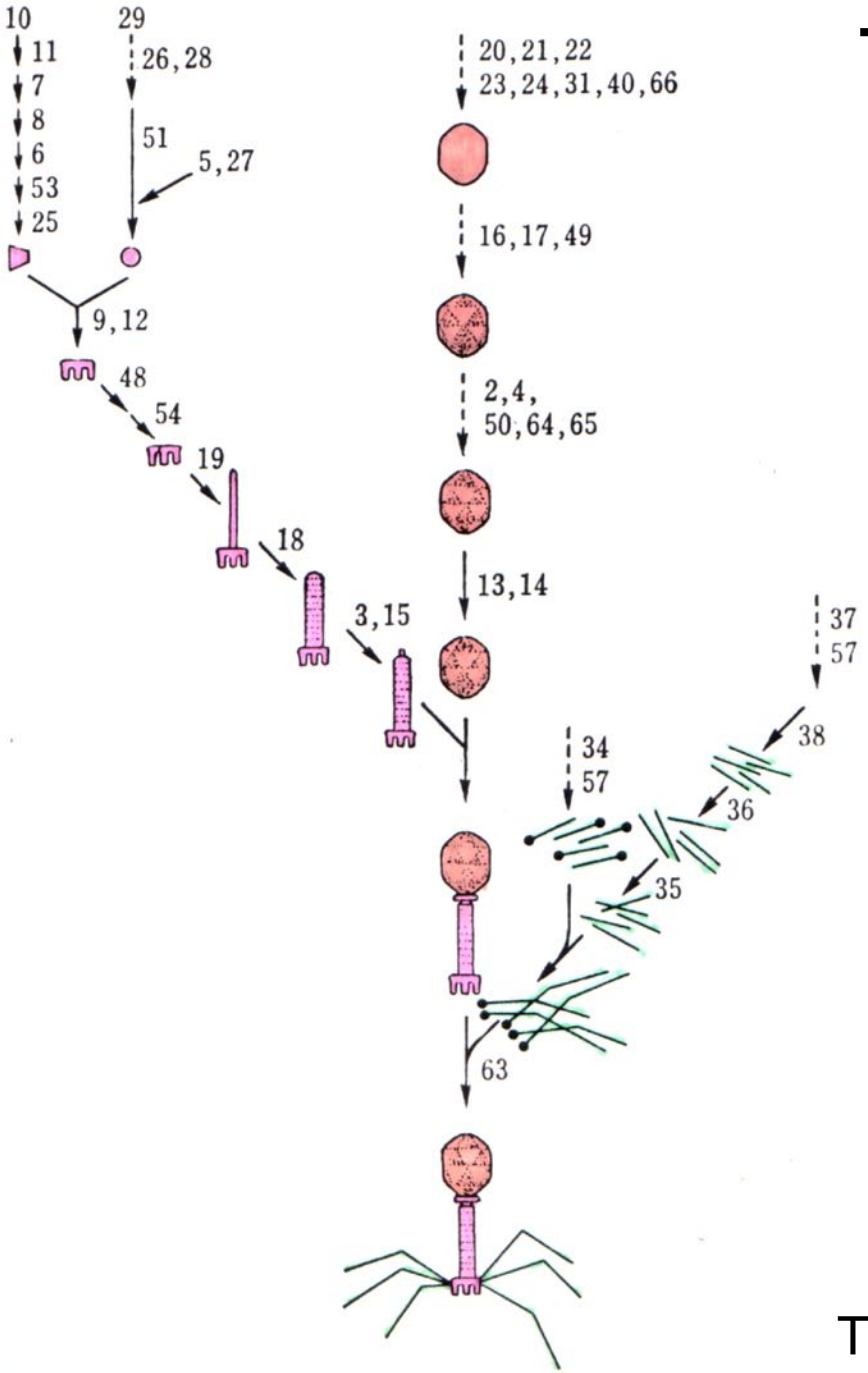
終止コドンとサプレッサー変異

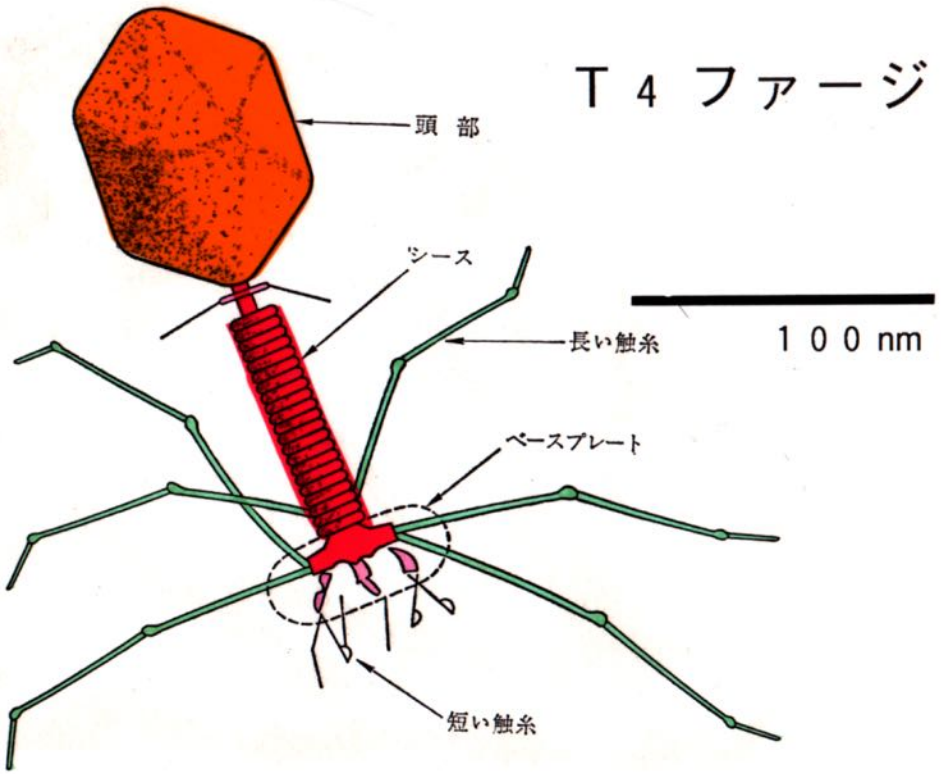
1番目の塩基	2番目の塩基 U（ウラシル）	2番目の塩基 C（シトシン）	2番目の塩基 A（アデニン）	2番目の塩基 G（グアニン）	3番目の塩基
U	UUU フェニルアラニン（Phe） UUC	UCU UCC UCA UCG セリン（Ser）	UAU チロシン（Tyr） UAC	UGU システイン（Cys） UGC	U C
	UUA UUG ロイシン（Leu）		UAA 終止コドン UAG 終止コドン	UGA 終止コドン UGG トリプトファン（Trp）	A G
C	CUU CUC CUA CUG ロイシン（Leu）	CCU CCC CCA CCG プロリン（Pro）	CAU ヒスチジン（His） CAC	CGU CGC CGA CGG アルギニン（Arg）	U C A G
			CAA グルタミン（Gln） CAG		
A	AUU AUC イソロイシン（Ile）	ACU ACC ACA ACG トレオニン（Thr）	AAU アスパラギン（Asn） AAC	AGU セリン（Ser） AGC	U C
	AUA AUG メチオニン（Met） 開始コドン		AAA リシン（Lys） AAG	AGA AGG アルギニン（Arg）	A G
G	GUU GUC GUA GUG バリン（Val）	GCU GCC GCA GCG アラニン（Ala）	GAU アスパラギン酸（Asp） GAC	GGU GGC GGA GGG グリシン（Gly）	U C A G
			GAA グルタミン酸（Glu） GAG		

