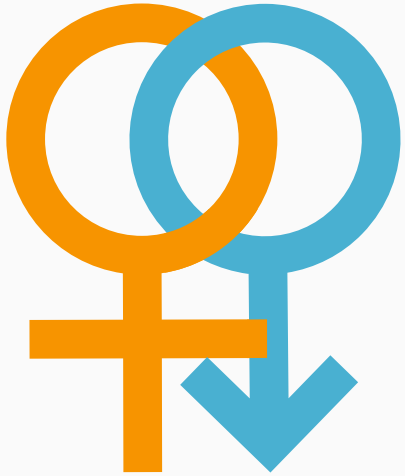


Introduction to PaJaMo: Bacterial genetics and the *lac* operon

ICB 5751 - The origins of Molecular Biology

Beny Spira

J. Mol. Biol. (1959) **1**, 165-178

The Genetic Control and Cytoplasmic Expression of "Inducibility" in the Synthesis of β-galactosidase by *E. Coli*†

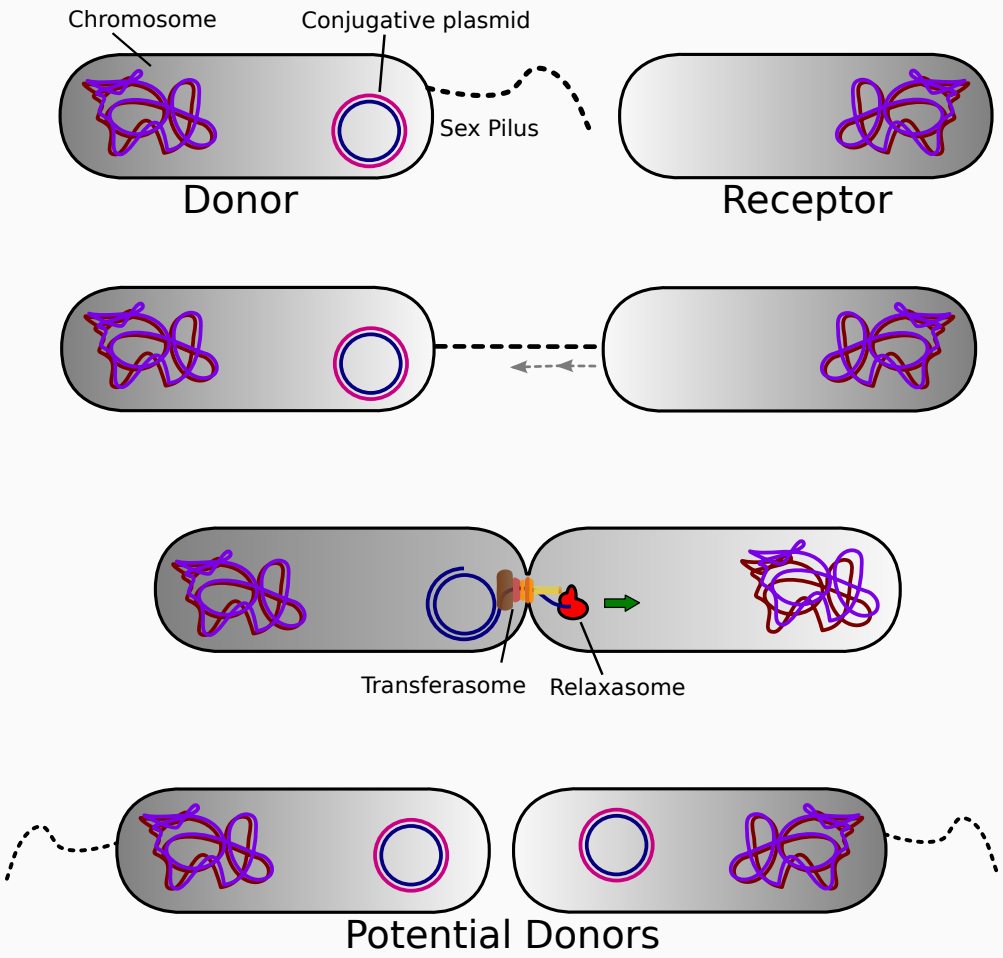
ARTHUR B. PARDEE‡, FRANÇOIS JACOB AND JACQUES MONOD

Institut Pasteur, Paris and University of California, Berkeley, California, U.S.A.

Exchange of genetic information between bacteria (sex)

1. Conjugation
2. Transduction
3. Transformation

Conjugation - main protagonists

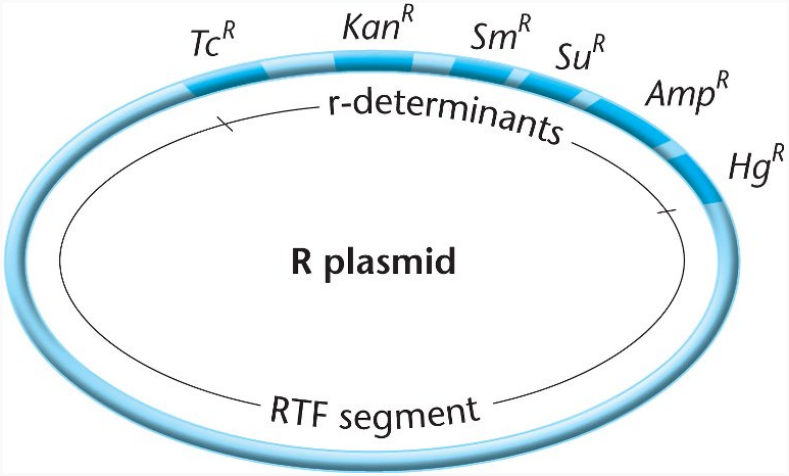


Joshua Lederberg

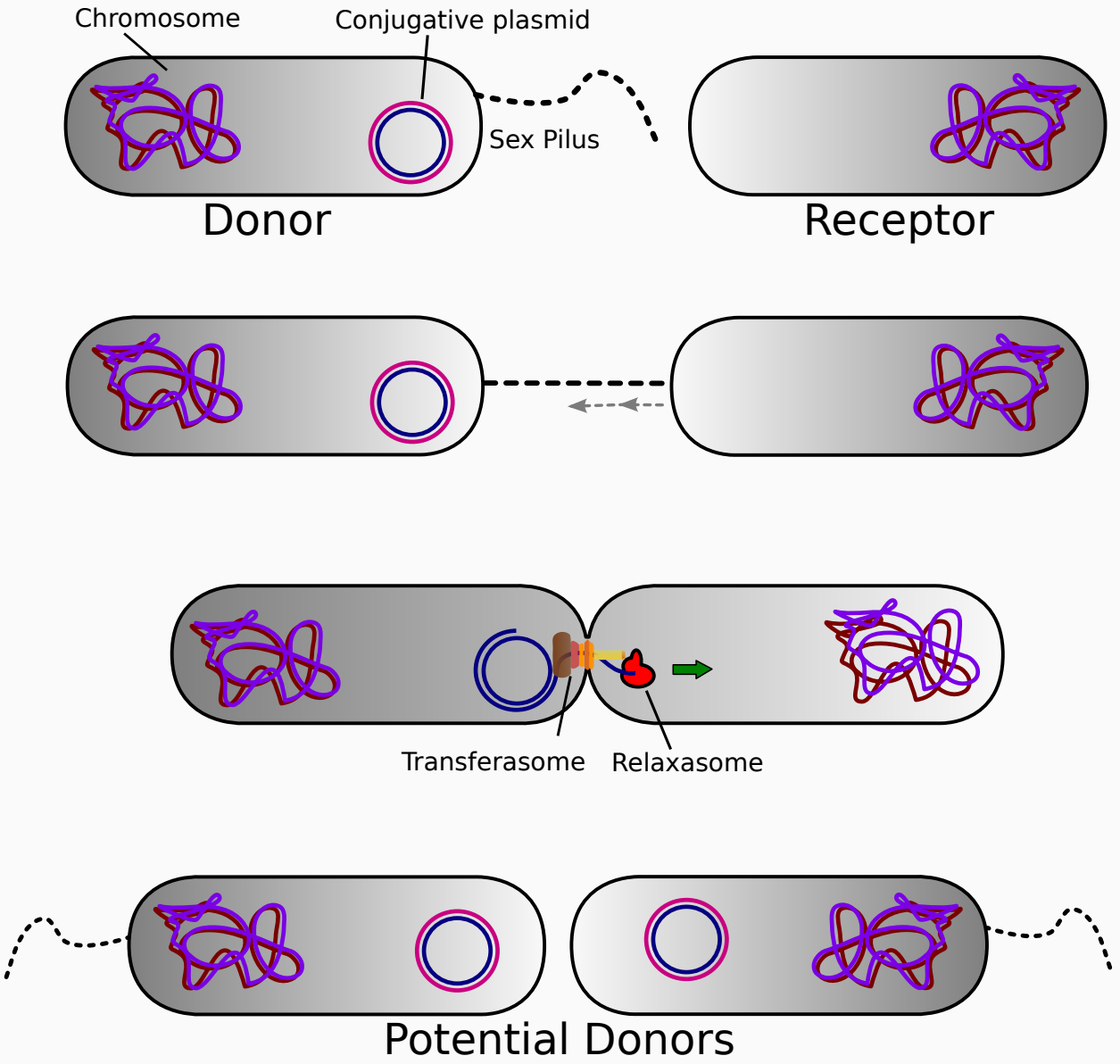
Edward Tatum

Efficiency of conjugation → up to 100% !!

