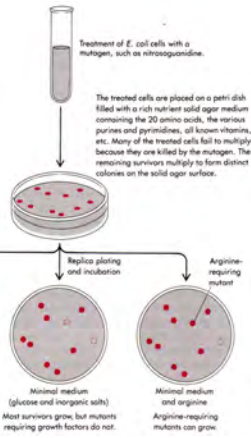


3. Isolation of mutant *E. coli* cells with a specific growth factor requirement

必須代謝物合成能に影響を与える変異体の分離

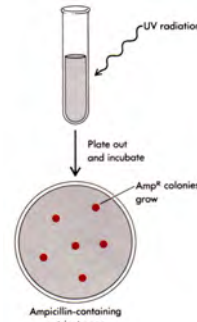
栄養要求株 (auxotroph)
原栄養株 (prototroph)

(1944年)

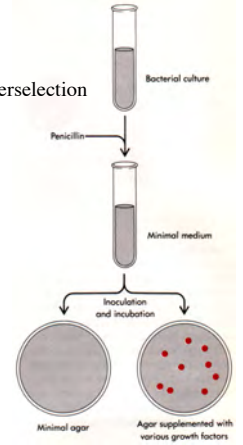


4. Enriching mutants I

direct selection

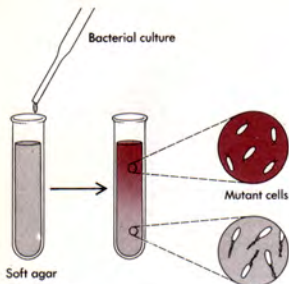


counterselection



5. Enriching mutants II

pH-sensitive dyes to detect metabolic mutants

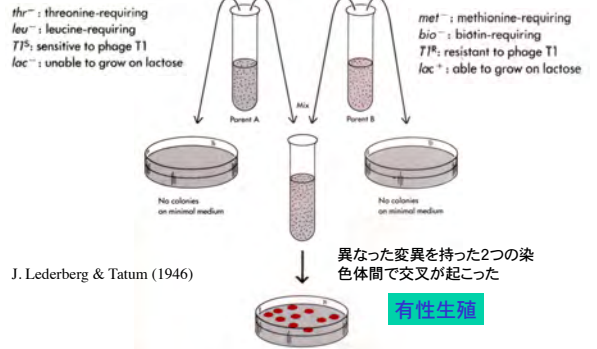


Brute force isolation
変異剤を使った後、1万個のコロニーを各々スクリーニングする。

8. The use of growth factor requirements 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

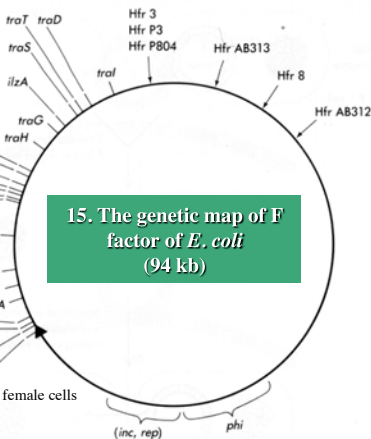


A very small fraction of the cells are *met*⁺, *bio*⁺, *thr*⁺, and *leu*⁺. They arise by genetic recombination, as shown by examination of the *lac* and *T1* markers. In addition to the parent *lac*⁻ *T1*^S and *lac*⁺ *T1*^R genotypes, there are found *lac*⁻ *T1*^R and *lac*⁺ *T1*^S cells.

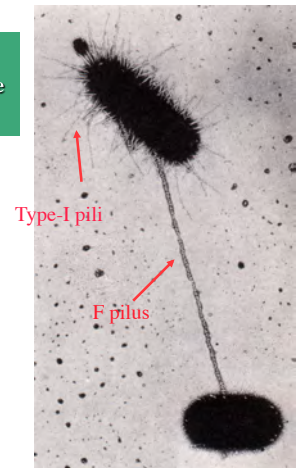
Hfr : the positions where insertion elements on F recombine with the bacterial chromosome

