

Stable propagation of 'selfish' genetic elements

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Extrachromosomal or chromosomally integrated genetic elements are common among prokaryotic and eukaryotic cells. These elements exhibit a variety of 'selfish' strategies to ensure their replication and propagation during the growth of their host cells. To establish long-term persistence, they have to moderate the degree of selfishness so as not to imperil the fitness of their hosts. Earlier genetic and biochemical studies together with more recent cell biological investigations have revealed details of the partitioning mechanisms employed by low copy bacterial plasmids. At least some bacterial chromosomes also appear to rely on similar mechanisms for their own segregation. The 2 μ m plasmid of *Saccharomyces cerevisiae* and related yeast plasmids provide models for optimized eukaryotic selfish DNA elements. Selfish DNA elements exploit the genetic endowments of their hosts without imposing an undue metabolic burden on them. The partitioning systems of these plasmids appear to make use of a molecular trick by which the plasmids feed into the segregation pathway established for the host chromosomes.

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1. Introduction

The overall success of social populations is influenced by the conflicting forces of 'selfishness' and 'altruism' among its members (Dawkins 1992; Sundstrom and Boomsma 2001; Thomas 2000). Since a living cell is made up of dynamic societies of interacting molecules, the rules of direct, individual fitness gains through selfishness and indirect, collective fitness gains through altruism are expected to operate on them at the molecular level.

1.1 Emergence of coexistence from conflicts and alliances

Consider a primitive cell containing two types of DNA molecules D1 and D2, each harbouring signals for the duplication and transcription of its genetic information (replication origins and promoters, respectively) (figure 1). Imagine further that D1 contains a stronger origin (a better template for replication) whereas D2 contains a stronger promoter (a better template for transcription). Finally, let D2 code for the replicase (R) and D1 for the trans-

criptase (T). In this scenario, D1 and D2 are in conflict with each other with respect to the utilization of the two enzymes but in potential alliance with each other with respect to their production. Under conditions of limited availability of raw materials (nucleotide substrates for DNA and RNA synthesis, for example), relative signal strengths for the origins and promoters can be optimized to obtain a steady state with a fixed relative abundance of D1 and D2. The genome copy numbers would be such that the dosage of the replicase and that of the transcriptase are sufficient for them to be partitioned between D1 and D2 to maintain homeostasis. Coexistence of D1 and D2 may thus be established through a delicate balance between competition and cooperation.

One might extend the simple D1–D2 analogy – with additional provisions – to the chromosome (D1) of a bacterium containing a drug resistance plasmid (D2) that relies at least partly on host-encoded functions for its survival. Under challenge by the drug in question, the host is doomed without the plasmid; and higher the drug concentration, the better it is for the host to house more copies of the plasmid. This mutual benefit, improved drug resi-

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stance for the host and enhanced copy number for the plasmid, has its limit. Beyond a certain copy number, by depleting metabolic resources, the plasmid begins to hurt the host and, in turn, itself. As we shall see later, even plasmids that apparently provide no added fitness to their hosts can maintain themselves stably in a cell population, provided their copy numbers are well regulated so as not to interfere with host physiology.

1.2 *Selfish nucleic acids in prokaryotes and eukaryotes*

DNA/RNA elements that fit the description of 'selfish genes' are widespread among prokaryotes and eukaryotes. In general, these have evolved by acquiring rather well understood survival and propagation functions, namely, replication, partitioning, multimer resolution, 'post-segregational killing' and conjugative transfer (Thomas 2000). While eukaryotic selfish elements are almost always in an integrated state in the chromosomes, they go through extrachromosomal intermediate stages during their spread.

