Plasmids and Plasmid Biology

By

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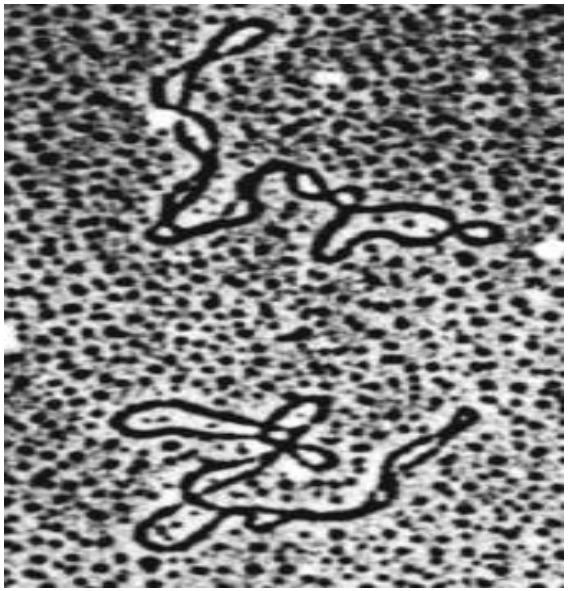


- Plasmid structure
- Plasmid replication and copy number control
- Plasmid transfer
- Plasmids as tools
- F plasmids

Plasmids

- Extrachromosomal DNA, usually circular-parasite?
- Usually encode ancillary functions for in vitro growth
- Can be essential for specific environments: virulence, antibiotics resistance, use of unusual nutrients, production of bacteriocins (colicins)
- Must be a replicon - self-replicating

Plasmids

- Plasmid DNA must replicate every time host cell divides or it will be lost
    a. DNA replication
    b. partitioning (making sure each progeny cells receives a plasmid)

- High copy plasmids are usually small; low copy plasmids can be large

- Partitioning is strictly controlled

Plasmids

- Plasmid replication requires host cell functions
- Copy number is regulated by initiation of plasmid replication
- Plasmids are incompatible when they cannot be stably maintained in the same cell because they interfere with each other's replication.

"Old School method of purifying plasmid"

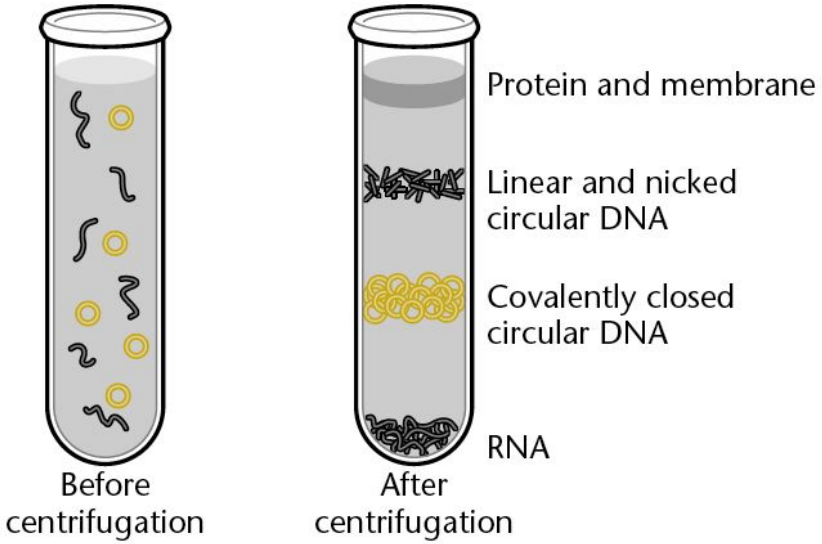
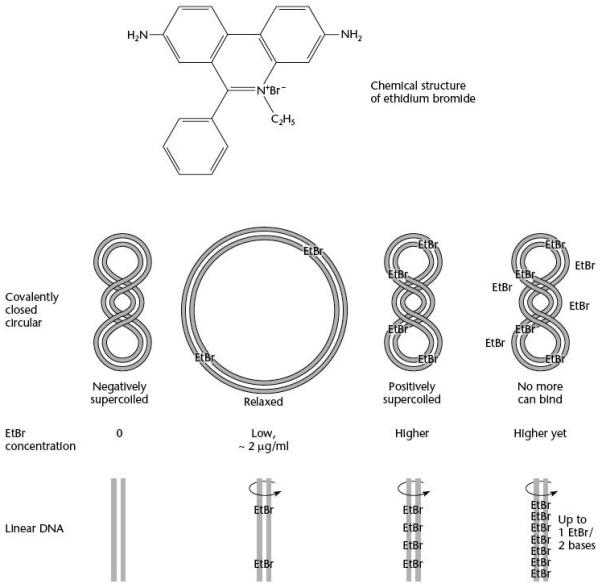
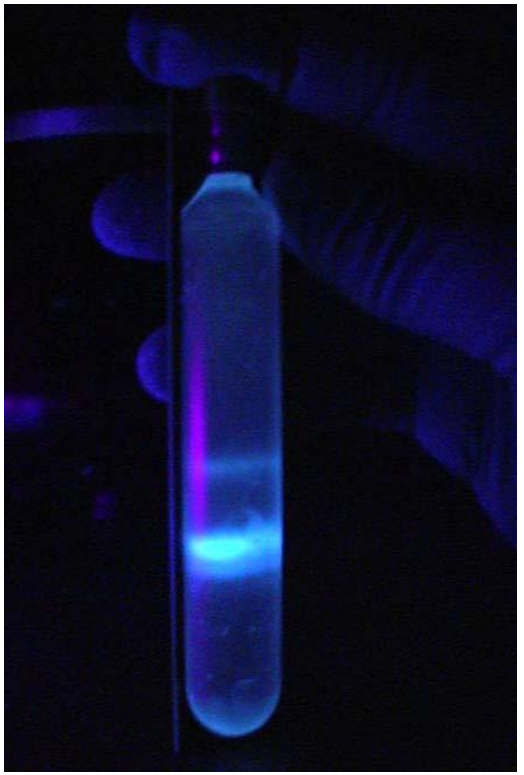


Figure 4.2



Three forms of plasmid DNA



CsCl gradient with ethidium bromide and UV light

TABLE 4.1 Some naturally occurring plasmids and the traits they carry

Plasmid	Trait	Original source
ColE1	Bacteriocin which kills *E. coli*	*E. coli*
Tol	Degradation of toluene and benzoic acid	*Pseudomonas putida*
Ti	Tumor initiation in plants	*Agrobacterium tumefaciens*
pJP4	2,4-D (dichlorophenoxyacetic acid) degradation	*Alcaligenes eutrophus*
pSym	Nodulation on roots of legume plants	*Rhizobium meliloti*
SCP1	Antibiotic methylenomycin biosynthesis	*Streptomyces coelicolor*
RK2	Resistance to ampicillin, tetracycline, and kanamycin	*Klebsiella aerogenes*

Virulence plasmids from *Salmonella, Shigella, Yersinia, B. anthracis, E.coli,* and others.