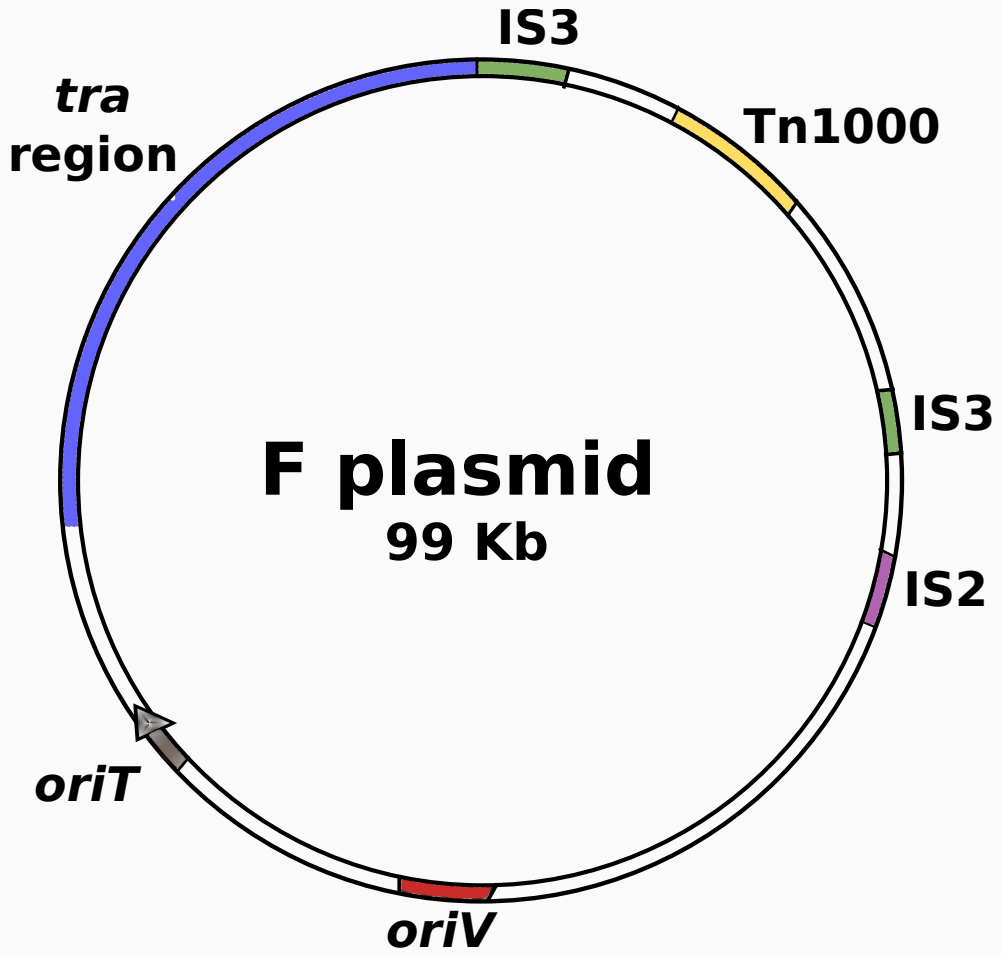
- Frequencies of conjugation in nature are probably several orders of magnitude higher than those under laboratory conditions
- Main mechanism for spreading of antibiotic resistance genes