ミスセンス変異とナンセンス変異

UAAーオーカー（黄土色）変異
UAGーアンバー(琥珀色）変異　Bernstein(ドイツ名：琥珀）
UGAーオパール変異

T4ファージの形成過程





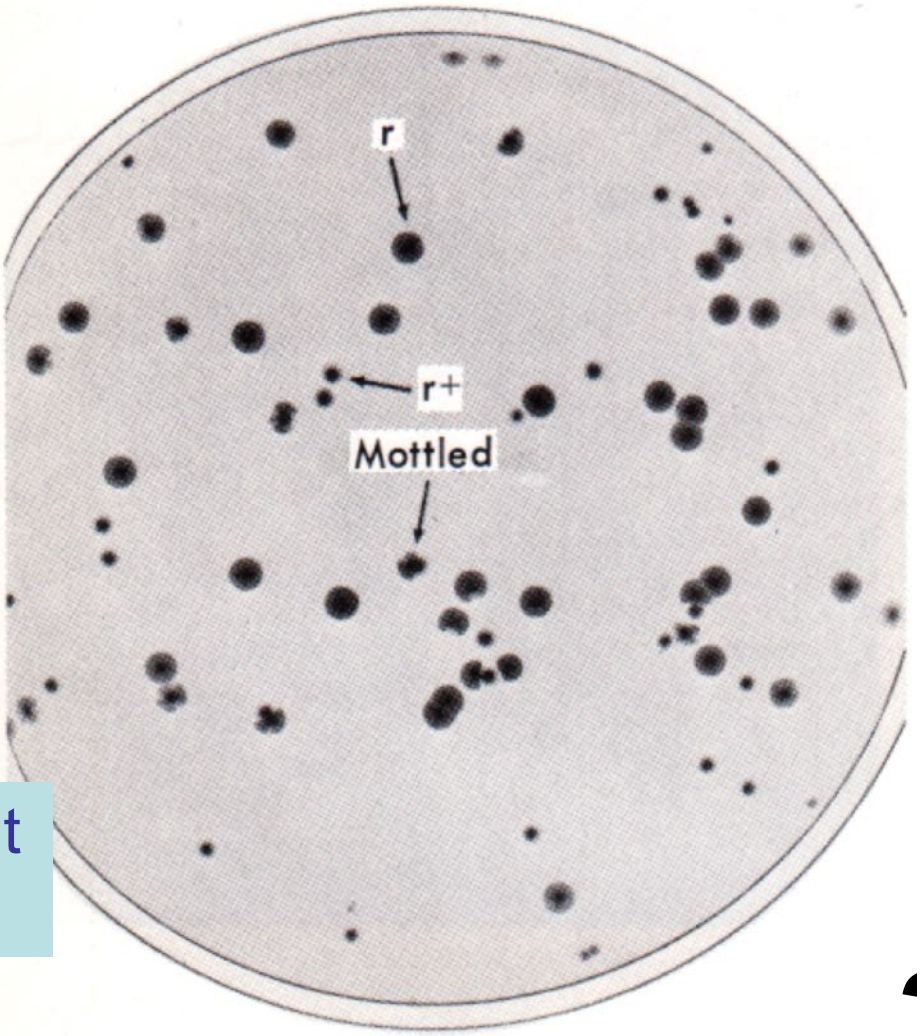
T4ファージのゲノムサイズ＝169Kbp

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

T2 phage

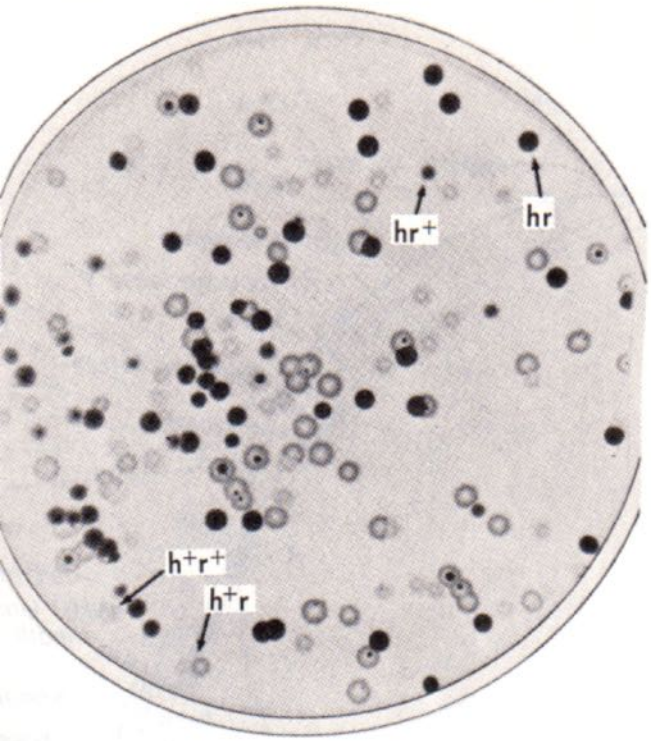
原因は分からないが、プラークの形をかえる変異を得ることが容易にできた

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



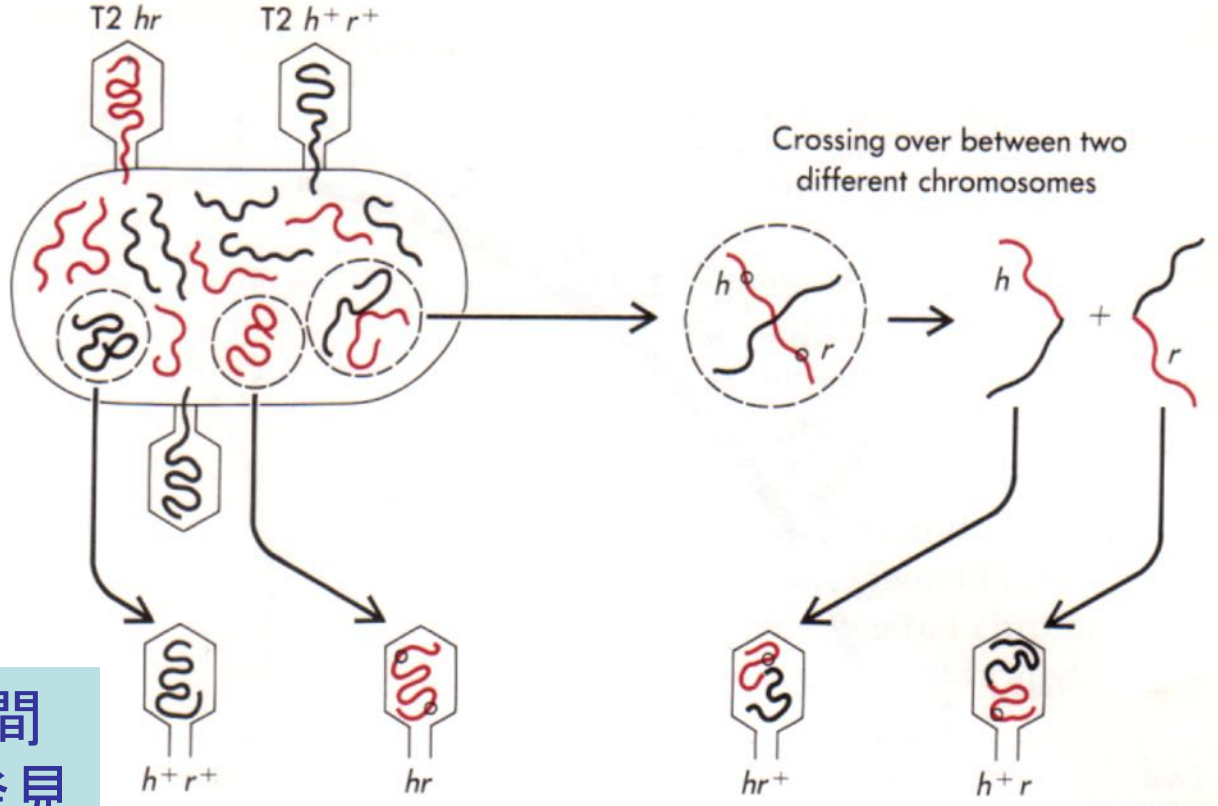
T2 mutant phage

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



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

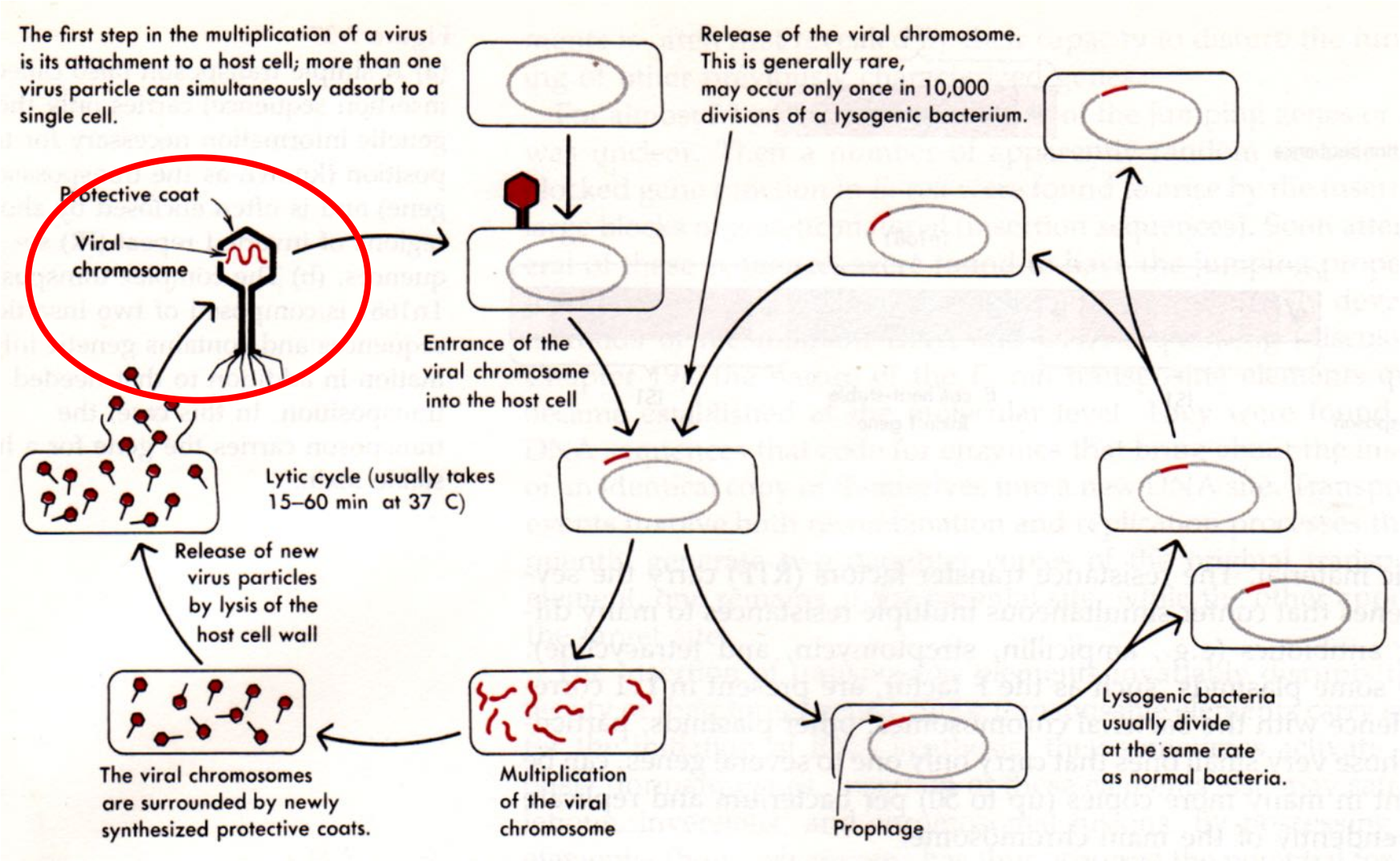
T2 r : rapid-lysis 変異株



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

1945年：変異株ファージ間での遺伝的組み換えの発見

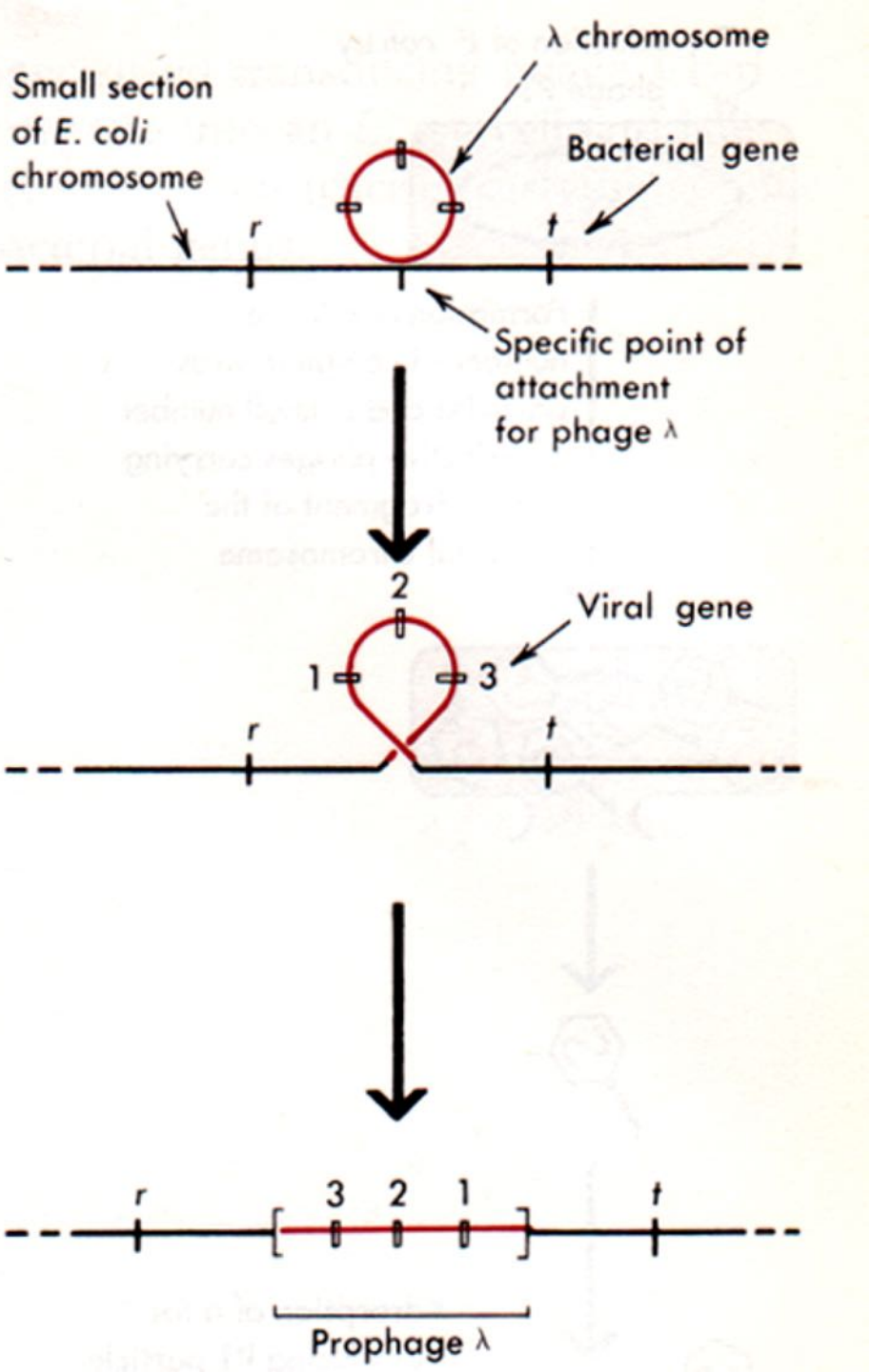
The life cycle of a lysogenic bacterial virus