TABLE 4.2 Copy numbers of some plasmids

Plasmid	Approximate copy number
F	1
P1 prophage	1
RK2	4–7 (in *E. coli*)
pBR322	16
pUC18	~30–50
pIJ101	40–300

Table 11-1 Examples of some plasmids and their properties

Plasmid	Size (Kb)	Number of copies per chromosome	Self-transmissible	Phenotypic features
Col plasmids				
ColE1	6.4	10–15	No	Colicin E1 disrupts energy gradient, host immunity to Colicin E1
ColE2	7.6	10–15	No	Colicin E2 is a DNase, host immunity to Colicin E2
ColE3	7.6	10–15	No	Colicin E3 is a ribosomal RNase, host immunity to Colicin E3
F plasmid	94.5	1–2	Yes	F-pilus, conjugation
R plasmids				
R100	106.7	1–2	Yes	Cam^r Str^r Sul^r Tet^r
RK2	56.0	5–8	Yes	Broad host range
pSC101	9.0	<5	No	Low copy number, compatible with ColE1-type plasmids, Tet^r
Phage plasmid				
λdv	6.4	50	No	λ genes *cro, cI, O, P*
Recombinant plasmids				
pBR322	4.4	20	No	Medium copy number, ColE1-type replication, Amp^r
pUC18	2.7	200–500	No	High copy number, ColE1-type replication with a mutation that increases the copy number, Amp^r
pACYC184	4.0	10–12	No	Cam^r Tet^r

Plasmid replication

- Plasmid replication requires host DNA replication machinery.
- Most wild plasmids carry genes needed for transfer and copy number control.
- All self replication plasmids have a *oriV*: origin of replication
- Some plasmids carry and *oriT*: origin of transfer. These plasmids will also carry functions needed to be mobilized or *mob* genes.

Plasmid replication

- Plasmid segregation is maintained by a *par* locus-a partition locus that ensures each daughter cells gets on plasmid. Not all plasmids have such sequences.

- There are 5 main "incompatibility" groups of plasmid replication. Not all plasmids can live with each other.

- Agents that disrupt DNA replication destabilize or cure plasmids from cells

Antisense RNA gene control.

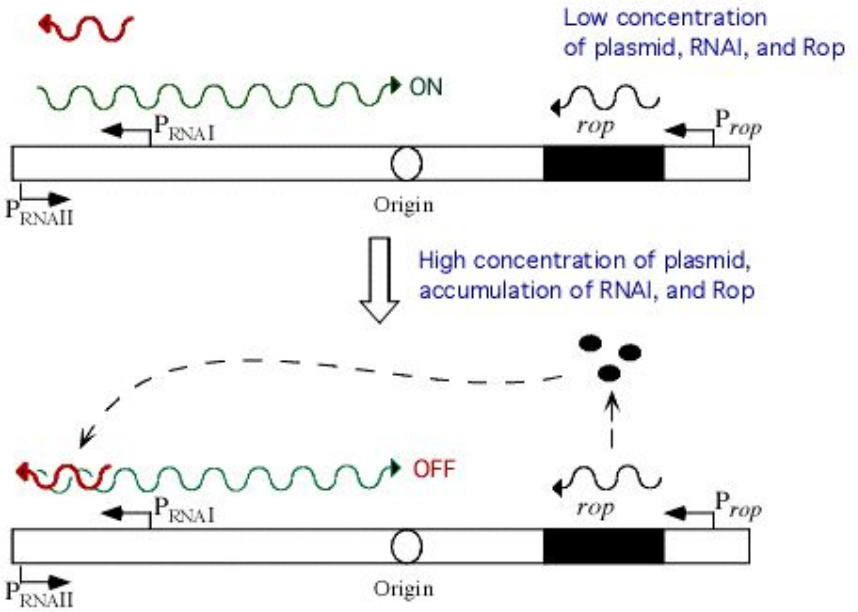
-the RNA-RNA hybrid is very stable

-blocks most translation and tanscription

-requires RNAases to degrade

-common theme in bacterial gene regulation as we are learning

Anti-sense RNA replication control



RNA I-small inhibitory RNA that binds to RNAII. RNAII will act as a primer for DNA replication

