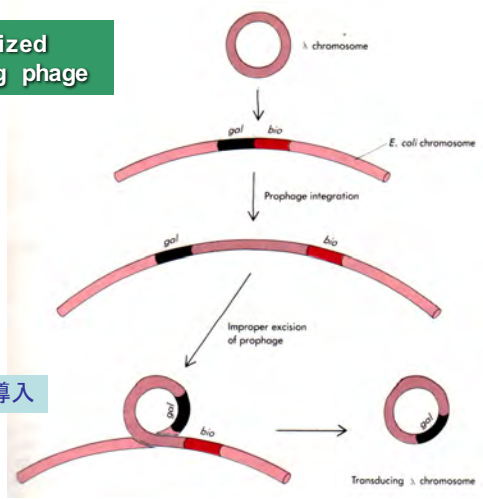
Transduction, the passive transfer of genetic material from one bacterium to another by means of carrier phage particles



普遍形質導入

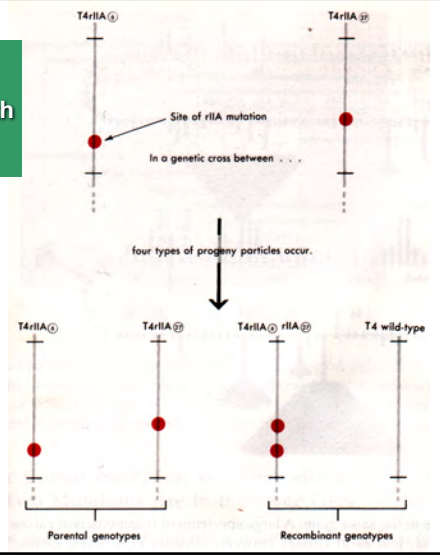
P1 トランスダクション

Specialized transducing phage

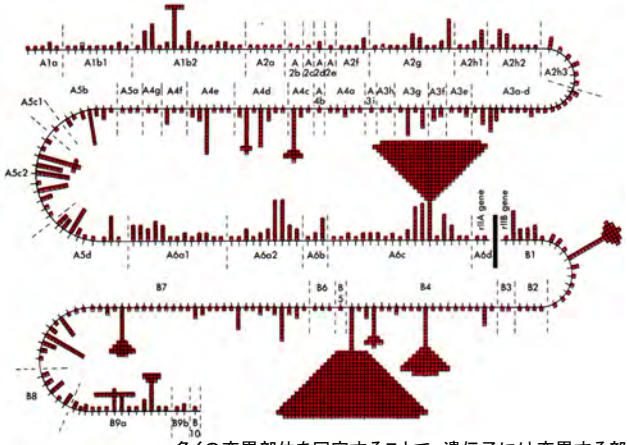


特殊形質導入

The demonstration of crossing over with in the gene



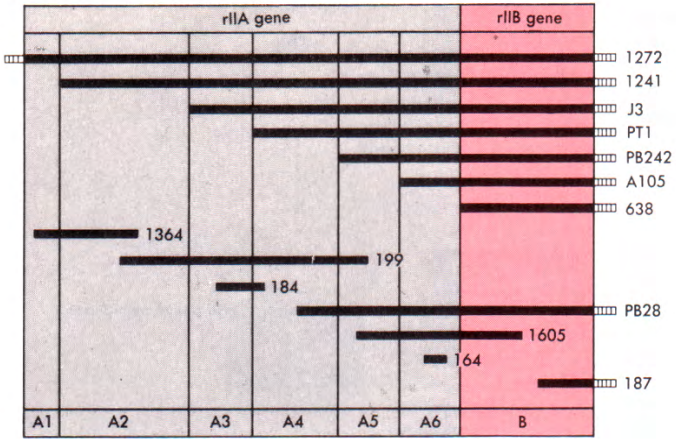
The genetic map of the *rIIA* and *rIIB* genes of phage T4