It has been suggested that a gain in fitness to ancient bacterial hosts conferred by accessory DNA elements provided the initial impetus for their evolution (Hartl *et al* 1984). Subsequent acquisition of selfish characters by these elements is believed to have accelerated their replication, horizontal transmission and dissemination. Further co-evolution with their host genomes is likely to have established specialized population structures and

sophisticated mechanisms of genetic exchange among bacteria. The invasive properties of prokaryotic and eukaryotic selfish elements appear to be evolutionarily interconnected, by divergence and/or convergence, in many respects. Several of the biochemical steps utilized by homing and mobile introns, retrons, transposons, and non-LTR and LTR retrotransposons that span phage, bacteria, fungi, plants, insects, flies, worms and mammals are well conserved among the two kingdoms (Belfort *et al* 2002; Eickbush 2002; Fedroff 2002; Mizuuchi and Baker 2002; Plasterk and van Luenen 2002; Rio 2002; Sandmeyer *et al* 2002; Yamanaka *et al* 2002).

2. Bacterial plasmids: strategies for stable propagation

Since plasmids are extrachromosomal elements (by definition), they cannot exist unless they can replicate autonomously. Plasmids utilize a fairly limited set of biochemical strategies for replication, yet the strategies are embellished with mechanisms for controlling copy number and coordinating their replication with cell growth. The problem of propagating the replicated molecules to daughter cells is dealt with in one of two ways. Provided the steady state copy number of the plasmid is reasonably high, and there are no constraints to free diffusion, the chances of a plasmid-free cell arising at any given generation is quite low. Deviations from the normal copy number due to unequal segregation are usually corrected by control mechanisms operating at the replication level. However, other things being equal, a low copy number would be favoured as it reduces the metabolic burden on the host. The potential disadvantage to the plasmid of mis-segregation, resulting from low copy number, is circumvented by the acquisition of an active partitioning system. Topological interlinking of daughter molecules resulting from DNA replication as well as the formation of dimers or multimers through homologous recombination pose additional threats to plasmid propagation. A cellular type II topoisomerase normally performs the unlinking function, and a site-specific recombination system resolves plasmid multimers into monomers (Barre and Sherratt 2002; Zechiedrich *et al* 2000). Finally, the horizontal spread and establishment of plasmids among bacteria would have been promoted or accelerated by the evolution of functions for mobilization and conjugative transfer.

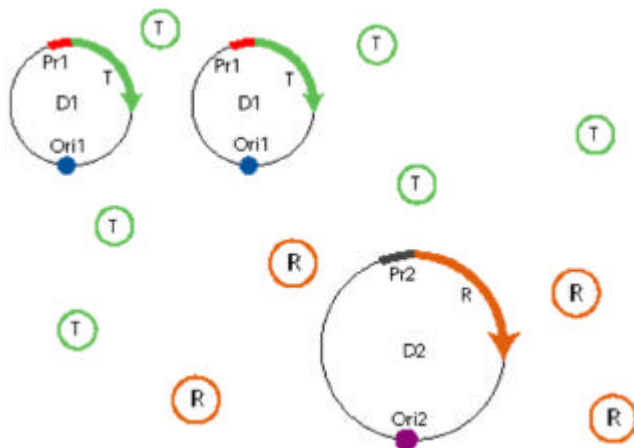


Figure 1. Coexistence of two genomes by balancing mutual competition and cooperation. In a primitive cell depicted here, a hypothetical steady state has been established. The genome D1 has a higher copy number than D2 because of its replication advantage (Ori1 stronger than Ori2). By balancing the promoter strength against copy number (Pr2 stronger than Pr1), the replicase R and the transcriptase T are made in amounts sufficient to propagate the steady state through sequential cell divisions. See text for further details.

2.1 *Plasmid maintenance by addiction and post-segregational killing*

A rather intricate scheme of plasmid selfishness, which results in eliminating competition from plasmid-free cells

arising in the population, is mediated through an 'addiction module' encoding a long-lived toxin and a short-lived antidote (Engelberg-Kulka and Glaser 1999; Gerdes *et al* 1997). Cells which lose the plasmid would be subject to post-segregational killing as they inherit the toxin in the absence of the antidote. An analogous behaviour is displayed by genetic elements (including plasmids) that harbour genes for type II restriction endonucleases and their cognate modification enzymes (Kobayashi 2001; Naito *et al* 1995). Cell descendants that have lost these genes fail to modify a sufficient number of genomic sites to ward off lethal attack from the remaining restriction enzyme molecules. Therefore the co-evolution and maintenance of restriction-modification gene pairs must have depended on their capacity to act as 'selfish symbionts'.