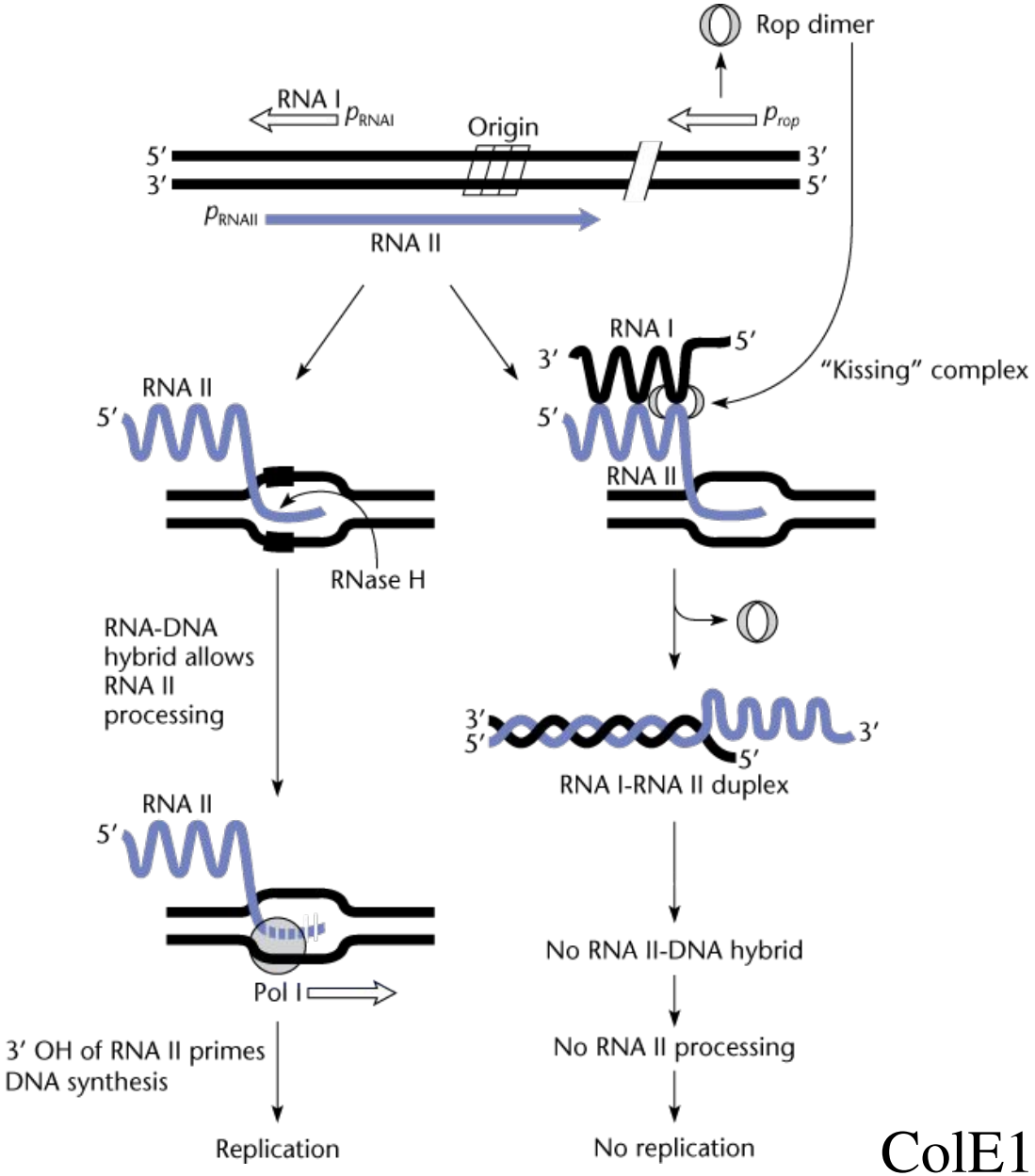
Rop: plasmid encoded proteins which stabilizes the RNAI-RNAII complex

Antisense RNA: RNA-RNA hybrid blocks replication

GGCUAAUUCC Antisense RNA is also used in euks called

CCGAUUAAGG siRNA

Blocking RNA priming for DNA PolI prevents replication

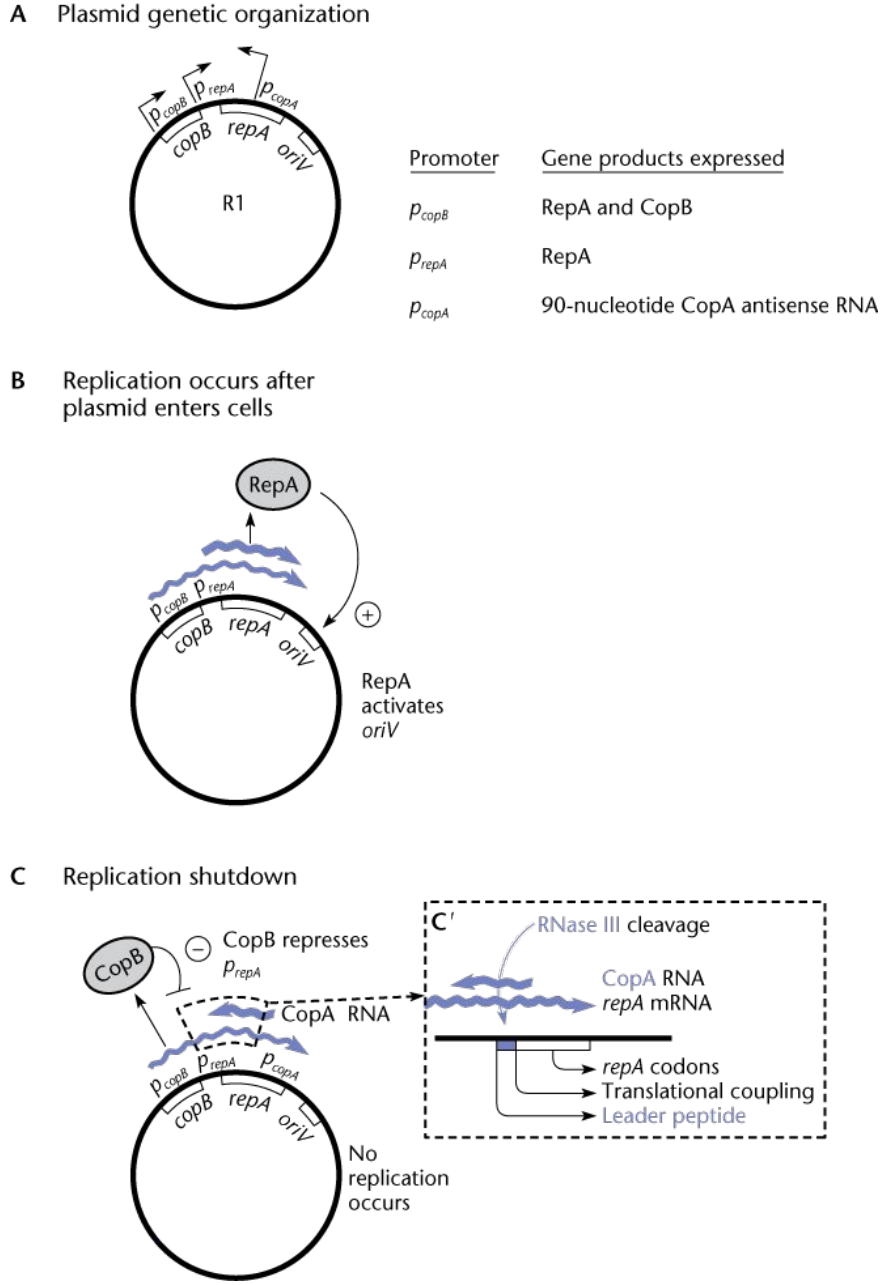


ColE1 Replication

Control-an example of primer control of replication

- RNAII will serve as a primer for the replication fork.
- The 3' end is processed by host RnaseH to allow efficient RNA-DNA hybrid to form
- The hybrid acts as a primer for host Pol1
- As the concentration of plasmid increases, Rop does also
- Rop stabilizes the RNA1-II complex
- No RNA for replication priming.

