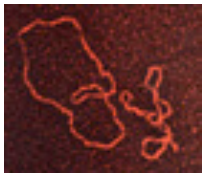
Outline



1. Plasmids: the basics
2. Replication control
3. Plasmids: no cure?
4. Partitioning

Reading assignment 25 Feb 2009

Gene transfer mechanisms: pp 243-266

Plasmids

Fundamental characteristics for each plasmid:

- Size/Copy number-from 3 to 200 Kb (or more) and from 1 to 100/cell; these are inversely related to each other
- Incompatibility group (Inc)-refers to whether 2 plasmids can be maintained in same cell
- Host range-bacteria where the plasmid will replicate
- Host range/Inc usually determined by features of plasmid replication/partitioning

Examples of bacterial plasmids

See Tables 4.1/4.2/4.4

Plasmid (Inc)	Size (Kb) / CN	Host range	Traits
F (IncF1)	100 / 1	Narrow (*E. coli*)	Conjugative
R1 (IncF2)	90 / 1	Enterics	Conjugative; drug resistance
RK2 (IncP)	60/4-7	Broad: Gram neg.	Conjugative; drug resistance
Ti (IncRh1)	200 / 1	*Agro*/*Rhizo*	Conjugative; plant tumor-inducing
ColEI	9/30	Narrow	Mobilizable; colicins

Plasmid replication

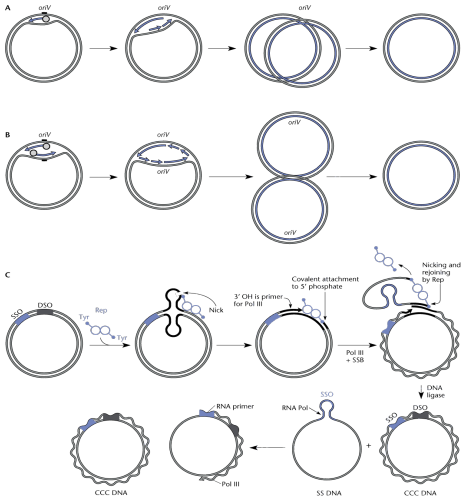
The replication strategy that a plasmid uses directly affects its copy number, host range, and incompatibility group.

- Plasmid replication relies on the normal host DNA replication machinery.
- Plasmids will carry distinct origins of replication (*oriV*) and genes that enable and control the frequency of their replication.

Fig. 4.4

Plasmid replication strategies

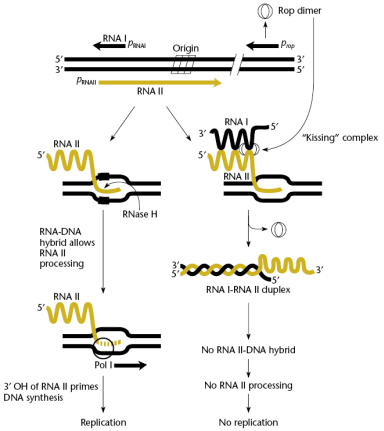
- Theta replication (A & B) common in G-plasmids like F, RK2, ColE1
- Rolling circle replication (in C; pT181) depends on Rep protein nicking at DSO; 3' OH primes synthesis of one strand; RNA pol makes primer for 2nd strand synthesis
- Rep protein used once, allowing control of initiation.



Controlling θ replication: ColE1

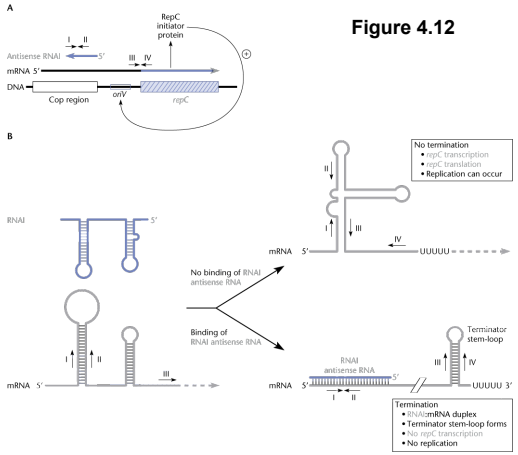
Fig. 4.8

- *oriV* replication initiation dependent upon RNA II hybridizing at origin; RNAse H trims RNA II; trimmed RNA II provides 3'OH for replication primer
- RNA I antagonizes RNA II processing: RNA I-RNA II complex is weak, made stronger by Rop
- Complete RNA I/II duplex removes *oriV* primer and prevents initiation



pT181: Another antisense RNA model

Figure 4.12

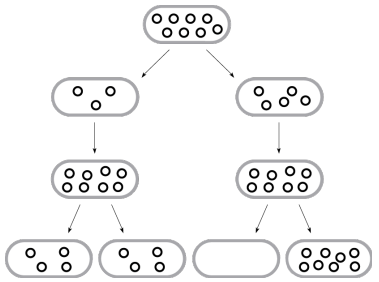


After replication: avoiding plasmid loss ("curing")

Different challenges faced by low and high copy number plasmids at time of cell division.

↪Plasmids with several copies/cell: safety in numbers.

Figure 4.17



After replication: keeping plasmids

Low copy number plasmids: plasmid multimers can change everything.

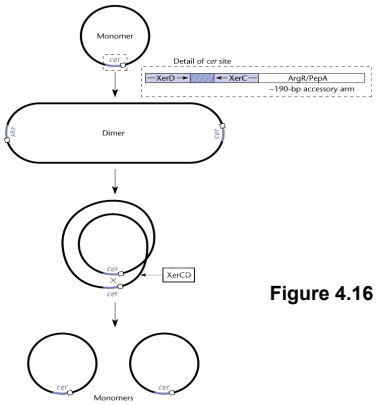


Figure 4.16

The best insurance: Partition

Table 4.3

TABLE 4.3 Partitioning systems

DNA	Par system	Centromere-like site[a]
ParA-ParB homologs		
P1 prophage	ParA-ParB	*parS*
F plasmid	SopA-SopB	*sopC*
RK2	IncC2-KorB	O_B3?
Ti	RepA-RepB	*inc2?*
B. subtilis	Soj-Spo0J	*parS*
C. crescentus	ParA-ParB	*parS*
ParM-ParR homologs		
R1	ParM-ParR	*parC*
CollB-P9	ParA-ParB	Unknown
C. crescentus	MreB	Unknown

[a] See Yao et al., Suggested Reading, and references therein.

Plasmid Partitioning

Figure 4.18