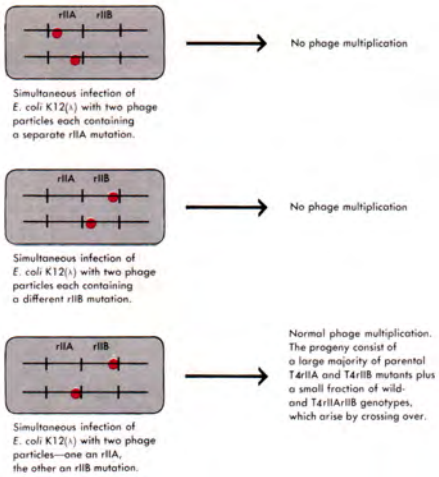
S. Benzer, PNAS, (1961)

多くの変異部位を同定することで、遺伝子には変異する部位がたくさんありそれが線状に配列していることが分かった。

Deletion mutations with in the *rII* region of T4



The *rII* region consists of two distinct genes that can complement each other during simultaneous infection



相補性検定
シス-トランス検定
シストロン

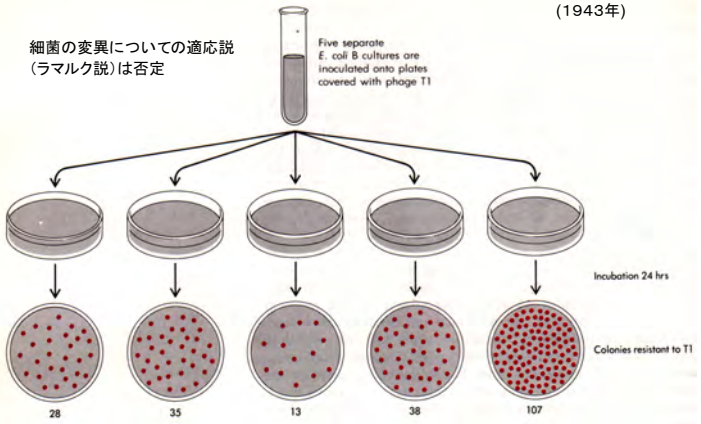
The Genetic Systems Provided by *E. coli*

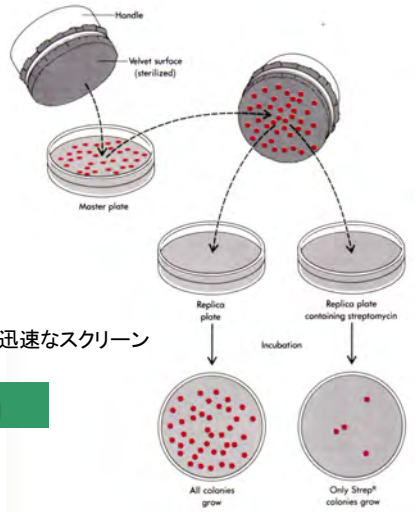
Molecular Biology of the Gene

1. Fluctuation analysis of bacterial resistance to phage

(1943年)

細菌の変異についての適応説
（ラマルク説）は否定





遺伝マーカーの迅速なスクリーン

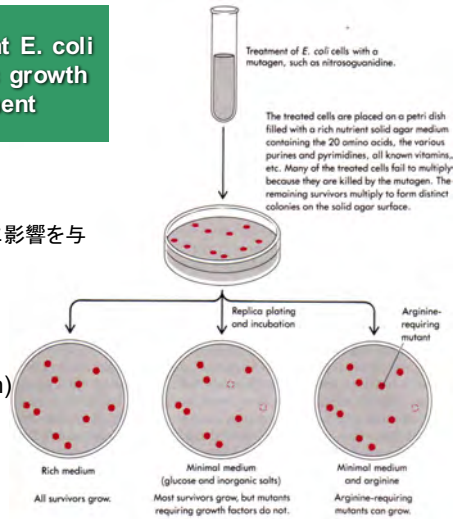
2. Replica plating

3. Isolation of mutant E. coli cells with a specific growth factor requirement

必須代謝物合成能に影響を与える変異体の分離

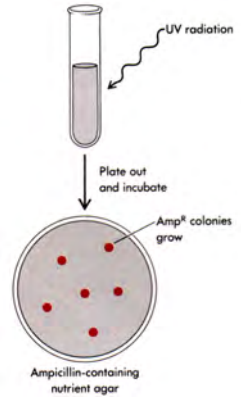
栄養要求株 (auxotroph)
原栄養株 (prototroph)

(1944年)