ColE1 replication does not need plasmid encoded rep proteins

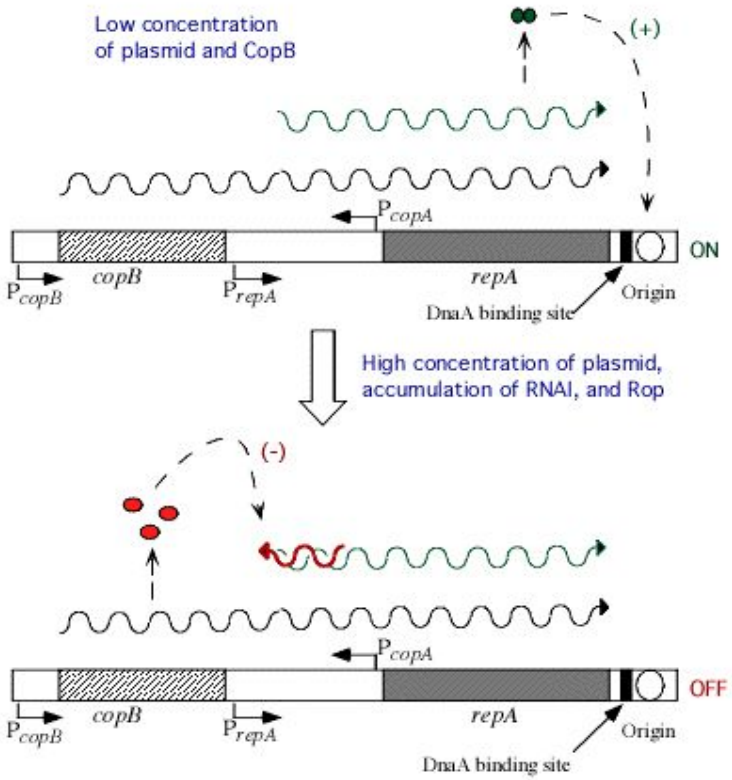


The events upon entry into a cell

- RepA mRNA is made from Prep until copy number becomes high
- CopB expression increase an Cop represses RepA expression at PrepA
- CopA now is made-a 90base antisense RNA
- CopA binds to 5-end of the RepA mRNA, forming dsRNA
- This is recognized by host RNAaseIII and degraded.

Thus concentration of RepA protein is maintained by rate of RNA-RNA hybrid formation.

Rep-protein control -R1 family of plamsids.



Plasmid copy control balanced by host RNaseIII activity and transcription from the plasmid

- Rep-protein expression controlled by antisense CopA
- PcopB-encodes Rep and CopB
- PcopA-encodes antisense RNA
- plasmid replicates to high level
- CopB levels rise, shutting off RepA production
- antisense RNA from PcopA made

Iteron Plasmids: Handcuffing RK2 and other broad host range plasmids

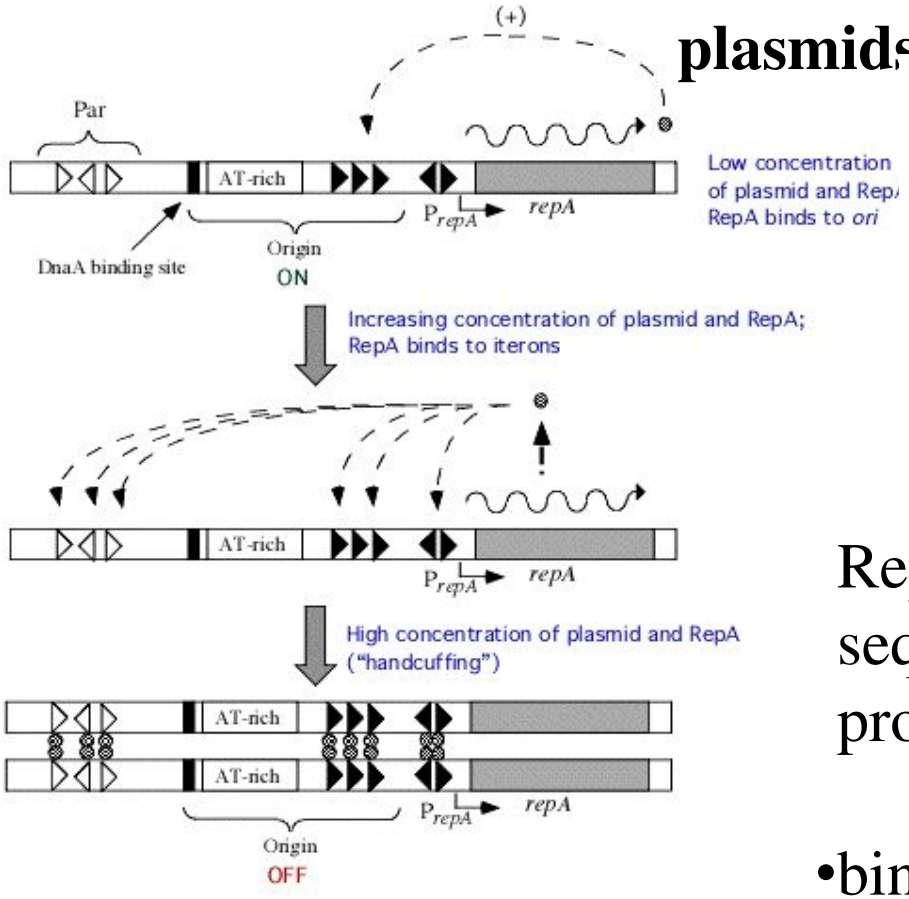
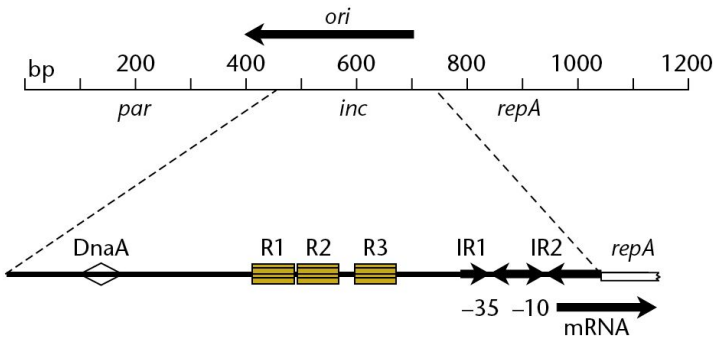


Figure 4.8



RepA is able to bind the repeat sequences upstream of the promoter region for *repA*.