fin : fertility inhibition
phi : phage inhibition

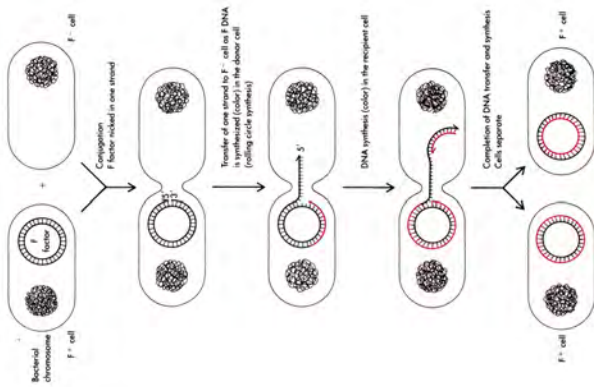
inc : incompatibility
rep : replication
tra : the transfer of the F factor to female cells
ilz : immunity to lethal zygosis
ori : origin of transfer replication



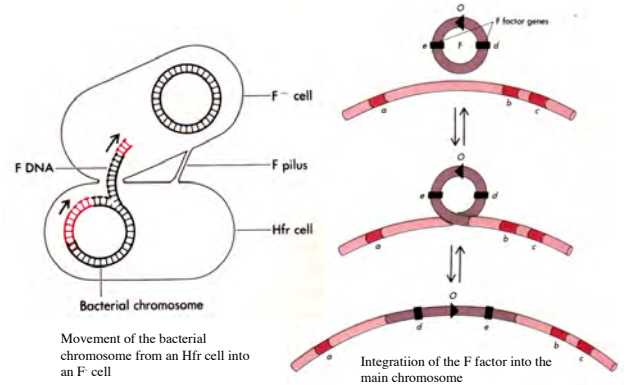
16. The attachment of a male F pilus to the surface of a female cell



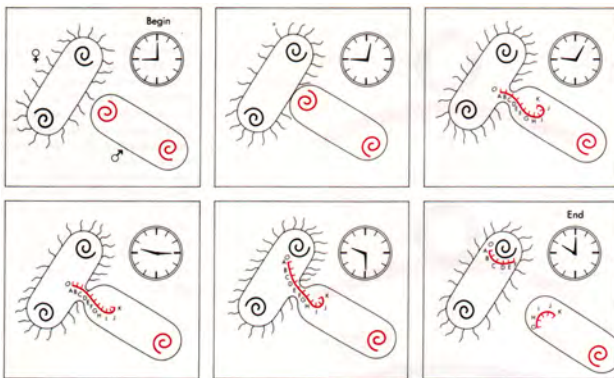
17. The transfer of F⁺ DNA to an F⁻ cell