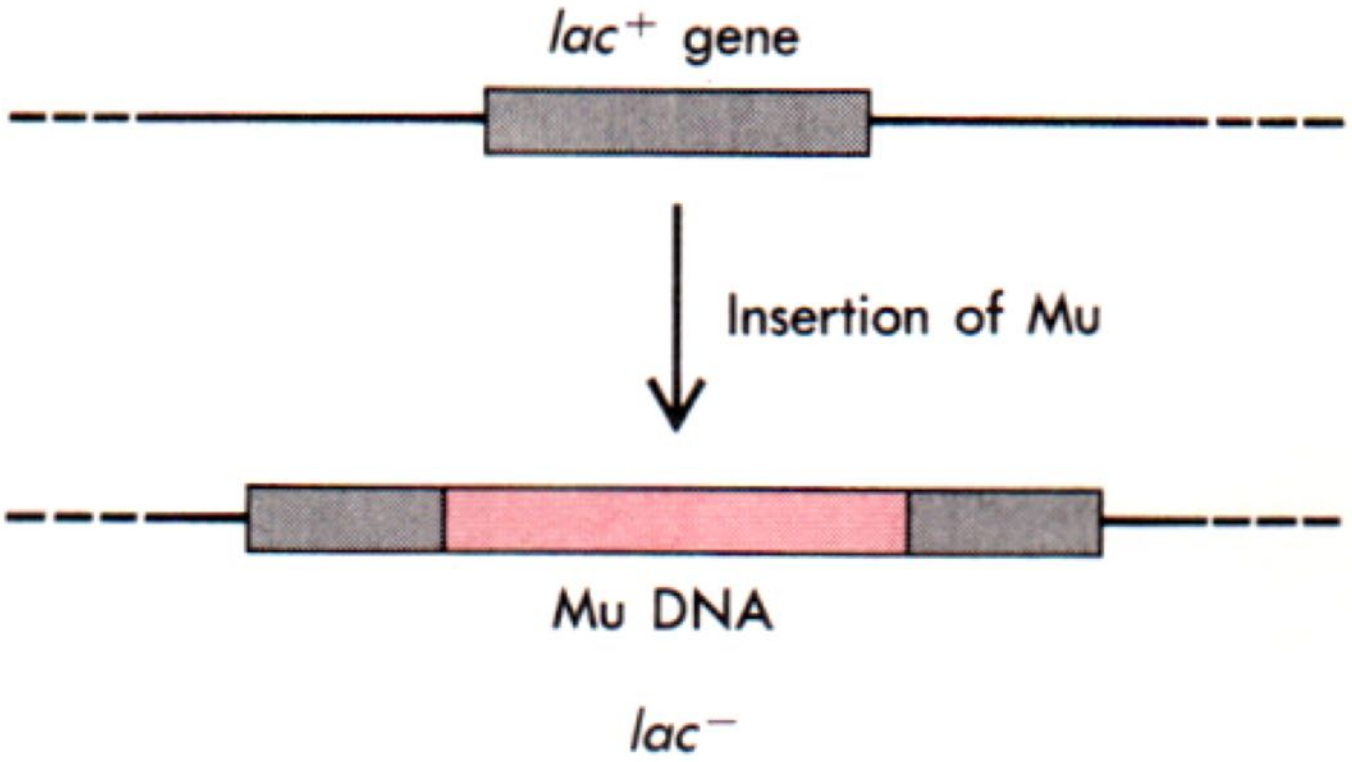
溶原化ファージ⇨λファージ⇨プロファージ

λファージのゲノムサイズ＝48Kbp

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



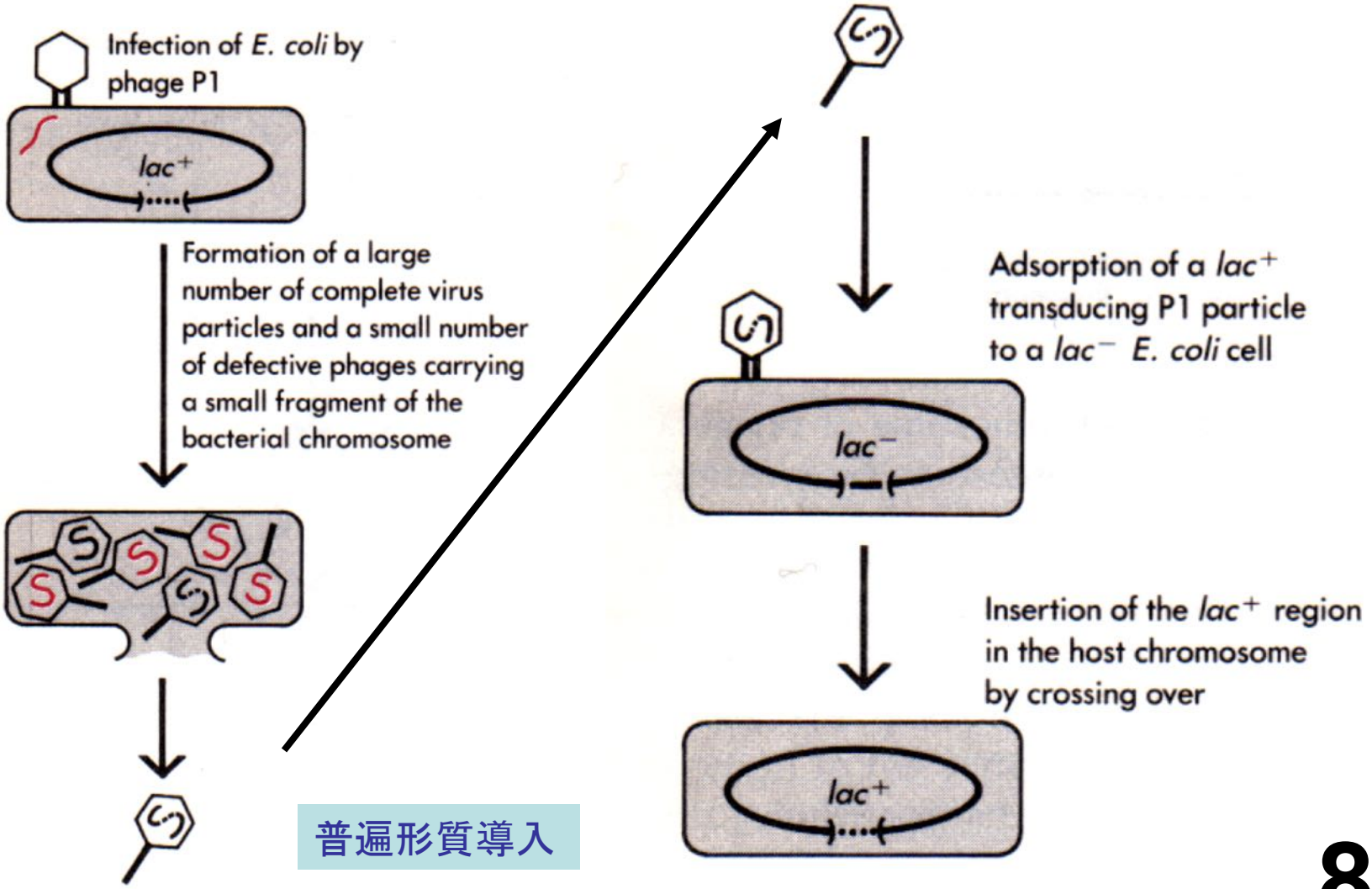
Insertion of Mu DNA



Muはλファージと異なり、挿入が起こる場所はランダムである

Muの名前はミュテーターから由来

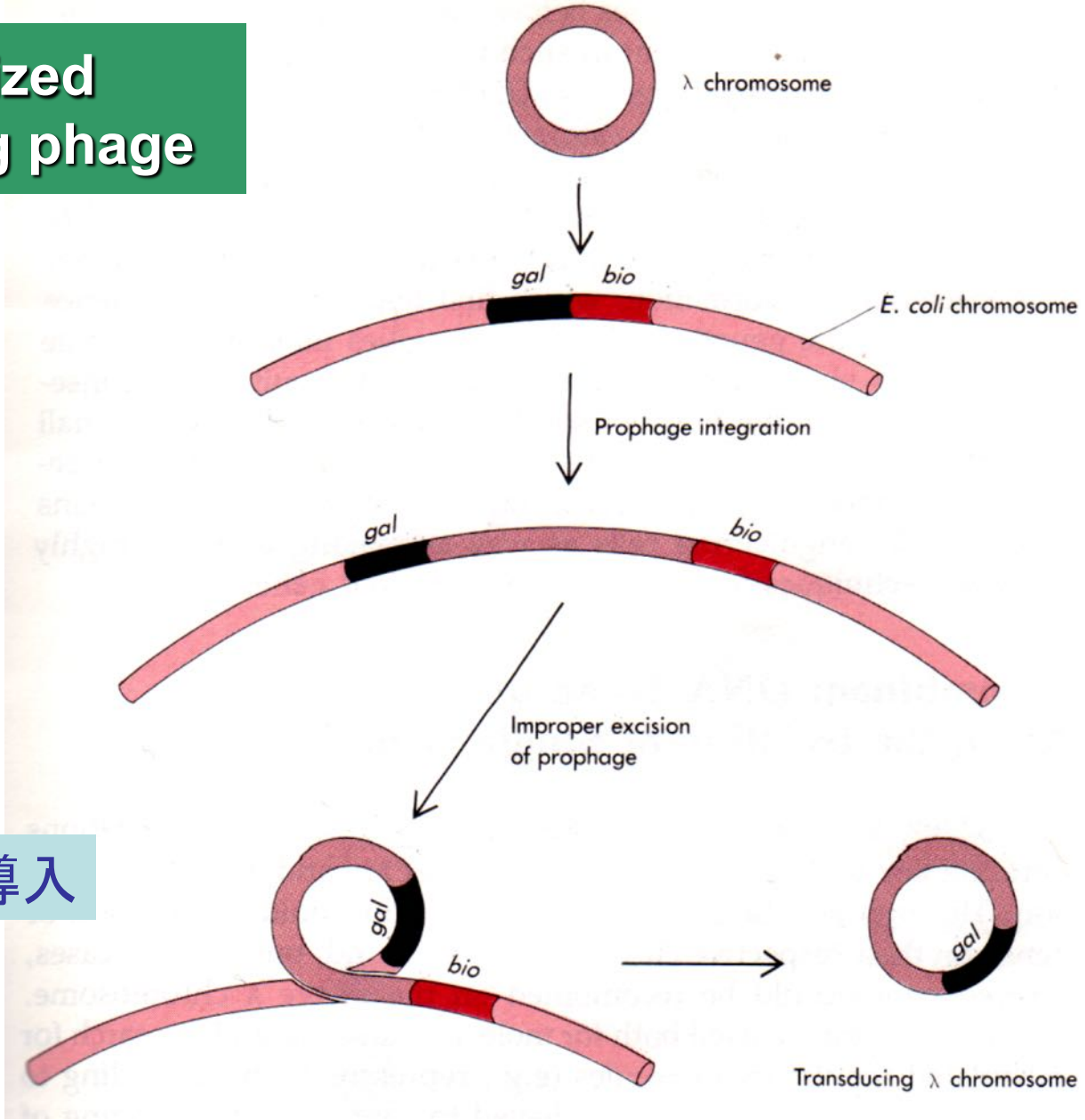
Transduction, the passive transfer of genetic material from one bacterium to another by means of carrier phage particles



普遍形質導入

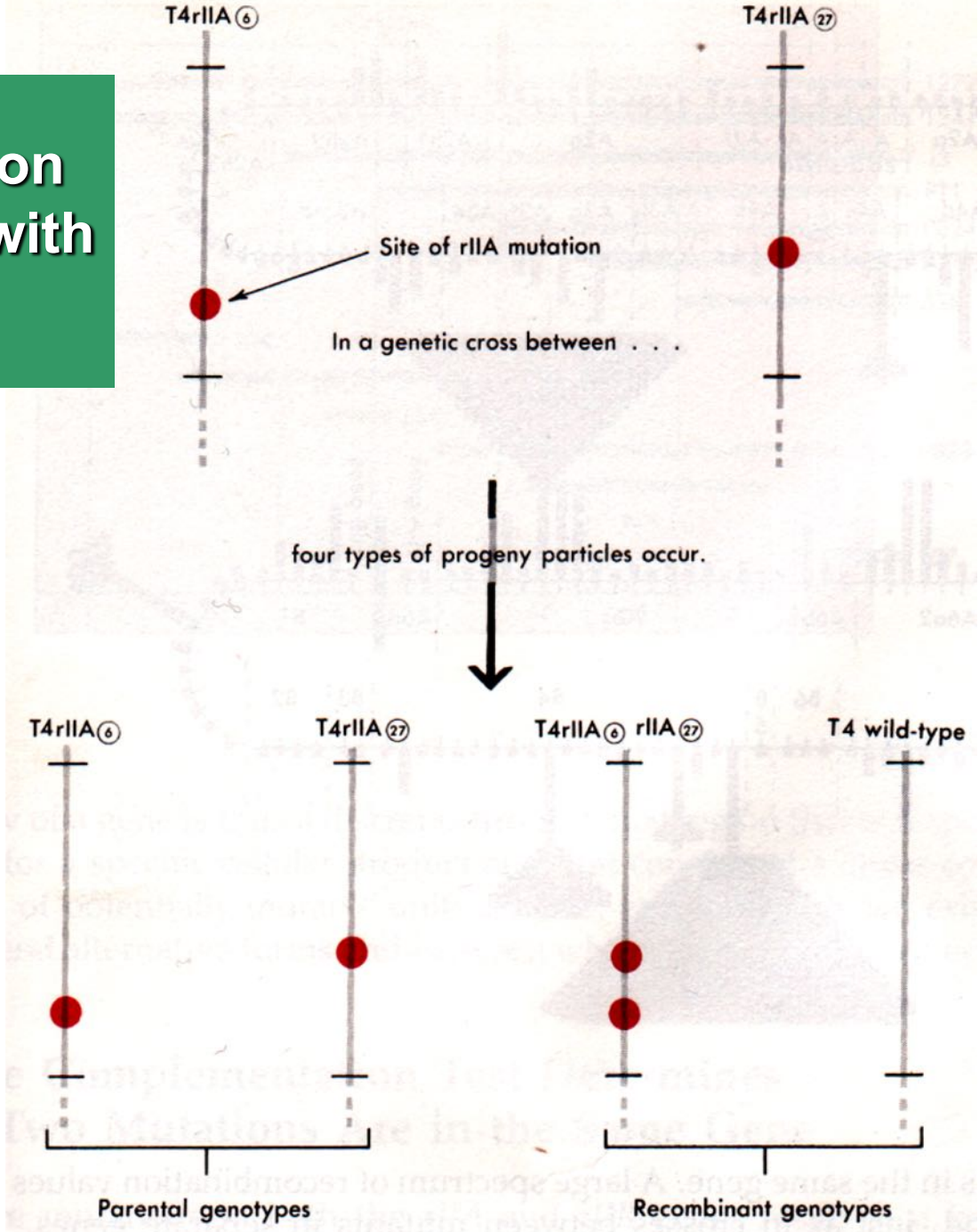
P1　トランスダクション　　P1ファージのゲノムサイズ＝93Kbp 2.2分

Specialized transducing phage

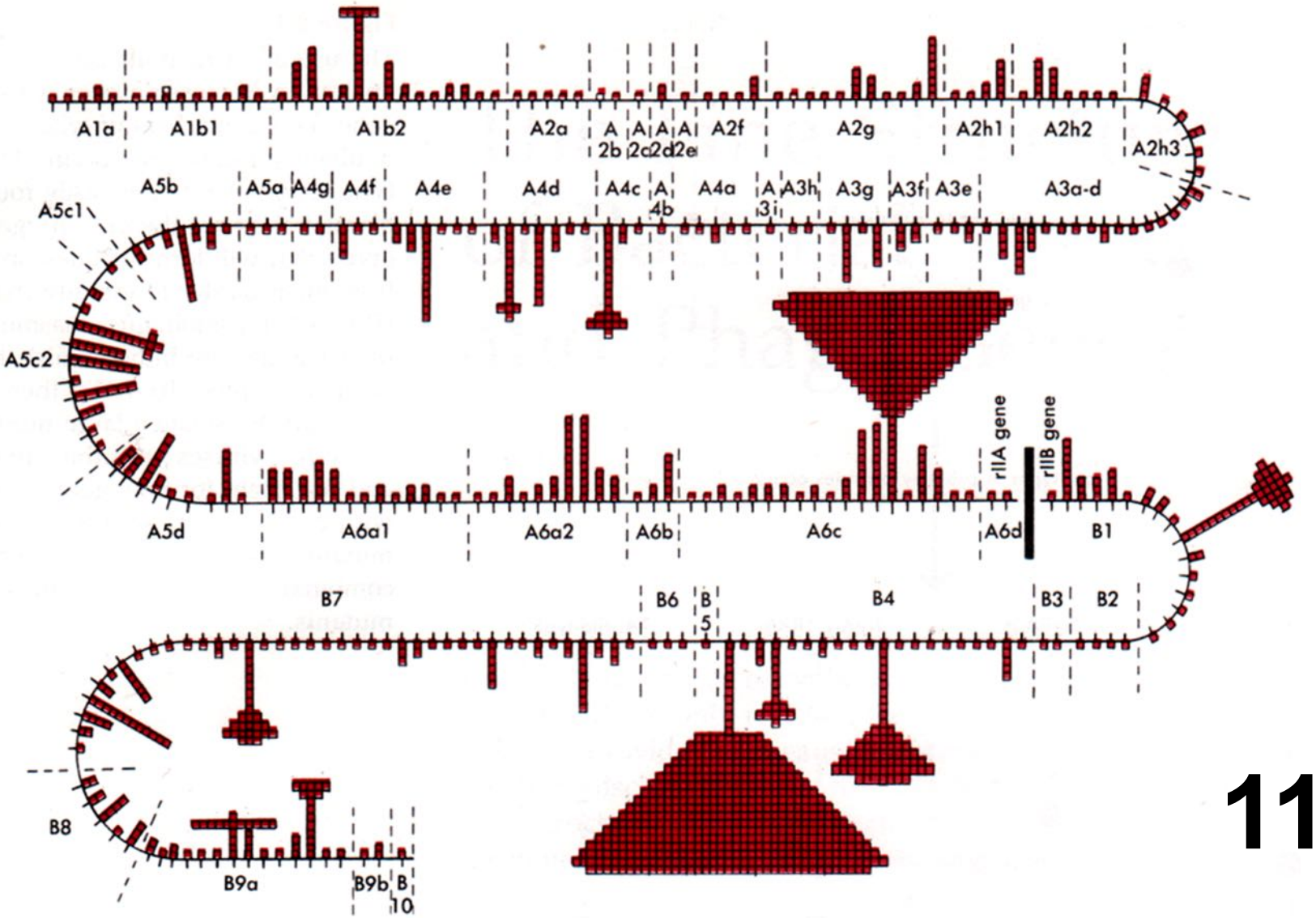


特殊形質導入

The demonstration of crossing over with in the gene



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

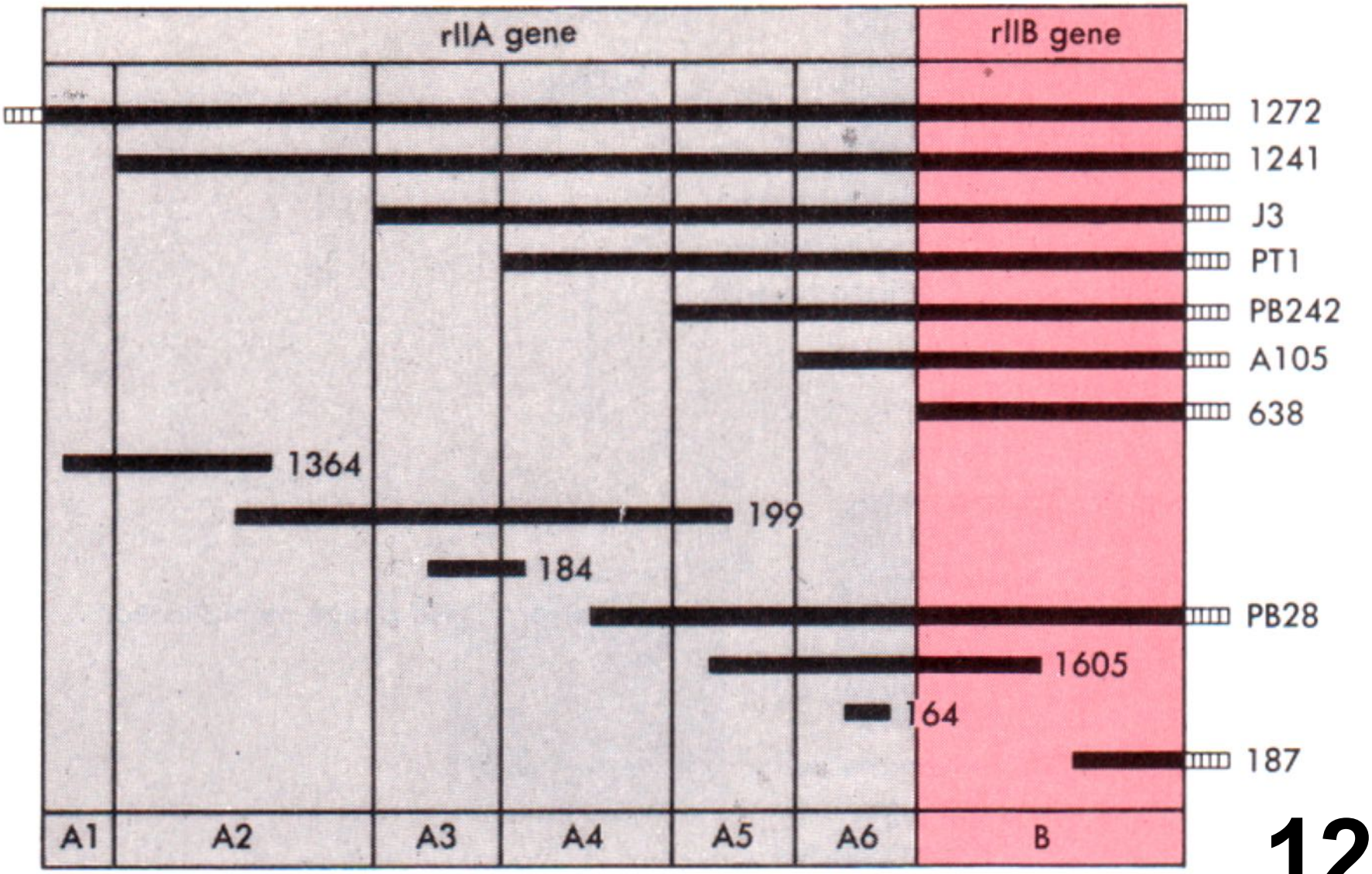


11

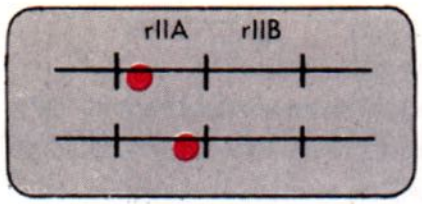
S. Benzer, PNAS, (1961)

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

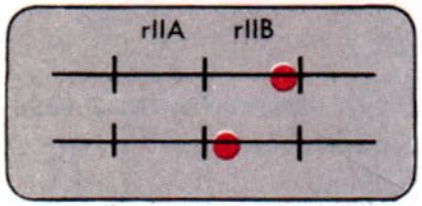


The *rII* region consists of two distinct genes that can complement each other during simultaneous infection



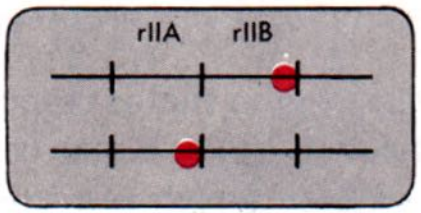
Simultaneous infection of *E. coli* K12(λ) with two phage particles each containing a separate rIIA mutation.