- binding causes two plasmid molecules to couple "handcuff"
- prevents replication.

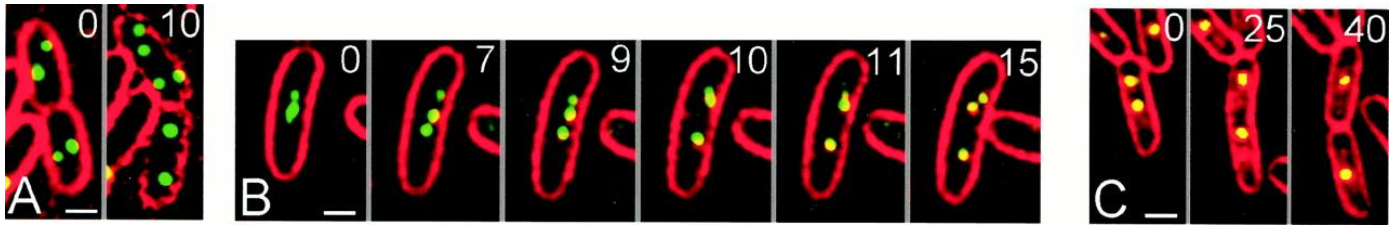
"copy up" mutants: mutations in RepA that are less able to bind to each other.

Incompatibility Groups

- Not all plasmids can live together.
- Plasmids that are able to coexist in the same cell do not interfere with each other's replication
- A single cell can have as many Inc group plasmids as it can tolerate and replicate!

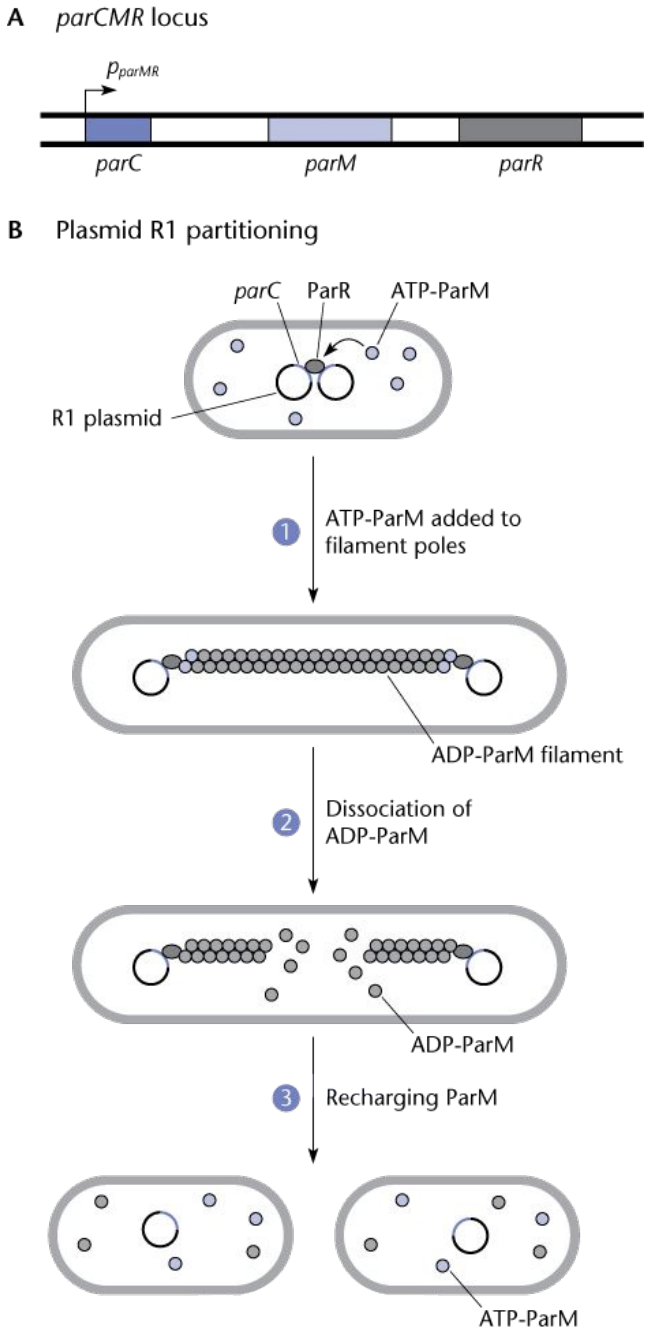
Partion Locus: a region on broad host range plasmids that binds to a structure on the inner membrane of the cell to ensure proper segregation.

Plasmids labeled with fluorescent protein
-move to each daughter cell during division.



Pogliano, Joe et al. (2001) Proc. Natl. Acad. Sci. USA 98, 4486-4491

Figure 4.18



Par locus

- think of this as a primitive centromere
- the growing filaments push the plasmids to the opposite poles of the cells

