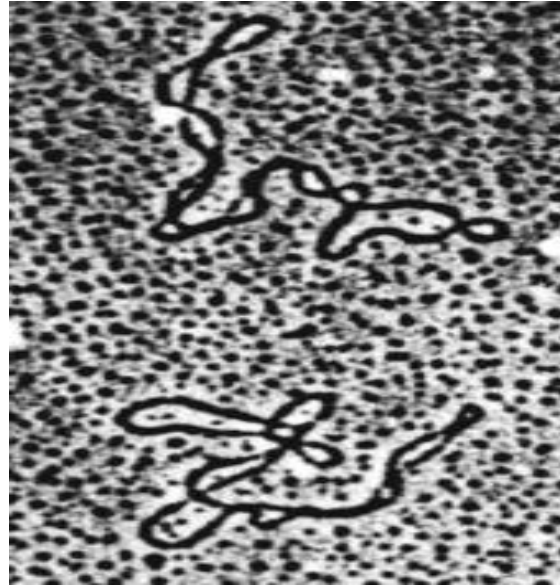


# Plasmids and Plasmid Biology

By

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# Plasmids and Plasmid Biology



- 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"

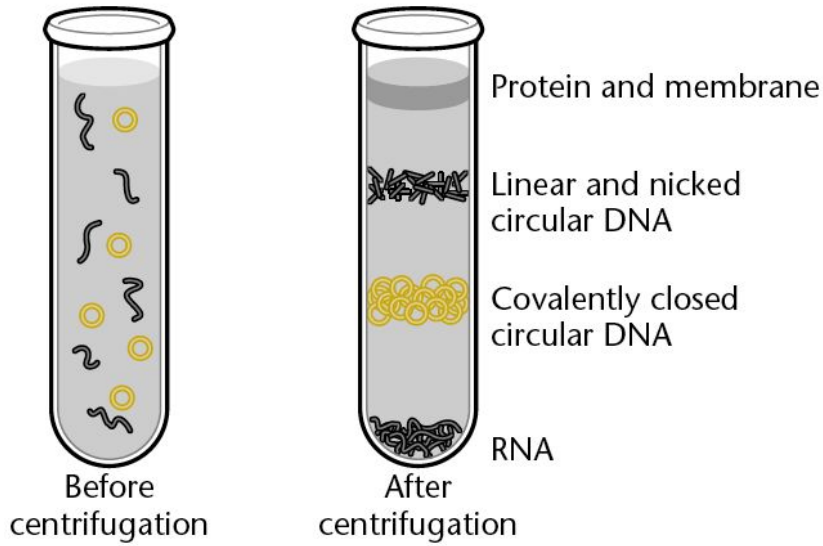
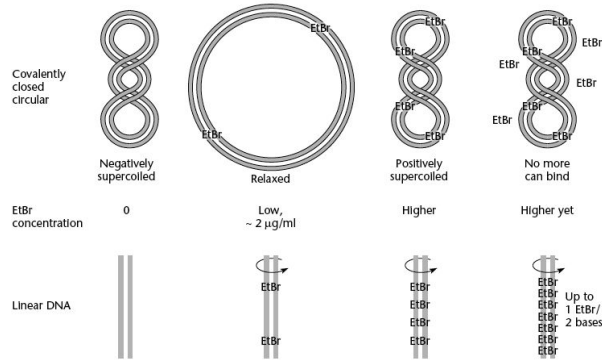
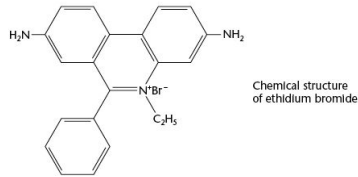


Figure 4.2



Three forms of plasmid DNA



CsCl gradient with ethidium bromide and UV light

<b>TABLE 4.1</b> Some naturally occurring plasmids and the traits they carry		
<b>Plasmid</b>	<b>Trait</b>	<b>Original source</b>
ColE1	Bacteriocin which kills <i>E. coli</i>	<i>E. coli</i>
Tol	Degradation of toluene and benzoic acid	<i>Pseudomonas putida</i>
Ti	Tumor initiation in plants	<i>Agrobacterium tumefaciens</i>
pJP4	2,4-D (dichlorophenoxyacetic acid) degradation	<i>Alcaligenes eutrophus</i>
pSym	Nodulation on roots of legume plants	<i>Rhizobium meliloti</i>
SCP1	Antibiotic methylenomycin biosynthesis	<i>Streptomyces coelicolor</i>
RK2	Resistance to ampicillin, tetracycline, and kanamycin	<i>Klebsiella aerogenes</i>