→ No phage multiplication



Simultaneous infection of *E. coli* K12(λ) with two phage particles each containing a different rIIB mutation.

→ No phage multiplication



Simultaneous infection of *E. coli* K12(λ) with two phage particles—one an rIIA, the other an rIIB mutation.

→ Normal phage multiplication. The progeny consist of a large majority of parental T4rIIA and T4rIIB mutants plus a small fraction of wild- and T4rIIArIIB genotypes, which arise by crossing over.

相補性検定
シス-トランス検定
シストロン

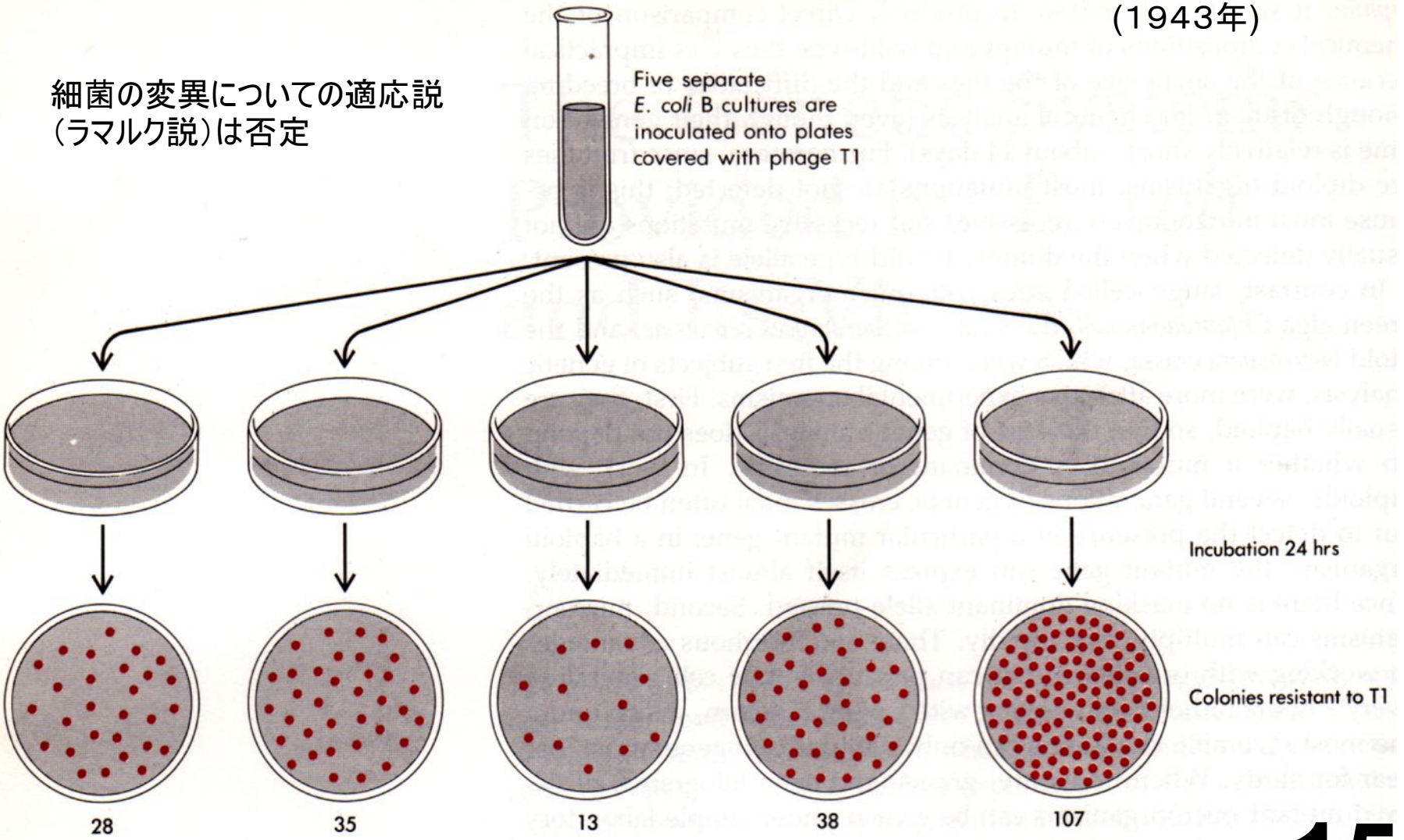
The Genetic Systems Provided by *E. coli*

Molecular Biology of the Gene

Fluctuation analysis of bacterial resistance to phage

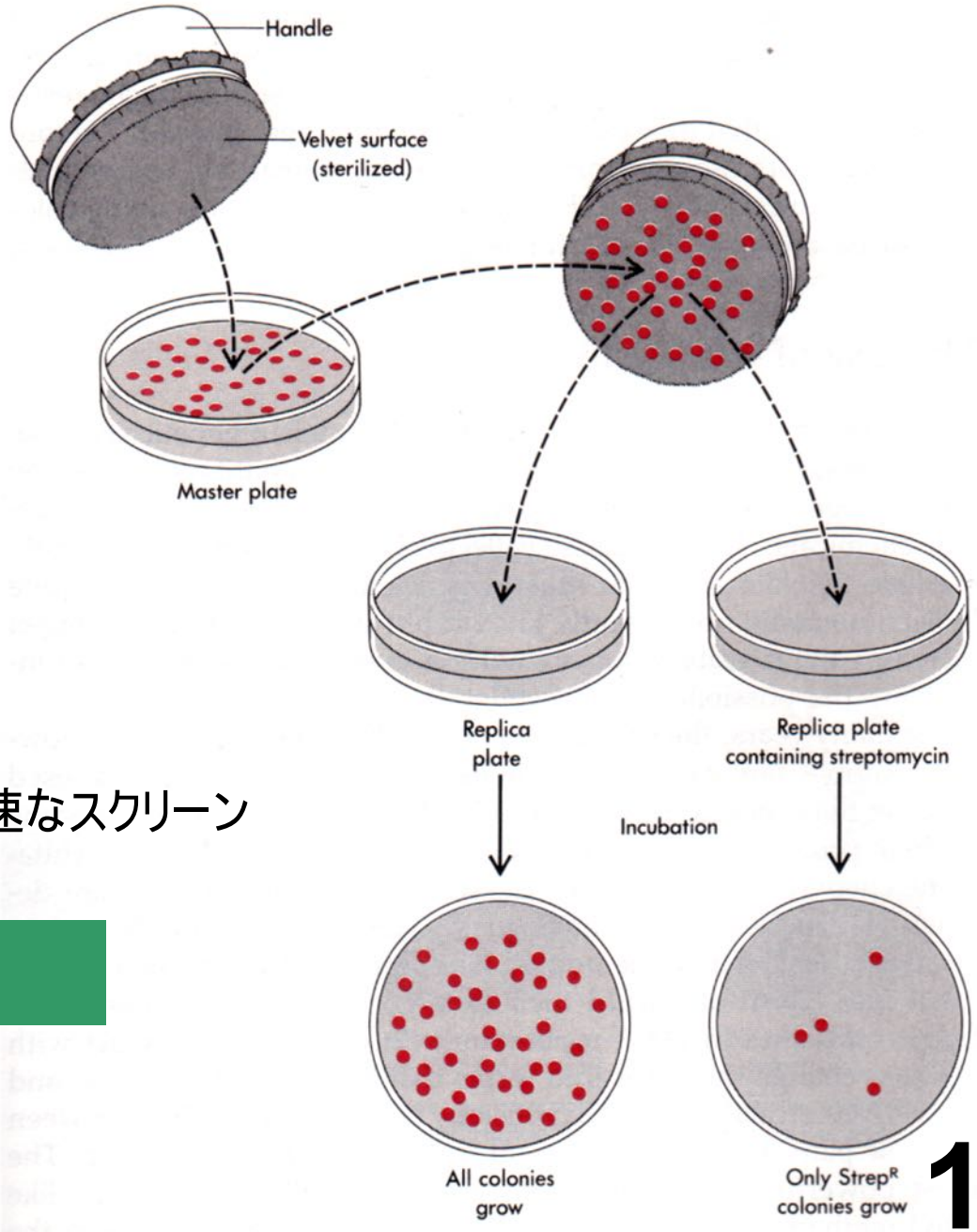
(1943年)

細菌の変異についての適応説
（ラマルク説）は否定



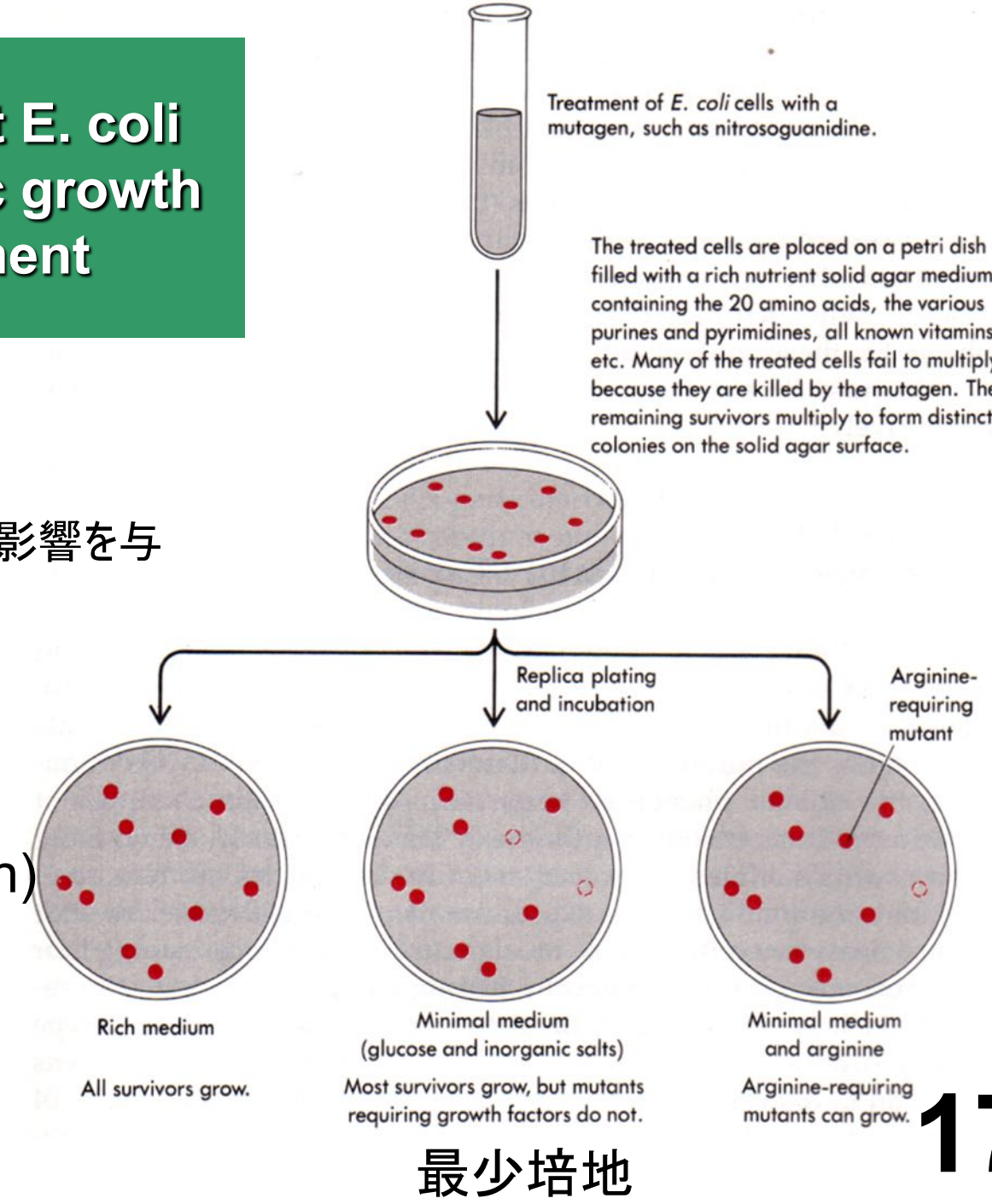
遺伝マーカーの迅速なスクリーン

Replica plating



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



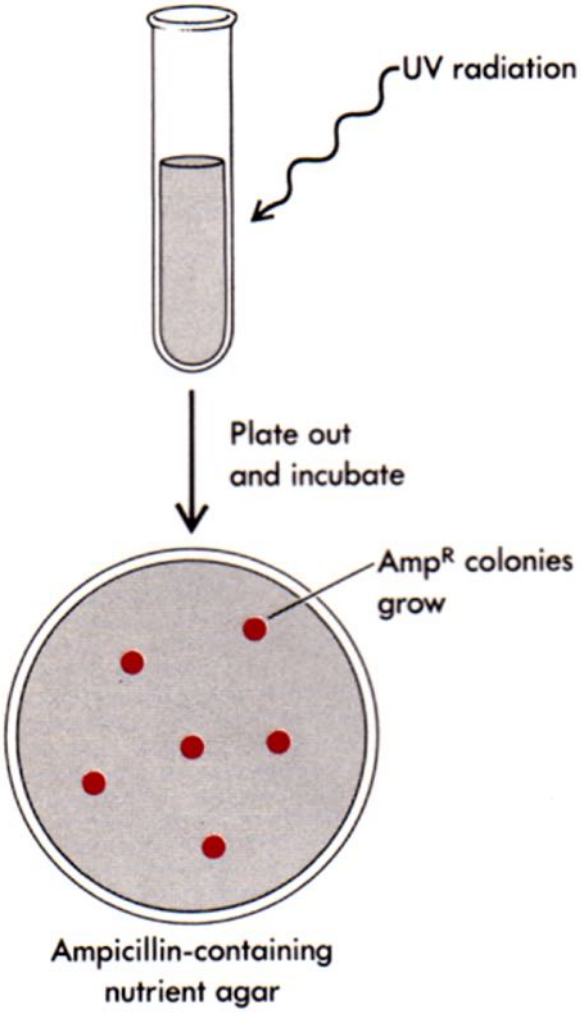
栄養要求株 (auxotroph)
原栄養株 (prototroph)