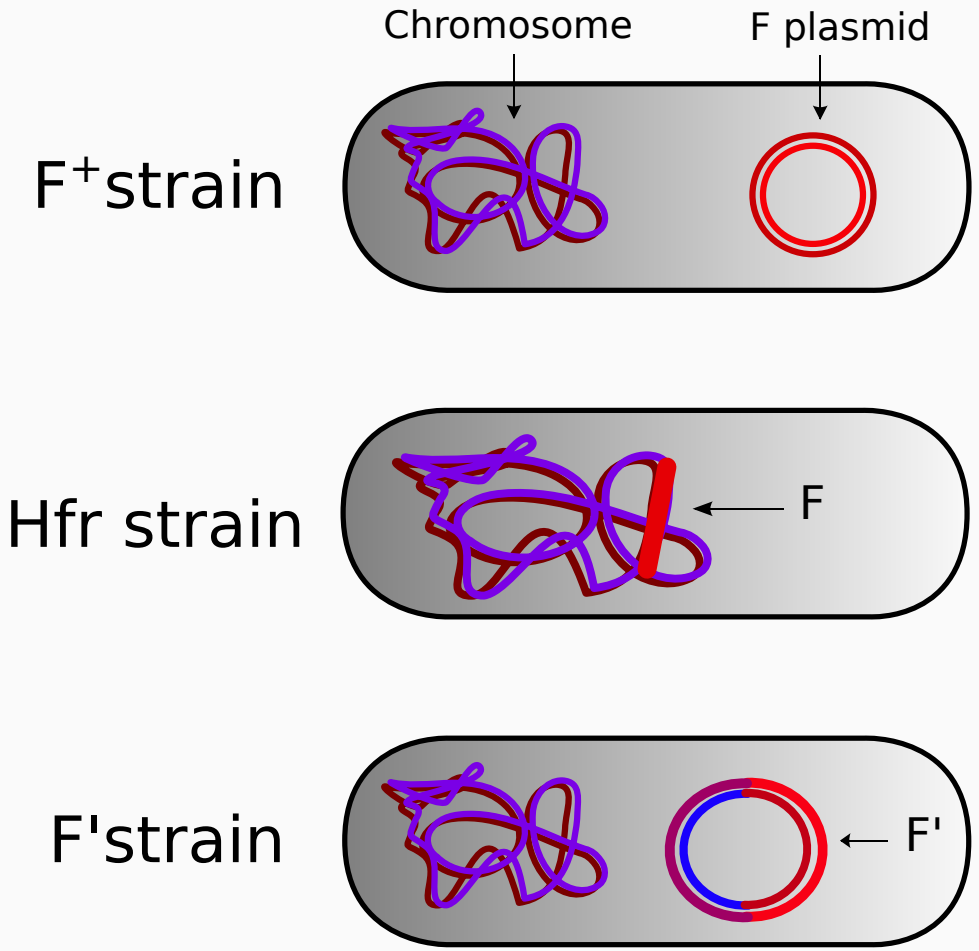
Conjugation - mechanism



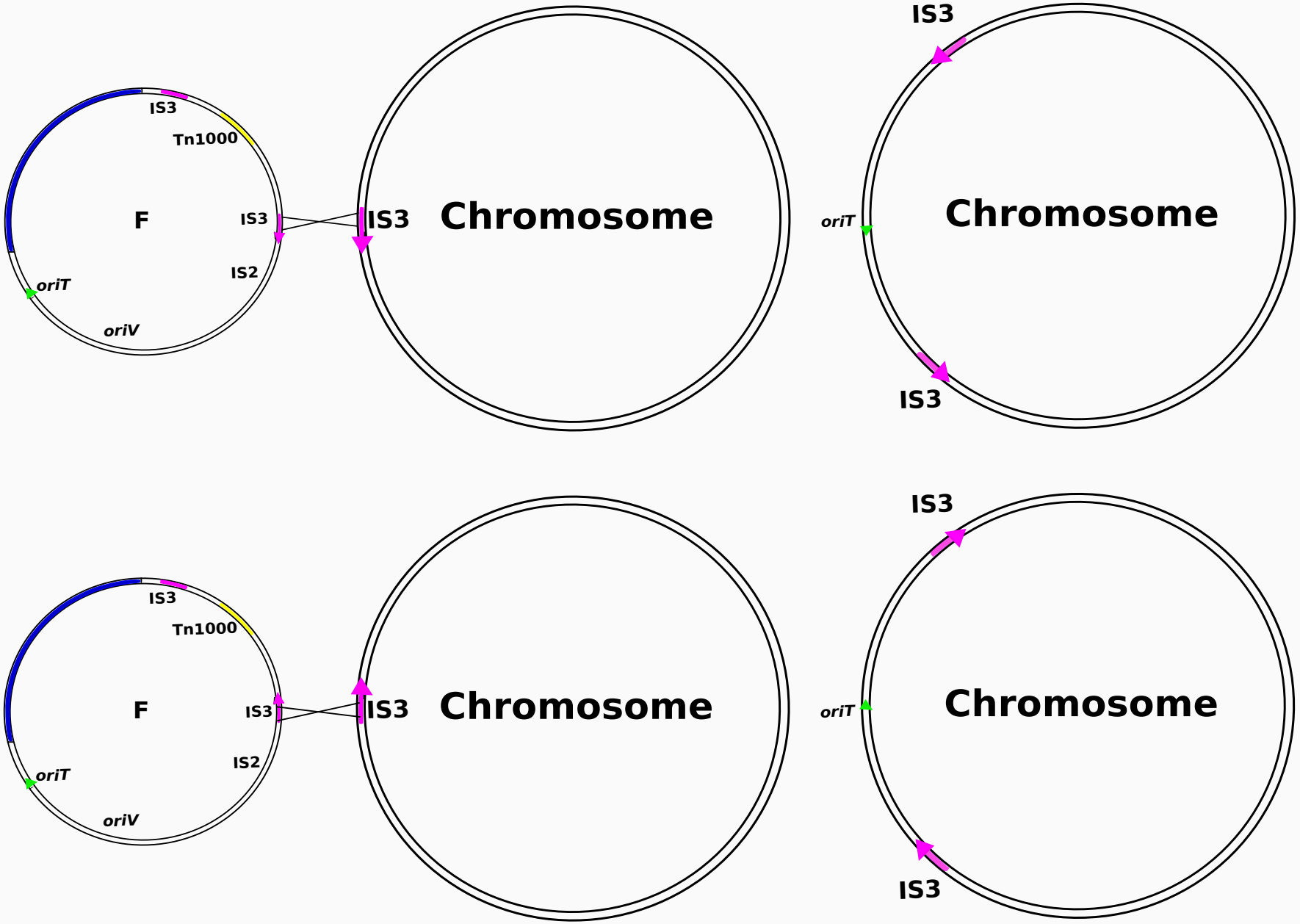
F factor



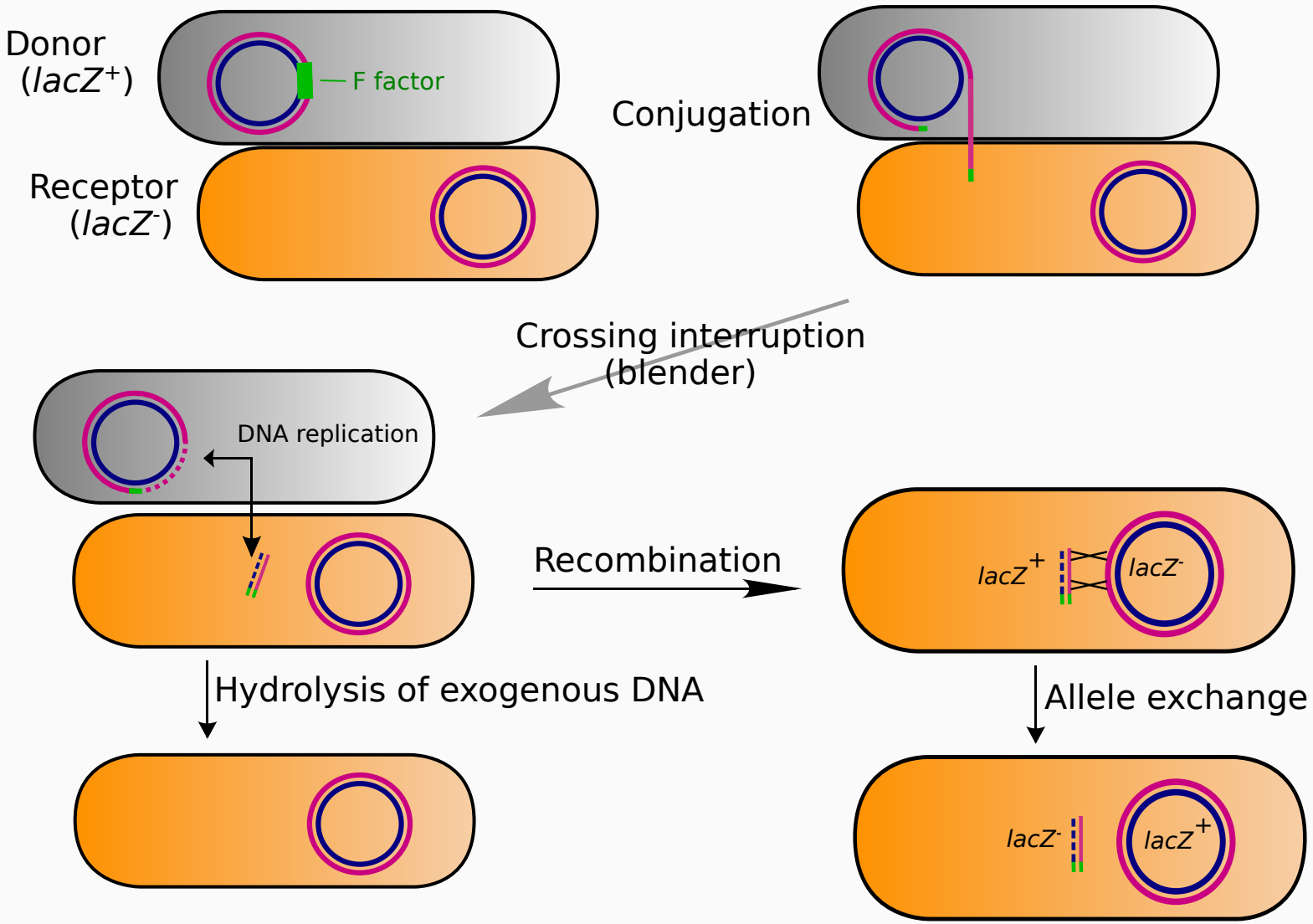
F factor - classification



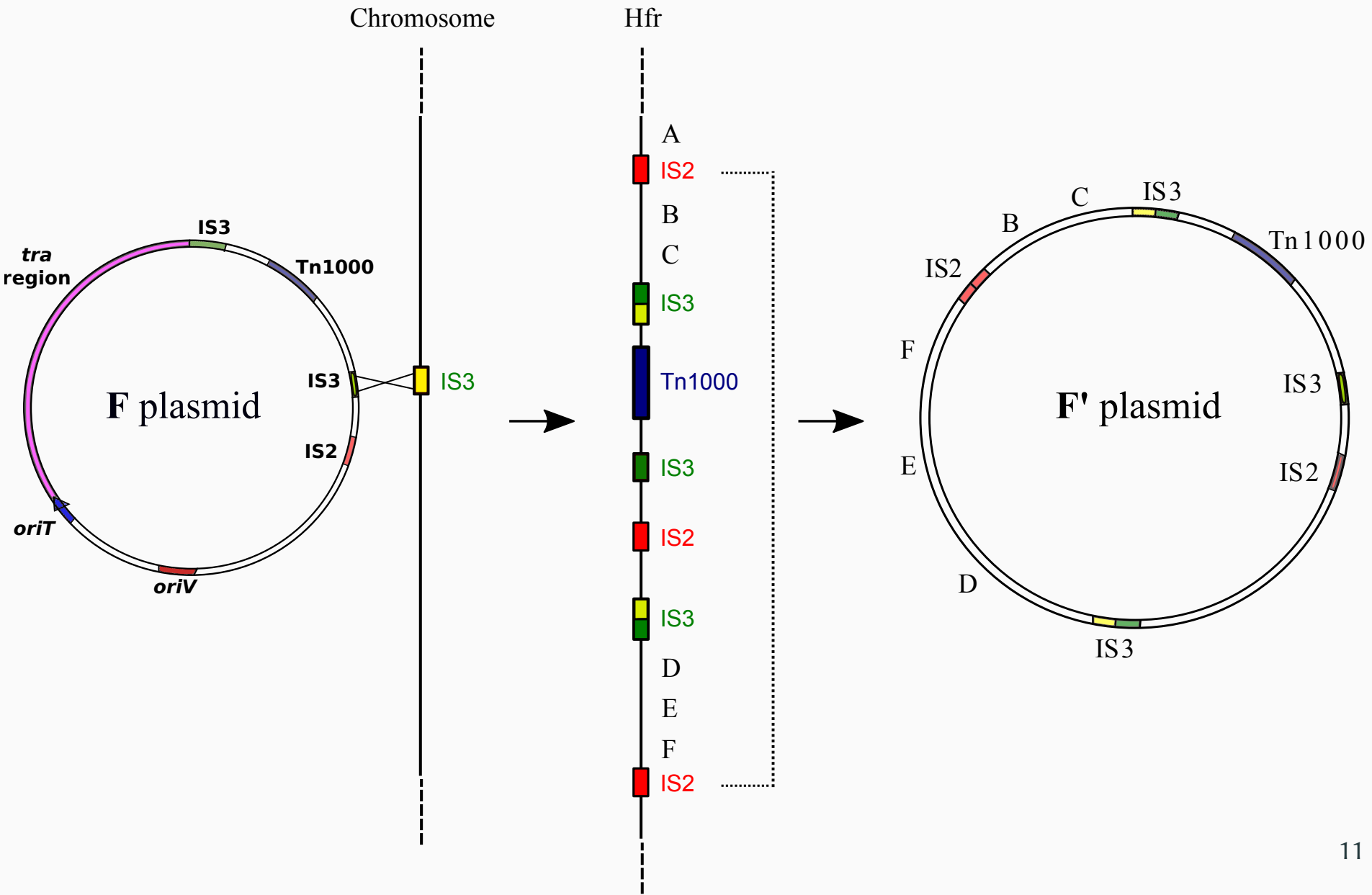
Formation of Hfr



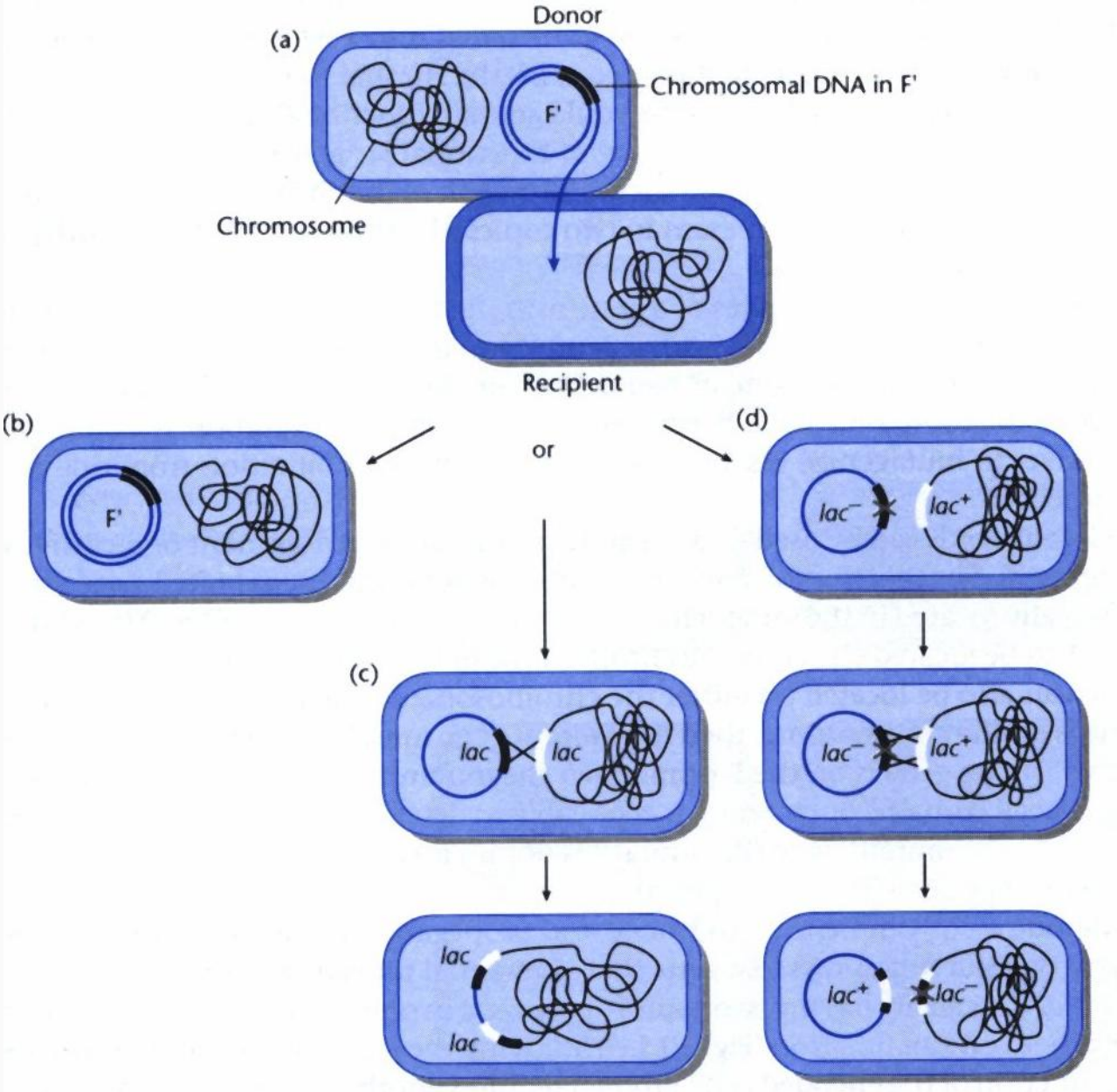
Hfr transfer



F’ formation


Chromosome
Hfr
IS3
tra region
Tn1000
F plasmid
IS3
IS2
oriT
oriV
IS3
A
IS2
B
C
IS3
Tn1000
IS3
IS2
IS3
D
E
F
IS2
C
IS3
B
Tn1000
IS2
F
F' plasmid
IS3
E
IS2
D
IS3