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

<b>TABLE 4.2</b> Copy numbers of some plasmids	
<b>Plasmid</b>	<b>Approximate copy number</b>
F	1
P1 prophage	1
RK2	4–7 (in <i>E. coli</i> )
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
<i>Col plasmids</i>				
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
<i>F plasmid</i>	94.5	1–2	Yes	F-pilus, conjugation
<i>R plasmids</i>				
R100	106.7	1–2	Yes	Cam <sup>r</sup> Str <sup>r</sup> Sul <sup>r</sup> Tet <sup>r</sup>
RK2	56.0	5–8	Yes	Broad host range
pSC101	9.0	<5	No	Low copy number, compatible with ColE1-type plasmids, Tet <sup>r</sup>
<i>Phage plasmid</i>				
$\lambda$ dv	6.4	50	No	$\lambda$ genes <i>cro</i> , <i>ci</i> , <i>O</i> , <i>P</i>
<i>Recombinant plasmids</i>				
pBR322	4.4	20	No	Medium copy number, ColE1-type replication, Amp <sup>r</sup>
pUC18	2.7	200–500	No	High copy number, ColE1-type replication with a mutation that increases the copy number, Amp <sup>r</sup>
pACYC184	4.0	10–12	No	Cam <sup>r</sup> Tet <sup>r</sup>



# 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 a *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 cell gets one 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

**A**

Antisense RNA

3'-UGUGCUCUCCAUAUGCUACCUUG-5'

← Antisense RNA

DNA

TATAATGCTACGTACACGAGGAGGTATACGATGGAACGCATTAGATTATA . . .  
 ATATTACGATGCATGTGCTCCTCCATATGCTACCTTGCGTAATCTAATAT . . .

←  $P_{\text{Antisense RNA}}$

$P_{\text{mRNA}}$  →

RNA

5'-ACACGAGGAGGUUAUACGAUGGAACGCAUUAGAUUAUA . . .

RNA →

**B**

Antisense RNA

3'-UGUGCUCUCCAUAUGCUACCUUG-5'

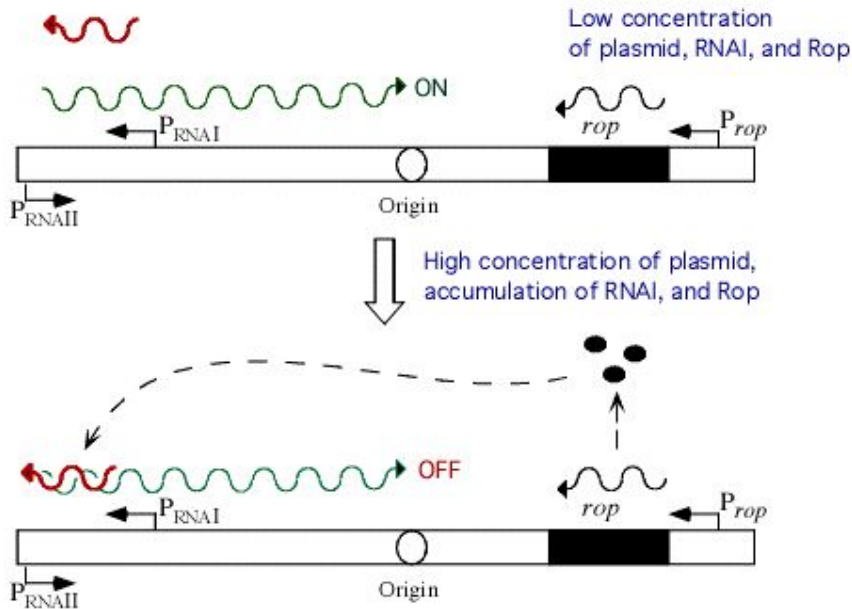
RNA

5'-ACACGAGGAGGUUAUACGAUGGAAC . . .