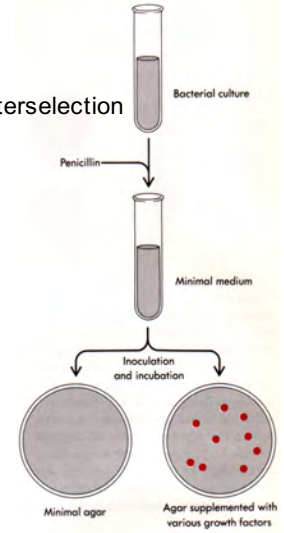


4. Enriching mutants I

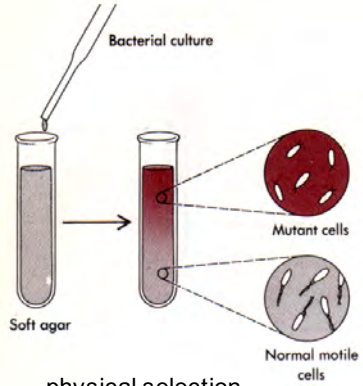
direct selection



counterselection

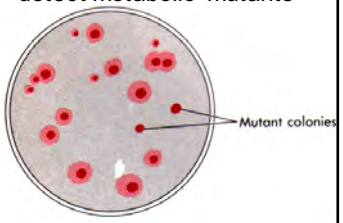


5. Enriching mutants II



physical selection

pH-sensitive dyes to detect metabolic mutants



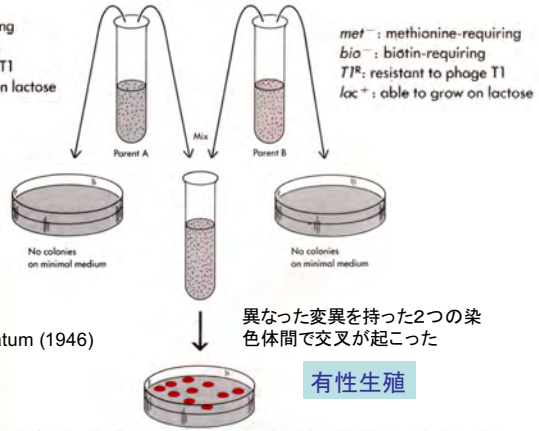
Brute force isolation

変異剤を使った後、1万個のコロニーを各々スクリーニングする。

8. The use of growth factor requirments to demonstrate sexuality in *E. coli*

thr⁻ : threonine-requiring
leu⁻ : leucine-requiring
*T1*ˢ: sensitive to phage T1
lac⁻ : unable to grow on lactose

met⁻ : methionine-requiring
bio⁻ : biotin-requiring
*T1*ᴿ: resistant to phage T1
lac⁺ : able to grow on lactose

