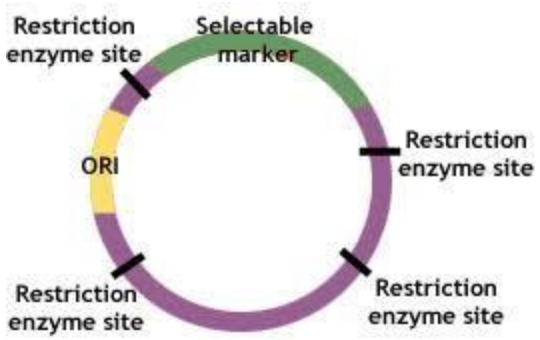
Uint 2- plasmid

What is plasmid

A plasmid is a small, circular,self replicating,extra chromosomal, double-stranded DNA molecule that is distinct from a cell's chromosomal DNA. Plasmids naturally exist in bacterial cells, and they also occur in some eukaryotes.



Episome

a genetic element inside some bacterial cells, especially the DNA of some bacteriophages, that can replicate independently of the host and also in association with a chromosome with which it becomes integrated.

Classification of plasmid

Plasmids may be classified in a number of ways. Plasmids can be broadly classified into

1. conjugative plasmids
2. non-conjugative plasmids

conjugative plasmids

Conjugative plasmids contain a set of transfer or *tra* genes which promote sexual conjugation between different cells. In the complex process of conjugation, plasmids may be transferred from one bacterium to another via sex pili encoded by some of the *tra* genes

non-conjugative plasmids

Non-conjugative plasmids are incapable of initiating conjugation, hence they can be transferred only with the assistance of conjugative plasmids.

Classify of plasmids by function.

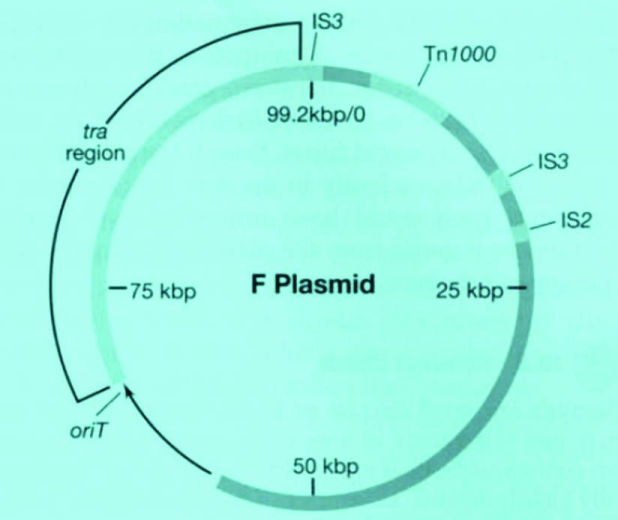
There are five main classes of plasmidd

1. F-plasmids(Fertility)
2. Resistance (R) plasmids
3. Col plasmids
4. Degradative plasmids
5. Virulence plasmids

F-plasmids(Fertility)

F is a circular DNAmolecule having a molecular weight of 62.5x 10^6(94.5kbp). it contains *tra* genes that allow the plasmids DNA to be transferred between cells. It is found in the bacterium *E. coli*; *E. coli* containing this F factor are known as F^+ and those without are known as F^-. The F stands for

fertility and the F factor is around 100000 bases in length. The F^+ cells have a tube-like structure called a pilus, which allows it to make contact with F^- cells. This joining via a pilus in order to transfer DNA between bacteria is known as conjugation. Therefore the F plasmid is known as a conjugative plasmid. Within the *E. coli* cells, the F plasmid has one or two copies making it a low-copy-number plasmid. During the cell cycle, it replicates once and segregates to both daughter cells.



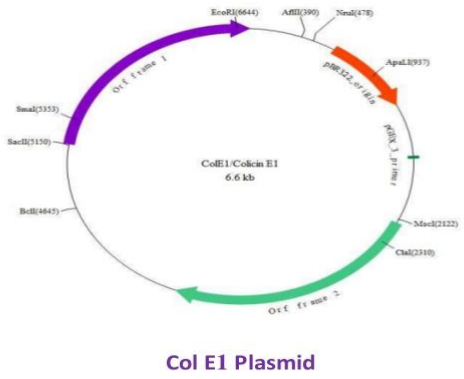
Resistance (R) plasmids

R plasmid is plasmid which contain genes that provide resistance against antibiotics or poisons.r plasmid consist of two contagious segment of DNA.one of this segment isthe resistance transfer factor (RTF) required for transfer of the plasmid between bacteria, and the r-determinants (genes conferring antibiotic resistance). R plasmids were first isolated from strains of Shigella dysenteriae that showed resistance to multiple antibiotics.

