Plasmids and Plasmid Biology

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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"



CsCl gradient with ethidium bromide and

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 <i>E. coli</i>)
pBR322	16
pUC18	~30–50
plJ101	40–300

Plasmid	Size (Kb)	Number of copies per chromosome	Self- transmissible	Phenotypic features
Col plasmids		3 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 -		
CoIE1	6.4	10-15	No	Colicin E1 disrupts energy gradient, host immunity to Colicin E1
CoIE2	7.6	10-15	No	Colicin E2 is a DNase, host immunity to Colicin E2
CoIE3	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' Str' Sul' Tet'
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				÷2
λdv	6.4	50	No	λ genes <i>cro, cl, O, P</i>
Recombinant plasmids				NY.
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

Table 11-1 Examples of some plasmids and their properties

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 A Plasmid genetic organization



moter	Gene products expressed
В	RepA and CopB
A	RepA
A	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 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.



•Rep-protein expression controlled by antisense CopA

- PcopB-encodes Rep and CopB
- PcopA-encodes antisense
 RNA

- Plasmid copy control balanced by host RNaseIII activity and transcription from the plasmid
- plasmid replicates to high level
- •CopB levels rise, shutting off RepA production
- •antisense RNA from PcopA made



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

•binding causes two plasmid molecules to couple "handcuff"

1200

•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



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. Plasm**Plasmer** 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

Alsgemerpeiploid reporteristrains 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?



The plasmid will replicate in a Pir⁺ host. Hence, the cell will be Put⁺ due to complementation from the *put4*⁺ gene encoded by the multicopy plasmid.

(b)



The plasmid cannot replicate in a Pir host. However, it is possible to select for integration into the chromosome by demanding Amp^R. Once integrated, the cell will be Put⁺ due to complementation from the copy of $putd^{+}$ 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 $putd^{4}$ gene lacks a promoter?)

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?

AmpR oriR6K DUITA put4 (Am) (Pir⁻)

F-plas mid

- 1. large (100 kb)
- 2. low copy (1-2 copies/cell)
- 3. self transmissible
- 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
- 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
traABCEFGHKLQ	UVWX Pilus biosynthesis, assembly
traGN	Mating-pair stabilization
traD	Coupling protein
tral	Relaxase
traYM	Accessories for relaxosome
traJ, finOP	Regulation of transfer
traST	Entry exclusion

F Pilus assembly



Genetic organization of F



F-transfer at fine detail





FIGURE 1 Approximate map positions of integrated sex factors (F, F₁₅-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.