18. Hfr (high frequency of recombination)

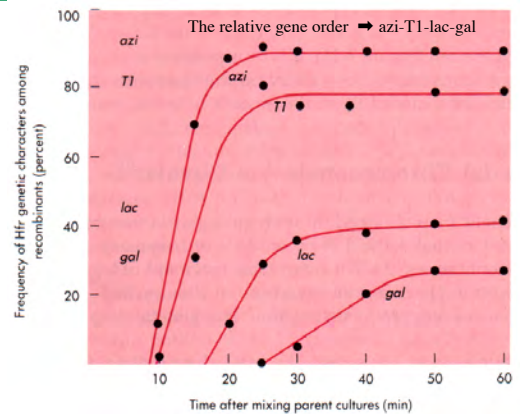


20. Conjugation between F⁻ and Hfr bacteria, as shown in a classic diagram



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

21. The frequency of donor Hfr marker genes

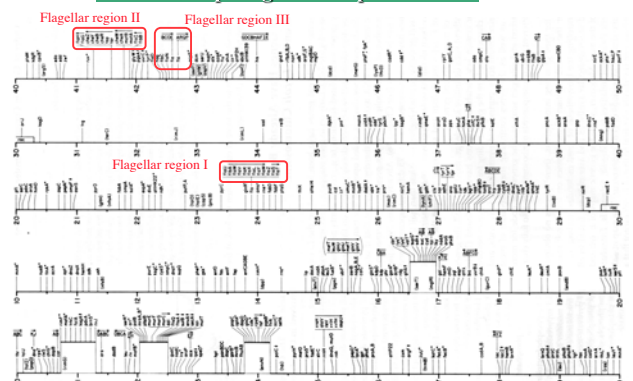


T1. Order of genes in conjugal transfer in different Hfr strains

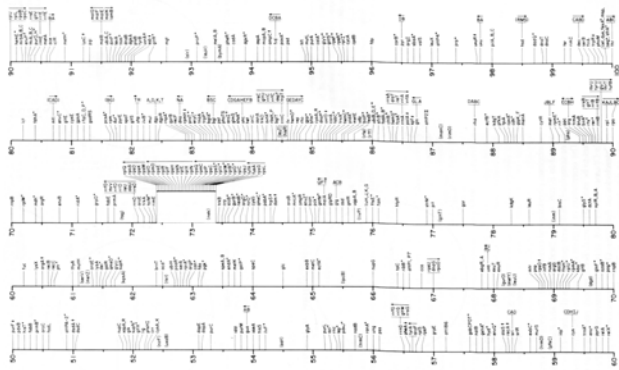
Hfr Strain	Order of Gene Transfer
Hayes	O (thr) leu-azi-ton-pro-lac-pur-gal-trp-his-gly-str-mal-xyl-mtl-ile-met-thi
Hfr 1	O-leu-(thr) thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac-pro-ton-azi
Hfr 2	O-pro-ton-azi-leu (thr) thi-met-ile-mtl-xyl-mal-str-gly-his-trp-gal-pur-lac
Hfr 3	O-pur-lac-pro-ton-azi-leu (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).

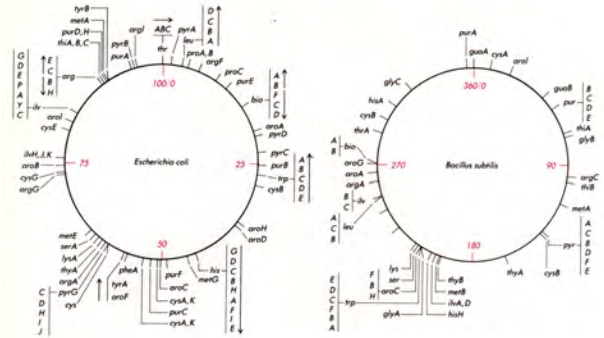
22-1. Complete genetic map of E. coli



22-2. Complete genetic map of E. coli



23. The genetic maps



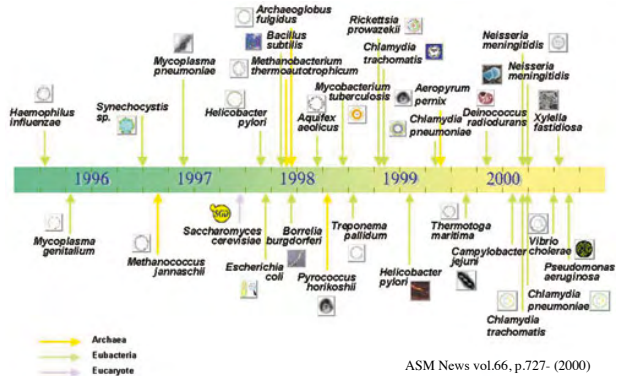
組み換えDNA技術からゲノム配列決定

1975年: カリフォルニアのアシロマにおいて、組換えDNA実験の安全性に関する激しい論争が、研究者の自主的な会議において展開され、組換えDNA実験の本格的な幕開けとなった。
 1979年: 3月我が国においても、組換えDNA実験の開拓に向け「大学等の研究機関等における組換えDNA実験指針」が文部省大臣告示。
 1980年: 東京大学医科学研究所および大阪大学微生物病研究所に、組換えDNA実験施設が設置された。
 1983年: 東京大学遺伝子実験施設を皮切りに、逐年、組換えDNA実験施設が整備されるようになった。(施設予定地から江戸時代の土器が出土)
 1995年: 独立生活を営む生物(細菌)の最初の完全なゲノム配列決定。
 2003年: ヒトのゲノム配列完成版が発表される。
 2010年: 本間研のピブリオ菌株VIO5の全ゲノム配列を外注で決定。
 2010年: 本間研のVIO5変異体の変異部位を次世代シーケンサーで決定。