(1944年)

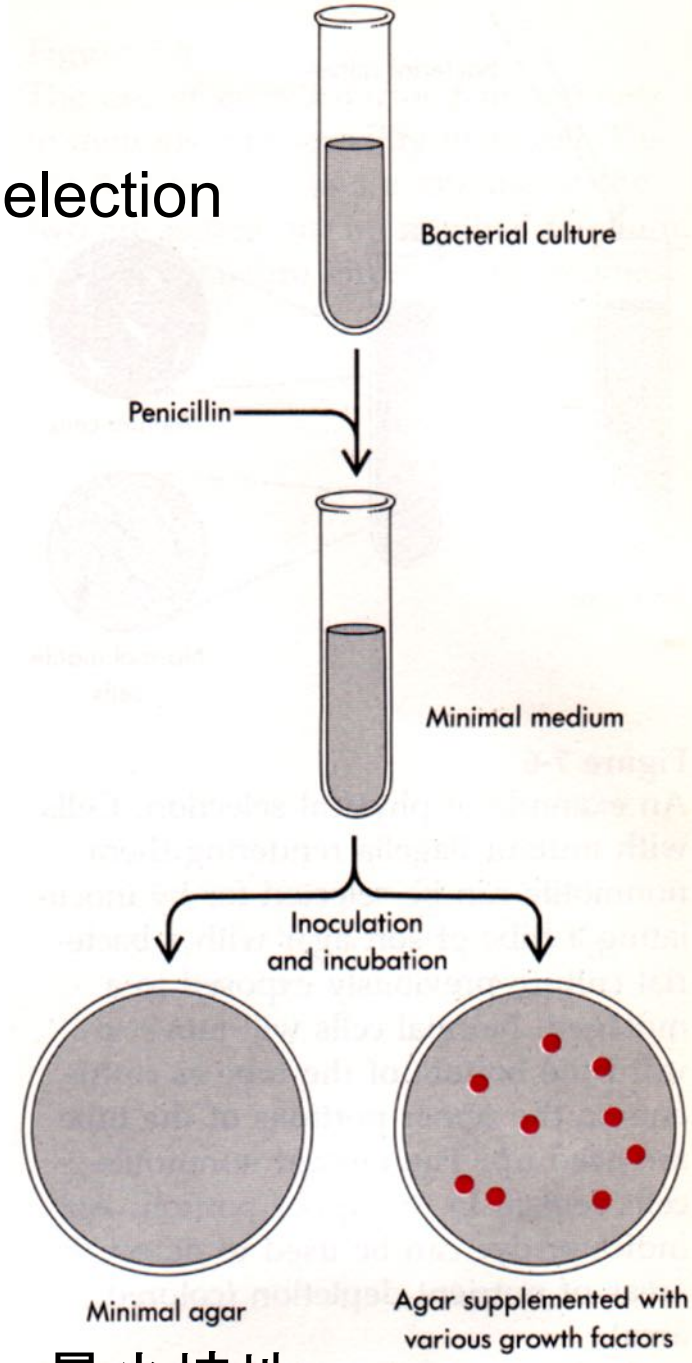
最少培地

Enriching mutants I

direct selection

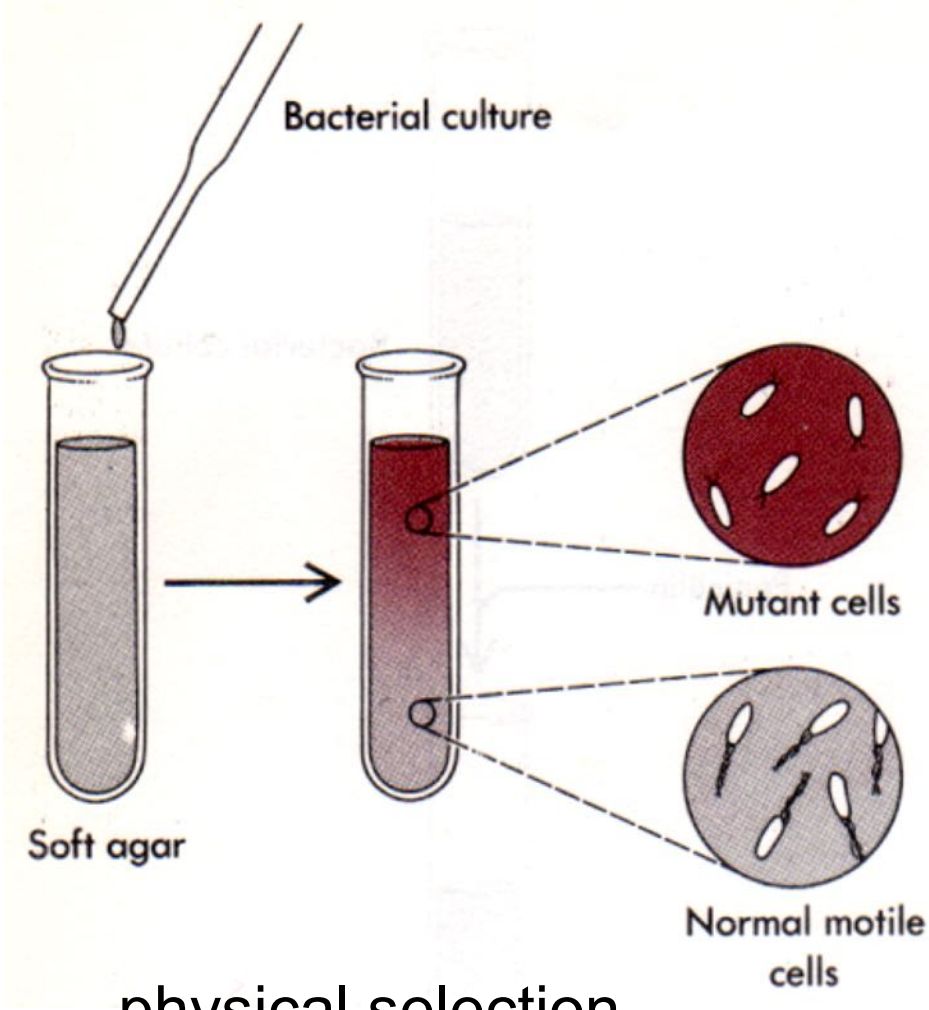


counterselection



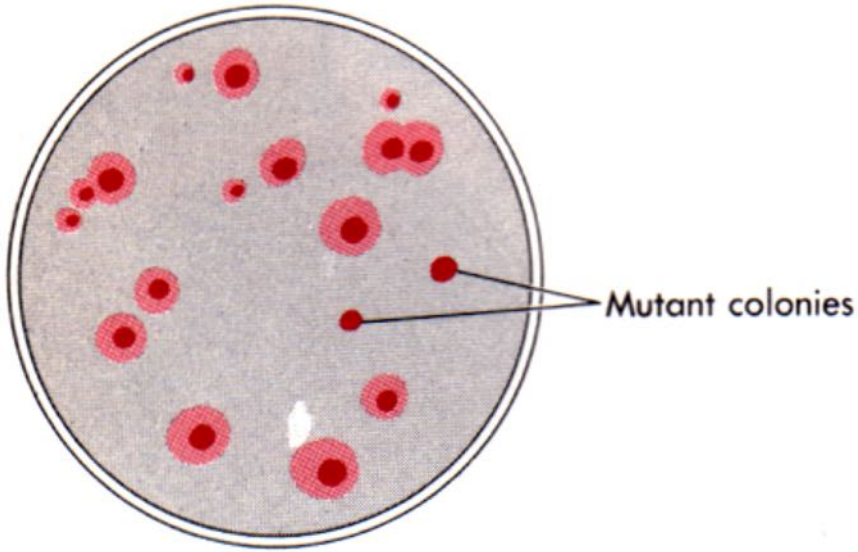
最少培地

Enriching mutants II



physical selection

pH-sensitive dyes to detect metabolic mutants



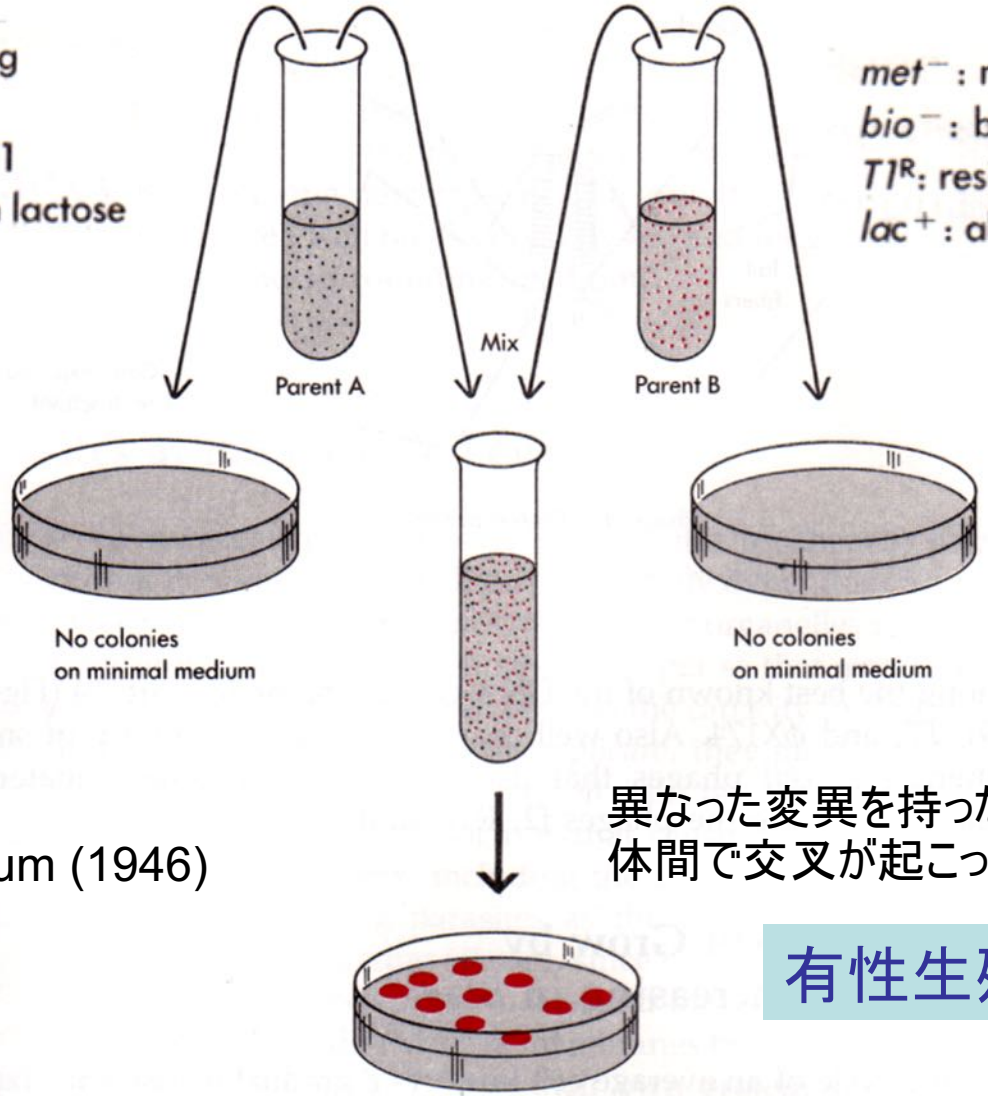
Brute force isolation

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。

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



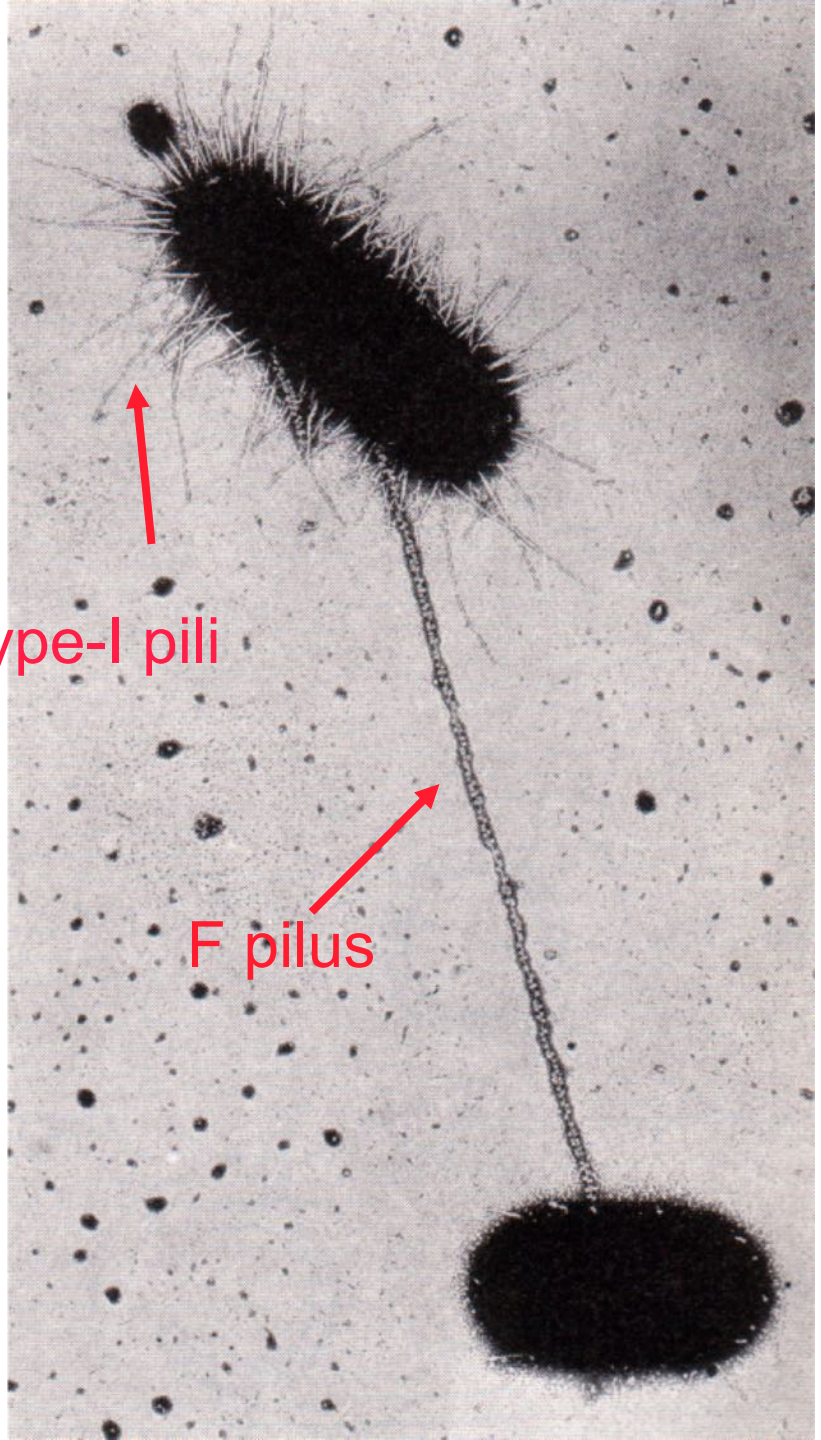
J. Lederberg & Tatum (1946)