For example, RP1, a plasmid that encodes resistance to ampicillin, tetracycline and kanamycin originated in a species of *Pseudomonas*, from the family Pseudomonadaceae, but can also be maintained in bacteria belonging to the family Enterobacteriaceae, such as *Escherichia coli*.[3]

Col plasmids(colicin)

Col plasmids contain genes that make bacteriocins (also known as colicins), which are proteins that kill other bacteria and thus defend the host bacterium. Bacteriocins are found in many types of bacteria including E. coli, which gets them from the **plasmid** ColE1.



Col E1 Plasmid

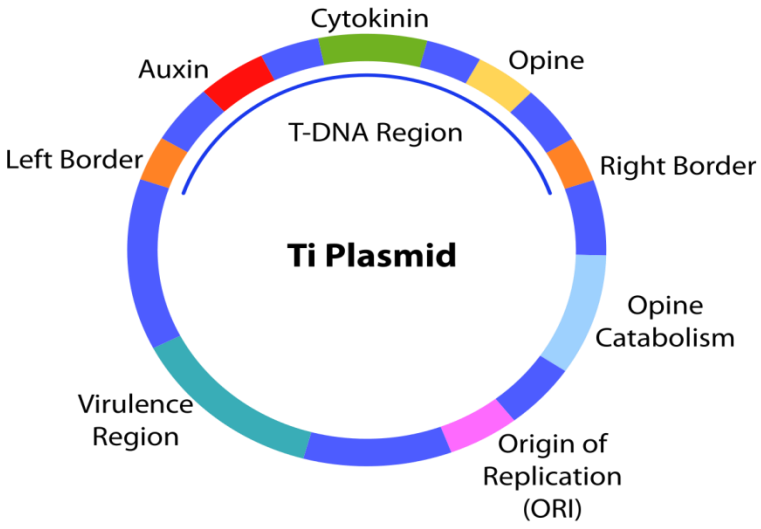
Degradative plasmids

Degradative plasmids help the host bacterium to digest compounds that are not commonly found in nature, such as camphor, xylene, toluene, and salicylic acid. These plasmids contain genes for special enzymes that break down specific compounds. Degradative plasmids are conjugative.

Example-Tol plasmid degrades toluene,xylane

Virulence plasmid

Virulence plasmids are usually large (>40 kb) low copy elements and encode genes that promote host–pathogen interactions. Although **virulence plasmids** provide advantages to bacteria in specific conditions, they often impose fitness costs on their host.it turn the bacterium into a pathogen. e.g. Ti plasmid in *Agrobacterium tumefaciens*



Ti plasmid

A **tumour inducing (Ti)** plasmid is a plasmid found in pathogenic species of *Agrobacterium*, including *A. tumefaciens*, *A. rubi* and *A. vitis*.

Evolutionarily, the Ti plasmid is part of a family of plasmids carried by many species of Alphaproteobacteria. Members of this plasmid family are defined by the presence of a conserved DNA region known as the *repABC* gene cassette, which mediates the replication of the plasmid, the partitioning of the plasmid into daughter cells during cell division as well as the maintenance of the plasmid at low

copy numbers in a cell. The Ti plasmids themselves are sorted into different categories based on the type of molecule, or opine, they allow the bacteria to break down as an energy source.

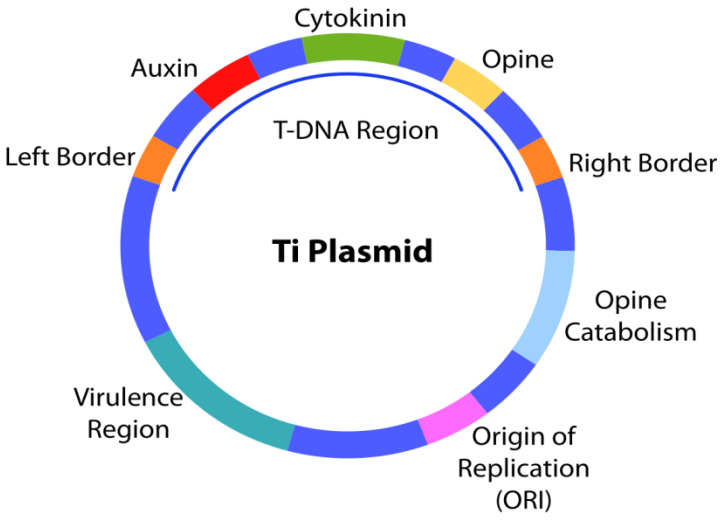
Classification of Ti plasmid

Ti plasmids have been classified based on the **type** of opine they catabolize, namely

1. nopaline-,
2. octopine- or mannityl-**types**, which are amino acid derivatives,
3. or agrocinopine-**type**, which are sugar phosphate derivatives.