3. Plasmid and chromosome segregation in prokaryotes

Thanks primarily to technological advances in cell biology that provide new perspectives to long-standing genetic, biochemical and molecular analyses of the phenomenon (Gerdes *et al* 2000; Moller-Jensen *et al* 2000; Pogliano 2002), recent years have seen rapid progress in our understanding of the mechanisms of chromosome and plasmid partitioning in prokaryotes. The ability to visualize either plasmids or chromosomes by fluorescence tagging in living or fixed cells has revealed hitherto unsuspected dynamic and spatial attributes of the segregation machinery. Not only does the DNA follow highly ordered localization patterns as a function of cell growth from birth to division, but also a number of proteins known to be involved in DNA partitioning display coincident localization patterns. The results are most easily explained by the organization of compact segregation complexes that are transferred to daughter cells in spatially and temporally orchestrated steps.

3.1 The partitioning loci of plasmids: ParA ATPase, ParB and centromere (parC)

Low copy bacterial plasmids, such as P1, F and R1, encode partitioning genes (*par*) that facilitate their faithful segregation at cell division. Since the different nomenclatures of genes in different systems can be confusing for the non-specialist, we will present here a simplified picture of the unifying features among these systems with reference to figure 2. The *par* loci specify three components: two *trans*-acting proteins and a *cis*-acting centromere-like partitioning locus that together form a nucleoprotein complex. The Par proteins are encoded by two genes within an operon which, generally speaking, is

autoregulated by these proteins via negative control of transcription. The polypeptide product of the upstream *par* gene, which we generalize here as ParA, is an ATPase; that of the downstream gene, designated as ParB, binds to the partitioning locus *parC* in a cooperative fashion. The ParA protein is recruited to the 'pre-partition' complex formed between ParB and *parC* to form the functional partition nucleoprotein complex. The *parC* locus is usually constituted by tandem arrays of repeat units (or iterons), and its position with respect to the *par* operon can be variable. For example, *parC* may harbour the promoter to the operon, or may reside distal to the promoter and downstream of the protein coding sequences. Some of the partitioning loci are characterized by multiple direct repeats upstream and downstream of the *par* genes, and the two sets may independently or cooperatively serve a centromere-like function.

The ATPase motif, the functional signature of the ParA proteins, is of the Walker type in the type I partitioning loci (P1 and F, for example) but fits the actin-like ATPases in the type II partitioning loci (R1 plasmid, for example) (Bork *et al* 1992; Koonin 1993a,b). A phylogenetic comparison of the Walker type partitioning ATPases has yielded two subgroups. The type Ia partitioning loci encompass one subgroup, the type Ib loci the other (see figure 2).

The type Ia ParA contains an amino-terminal DNA binding motif that associates with the *par* operator region and negatively regulates it. The level of repression is usually augmented by the ParB protein acting as a co-repressor. In the case of F, maximal repression also requires the centromere locus, suggesting a role for DNA looping in this process (Yates *et al* 1999). *In vitro* studies demonstrate that the P1 ParA in its ADP associated form binds to the promoter, whereas the same ParA in its ATP associated form interacts with the centromere-ParB pre-partition complex (Bouet and Funnell 1999; Yates *et al* 1999). Thus the function of ParA, as a transcription regulator or as a partitioning agent, appears to be subject to allosteric regulation by the bound form of the nucleotide ligand. ParA proteins of type Ib loci appear to lack a DNA binding domain. The autoregulation of the operon in this case is perhaps mediated by ParB interaction with the centromere, within which the promoter is embedded.