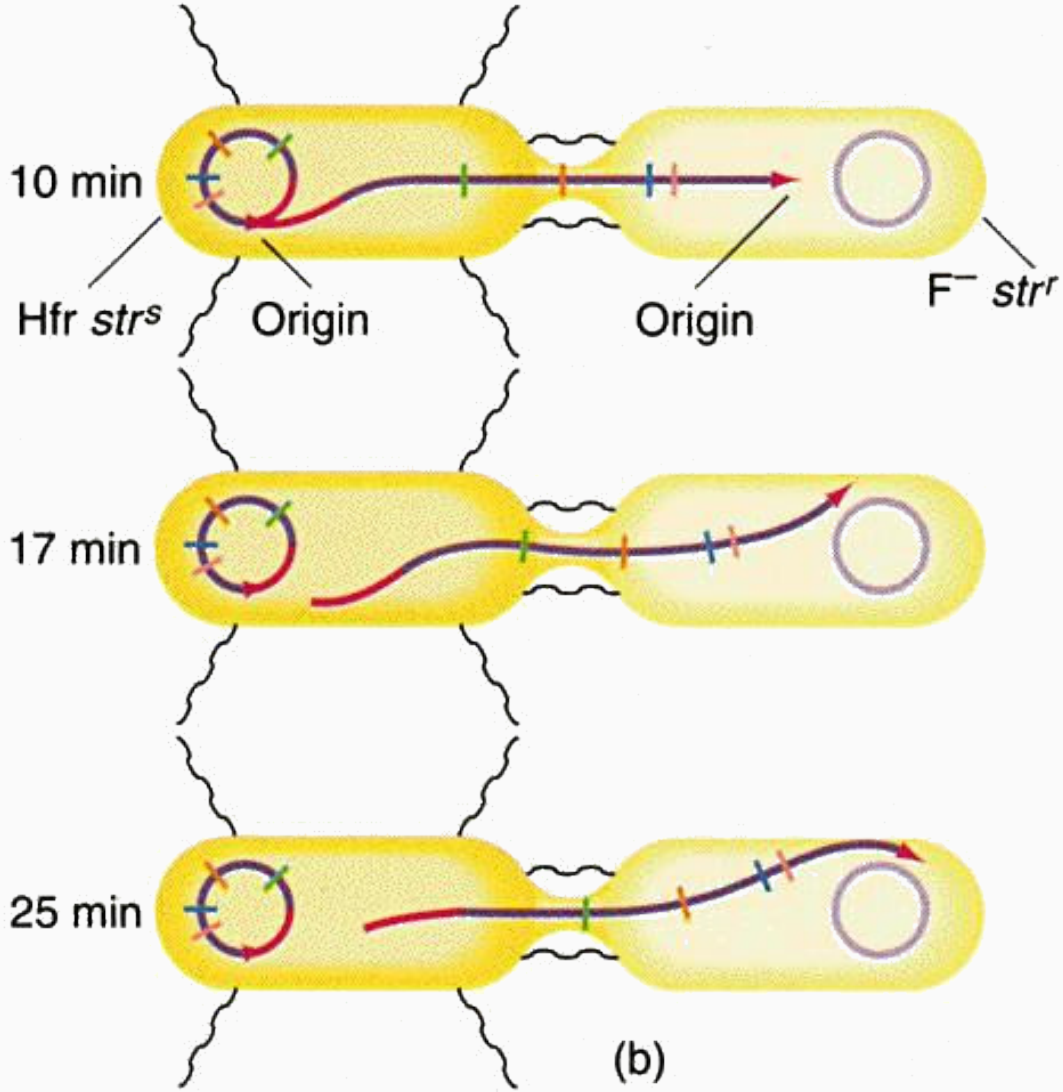
F’ transfer


Donor
(a)
Chromosomal DNA in F'
F'
Chromosome
Recipient
(b)
or
(d)
F'
lac⁻
lac⁺
(c)
lac
lac
lac⁻
lac⁺
lac
lac
lac⁺
lac⁻

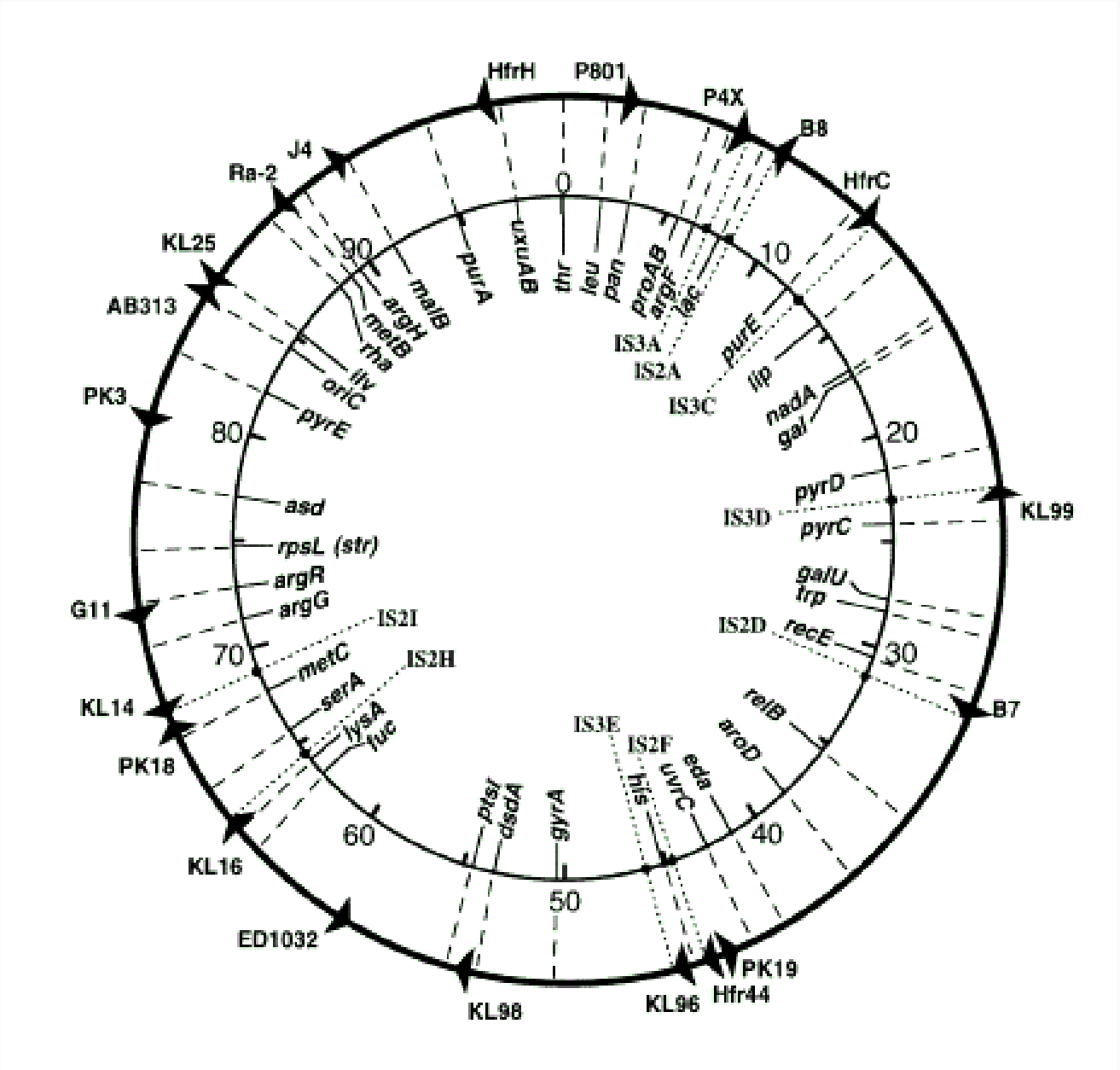
F factor - Table

Name	F Status	Features
F^-	None	F Receptor
F^+	Free F factor	Efficiently transfers F to a receptor
F'	F factor carries chromosomal DNA segments. Ex.: F' lac	Transfers F and chromosomal segments; may occur (usually not) homologous recombination with chromosome
Hfr	F factor integrated in bacterial chromosome	Transfers chromosomal regions from a fixed point (according to Hfr location) with high efficiency

Coitus interruptus = building a chromosome map (Wollman & Jacob, 1956)



An early chromosomal map of *E. coli*



Conjugation problem

Donor: StrS Pro^+ (F' *lac proAB*) × Receptor: StrR Pro^- (F^-)

Recombinant: StrR Pro^+ (F' *lac proAB*)

Selection: plates with streptomycin, no proline

Another problem

Four Hfr strains are able to conjugate the following genes in this order:

Strain 1:	M Z X W C
Strain 2:	L A N C W
Strain 3:	A L B R U
Strain 4:	Z M U R B

All Hfr strains come from the same F^+.

What is the order of the genes in the chromosome of the original F^+ strain?

Another one

An Hfr strain with a^+ b^+ c^+ d^- Str^S genotype crosses with a F^- strain, whose genotype is a^- b^- c^- d^+ Str^R. The cross is stopped at different time intervals. The transconjugants were then plated onto three different plates (1, 2 or 3) having one or more of the following supplements: A, B, C and D. An a^- bacterium can grow only on a plate containing supplement A ; a b^- bacterium can grow only on a plate containing supplement B and so on.

Plate	Str	A	B	C	D
1	+	+	+	-	+
2	+	-	+	+	+
3	+	+	-	+	+

(a) What genes were selected by each plate type?

Oh No! More problems!

The following table shows the number of colonies of each type in samples harvested at various times after the start of the conjugation above.