Virulence of Ti plasmid

The presence of this Ti plasmid is essential for the bacteria to cause crown gall disease in plants This is facilitated via certain crucial regions in the Ti plasmid, including the *vir* region, which encodes for virulence genes, and the transfer DNA (T-DNA) region, which is a section of the Ti plasmid that is transferred via conjugation into host plant cells after an injury site is sensed by the bacteria. These regions have features that allow the delivery of T-DNA into host plant cells, and can modify the host plant cell to cause the synthesis of molecules like plant hormones (e.g. auxins, cytokinins) and opines and the formation of crown gall tumours.



Linear plasmid

Linear plasmids are commonly present in both pro- and eukaryotes, and belong to one of two types, those. with covalently closed ends and those with proteins. bound to their 5' termini.

Types

Two types of linear plasmids exist,

1. hairpin plasmids with covalently closed ends and those with proteins bound to their 5' termini. Hairpin plasmids are common in human-pathogenic Borrelia spirochetes, in which they are instrumental in escape from the immunological response;
2. cryptic hairpin elements are present in mitochondria of the plant pathogenic fungus Rhizoctonia solani.

About linear plasmid

Plasmids with 5' attached proteins constitute the largest group. In actinomycetous bacteria they are conjugative and usually confer advantageous phenotypes, e.g. formation of antibiotics, degradation of xenobiotics, heavy-metal resistance and growth on hydrogen as the sole energy source. in contrast, the majority of linear plasmids from eukaryotes are cryptic, with only a few exceptions. In some yeasts a killer phenotype may be associated, the most thoroughly investigated elements being those from Kluyveromyces lactis killer strains. In Neurospora spp. and in Podospora anserina, senescence and longevity respectively are correlated with linear plasmids

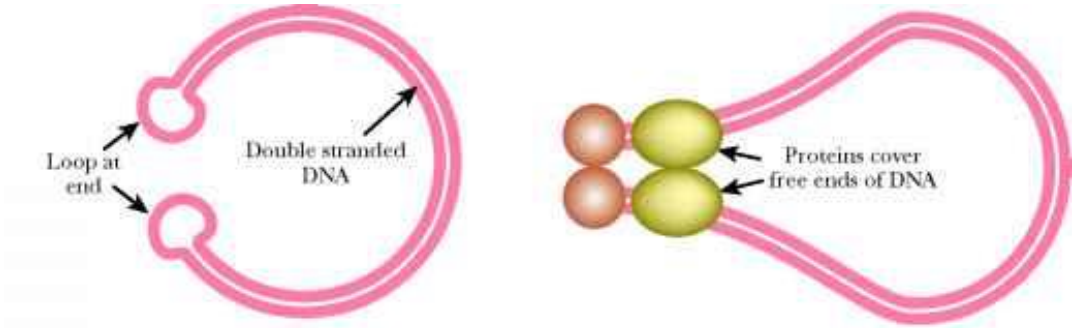
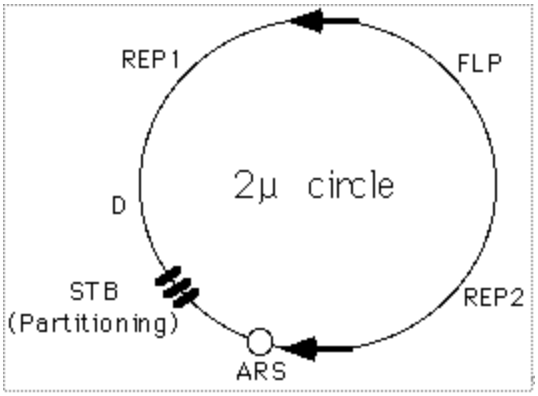


FIGURE 16.04 ***End Structures of Linear Plasmids***

2 micron plasmid

The 2 micron plasmid of *Saccharomyces cerevisiae* is a relatively small multi-copy selfish DNA element that resides in the yeast nucleus at a copy number of 40–60 per haploid cell.the DNA of 2 micron plasmid is 2 micromitereand a molecular weight about $4x10^6$.the plasmid DNA is coated with histones .the plasmid does not apparently integrate in to host DNA.the base sequence of this plasmid includes two inverted repeat each containing 599 bp,these are separated by two sequence containing 2346 and 2774 bp. This plasmid contain Flp gene. The Flp system corrects any decrease in plasmid population by promoting plasmid amplification via a recombination induced rolling circle replication mechanism. This plasmid coded proteins, Rep1 and Rep2, and a partitioning locus STB. examination of these base sequence of these plasmid shows unique terminal structure ,such as palindromes, hairpin and repetitive sequence,much like telomeres of yeast.