## **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



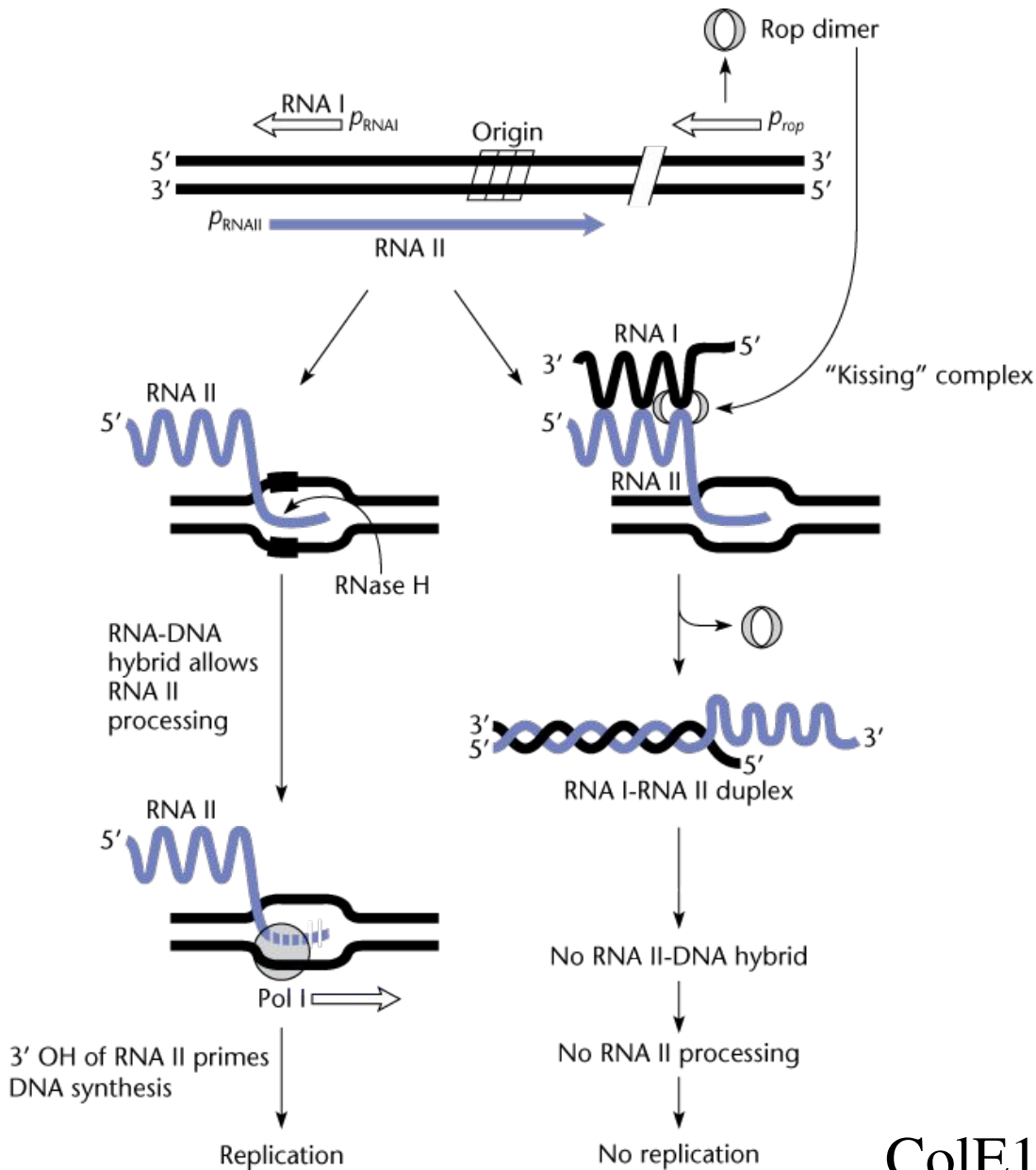
RNA I-small inhibitory RNA that binds to RNAII. RNAII will act as a primer for DNA replication  
 Rop: plasmid encoded proteins which stabilizes the RNAI-RNAII complex

Antisense RNA: RNA-RNA hybrid blocks replication

GGCUAAUUC      Antisense RNA is also used in euks  
 called

CCGAUUAAGG      siRNA

Blocking RNA priming for DNA PolI prevents replication



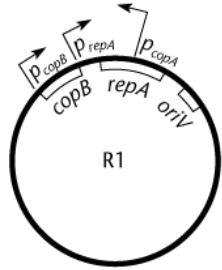
## ColE1 Replication

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.

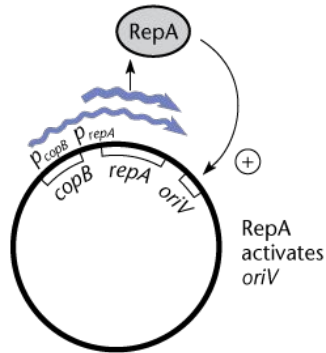
ColE1 replication does not need plasmid encoded rep proteins

A Plasmid genetic organization

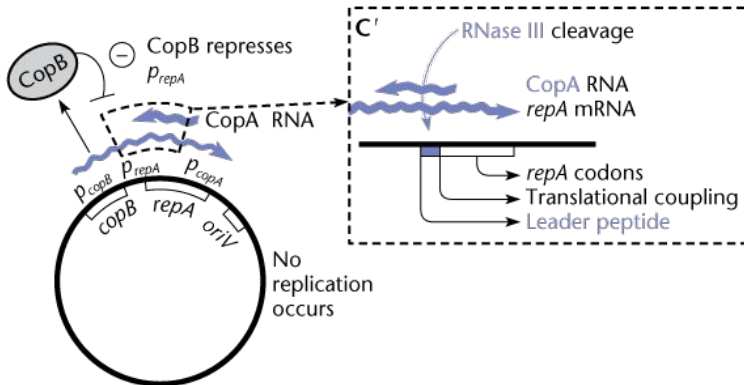


Promoter	Gene products expressed
$P_{copB}$	RepA and CopB
$P_{repA}$	RepA
$P_{copA}$	90-nucleotide CopA antisense RNA