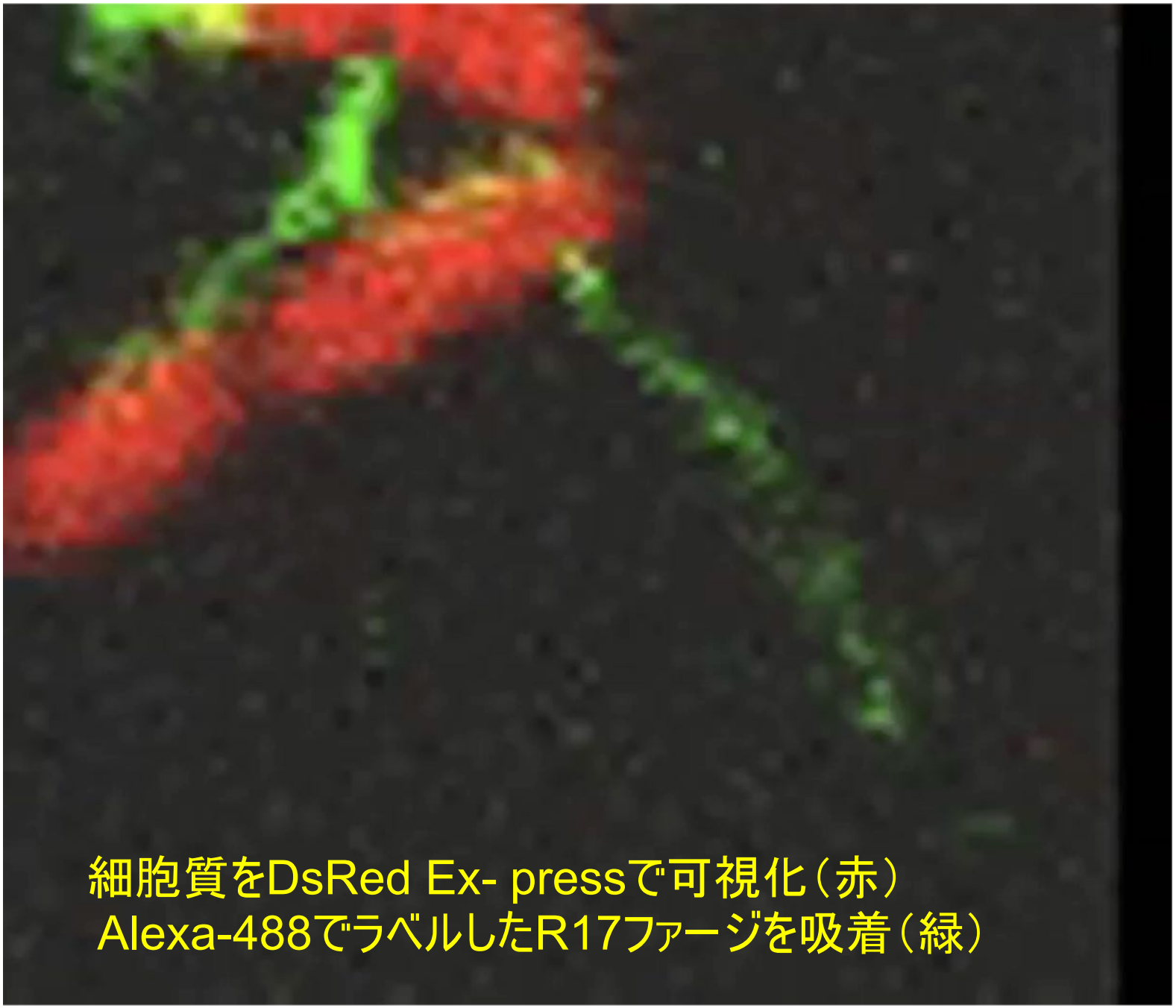
異なった変異を持った2つの染色体間で交叉が起こった

有性生殖

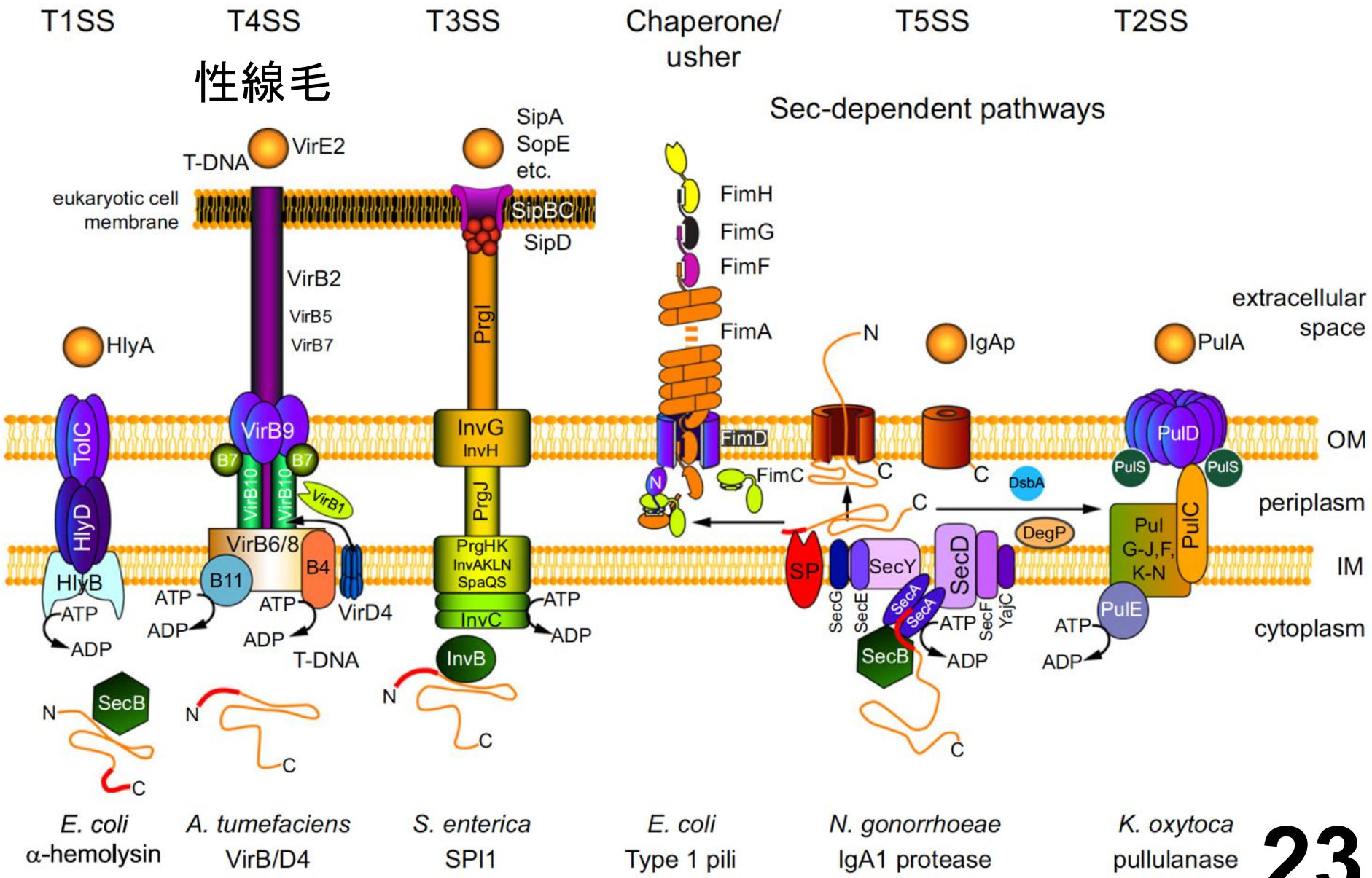
A very small fraction of the cells are *met*$^+$, *bio*$^+$, *thr*$^+$, and *leu*$^+$. They arise by genetic recombination, as shown by examination of the *lac* and *T1* markers. In addition to the parent *lac*$^-$ *T1*^{S} and *lac*$^+$ *T1*^{R} genotypes, there are found *lac*$^-$ *T1*^{R} and *lac*$^+$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

The attachment of a male F pilus to the surface of a femail cell

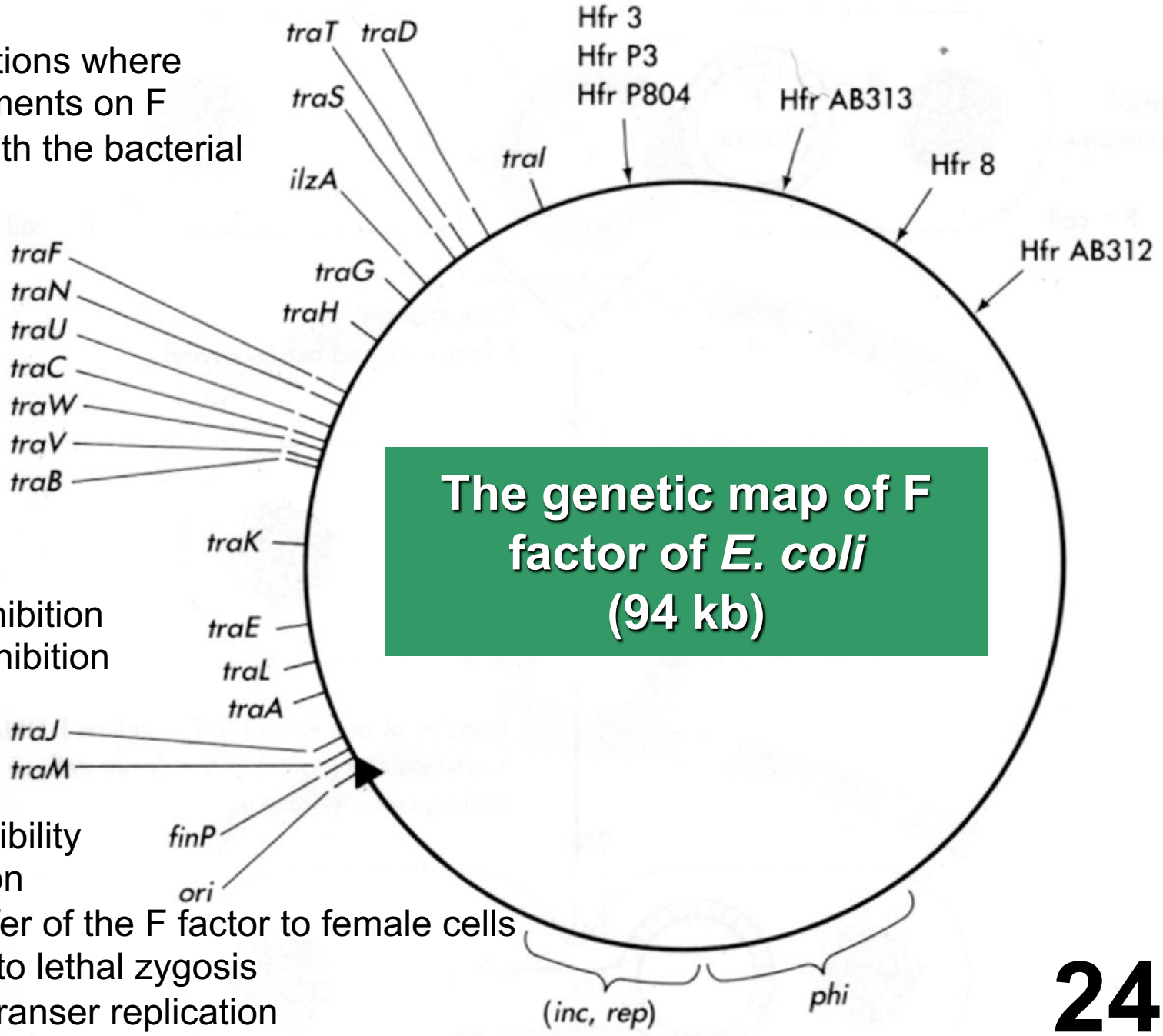



細胞質をDsRed Ex- pressで可視化（赤）
Alexa-488でラベルしたR17ファージを吸着（緑）

グラム陰性細菌の分泌機構



Hfr : the positions where insertion elements on F recombine with the bacterial chromosome



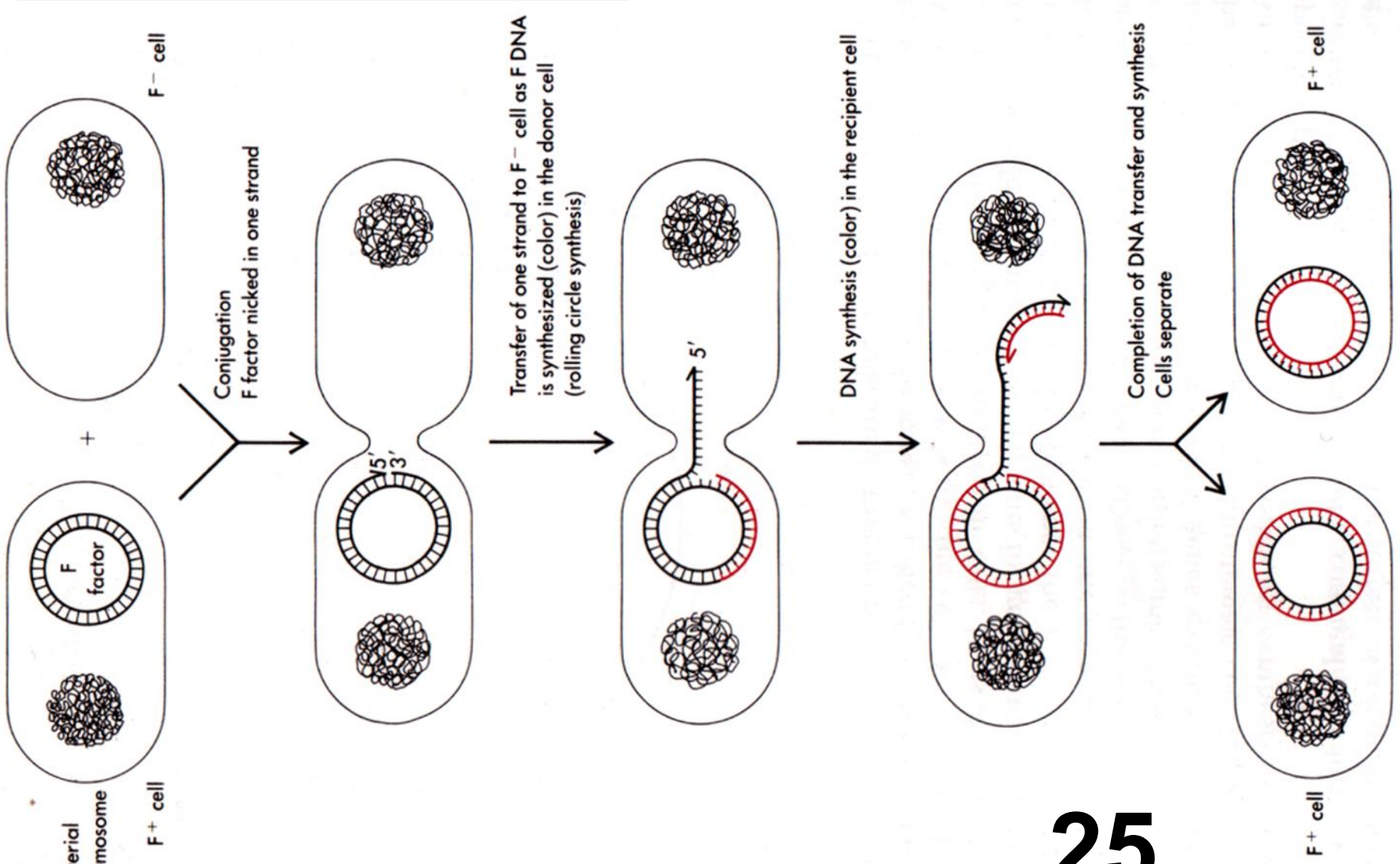
The genetic map of F factor of *E. coli* (94 kb)