Plasmid replication

Bacterial plasmid replication is not dependant on its nuclear genome replication with long intermissions between replication proceedings occurring during the course of cell division. Definite plasmid copy number depends on plasmid type, host organism and the growth conditions. Unintended aberrations from normal copy number are attuned. However dominant and recessive copy mutants to the wild type do exist

Plasmid replication mechanisms

There are three types of plasmid replication namely

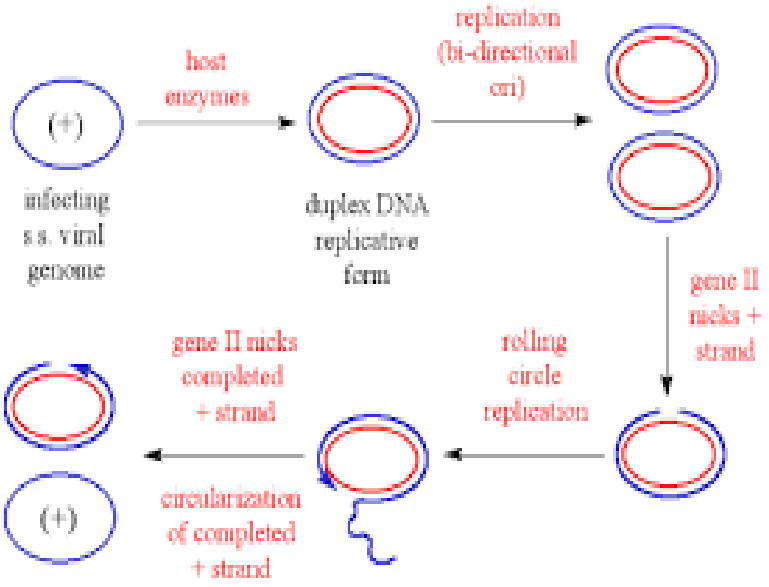
1. rolling circle,
2. Col E1 type
3. Iteron contain replication

Rolling circle

Rolling circle replication mechanism is specific to bacteriophage family m13 and the fertility F factor which encodes for sex pili formation during recombination by means of conjugation. Fragments smaller than 10 kilo base usually replicate by this replication mechanism as reported in some gram positive bacteria. It allows the transfer of single stranded replication product at a faster rate to the recipient cell through pilus as in case of fertility factor or to the membrane in case of phage.

Mechanism

Rolling circle occurs to a covalently closed circular piece of double-stranded DNA. A nick is produced in one of the strands by enzyme nickases creating a 5' phosphate and a 3' hydroxyl. Free 3' hydroxyl will be used by DNA polymerase to make new DNA pushing the old nicked strand off of the template DNA



Col E1 type replication

Col E1 replication is a negative regulation mechanism which enables the plasmid to control its own copy numbers by involving RNA type I, RNA type II, Rom protein, and the plasmid itself. Col E1 replication is initiated by means of RNA-RNA interactions and does not rely on replication initiation protein encoded by the plasmid to regulate its copy numbe.

Mechanism

RNA type II that originates 555 base pairs upstream from the replication origin of Col E1 plasmid is transcribed which marks the start of Col E1 replication. A determined hybrid with the DNA strand is formed by a loop enriched in G nucleotide positioned 290 of RNAII and a C-rich region on the template strand positioned 20 nucleotides upstream from the origin [8]. Several stems and loops are exhibited by the newly formed secondary structure. A DNA/RNA hybrid is recognized by enzyme RNase and dissociates the RNA hybrid to the 3' end of RNAII. The resultant RNA primer is linked to the plasmid with a free 3' hydroxyl group. This RNA enables replication of DNA to begin by providing DNA polymerase a specific site to initiate nucleotides synthesis. Consequently DNA synthesis is commenced with the leading strand is happening.