ParA proteins of type II loci share homology with the superfamily of ATPases that encompasses actin, hsp70/DnaK and the bacterial cell cycle proteins FtsA and MreB, among others (Bork *et al* 1992). The type II operons, like the type Ib operons, are also regulated by ParB binding to the centromere, apparently with no co-repressor function provided by ParA. Strikingly, the type Ib and type II operons have a similar organization, with the promoter being nested within the centromere. Assays using electron microscopy or based on the kinetics of ligation reveal

that two *parC* containing DNA molecules belonging to the type II system can be paired by ParB, and the pairing efficiency can be increased in the presence of ParA and ATP and by the substrates being supercoiled plasmids (Jensen *et al* 1998). This pairing, occurring concomitant with or in a post-replication manner, has implications for plasmid partitioning as well as for *parC* mediated plasmid incompatibility. Despite the strong similarity between type Ib and type II loci in their genetic organization, the ATPase family encoded by one does not show sequence conservation with that encoded by the other. As noted before, the type I ATPases are of the Walker type and the type II ATPases of the actin type. It has been suggested that the two types of *par* loci could have arisen independently by convergent evolution or could have exchanged components during their evolution (Gerdes *et al* 2000).

3.2 Partitioning loci of bacterial chromosomes

Genes encoding homologues and orthologues of ParA and ParB have been identified in the chromosomes of *Bacillus subtilis* and *Caulobacter crescentus* (Ireton *et al* 1994; Lin and Grossman 1998; Mohl and Guber 1997). The *subtilis* genes are called *soj* (*parA*) and *spoJ* (*parB*). Experimental evidence supports a role for these genes in chromosome partitioning in both bacteria. Deletions of *soj* and *spoJ*, but not of *soj* alone, result in a large increase in the frequency of anucleate cells (Ireton *et al* 1994). The SpoJ protein binds to eight *parC*-like sequences

located close to the chromosomal replication origin (Lin and Grossman 1998). *Caulobacter parA* and *parB* are essential genes, and their overexpression leads to chromosome missegregation. An AT-rich binding site for the *Caulobacter* ParB protein is located downstream of the gene, and is likely the *Caulobacter parC*.

Phylogeny of chromosomal ParA proteins from Gram positive and Gram negative bacteria places them in a distinct subgroup that contains the A, A' and B boxes of the 'deviant' Walker-type ATPases (Koonin 1993a,b). Within this subgroup, there is large scale sequence divergence among the members, with indications of horizontal gene transfer. Whereas some bacterial chromosomes reveal the presence of two or more *parAB* loci, others contain, in addition to one complete *parAB*, extra *parA* homologues without their typical *parB* partners.

What comes as a surprise is that the typical *par* locus is absent in some bacteria including *Escherichia coli* and *Haemophilus influenzae*. Do these bacteria harbour novel partitioning loci for segregating their chromosomes? If the answer is yes, the identities of the genes are unknown at present.

3.3 Cellular location of bacterial plasmids and their segregation dynamics

The ability to tag either the bacterial chromosome or reporter plasmids using fluorescent probes has facilitated