B Replication occurs after plasmid enters cells



C Replication shutdown

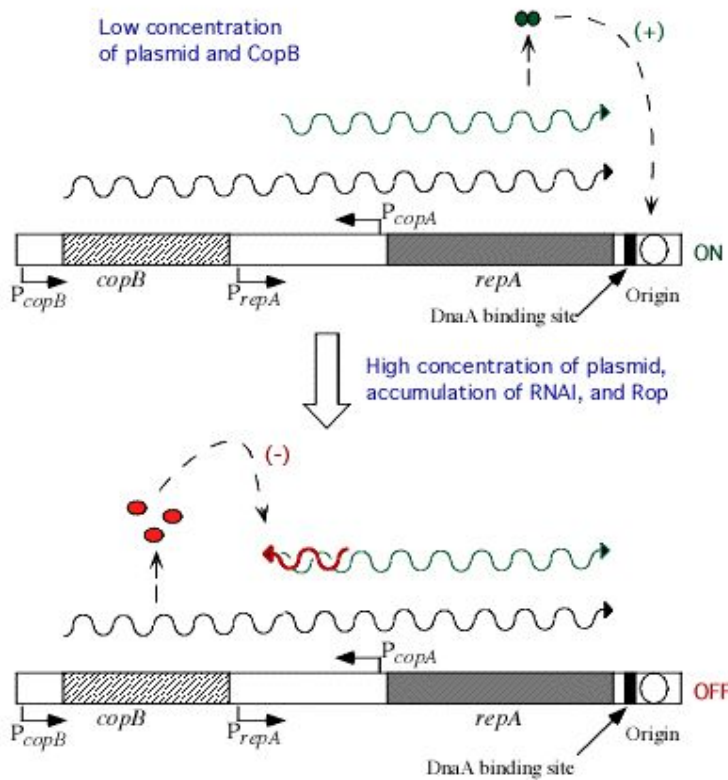


The events upon entry into a cell

- RepA mRNA is made from  $P_{repA}$  until copy number becomes high
- CopB expression increases and CopB represses RepA expression at  $P_{repA}$
- CopA now is made—a 90-base 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.

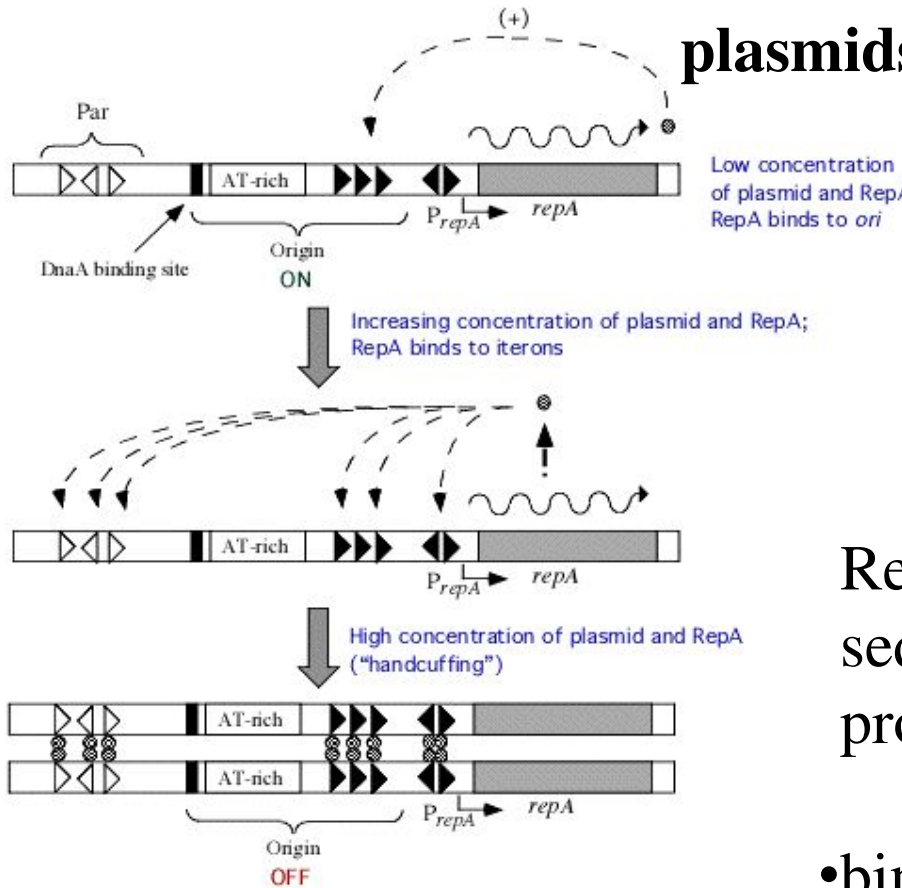
# Rep-protein control -R1 family of plasmids.



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

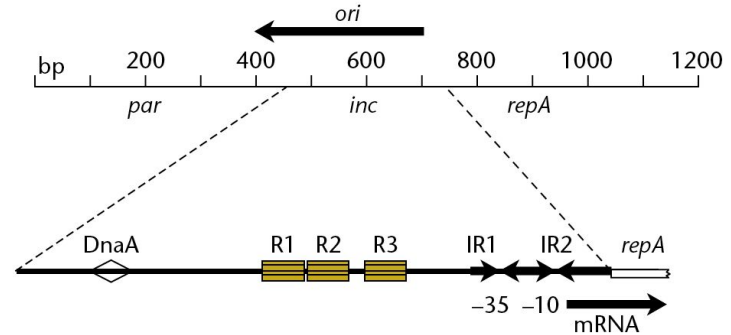
- Rep-protein expression controlled by antisense CopA
- $P_{copB}$ -encodes Rep and CopB
- $P_{copA}$ -encodes antisense RNA
- plasmid replicates to high level
- CopB levels rise, shutting off RepA production
- antisense RNA from  $P_{copA}$  made

# Iteron Plasmids: Handcuffing RK2 and other broad host range plasmids



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

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.