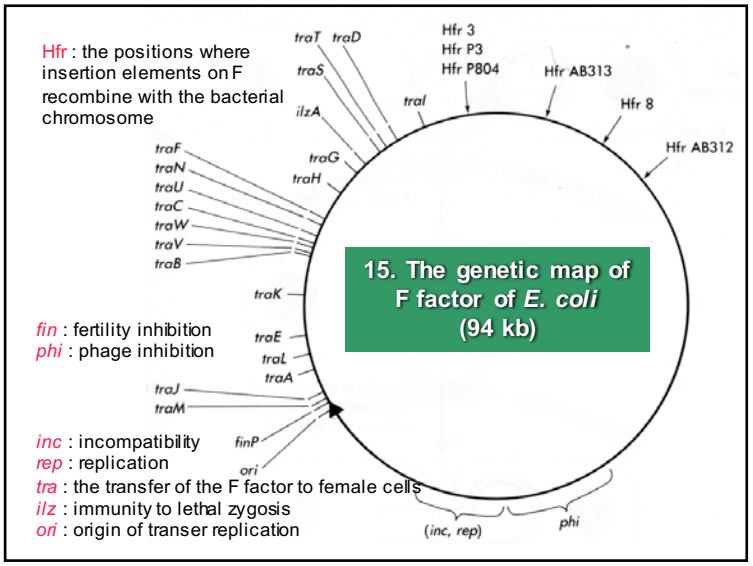


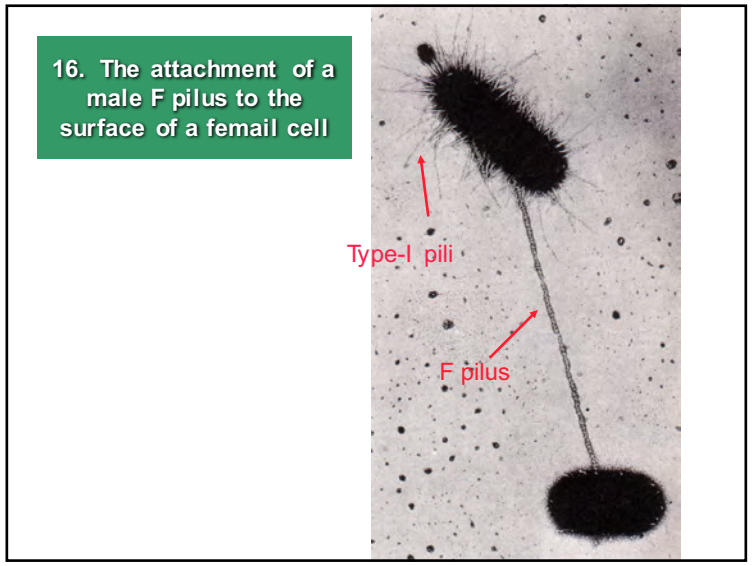
J. Lederberg & Tatum (1946)

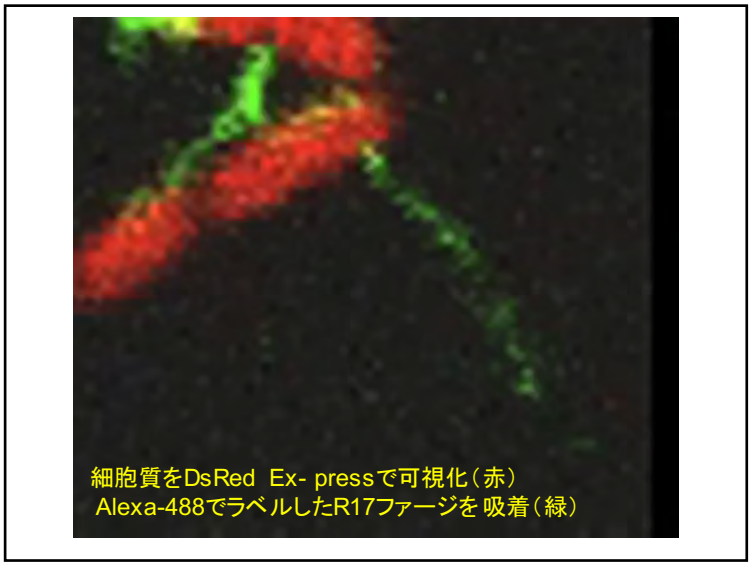
異なった変異を持った2つの染色体間で交叉が起こった

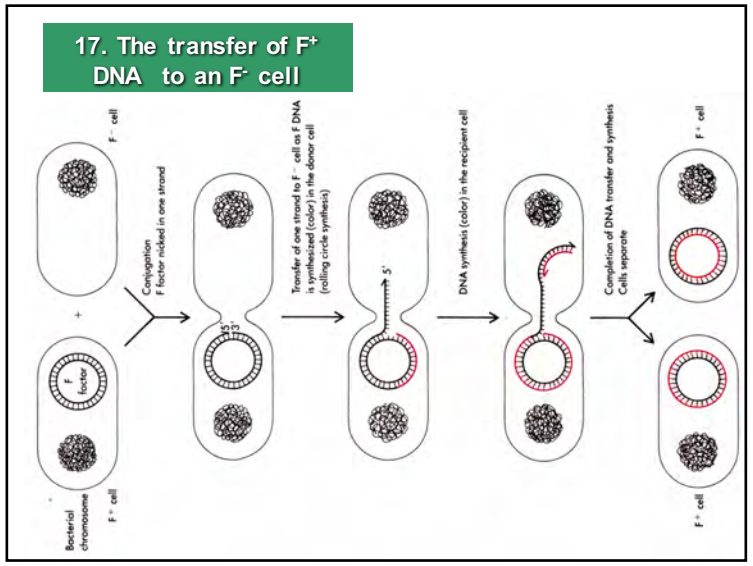
有性生殖

A very small fraction of the cells are *met*⁺, *bio*⁺, *thr*⁺, and *leu*⁺. They arise by genetic recombination, as shown by examination of the *lac* and *T1* markers. In addition to the parent *lac*⁻ *T1*ˢ and *lac*⁺ *T1*ᴿ genotypes, there are found *lac*⁻ *T1*ᴿ and *lac*⁺ *T1*ˢ cells.