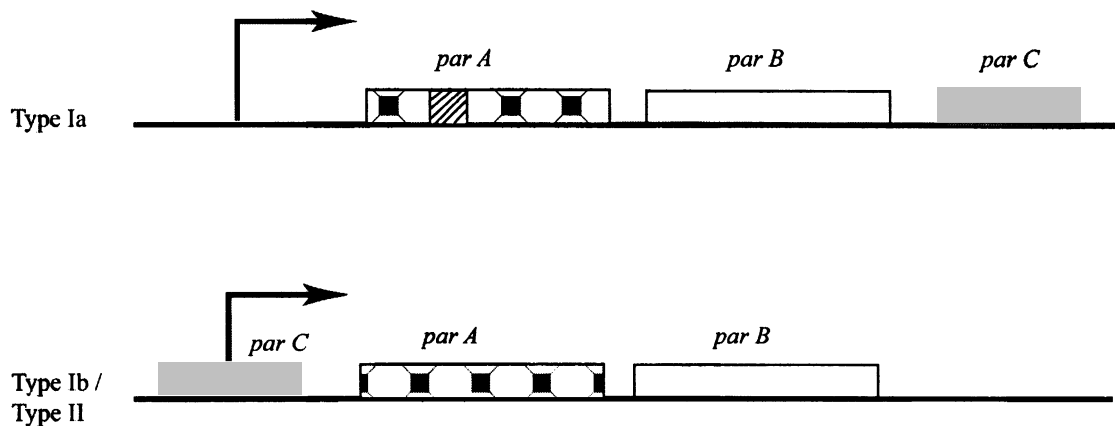


Figure 2. Organization of plasmid partitioning loci in bacteria. Based on the arrangement of the centromere-like *cis*-acting locus and the genes coding for the partitioning proteins, the *par* loci may be divided into two general classes: type Ia and type Ib/II. We have generalized here the upstream gene as *parA*, the downstream one as *parB* and the *cis*-acting sequence as *parC*. In the literature these names for the different systems are not constant. The ParA proteins in the type Ia (plasmids P1 and F) and Ib (the Ti plasmid of *Agrobacterium tumefaciens*) systems are ATPases with the standard Walker motifs; ParA proteins of the type II loci (R1 plasmids) are actin-like ATPases. The type Ia ATPases contain an amino-terminal DNA binding domain (indicated by the stippled box). The ParB proteins in general bind to *parC*, which includes repeated DNA elements (iterons). In several type Ib loci, in addition to the upstream *parC*, direct multiple repeats are also present downstream of the *par* genes, and may contribute to plasmid partitioning. The location of the promoters and the direction of transcription are indicated by the arrows.

their tracking by fluorescence microscopy during cell growth and division. In one widely used approach, fluorescent nucleic acids are hybridized *in situ* to target DNAs after fixing cells (FISH; see Moller-Jensen *et al* 2000). In a second approach, DNA molecules harbouring a cloned array of an operator sequence and rendered fluorescent by binding of the GFP-repressor are visualized in live cells (Pogliano 2002).

Unit copy plasmids, such as P1 and F, normally localize to the midpoint of a new-born cell. Following replication, one molecule moves to the one-quarter cell position, while the second one moves in the opposite direction to the three-quarter position (figure 3A). These are the midpoints of the future daughter cells. Occasionally, the plasmids may duplicate once more prior to cell division, so that the daughter cells, when formed, will contain two plasmid copies. Studies with the F plasmid indicate that the two progeny molecules formed by DNA replication separate from each

other within minutes after the completion of replication (Onogi *et al* 2002). This is in contrast to chromosomal replication origins that appear to remain associated with each other for a much longer time, into the late replication period, after they have been duplicated (Sunako *et al* 2001).

Reporter plasmids which carry the R1 partitioning system reveal a characteristic localization pattern that is distinct from that of P1 and F (figure 3B). A single plasmid focus in a cell is located close to one pole or at the mid-cell position. When two plasmid foci are present in a cell, each one is located proximal to each of the two cell poles. These observations suggest that plasmids replicate at the mid-cell position, and the daughter molecules move rapidly to the opposite cell poles. Since the R1 plasmids have an average copy number of 4–5 molecules per cell, the cytological observations imply that these molecules remain clustered as a single unit during replication and segregation.