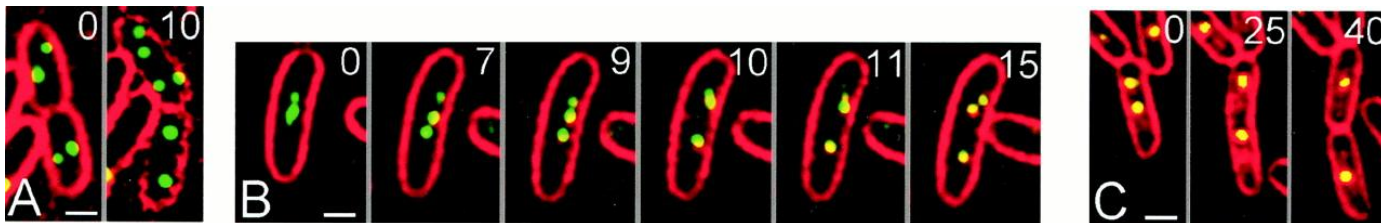


## Incompatibility Groups

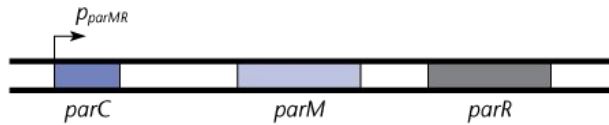
- Not all plasmids can live together.
- Plasmids that are able to coexist in the same cell do not interfere with each other's replication
- A single cell can have as many Inc group plasmids as it can tolerate and replicate!

**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.

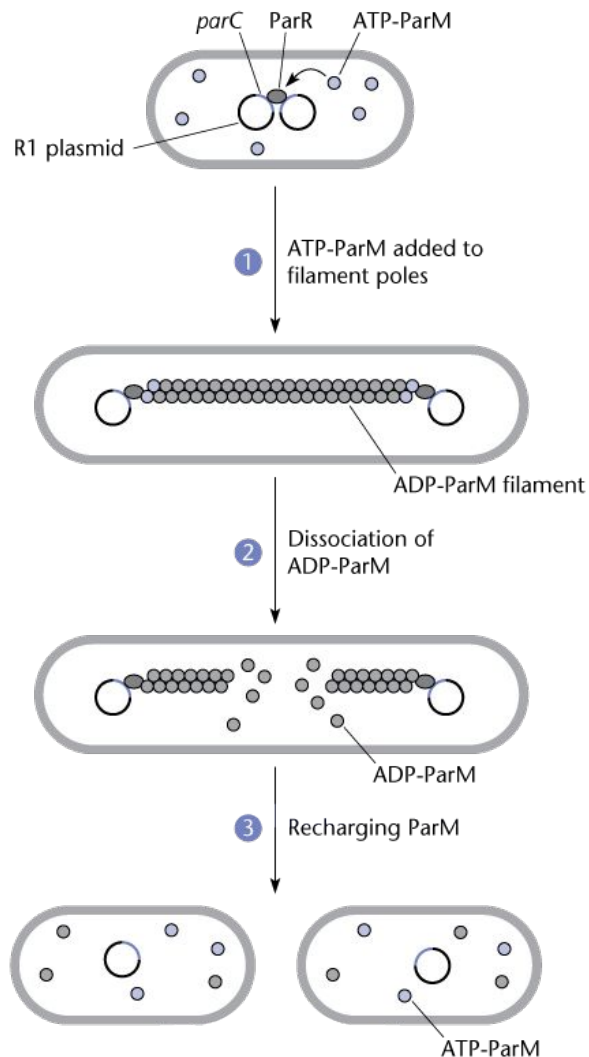
Plasmids labeled with fluorescent protein  
-move to each daughter cell during division.



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

A *parCMR* locus

## B Plasmid R1 partitioning



## 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

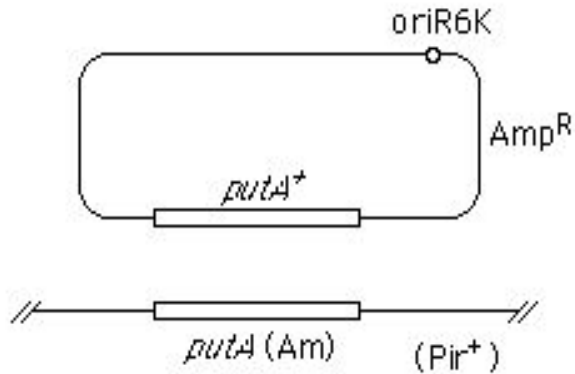
1. Plasmids must be unable to replicate without essential replication proteins provide *in trans*.
2. It helps if the plasmid can be mobilized-*oriT* required
3. Need a selectable marker
4. Large or small region of homologous DNA cloned that will integrate into the chromosomal target.
5. Need a counter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

6. **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?

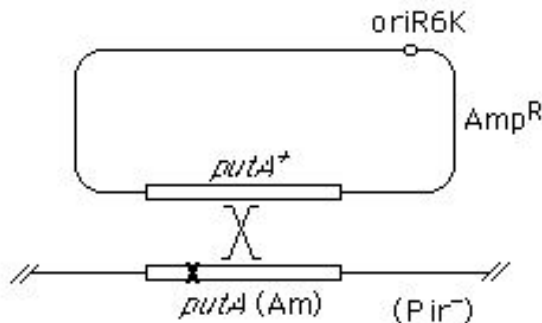
(a)



The plasmid will replicate in a  $Pir^+$  host. Hence, the cell will be  $Put^+$  due to complementation from the *putA<sup>+</sup>* gene encoded by the multicopy plasmid.