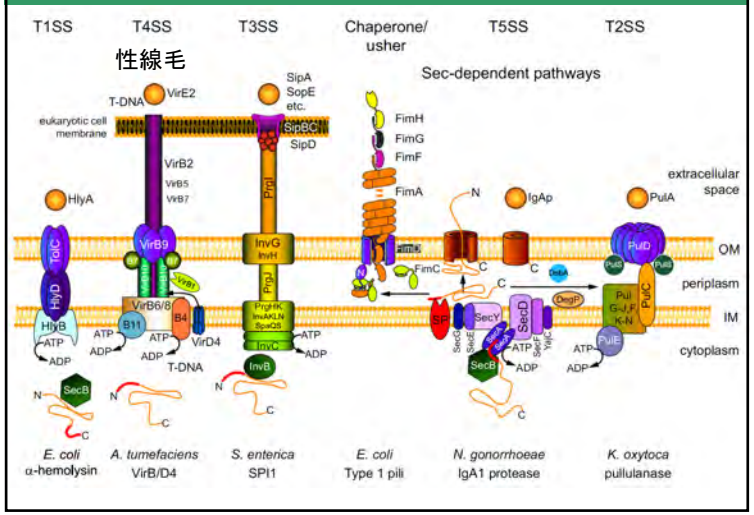

Hfr : the positions where insertion elements on F recombine with the bacterial chromosome
traT
traD
traS
ilzA
traF
traN
traU
traC
traW
traV
traB
traK
traE
traL
traA
traJ
traM
finP
ori
traG
traH
traI
Hfr 3
Hfr P3
Hfr P804
Hfr AB313
Hfr 8
Hfr AB312
fin : fertility inhibition
phi : phage inhibition
inc : incompatibility
rep : replication
tra : the transfer of the F factor to female cells
ilz : immunity to lethal zygosis
ori : origin of transer replication
(inc, rep)
phi
15. The genetic map of F factor of E. coli (94 kb)


16. The attachment of a male F pilus to the surface of a femail cell
Type-I pili
F pilus

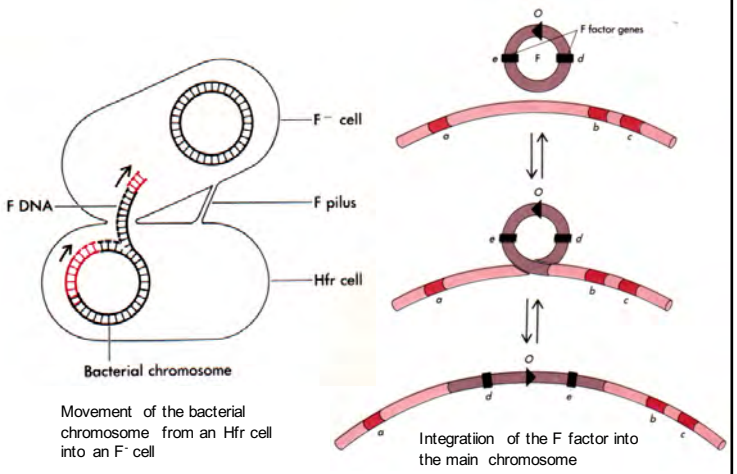

細胞質をDsRed Ex- pressで可視化（赤）
Alexa-488でラベルしたR17ファージを 吸着（緑）


17. The transfer of F+ DNA to an F- cell
F⁻ cell
Conjugation
F factor nicked in one strand
Transfer of one strand to F⁻ cell as F DNA is synthesized (color) in the donor cell (rolling circle synthesis)
DNA synthesis (color) in the recipient cell
Completion of DNA transfer and synthesis
Cells separate
F+ cell
Bacterial chromosome
F factor

グラム陰性細菌の分泌機構



18. Hfr (high frequency of recombination)



Movement of the bacterial chromosome from an Hfr cell into an F⁻ cell

Integratiion of the F factor into the main chromosome