fin : fertility inhibition
phi : phage inhibition

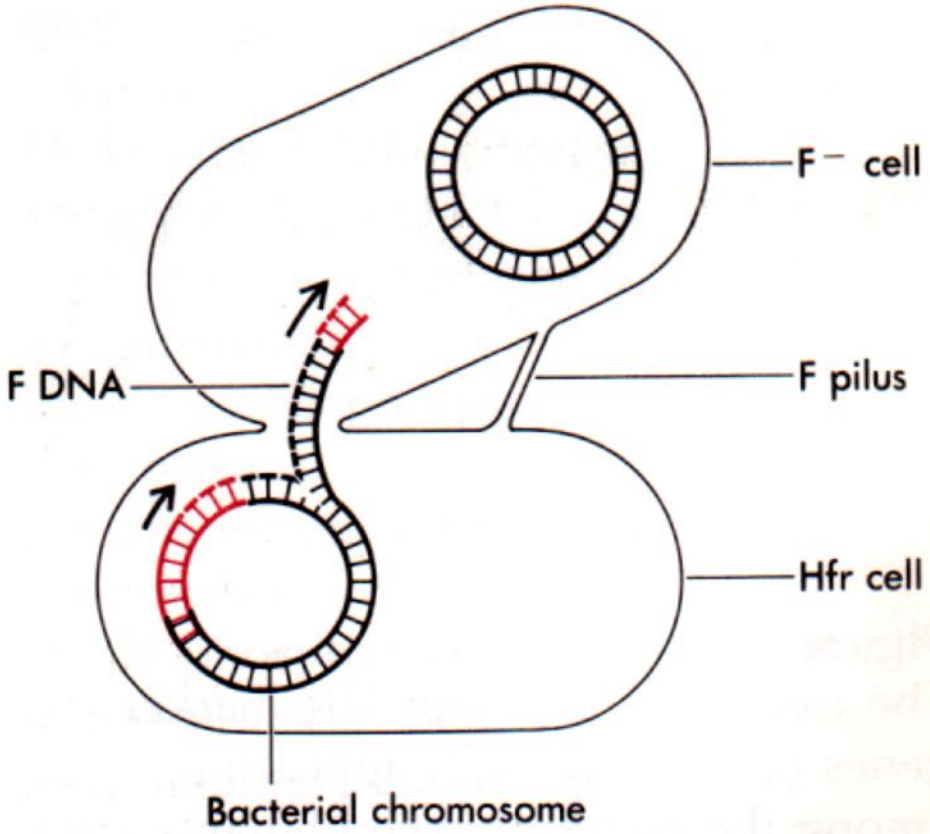
inc : incompatibility
rep : replication
tra : the transfer of the F factor to female cells
ilz : immunity to lethal zygosis
ori : origin of transer replication

The transfer of F+ DNA to an F- cell

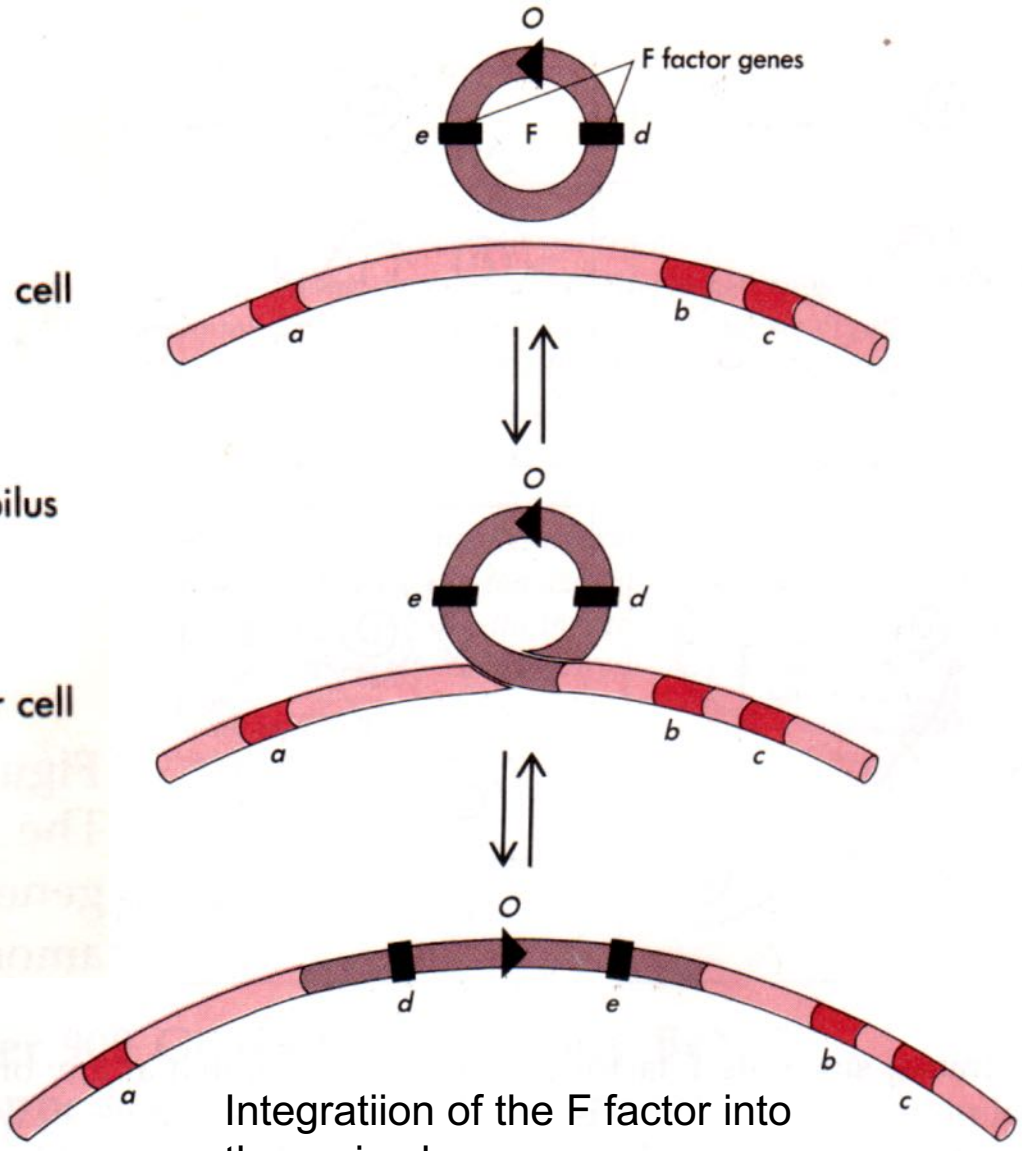
接合伝達



Hfr (high frequency of recombination)

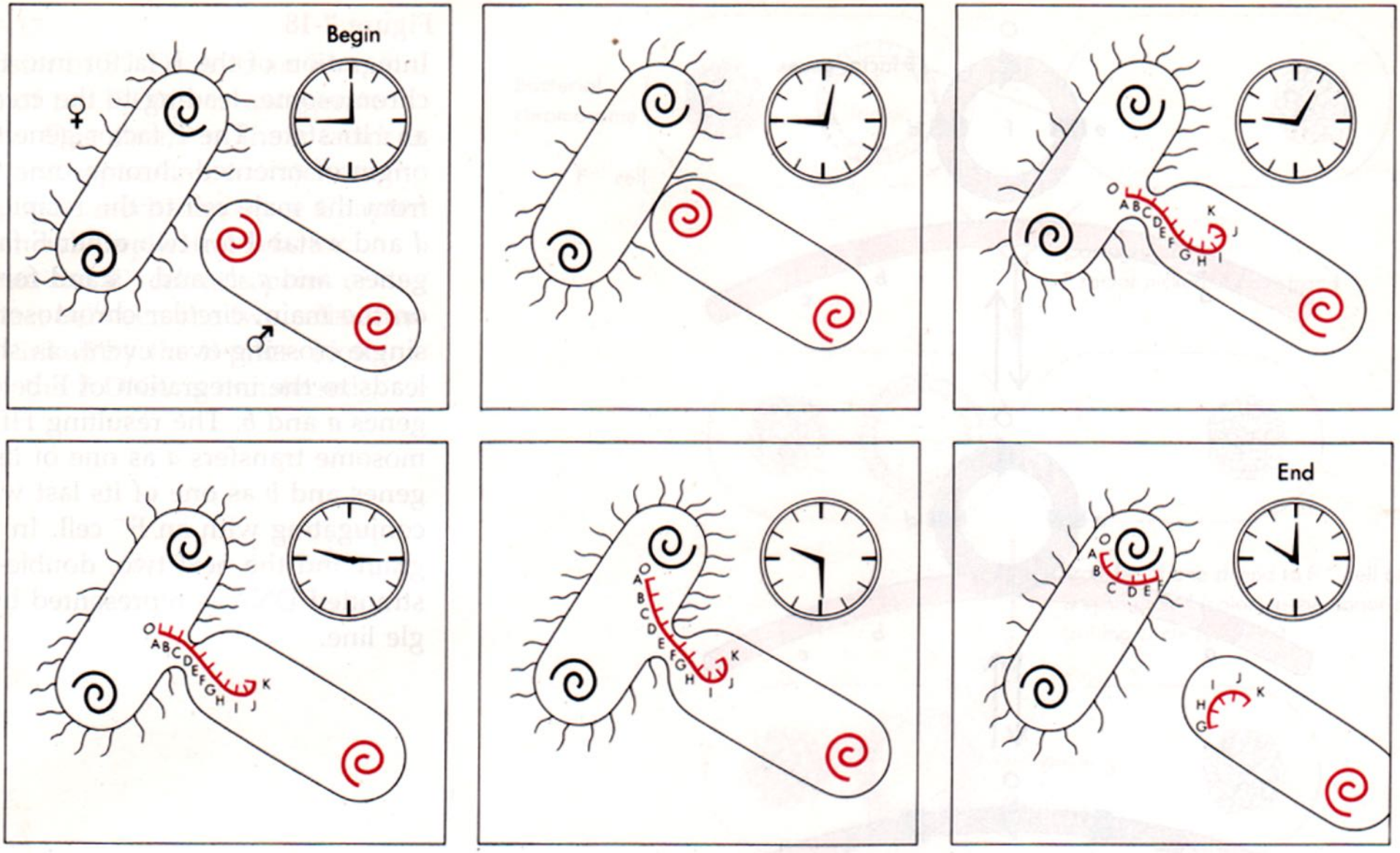


Movement of the bacterial chromosome from an Hfr cell into an F^- cell



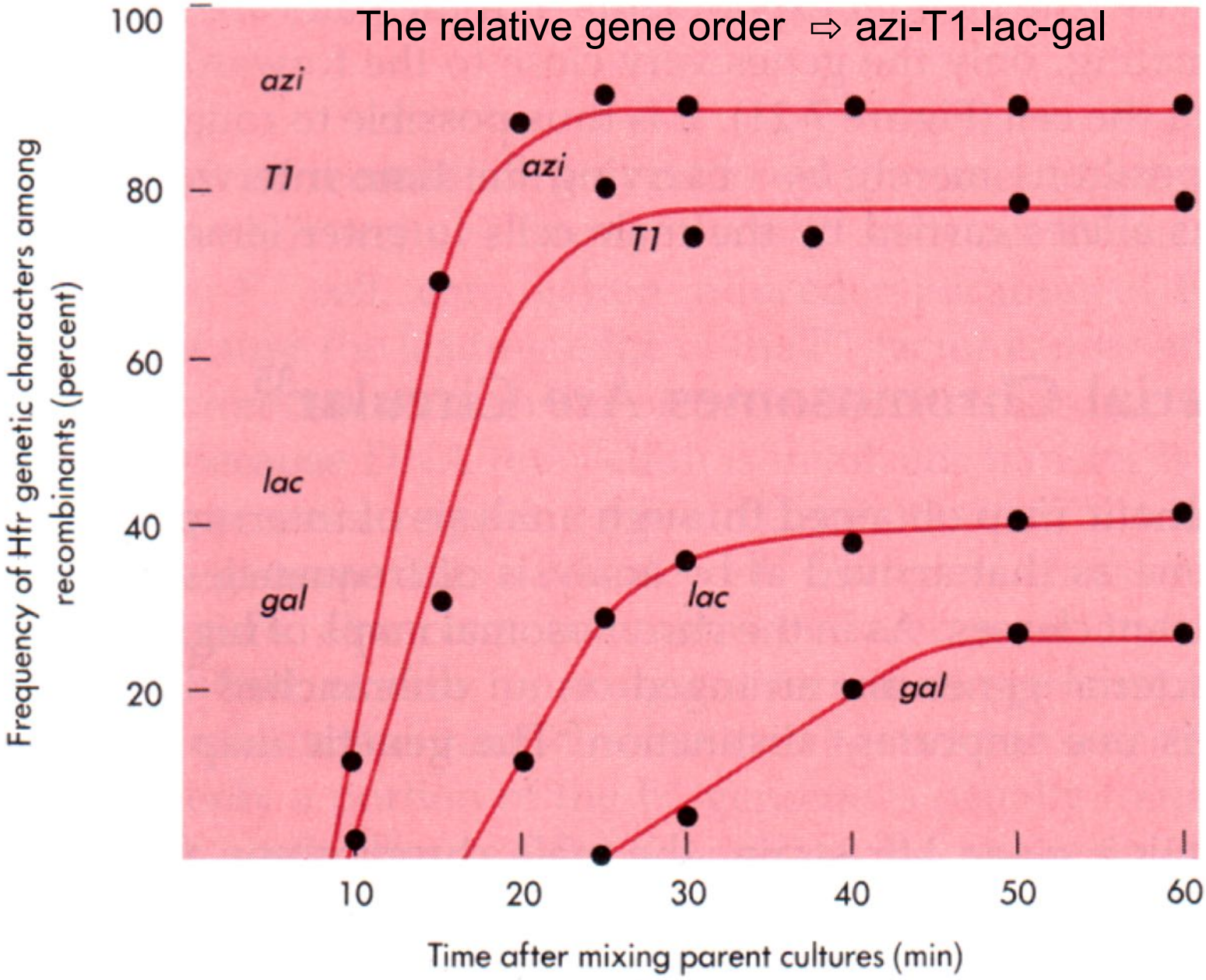
Integratiion of the F factor into the main chromosome