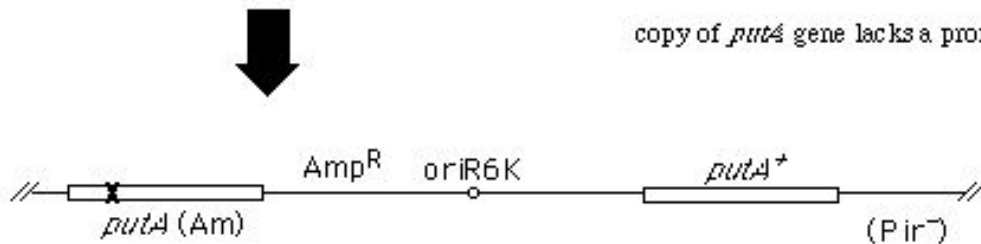
R6K: broad host plasmid.  
-Pir is the essential replication protein  
-*pir* mutants cannot replicate unless supplied *in trans*.

(b)



The plasmid cannot replicate in a  $Pir^-$  host. However, it is possible to select for integration into the chromosome by demanding *Amp<sup>R</sup>*. Once integrated, the cell will be  $Put^+$  due to complementation from the copy of *putA<sup>+</sup>* encoded by the integrated plasmid. (This is only true if the plasmid copy has its own promoter. What would happen if the plasmid copy of *putA* gene lacks a promoter?)

-integration into the chromosome is selected for by growth on ampicillin



How could you make targeted mutant using this method?