Minutes of conjugation	Colonies/plate		
	1	2	3
0	0	0	0
5	0	0	0
7.5	100	0	0
10	200	0	0
12.5	300	0	75
15	400	0	150
17.5	400	50	225
20	400	100	250
25	400	100	250

(b) What is the order of genes *a*, *b* and *c* in the chromosome.

(c) 100 colonies from each 25 minutes plate were restreaked and transferred to a plate containing all supplements except D. 89 colonies from plate 1, 8 from plate 2 and 51 from plate 3 grew on that plate. What is position of gene *d* in the sequence *a, b* and *c*.

This is the last one, I promise

Two *E. coli strains* with the following genotypes were crossed:

Strain A: F^- lac^- arg^- his^- pyr^- lys^- Ton^R
Strain B: Hfr lac^+ arg^+ his^+ pyr^+ lys^+ Ton^S

Ton^R= resistance to phage T1

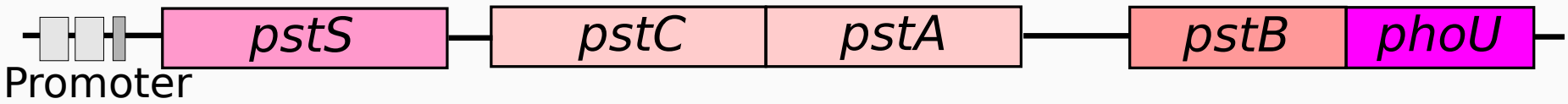
(a) What supplements should be added to the culture medium to select recombinants lac^+ arg^+?
(b) What is the likely genotype of the recombinants selected on minimal medium plates containing glucose, arginine and phages T1?
(c) Cultures of strains A and B were mixed and crossed for 2 hours. The bacteria were plated and the results are in the table below. What genetic marker was transferred first? Who was transferred last?

Selection	Recombinants per 1000 Hfr bacteria
Arg^+	350
Pyr^+	100
Lac^+	25
Lys^+	350
His^+	500

The concept of operon

Definition

Genomic structure, found primordially in prokaryotes, having two or more genes *in tandem*, transcribed in a single transcriptional unit



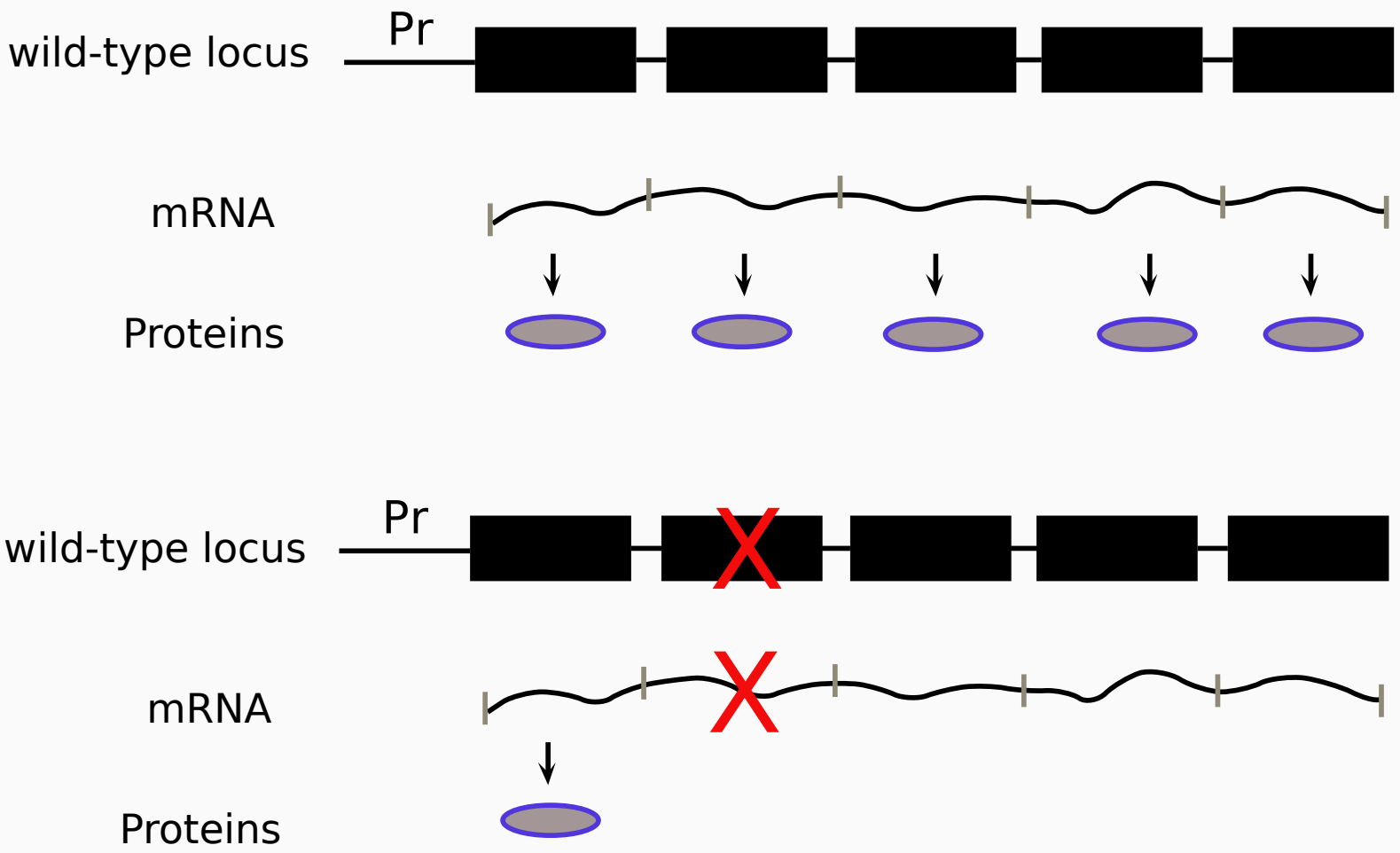
More definitions

Promoter: RNA polymerase binding site, where transcription starts

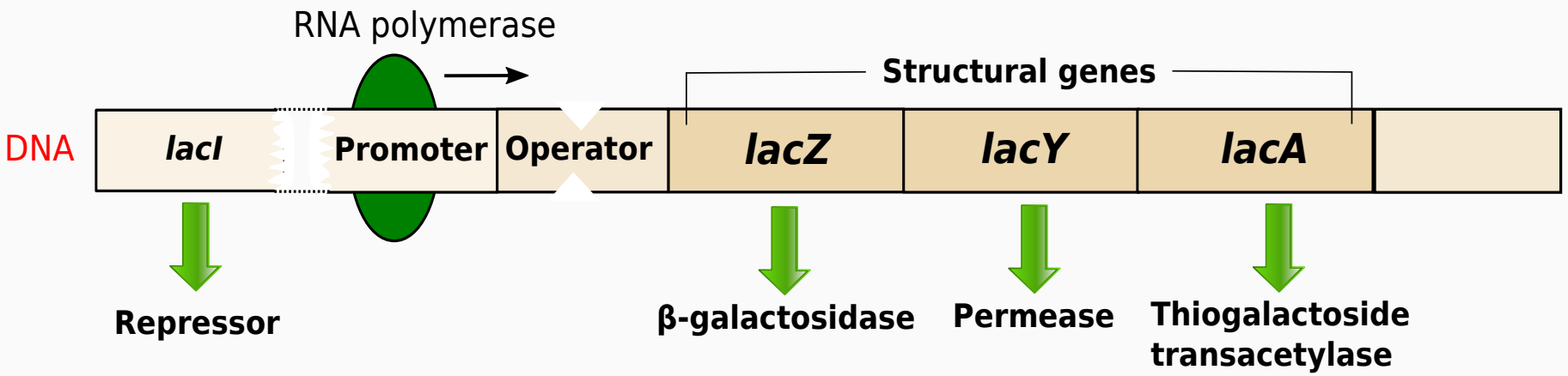
Operator: repressor (if any) binding site