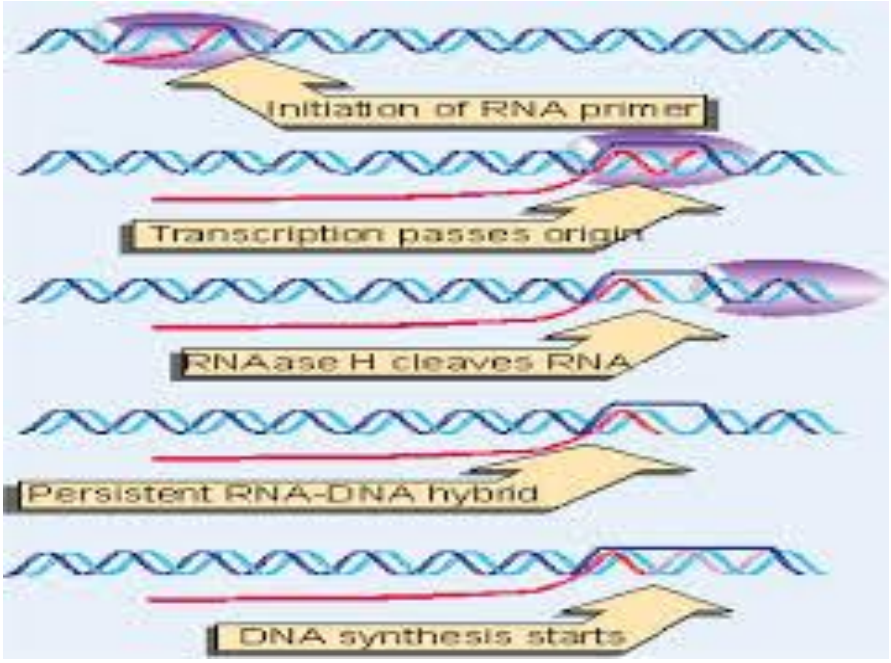
Iteron-containing replicons

This replicon consists of a gene that encodes Rep protein for plasmid replication initiation, set of direct repeat sequences called iteron, adjacent AT-rich region and Dna boxes which is a protein required for bacterial chromosome replication initiation. However length of adjacent AT-rich region and number of iterons and DnaA boxes differs in a replicon

Mechanism

Iteron contain replication begins with the binding of Rep proteins to the iteron being organized in the same orientation of the DNA helix. And by binding to the DnaA boxes in the replicon the Rep-DnaA-DNA assembly promotes melting of the strand at the nearby AT-rich region to which host replication factors subsequently gain access and promote leading and lagging strand synthesis in a manner analogous to initiation of replication at the chromosomal origin, oriC .

Plasmids copy number is controlled principally at the beginning of replication initiation. The frequency with which initiation of replication of iteron-containing plasmids occurs is modulated in part by sequestration of the origin region in nucleoprotein complexes and intermolecular pairing of complexes on different plasmids, which is referred to as "handcuff ”



Incompatibility

Pairs of two closely related plasmid usally cannot be stably maintained in the progeny of a single cell ,such plasmid are said to be incompatible.

Let us consider a cell that contains two plasmid says F and ColE1 having different inhibitors .repilcation of each pkasmid will proceed independently of one another since the inhibitor of one type(F) does not regulate the replication of the other type(ColE1) .thus ,F and ColE1 are compatible .alternatively ,one says that they belong to different incompatibility.

The situation is quite different with two plasmid Aand B whose inhibitors are either identical or are similar enough that the inhibitor of A can regulate replication of B and vice versa.let us consider a cell havig one copy of A and one copy of B and which has enlarged sufficiently that initiation occurs.since two plasmid copies are selected at random for replication event is a cell having either one copy of A and two copy of B (1A,2B) or one copy of B and two copy of A(2A ,1B).WHEN the second replication event occur ,each cell will have four plasmid ,but depending on the plasmid that is replicated ,the plasmid composition may be (1A,3B) ,(2A,2B),(3A,1B).at this point the cell which has twice the initial number of plasmid ,can devidethe plasmid composition of two doughter cell will be one of the following

(1A,3B)becomes(1A,1B)+(1B,1B)

(2A,2B)becomes (1A,1A)+(1B,1B)or(1A,1B)+(1A,1B)

(3A,1B)becomes (1A,1A)+(1A,1B)

Not that two possible types of cells, namely ,(1A,1A)and (1B,1B),contain only one of the two kind of the two kinds of plasmid ,doughter cell obtained from these cells will of curese continue to have only one kind of plasmid.