Plasmids as genetic tools: Construction of Mutants

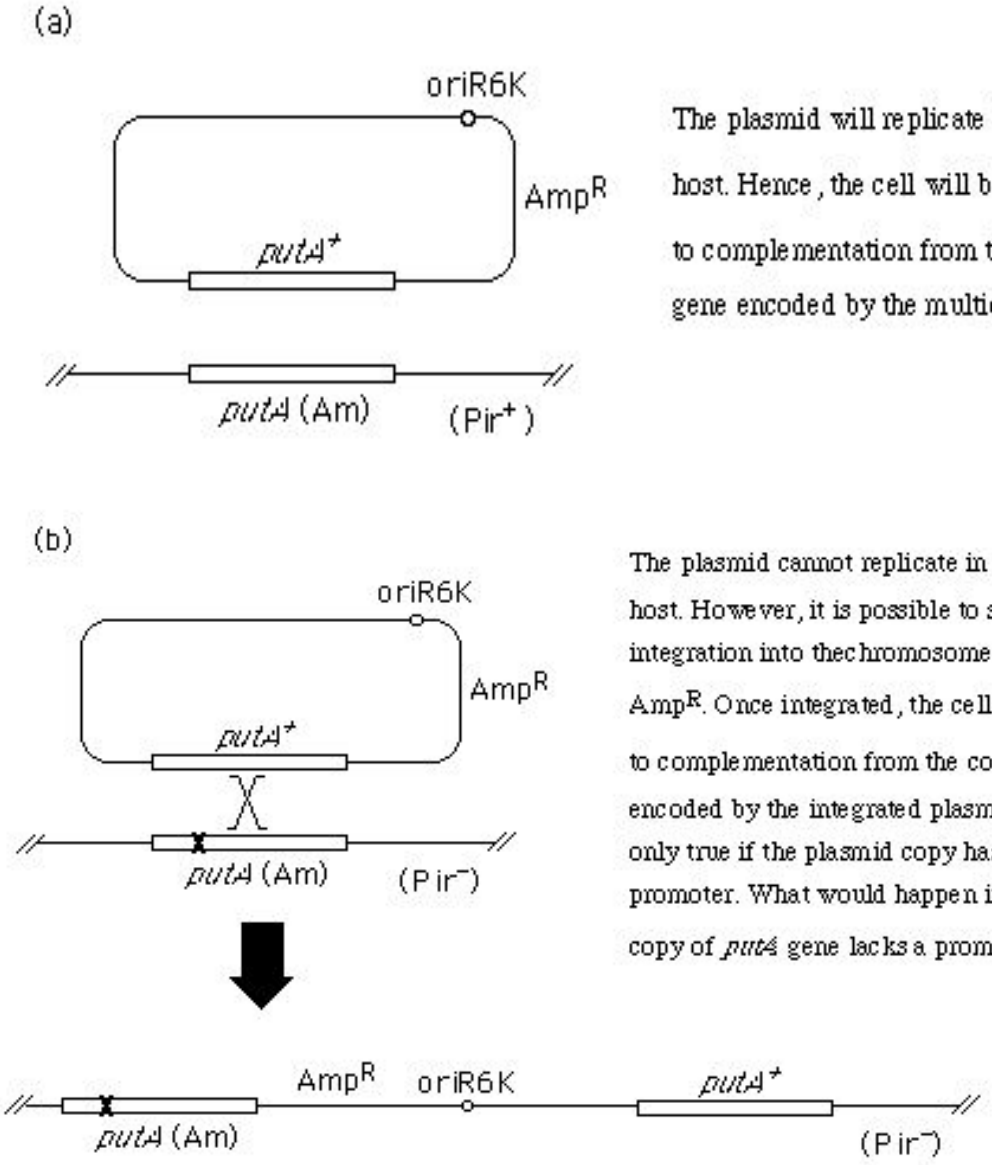
Site-directed mutation: Suicide plasmds

1. Plasmid must be unable to replicate without essential replication proteins provide *in trans.*
2. It helps if the plasmid can be mobilized-*oriT* required
3. Need a selectable marker
4. Large or small region of homologous DNA cloned that will integrate into the chromosomal target.
5. Need a counter selection method to kill the donor cells
6. Screen for what you think is correct.

Also, merodiploid reporter strains can be constructed in this manner

1. Make a *lacZ* fusion to your promoter of interest
2. Clone into a suicide plasmid
3. Mate into recipient.
4. Resulting strain will harbor a duplication of the promoter region:*lacZ* and still have a functional copy of the gene.

Why would this be important?



R6K: broad host plasmid.
-Pir is the essential replication protein
-*pir* mutants cannot replicate unless supplied *in trans*.
-integration into the chromosome is selected for by growth on ampicillin

How could you make targeted mutant using this method?