Terminator: transcription end site

Polar mutation

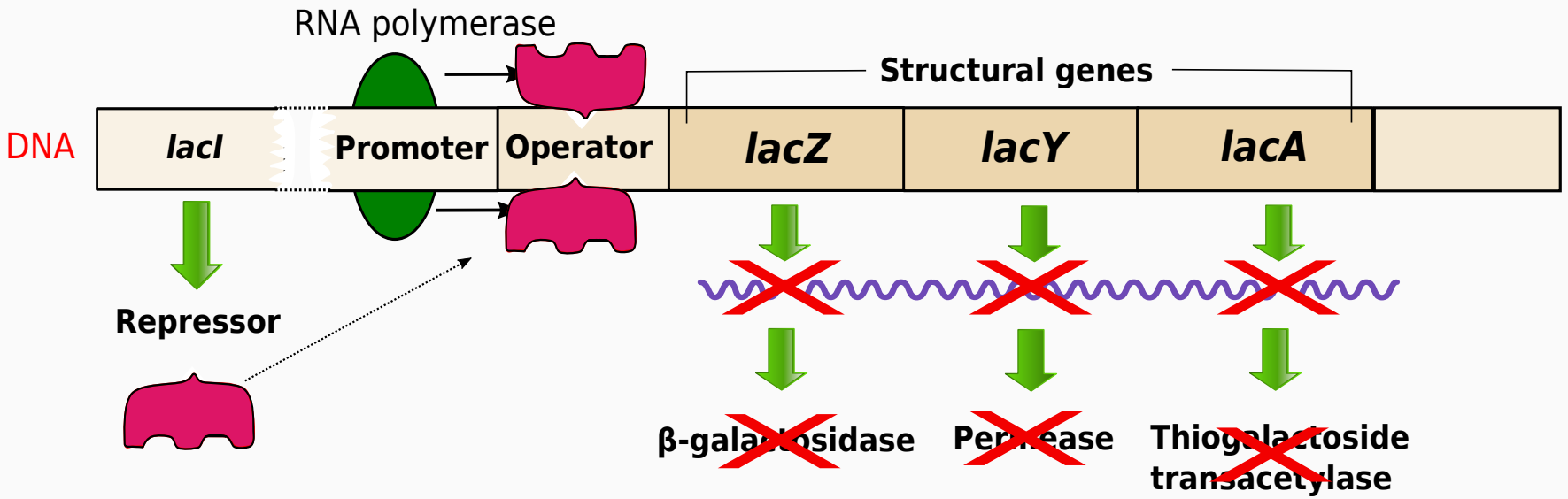


The *lac* operon

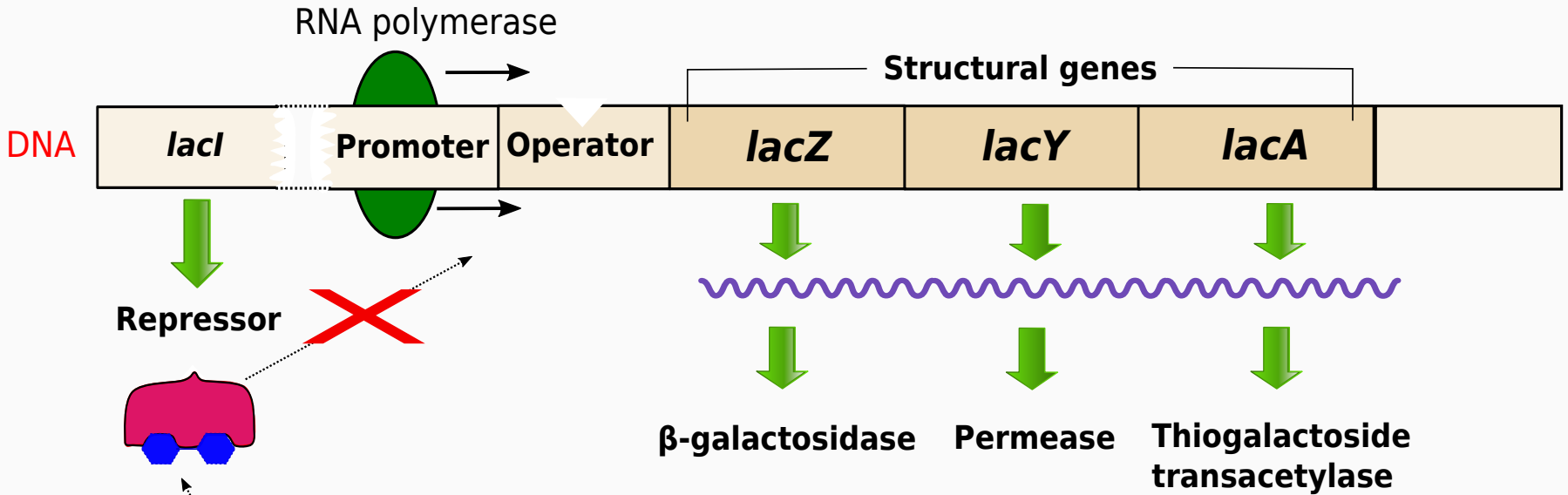


lac regulation

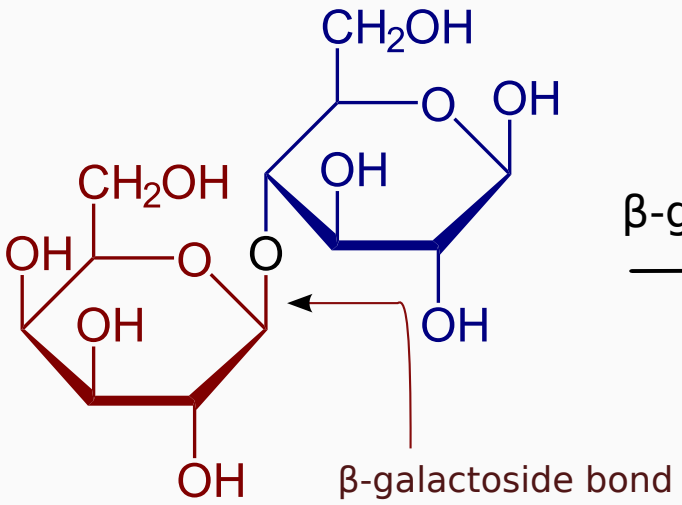
Absence of inducer:



Presence of inducer:



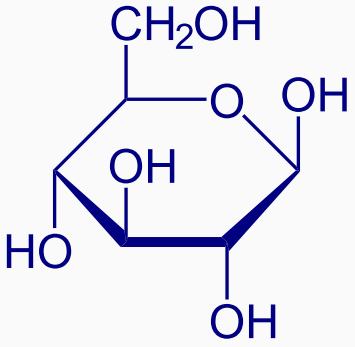
β-galactosidase substrates



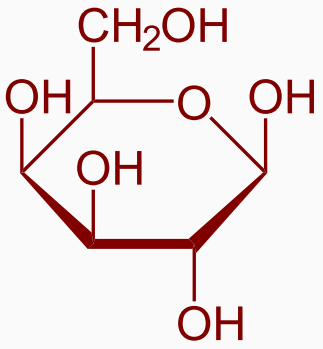
lactose

β-galactosidase

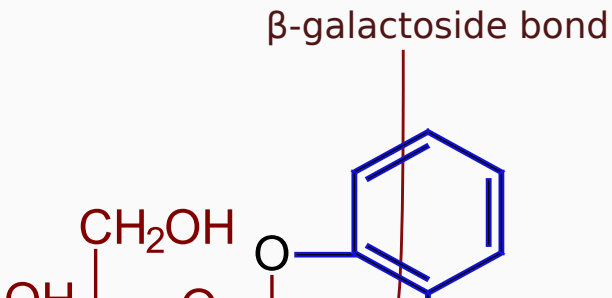
+ H_2O

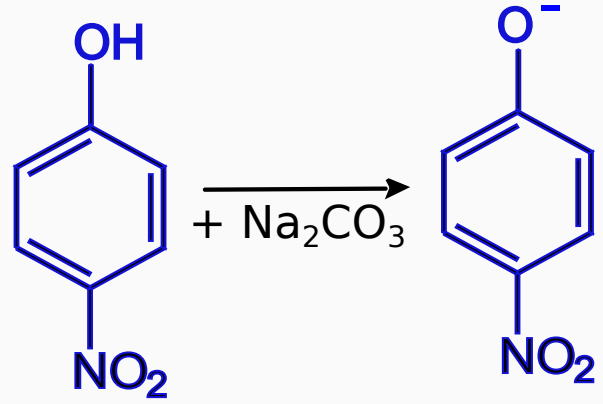


glucose



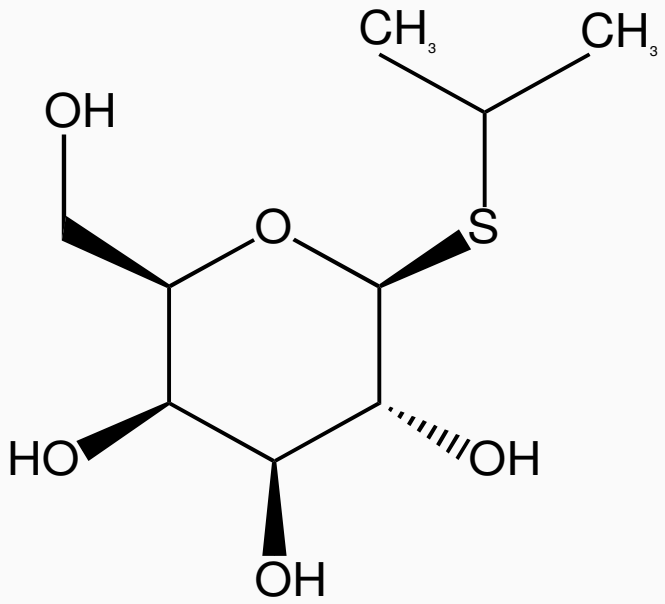
galactose





ortho-nitrophenol

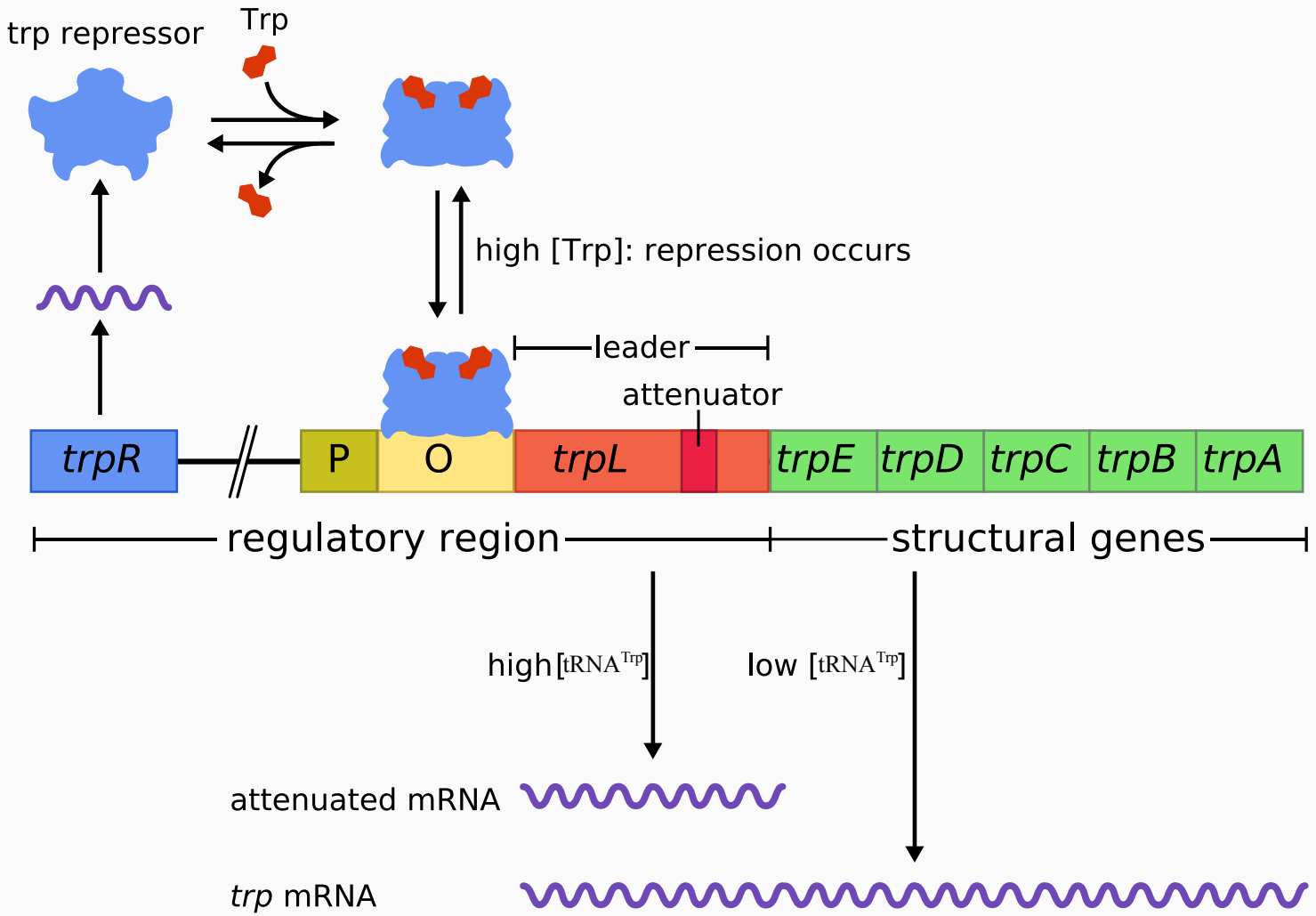
Gratuitous inducer of β-galactosidase



Isopropil-thio-galactoside

(IPTG)