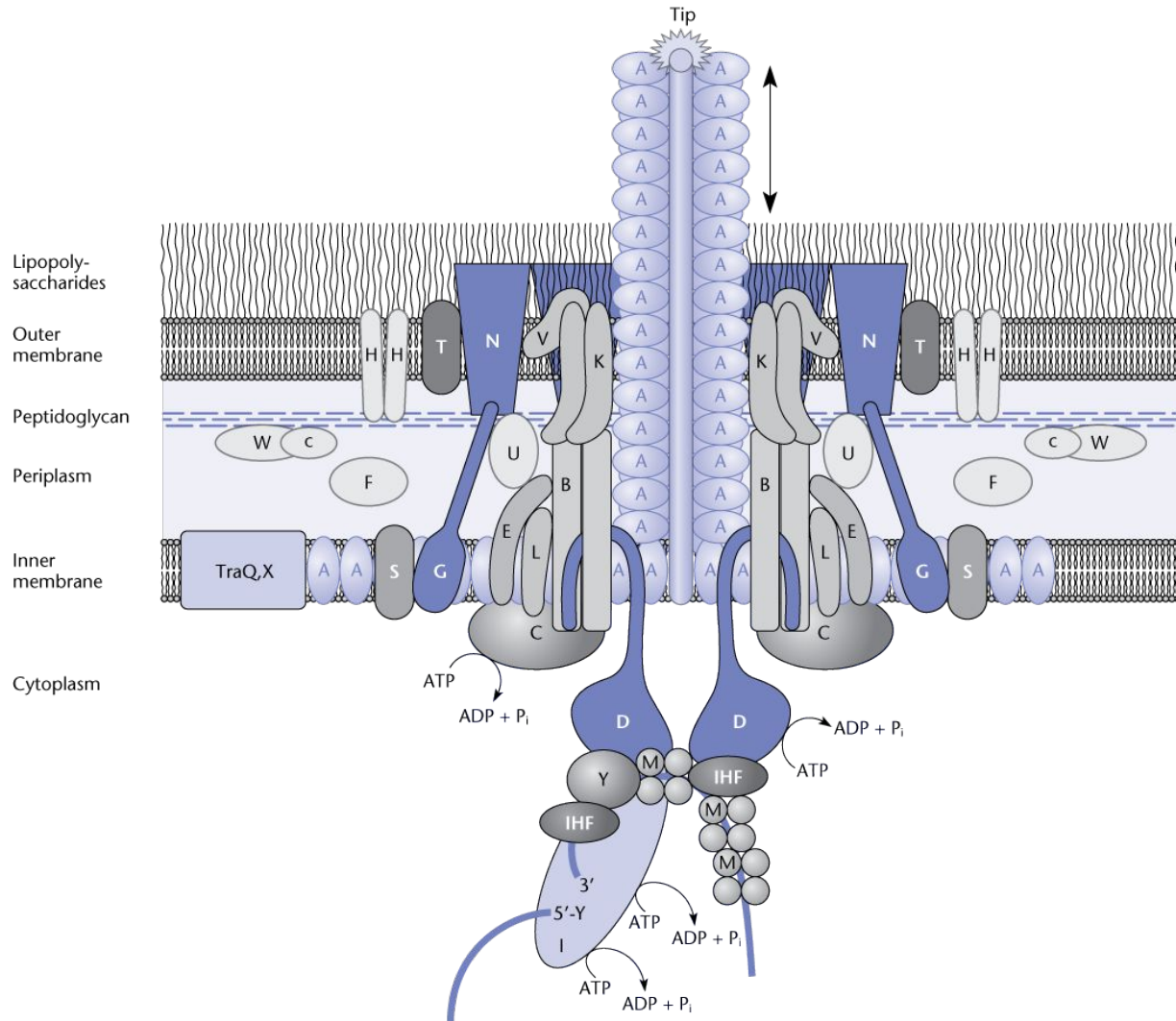
## F-plas mid

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

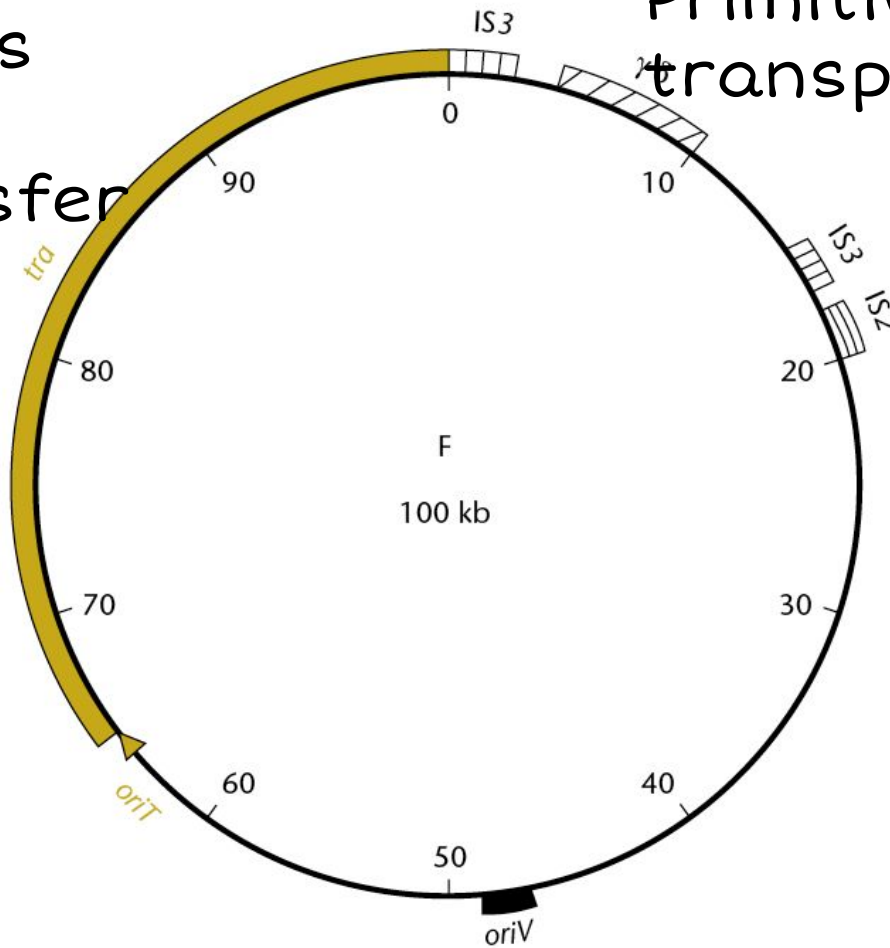
# F Pilus assembly



# Genetic organization of F

Figure 5.6

30+ genes  
needed  
For transfer



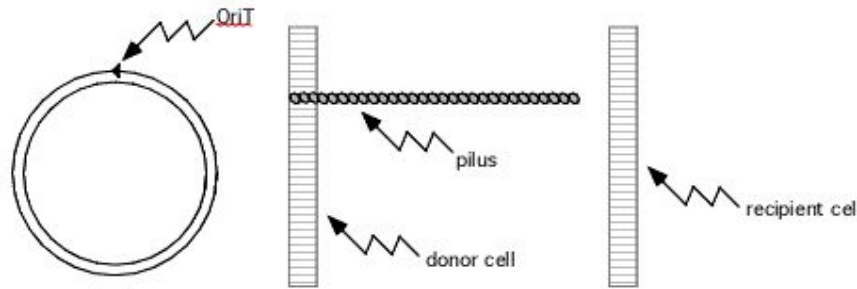
Primitive  
transposon

IS  
elements

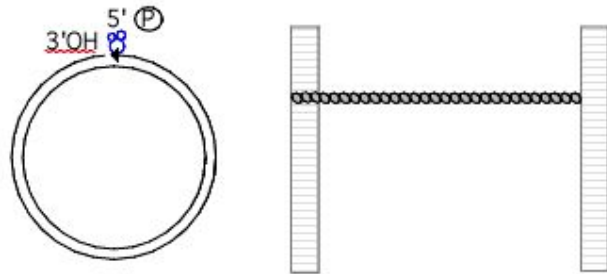
replicati  
on



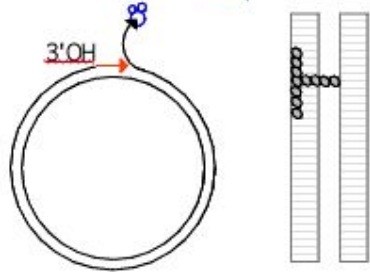
# F-transfer at fine detail



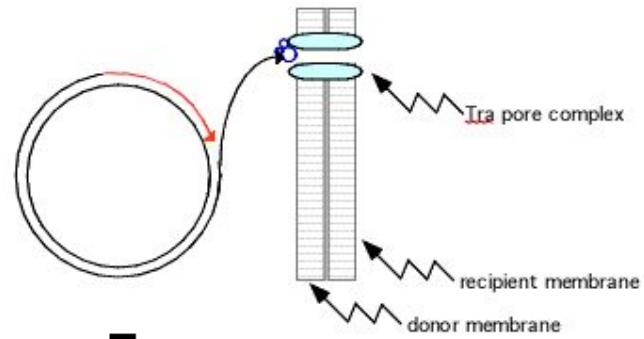
↓ Contact between donor and recipient cells.  
DNA relaxase (⊗) nicks at oriT and covalently binds to 5' Ⓟ