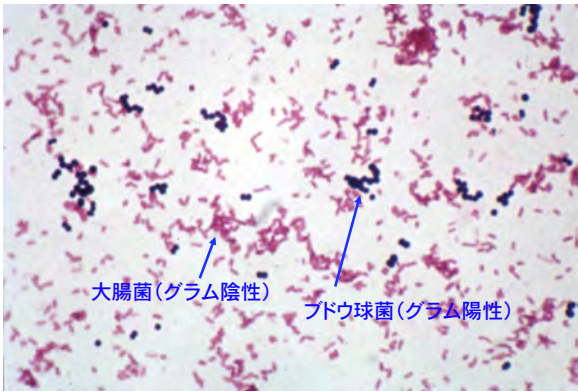
ゲノミクス から プロテオミクス
 配列解読 から インフォマティクス

結局 生命現象理解のために、
 タンパク質の機能解析をおこなう

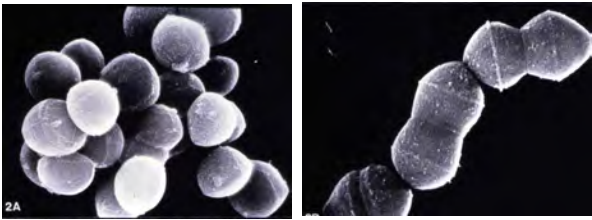
細菌ゲノム決定のタイムテーブル



1. グラム染色



2. 細菌の形 I



ブドウ球菌
 Staphylococcus
 (S. aureus: 黄色ブドウ球菌)

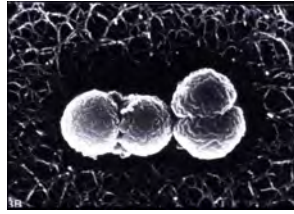
レンサ球菌
 Streptococcus
 (S. pneumoniae: 肺炎レンサ球菌)

3. 細菌の形 II



マイクロコッカス(八連菌)

Micrococcus



淋菌(双球菌)

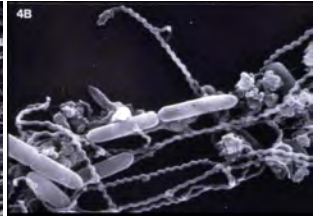
Neisseria gonorrhoeae

4. 細菌の形 III



枯草菌

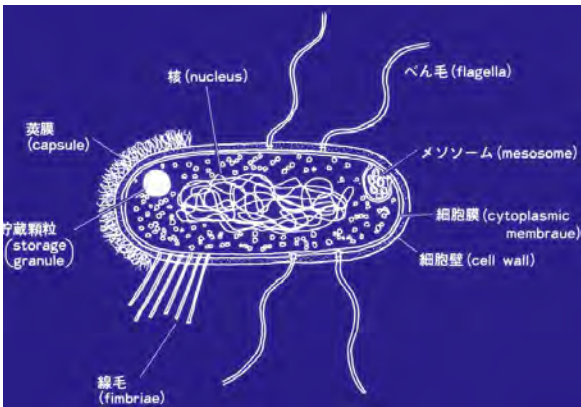
Bacillus subtilis



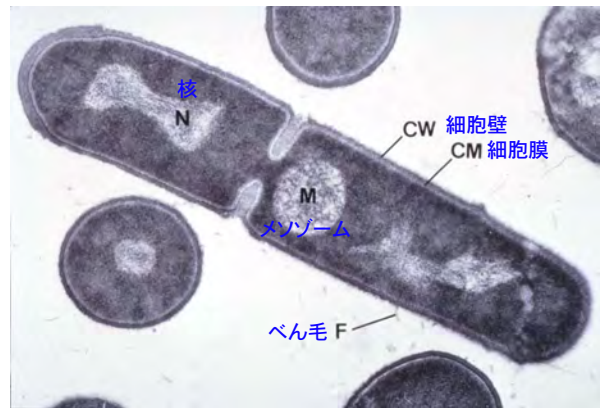
枯草菌とレプトスピラ

Leptospira
(スピロヘータ)

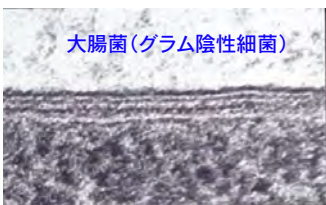
5. 細菌の構造



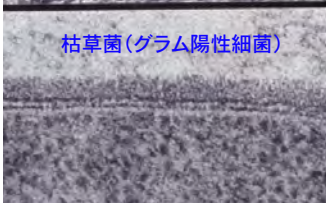
6. 枯草菌の超薄切片像



7. 超薄切片法で見た細胞壁の構造



大腸菌(グラム陰性細菌)



枯草菌(グラム陽性細菌)