The *trp* operon: a paradigm for regulation of anabolic operons



A bit of history

- Crick model about gene expression: rRNA (ribosomal RNA) is transcribed at the nucleus and transferred to the cytoplasm, where it interacts with the ribosomal proteins and "oversees" the synthesis of proteins
- On Good Friday (15th April 1960) an informal meeting at King's College dorms in London with: Sydney Brenner, Francis Crick, François Jacob, Ole Maaløe (Denmark) and Alan Garen (MIT) took place.
- Jacob described his and Monod's last results about the operon *lac*, Crick (as usual) asked many questions.

Crick own words:

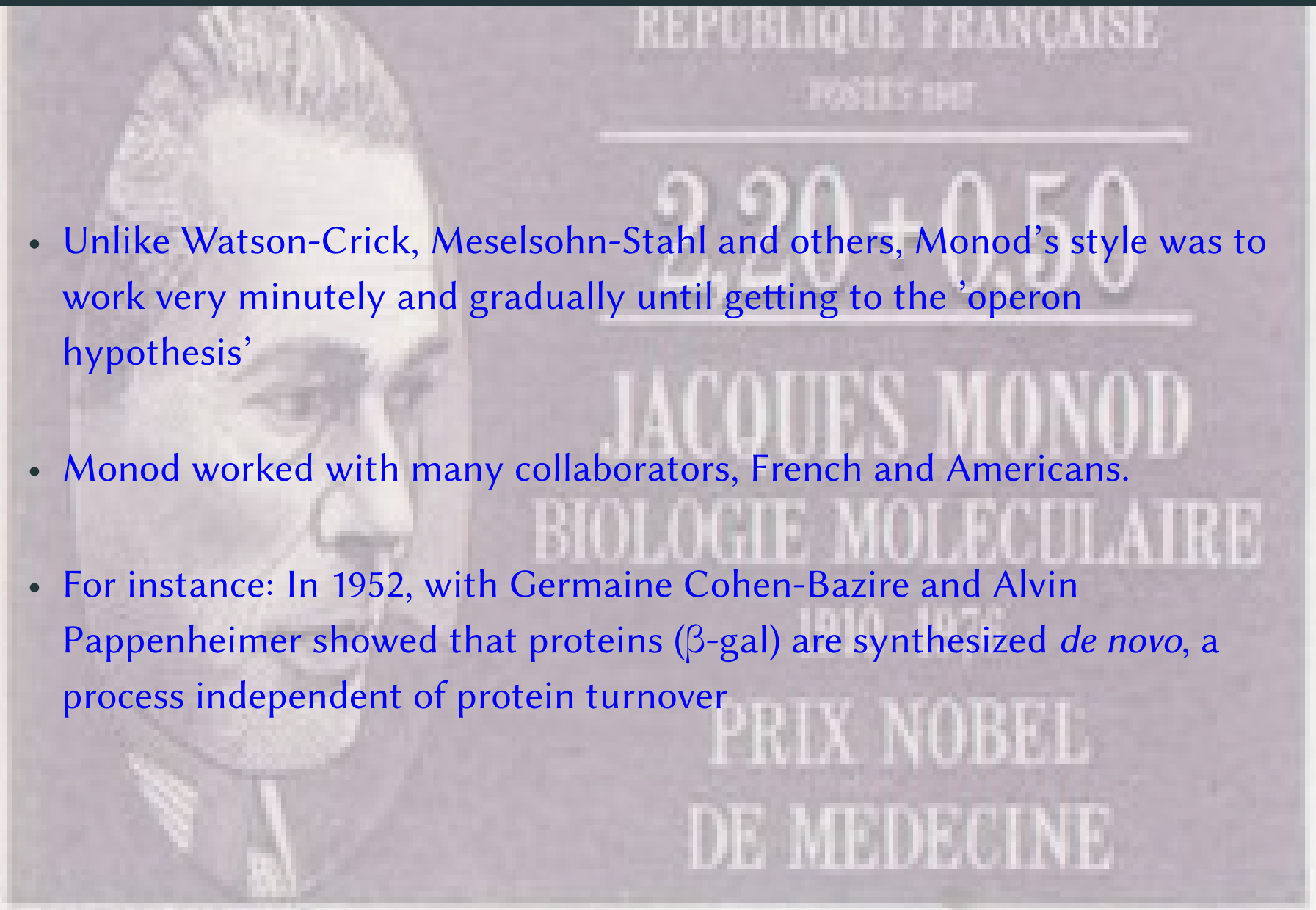
> *Just a single wrong assumption (that the ribosomal RNA was the messenger RNA) had completely messed up our thinking, so that it appeared as if we were wandering in a dense fog. I woke up that morning with only a set of confused ideas about the overall control of protein synthesis. When I went to bed all our difficulties had resolved and the shining answers stood clearly before us.*

- In the summer, Brenner, Jacob and Meselsohn conducted together an experiment that demonstrated the existence of mRNA (unstable RNA)

Jacques Monod (1910-1976)

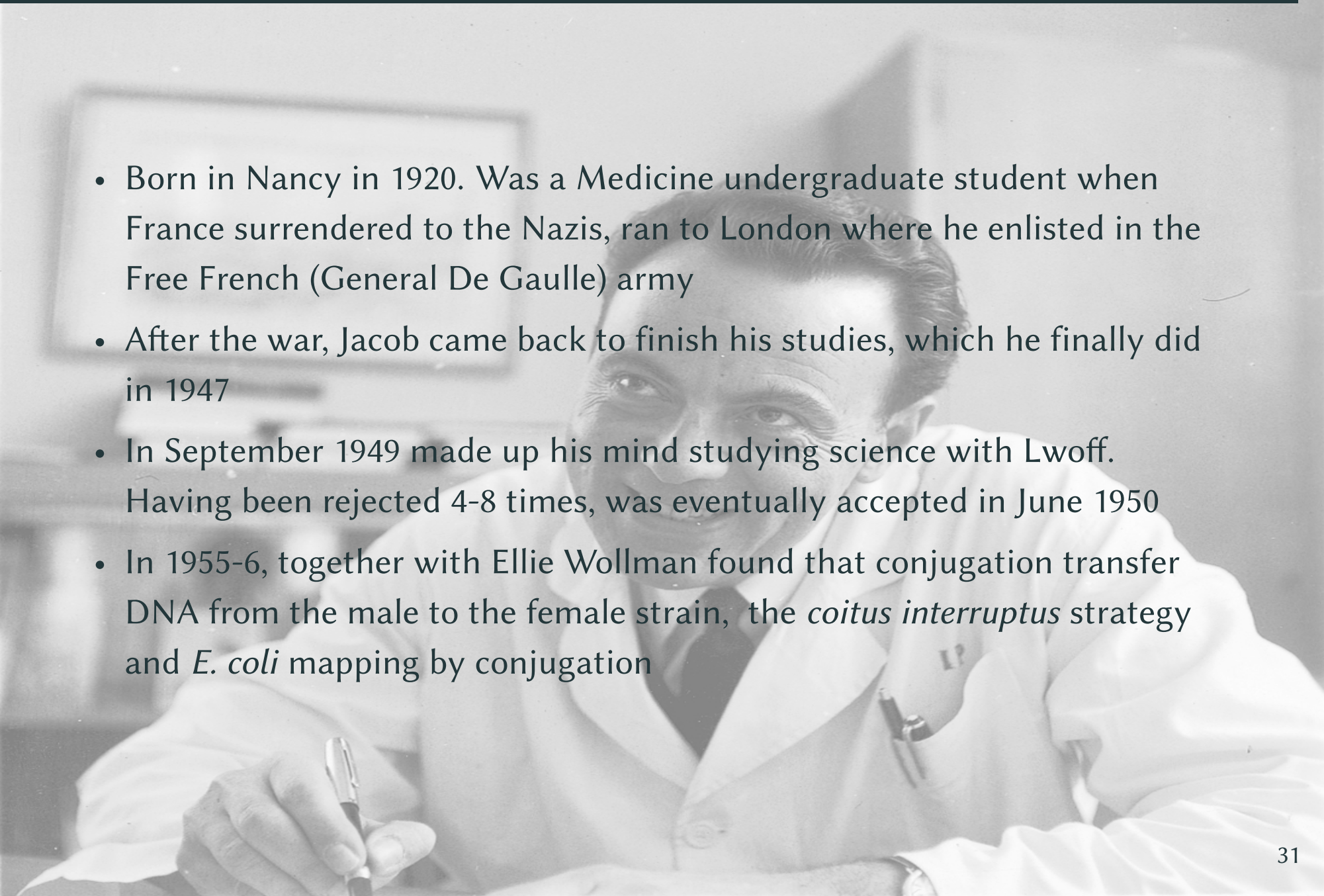
- Monod was born in Paris in 1910, from a French father and an American mother
- 1928-1931 undergraduation in Natural Science
- Spent 8 months of 1936 in Thomas Morgan's Laboratory at Caltech
- In 1937 began working with *E. coi* (cell growth) at the Pasteur Institute
- Found out the phenomenon of two-phases growth (diauxic growth)
- *"L'adaptation enzymatique? Connais pas!"*
- Joined the French Resistánce in 1940. In 1943 became the chief of resistance operations in Paris
- In 1941 received his Ph.D. with the thesis: 'Recherche sur la croissance des cultures bactériennes'