Thus compatibility is a result of

1. Two plasmid having a similar inhibition
2. The random selection of plasmid for DNA replication.

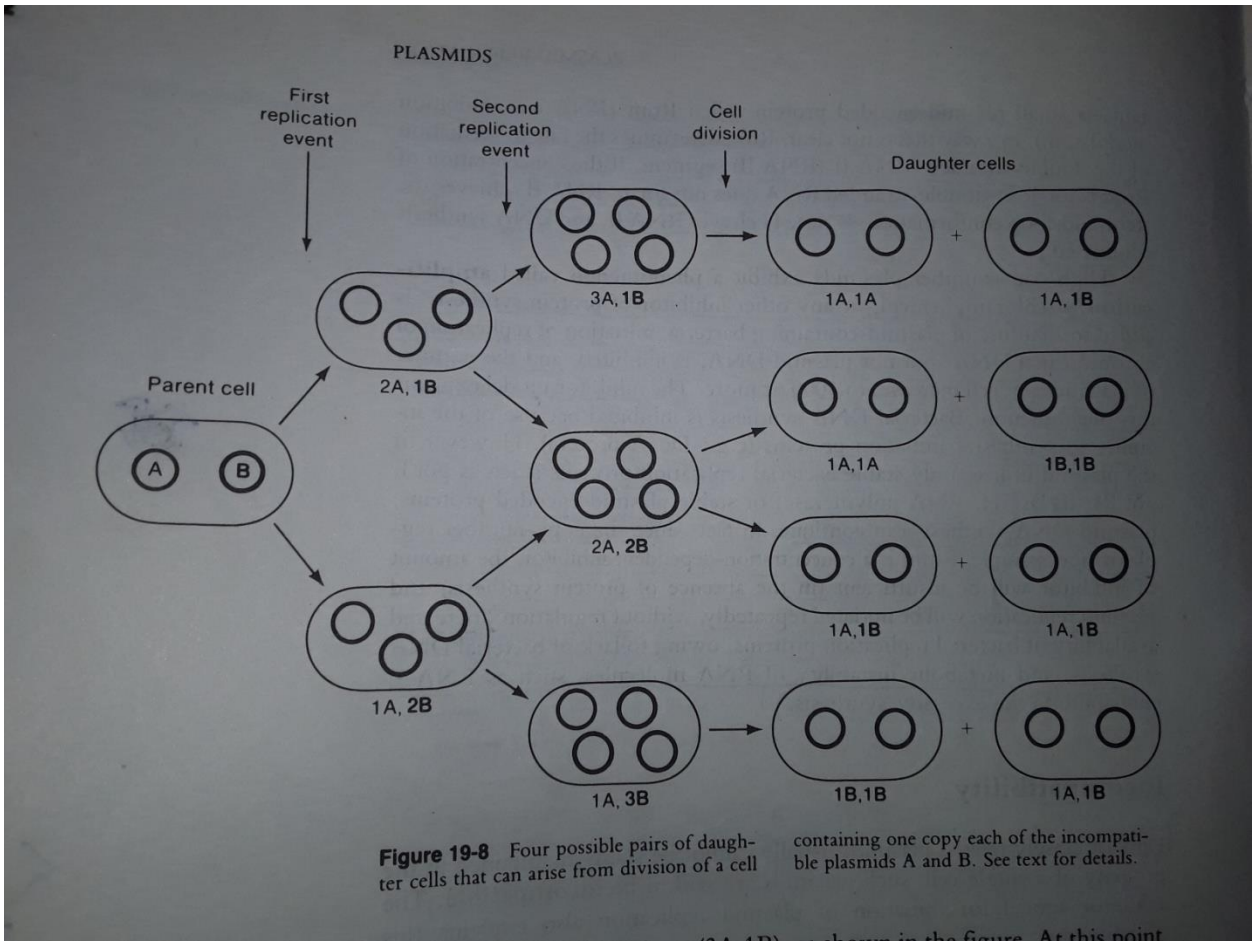
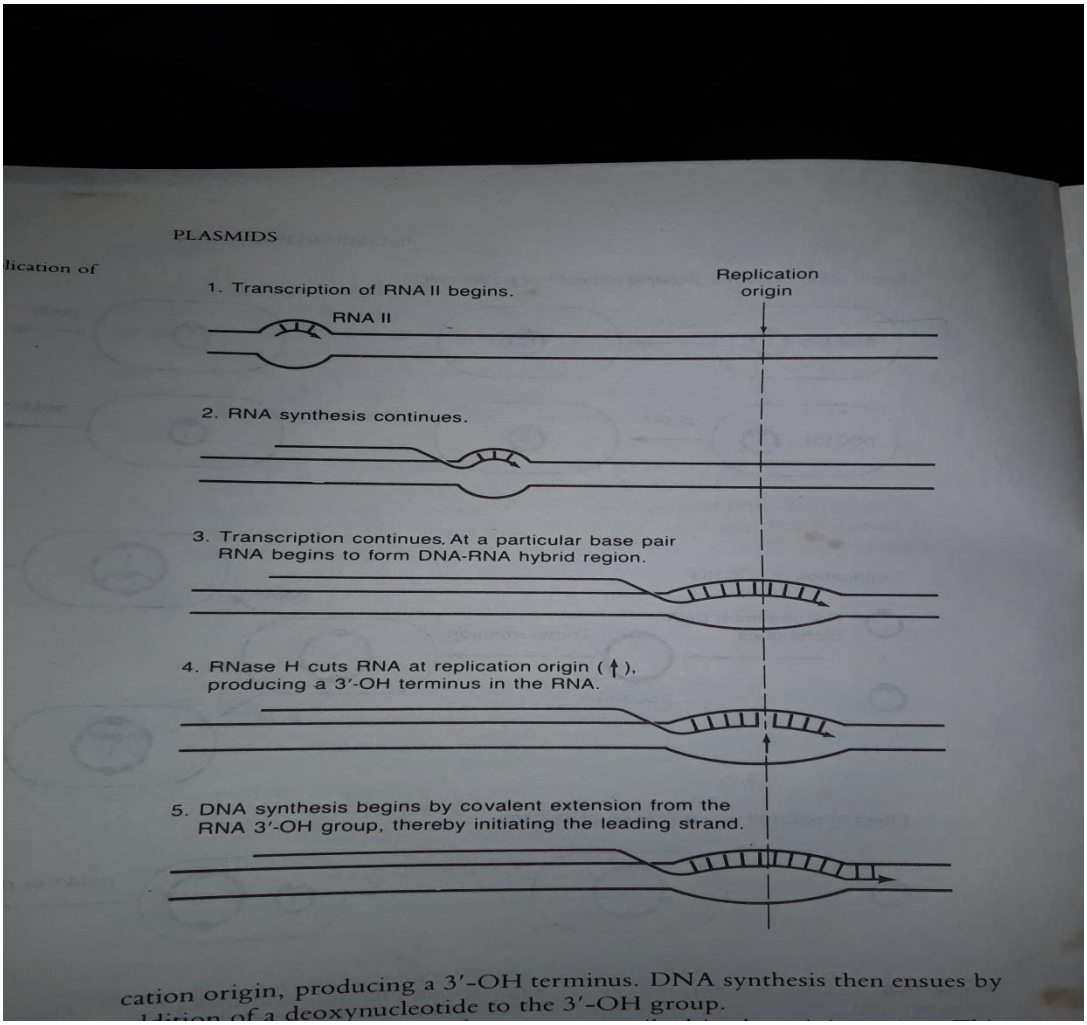