Conjugation between F⁻ and Hfr bacteria, as shown in a classic diagram



F. Jacob & E.L. Wollman, Sexality and the Genetics of Bacteria, (1961)

The frequency fo donor Hfr marker genes

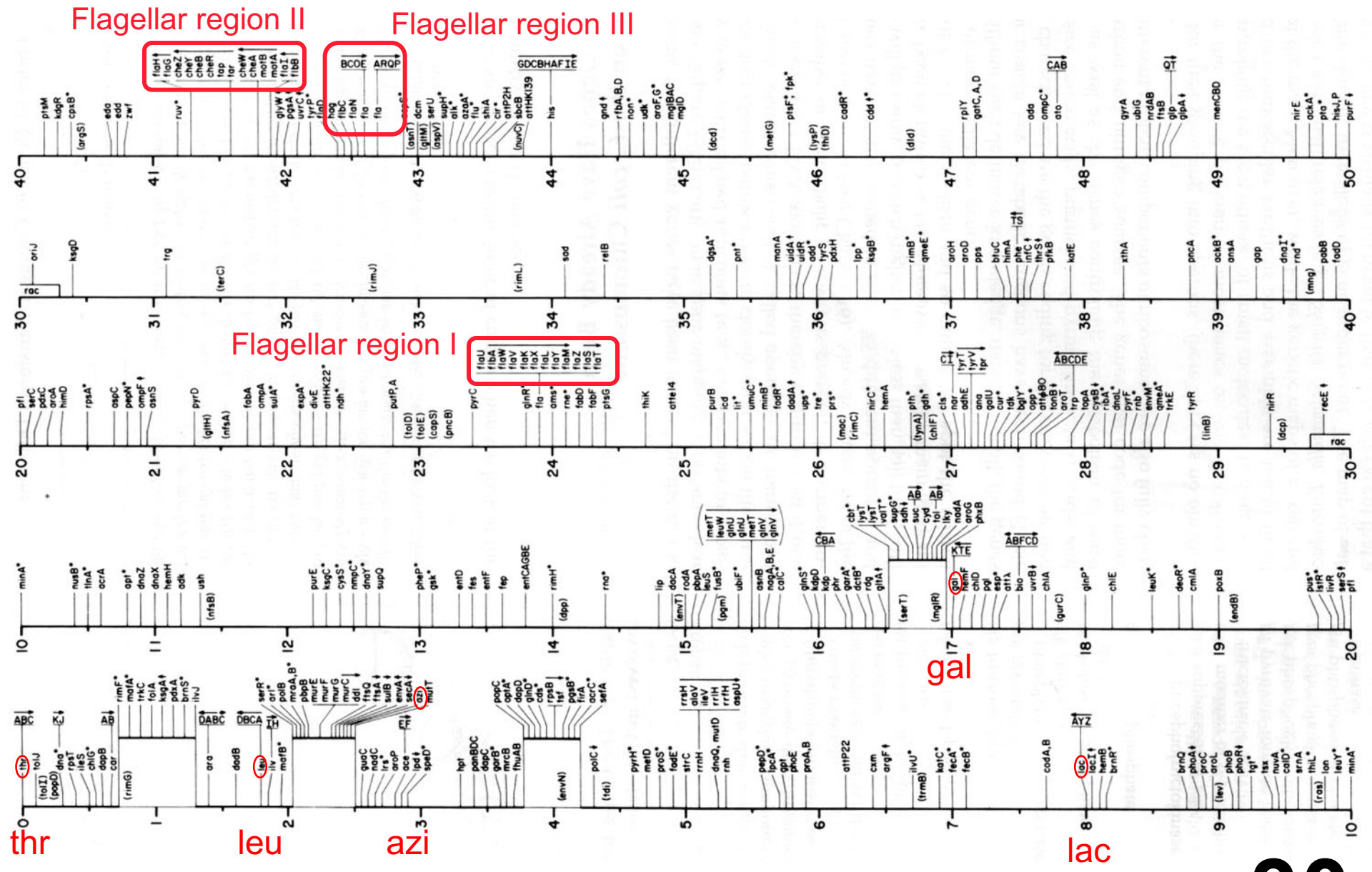


Order of genes in conjugal transfer in different Hfr strains

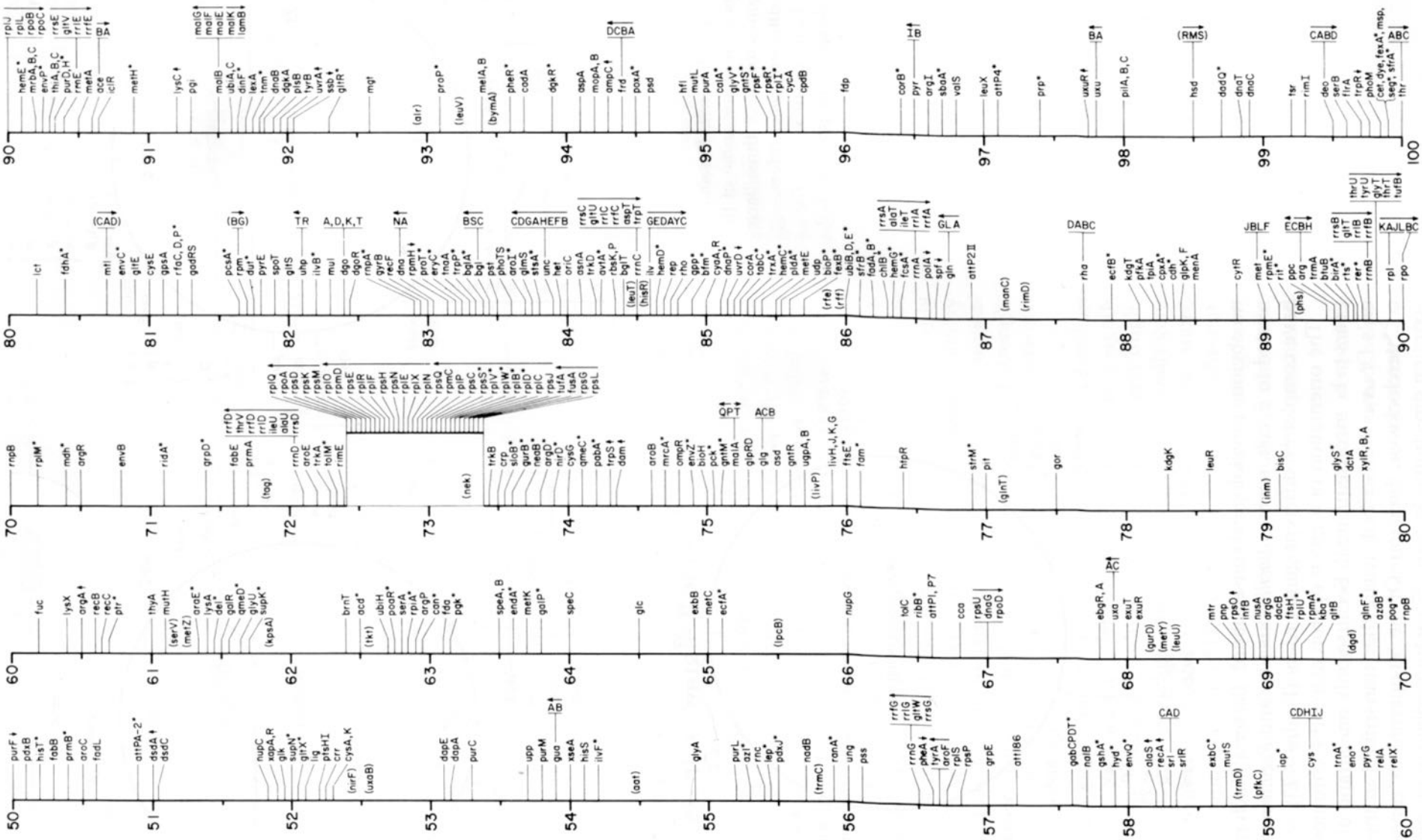
Hfr Strain	Order of Gene Transfer
Hayes	*O-thr-leu-azi-ton-pro-lac-pur-gal-trp-his-gly-str-mal-xyl-mtl-ile-met-thi*
Hfr 1	*O-leu-thr-thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac-pro-ton-azi*
Hfr 2	*O-pro-ton-azi-leu-thr-thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac*
Hfr 3	*O-pur-lac-pro-ton-azi-leu-thr-thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal*
Hfr 4	*O-thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac-pro-ton-azi-leu-thr*
Hfr 5	*O-met-thi-thr-leu-azi-ton-pro-lac-pur-gal-trp-his-gly-str-mal-xyl-mtl-ile*
Hfr 6	*O-ile-met-thi-thr-leu-azi-ton-pro-lac-pur-gal-trp-his-gly-str-mal-xyl-mtl*
Hfr 7	*O-ton-azi-leu-thr-thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac-pro*
AB311	*O-his-trp-gal-pur-lac-pro-ton-azi-leu-thr-thi-met-ile-mtl-xyl-mal-str-gly*
AB312	*O-str-mal-xyl-mtl-ile-met-thi-thr-leu-azi-ton-pro-lac-pur-gal-trp-his-gly*
AB313	*O-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac-pro-ton-azi-leu-thr-thi-met-ile*

SOURCE: From F. Jacob and E. L. Wollman, *Sexuality and the Genetics of Bacteria* (New York: Academic Press, 1961).

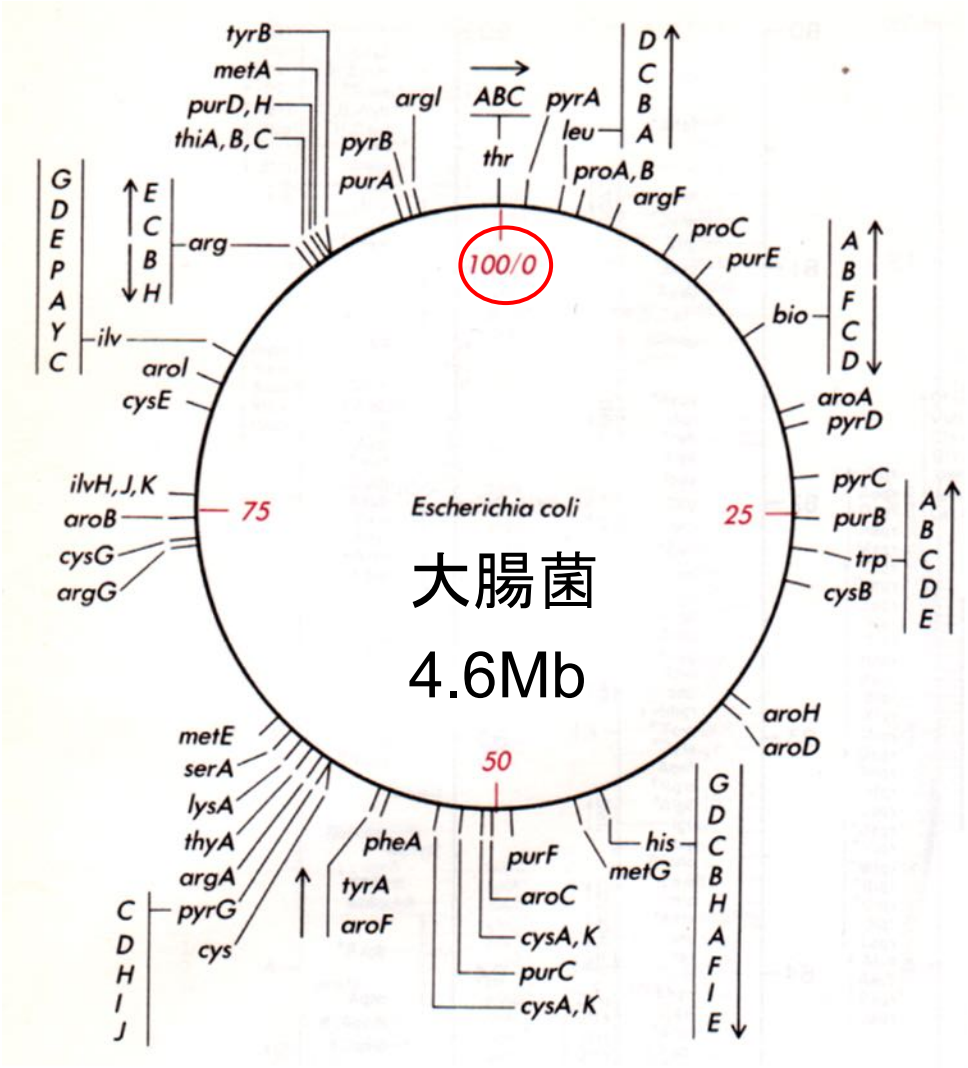
Complete genetic map of *E. coli* I

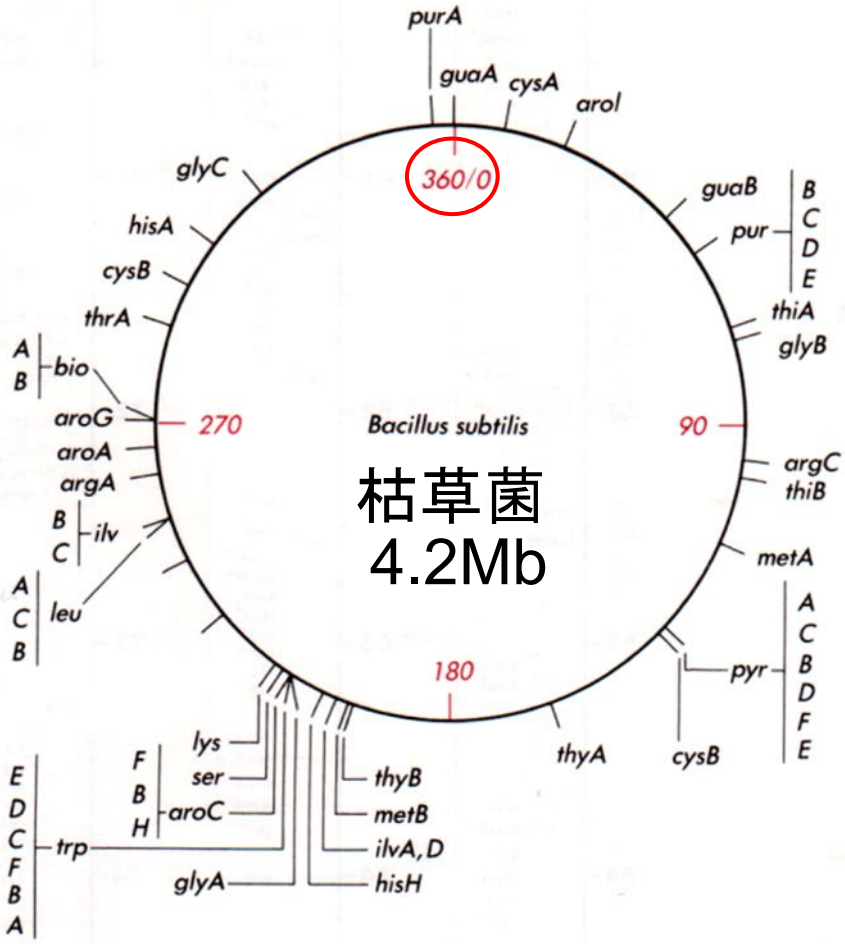


Complete genetic map of *E. coli* II



The genetic maps





課題

I）サプレッサー変異について説明せよ。アンバーサプレッサーって何のこと？（問1）Ts変異ってなんのこと？（問2）バクテリオファージでこれらの変異が頻繁に使われたが、どうしてなの？）（問3）

II）大腸菌の遺伝子地図はどのように作られたのか？（問4）

ファイルで提出する場合は、ファイル名に名前を入れてくれると助かります。