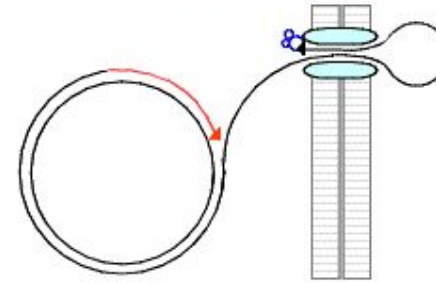
↓ Pilus retracts, bringing donor and recipient into close proximity and Ira proteins form a pore complex that spans the membranes



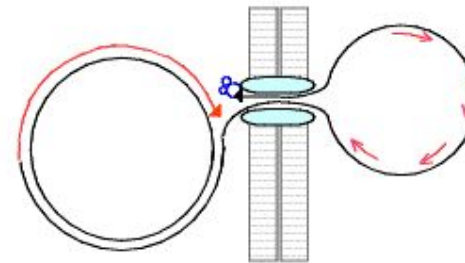
↓ Rolling circle DNA replication initiates at 3'OH and proceeds 5' to  
Membranes brought into close proximity to form mating bridge.  
Relaxase interacts with membrane Ira pore complex



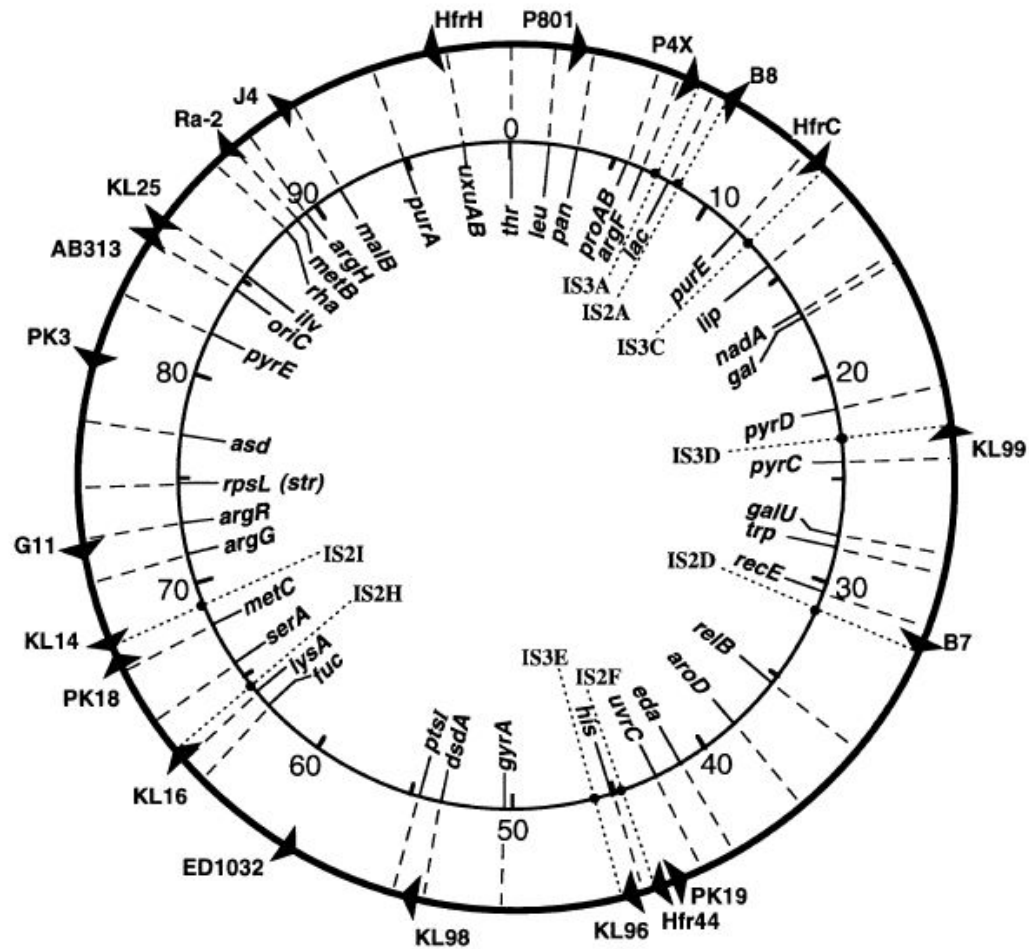
↓ DNA replication pushes the ssDNA into the recipient cell



↓ 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<sub>is</sub>-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 *uxuAB*, *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.