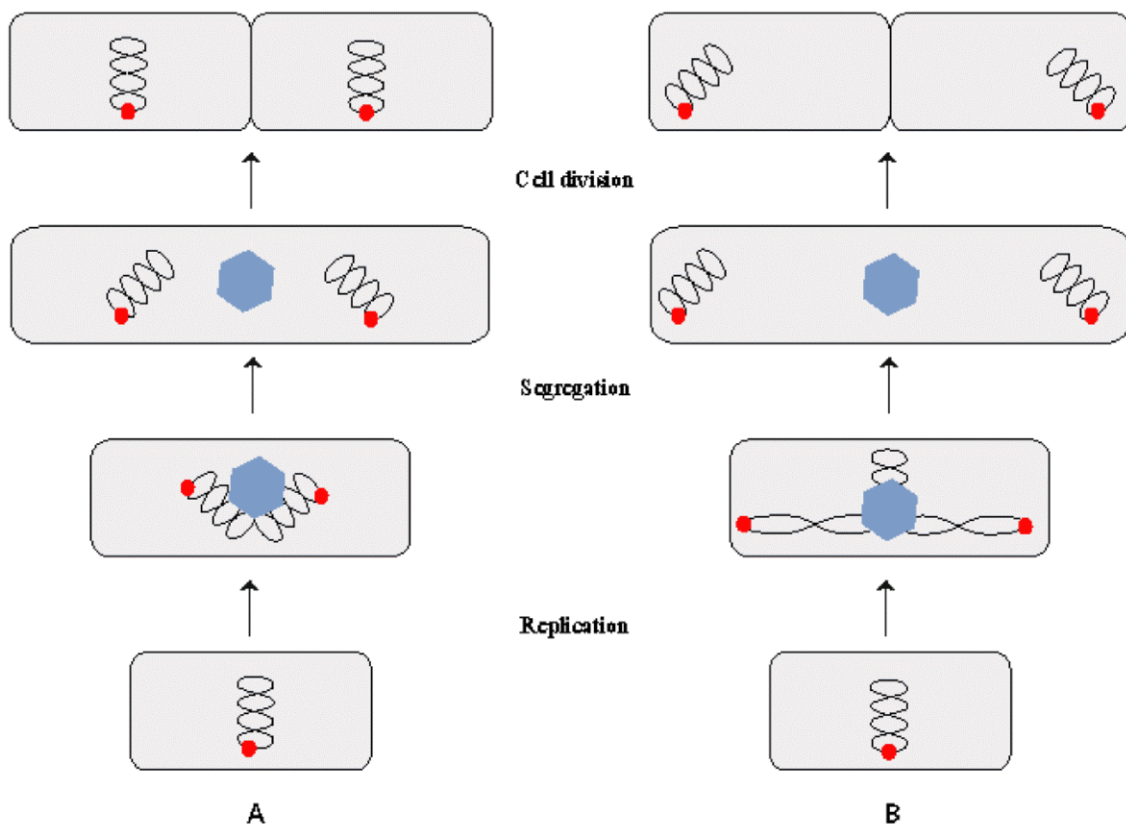


Figure 3. Plasmid localization and spatial control of replication and partitioning in bacteria. (A) One class of plasmids such as P1 and F localize to the mid-cell position, where the ‘replication factory’ is presumably resident. Following replication, the product molecules move to the quarter and three-quarter positions, which are the future mid-cell positions of the daughter cells. (B) A second class of plasmids (R1, for example) is normally localized at one pole of the bacterial cell. They move to the center of the cell for replication, and the daughter molecules move away from each other so as to occupy their polar locations. A similar pattern of dynamic localization is also seen with replication origins of some bacterial chromosomes.

High copy number plasmids, such as ColE1 derivatives, do not encode a partitioning system, and the long held belief has been that they segregate by a random mechanism. The finding that pBR322 plasmids may have a clustered organization, based on direct visualization and X-ray scattering analysis, has challenged this assumption (Eliasson *et al* 1992; Reich *et al* 1994). Recent observations with pUC19 derived plasmids indicate that they may exist either in the clustered state or as randomly distributed molecules (Pogliano *et al* 2001). The clusters, usually located at the mid-cell or quarter cell positions, are dynamic; they can be seen to assemble and disassemble when observed by time-lapse microscopy. The results are consistent with the possibility that the primary segregation mechanism for these high copy plasmids is still random diffusion. However, specific localization and segregation may operate as a secondary mechanism, and is likely to be inefficient in comparison to the par-mediated partitioning processes described for unit or low copy plasmids.