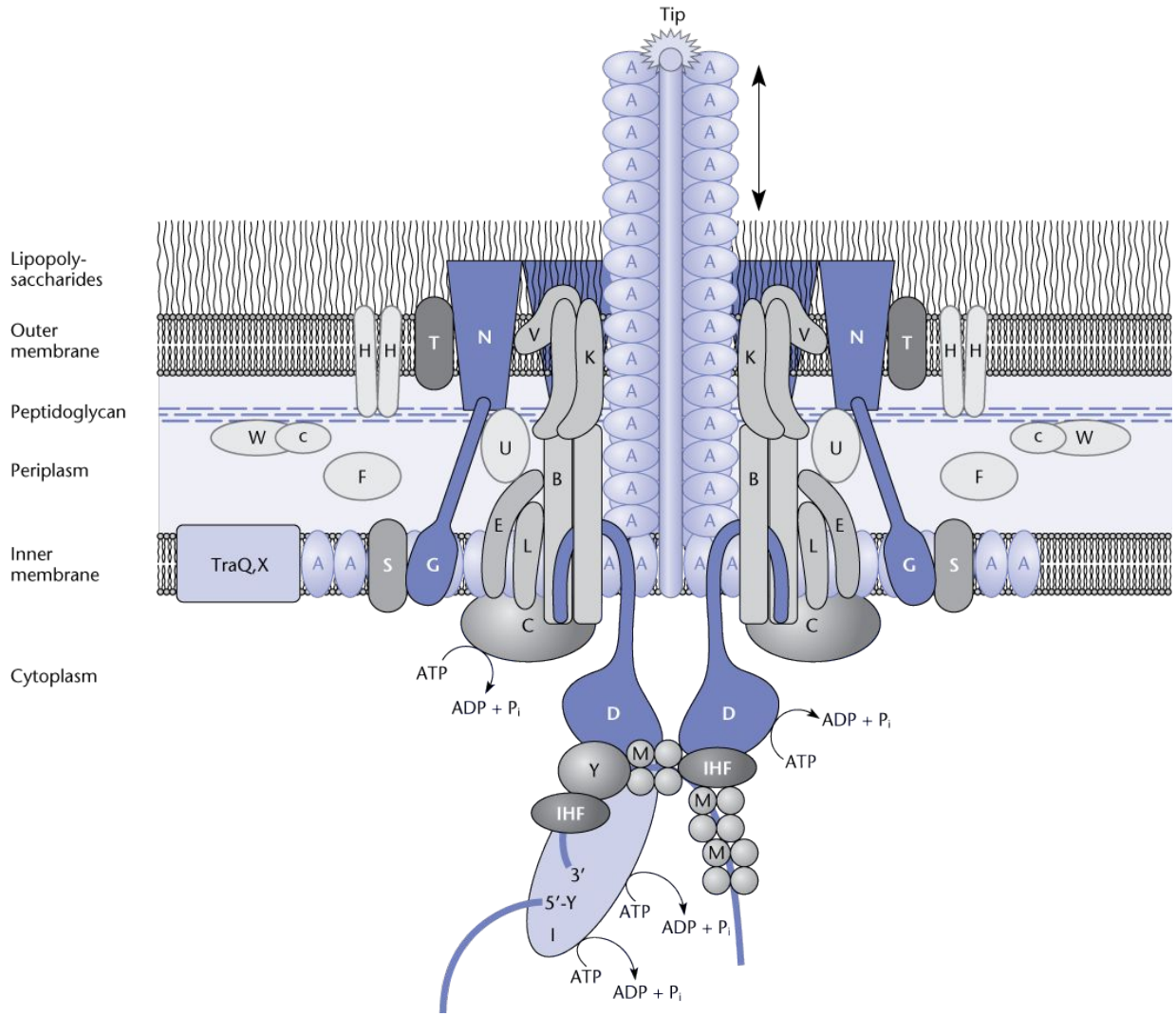
F-plas
mid

1. large (100 kb)
2. low copy (1-2 copies/cell)
3. self transmissible
4. requires protein synthesis (chloramphenicol-sensitive)
5. *repE* gene encodes RepE protein
6. RepE protein binds to origin of replication (*oriS*) and initiates DNA replication
7. RepE binds to the *repE* promoter and activates transcription
8. RepE binds to the *copA/incC* locus binding copies of F together via RepE - inhibiting replication (coupling)

TABLE 5.1 Some F-plasmid genes and sites

Symbol	Function
ccdAB	Inhibition of host cell division
incBCE	Incompatibility
oriT	Site of initiation of conjugal DNA transfer
oriV	Origin of bidirectional replication
sopAB	Partitioning
traABCEFGHKLQUVWX	Pilus biosynthesis, assembly
traGN	Mating-pair stabilization
traD	Coupling protein
traI	Relaxase
traYM	Accessories for relaxosome
traJ, finOP	Regulation of transfer
traST	Entry exclusion