Figure 19-8 Four possible pairs of daughter cells that can arise from division of a cell containing one copy each of the incompatible plasmids A and B. See text for details.

Fig: plasmid Incompatibility

Plasmid amplication

High copy number plasmid exhibit a phenomenon s called amplification. if chloramphenicol or any other inhibitor of protein synthesis ,is added to a culture of a plasmids containing bacteria ,initiation of replication of chromosomal DNA but not plasmid DNA ,is inhibited and the number of plasmid per cell increases to 1000 or more .the inhibitor model explain this phenomenon. bacterial DNA synthesis is inhibited because of inability to synthesize initiation proteins (DnaA protein)however if the plasmid utilize only stable bacterial replication protein or stable plasmid encoded proteins ,plasmid DNA synthesis can continue. since the copy number regulator is a concentration dependent inhibitor ,the amount of inhibitor will be insufficient and the plasmid replication will be initiated repeatedly ,without regulation. increased availability of bacterial replication proteins ,owing to lack of bacterial DNA synthesis ,and metabolic instability of RNA molecules ,such as RNA I,CONTRIBUTE TO THE EXCESSIVE SYNTHESIS



Regulatin of copy number

Some plasmid are present in cells in low –copy number------one or few per cell---whereas other exist in large numbers –from 10 to 100 per cell.the copy number is established and regulated by controlling the rate of initiation of DNA replication.if a cell is transformed with a single copy of a low copy number plasmid,the plasmid DNA replicates only once or twice before cell division.how ever ,if a cell is transformed with a single DNA molecule of a high copy number plasmid,the plamid DNA replicates repeatedly until the proper copy number is reached.

The general accepted explanation for the regulation of copy number is that there is plasmid –encoded inhibitor that is a negative regulator of the initiation of replication. we avoid the term repressor because ,as we will see ,in at least one case the inhibitor does not act on DNA.The activity of inhibitor dependent on inhibitor concentration. thus as cell grows ,the inhibitor concentration drops ,replication is not inhibited ,and the number of plasmid DNA molecules doubles. at this point exist twice initial number of point there will exist twice the initial number of inhibitor genes, therefore ,by protein synthesis the inhibitor concentration also doubles, causing replication stop .a similar event would occur if there were initially only one copy a high copy number plasmid –that is ,replication would continue there is sufficient number plasmid –that is ,replication would continue until there is sufficient inhibitor to turn off synthesis. the most likely possibilities is that for the high copy number plasmid the inhibitory activity requires a high concentration of inhibitor than is the case for the low copy number plasmid. The following experiment providing evidence that each plasmid controls its own copy number.

A hybrid plasmid pSC134 was constructed that consist of a complete copy of each two plasmid ,ColE1 and Psc101.the copy number for that plasmid are 18 and 6 respectively .

1. Plasmid pSC134 replicates from ColE1 origin and has a copy number of 16 – roughly equal to that for ColE1.
2. If pSC134 is put into polA$^-$ cell(ColE1 cannot replicate in a polA$^-$ cell)the pSC101origin is used and the copy number become 6 ,the

value of pSC101 .these two result show that the copy number correlates with the replication origin that is being used.