Jacques Monod (1910-1976)



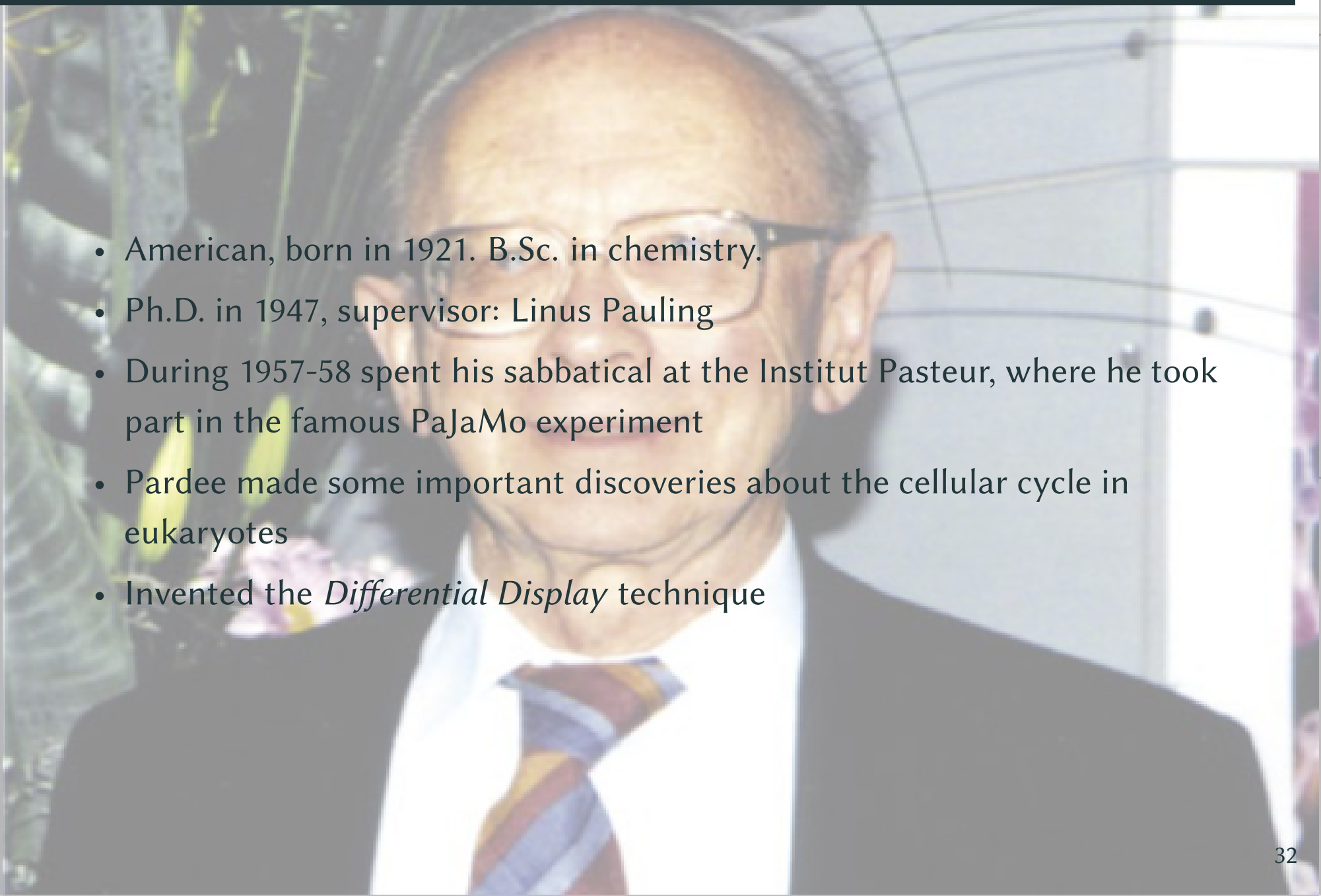
- Unlike Watson-Crick, Meselsohn-Stahl and others, Monod's style was to work very minutely and gradually until getting to the 'operon hypothesis'
- Monod worked with many collaborators, French and Americans.
- For instance: In 1952, with Germaine Cohen-Bazire and Alvin Pappenheimer showed that proteins (β-gal) are synthesized *de novo*, a process independent of protein turnover

François Jacob (1920-2013)



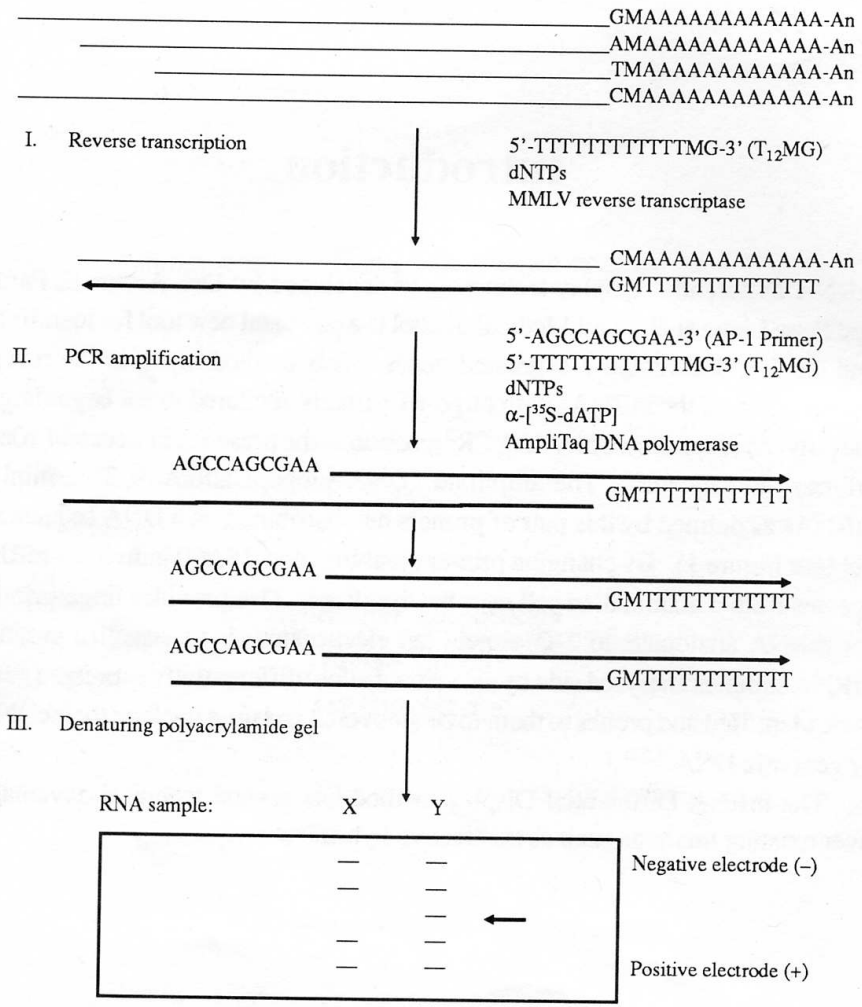
- Born in Nancy in 1920. Was a Medicine undergraduate student when France surrendered to the Nazis, ran to London where he enlisted in the Free French (General De Gaulle) army
- After the war, Jacob came back to finish his studies, which he finally did in 1947
- In September 1949 made up his mind studying science with Lwoff. Having been rejected 4-8 times, was eventually accepted in June 1950
- In 1955-6, together with Ellie Wollman found that conjugation transfer DNA from the male to the female strain, the *coitus interruptus* strategy and *E. coli* mapping by conjugation

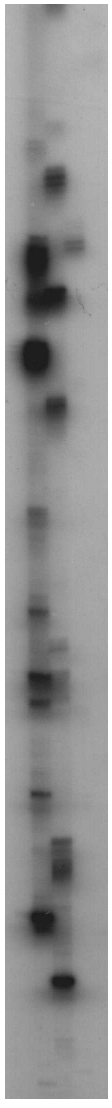
Arthur Pardee



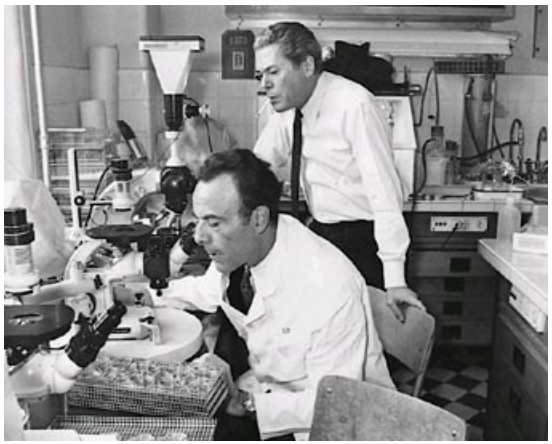
- American, born in 1921. B.Sc. in chemistry.
- Ph.D. in 1947, supervisor: Linus Pauling
- During 1957-58 spent his sabbatical at the Institut Pasteur, where he took part in the famous PaJaMo experiment
- Pardee made some important discoveries about the cellular cycle in eukaryotes
- Invented the *Differential Display* technique

Differential display

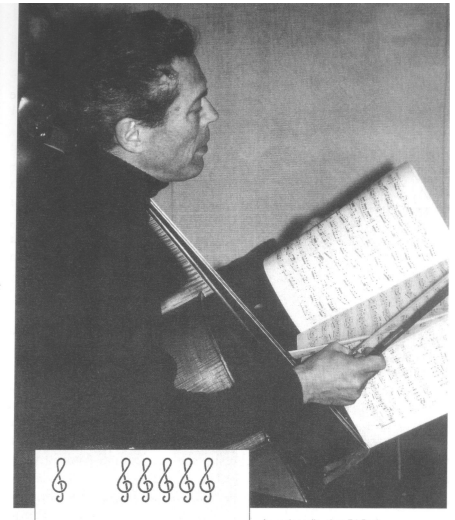




Photographs



Photographs



Au violoncelle, chez Ed Fischer, à Seattle en 1969.

« Science, musique et philosophie. »