F Pilus assembly



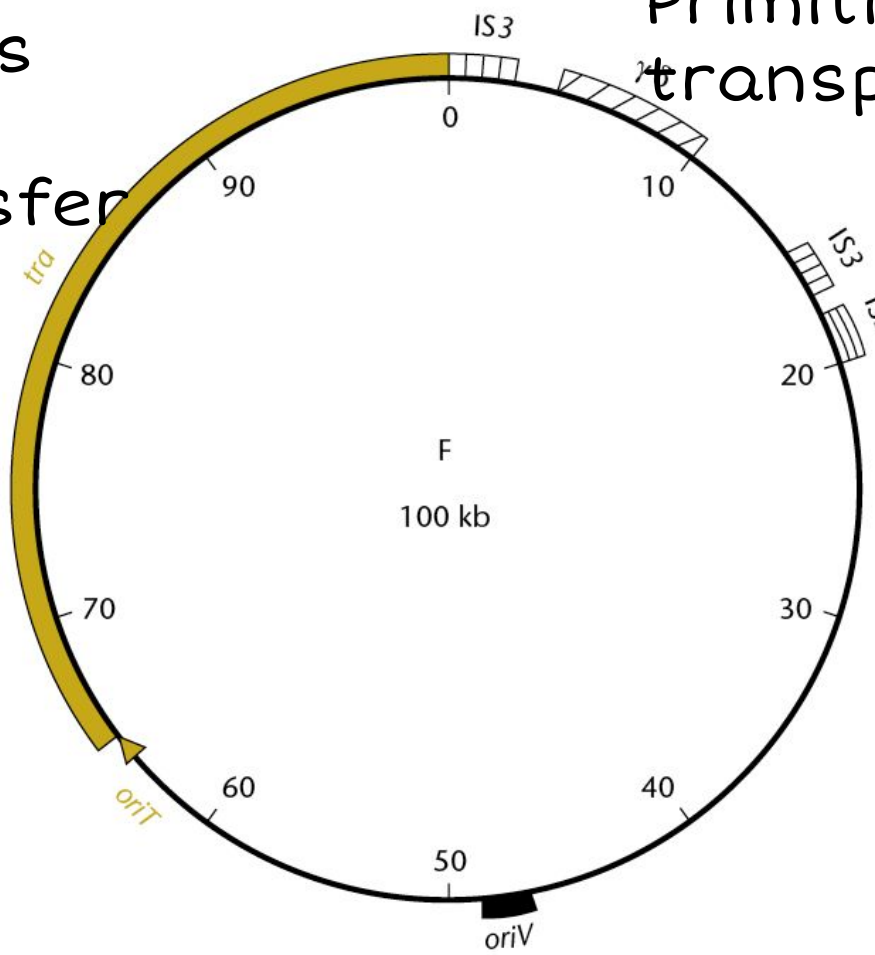
Genetic organization of F

Figure 5.6

30+ genes needed For transfer

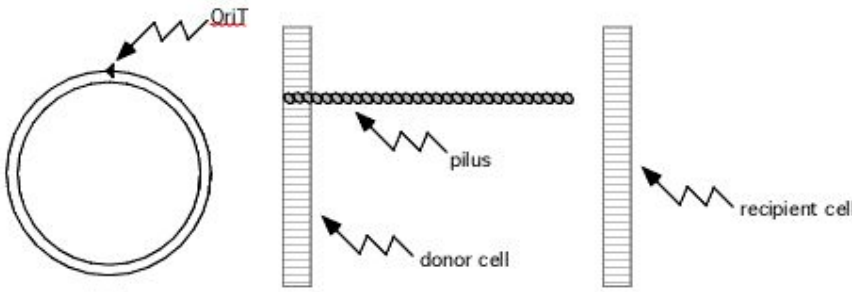
Primitive transposon

IS elements

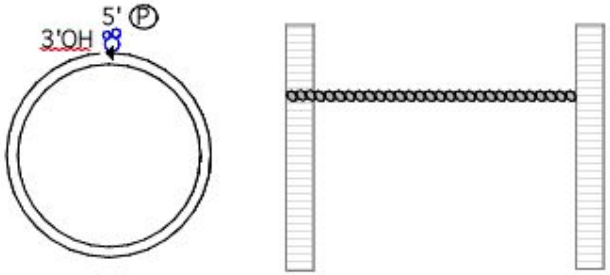


replicati on

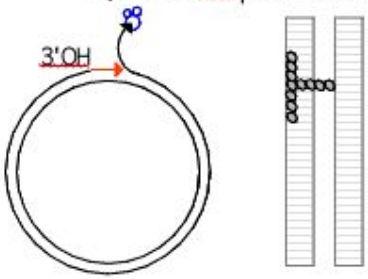
F-transfer at fine detail



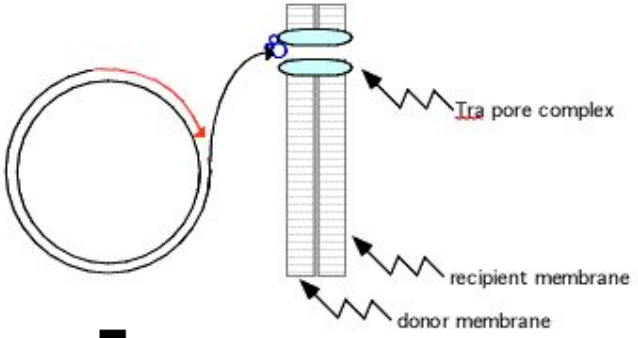
Contact between donor and recipient cells.
DNA relaxase ( ) nicks at oriT and covalently binds to 5' Ⓟ



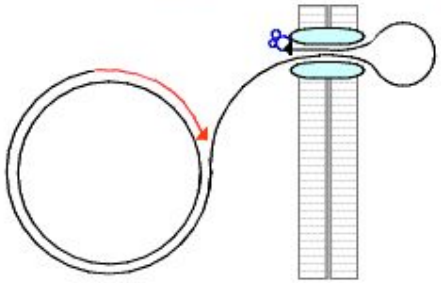
Pilus retracks, bringing donor and recipient into close proximity and Tra proteins form a pore complex that spans the membranes



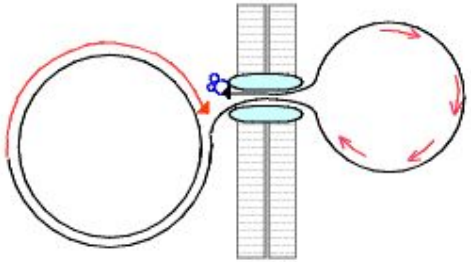
Rolling circle DNA replication initiates at 3'OH and proceeds 5' to
Membranes brought into close proximity to form mating bridge.
Relaxase interacts with membrane Tra pore complex



DNA replication pushes the ssDNA into the recipient cell



Lagging strand DNA replication in recipient cell converts ssDNA to dsDNA



Upon complete replication of plasmid, the old and new oriT sites "collide", and nicking between oriT sites occurs

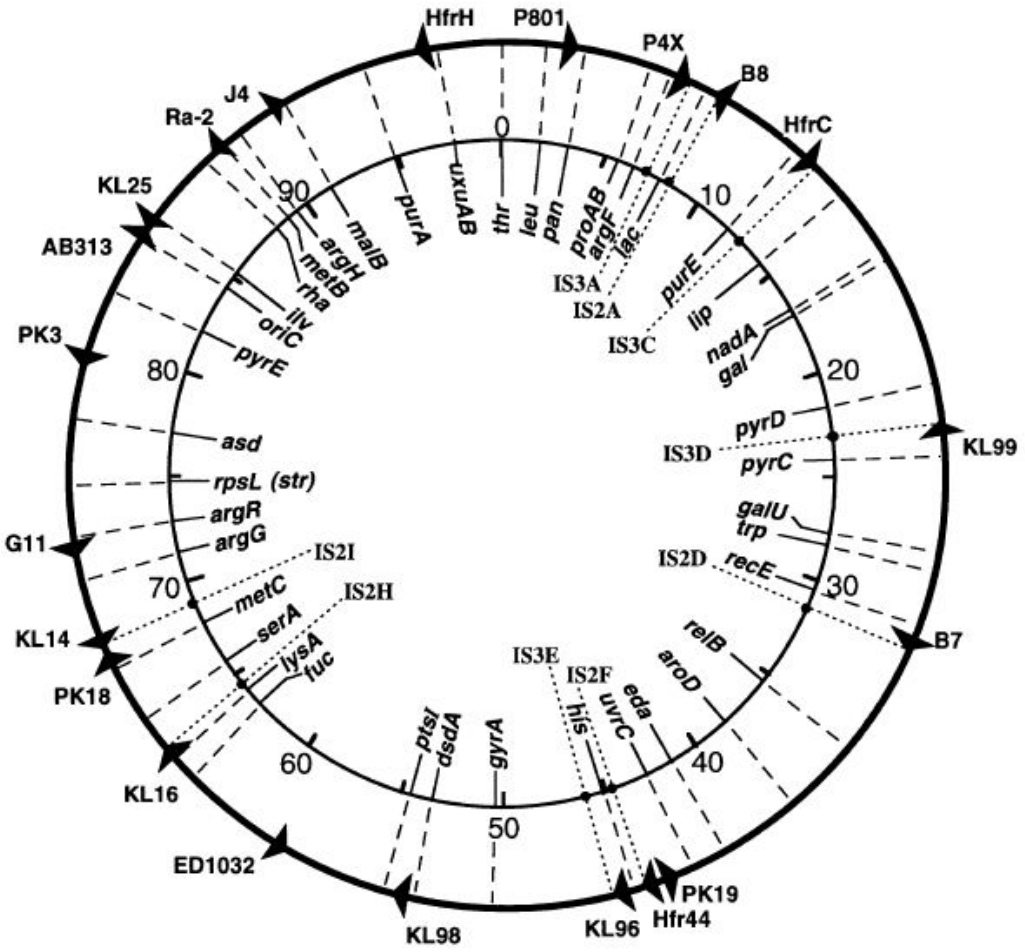


FIGURE 1 Approximate map positions of integrated sex factors (F, F_{ts}-lac, of ColV) for some Hfr strains. See Table 1 for commonly used derivatives of these strains. The sequence of chromosomal genes transferred from a given strain begins behind the arrowhead; e.g., HfrH transfers genes in the order *uxu*AB, *thr*, *leu*, etc. The positions of the IS sequences which appear to correlate with the sites of F insertion for some of the Hfrs are indicated and can be found on the physical map in chapter 129.