3. If Psc101 DNA IS TAKEN INTO A BACTERIUM CONTAINONG 16 copies of P**sc**134,the psc101 cannot replicate .the lack of replication shows that the psc101 inhibitor is being made by psc134

The interpretation of these result is following .if there were a copy number less than 6 ,both psc101 and colE1 would be active and replication from both origins would increase the number.

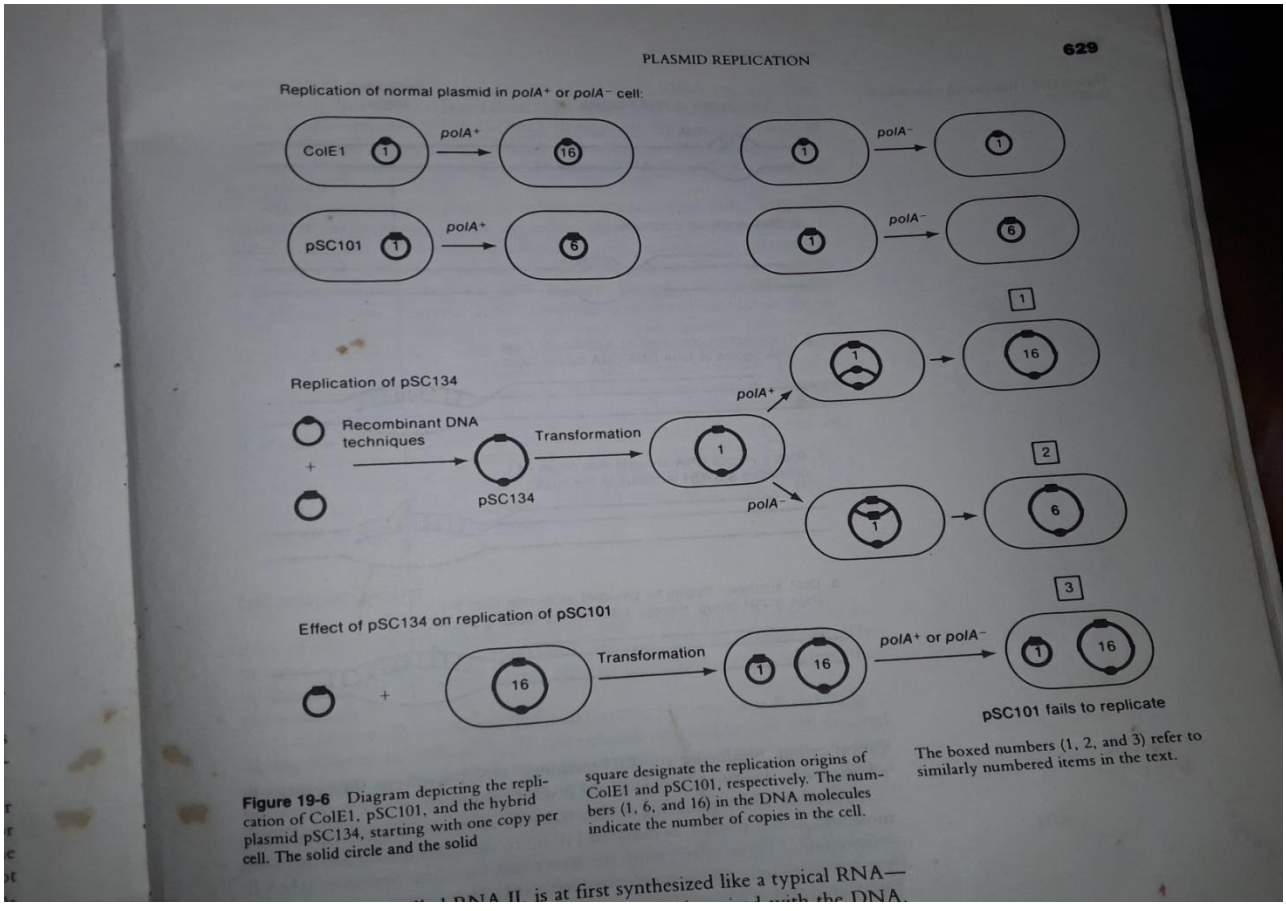


Figure 19-6 Diagram depicting the replication of ColE1, pSC101, and the hybrid plasmid pSC134, starting with one copy per cell. The solid circle and the solid square designate the replication origins of ColE1 and pSC101, respectively. The numbers (1, 6, and 16) in the DNA molecules indicate the number of copies in the cell. The boxed numbers (1, 2, and 3) refer to similarly numbered items in the text.

RNA II is at first synthesized like a typical RNA—