A striking recent finding is that the ParA ATPase of the R1 plasmid (ParM) can assemble into actin like filaments running nearly the entire length of the *E. coli* cell. This adds a new twist to the current thinking on plasmid and chromosome partitioning in prokaryotes (Engelman 2003; Moller-Jensen *et al* 2002; Stephens 2002). Recall that replicated R1 plasmids can be paired by the ParA–ParB–*parC* complex (same as ParM–ParR–*parC*) prior to their movement from the mid-cell position to symmetrical positions near the cell poles. Perhaps, subsequent to replication and pairing of the daughter molecules, the ParM protein initiates filament formation in the form of protofilament bundles attached to the plasmids. Filament growth by recruitment of new ParM molecules can generate the mechanical force required to separate and push each plasmid away from the center of the cell in opposite directions.

An interesting question is whether chromosomes might also utilize an active filament mechanism to segregate their origins towards the cell poles and away from the cell center, where they are positioned for replication. Evidence from *B. subtilis* and *E. coli* suggest that a replication factory positioned at the mid-cell point could act as a pump drawing in unreplicated DNA and extruding the replicated molecules in a directional fashion. However, the factory model is not altogether consistent with the observations that chromosomal origins remain paired for a significant length of time prior to separation and movement away from each other. Does the replication factory also utilize a filament mediated segregation machine? The answer must await future experiments.

Until recently, bacterial cells were believed entirely to lack cytoskeletal structures analogous to the dynamic scaffolding that regulates cell shape, movement and division in eukaryotes. The discovery of a ring structure

formed by the FtsZ protein, a tubulin homolog, at the division septum in bacteria (van den Ent *et al* 2001a,b) suggested that this long held notion may be suspect. This was followed by the finding that actin-like helical filaments may control cell shape in non-spherical bacteria (Moller-Jensen *et al* 2002). The MreB protein from *B. subtilis* can polymerize *in vitro* into filaments that resemble F-actin (Jones *et al* 2001) and form long helical bands *in vivo* underlying the cytoplasmic membrane. The specter of ParM forming bundles of protofilaments attached to plasmid molecules that can then be segregated by subsequent extension of the filaments suggests a mechanism for how cytoskeletal structures may promote faithful segregation of genetic information in bacteria without recourse to the elaborate spindle based mechanism utilized by eukaryotes.

4. Mechanisms for plasmid segregation in yeasts

The multicopy yeast plasmid 2 μ m circle (figure 4A), nearly ubiquitously present in *Saccharomyces* yeast strains, is a selfish extrachromosomal DNA element which encodes a simple, yet highly efficient partitioning system (Velmurugan *et al* 2003). The circular plasmid has a mean copy number of approximately 60 per cell, and each plasmid molecule is duplicated once per cell cycle by the host replication machinery. The product molecules are then distributed evenly (or nearly evenly) to the daughter cells with the help of the partitioning system. The plasmid also encodes an amplification system, consisting of a site-specific recombinase and a pair of target sites that are arranged in inverted orientation within the plasmid genome. The amplification system is normally kept repressed, and is triggered into action only when a rare missegregation event decreases the copy number in one of the daughter cells. The proposed model for amplification requires a temporally controlled site-specific recombination event during bidirectional replication of the plasmid. The crossover between one of the duplicated copies of the target site proximal to the origin and the unduplicated site distal to it (Futcher 1986; figure 4B) causes one of the replication forks to change its direction. The unidirectional forks chase each other around the circular template to generate an amplicon containing multiple tandem copies of the plasmid. Resolution of the multimer into single molecules can be mediated either by the site-specific recombination system or by the general recombination machinery of the host. Circular plasmids identified in a limited set of yeast strains belonging to a common genus have structural and genetic organizations that closely resemble that of the 2 μ m plasmid, even though there is a large divergence in their DNA sequences. All of these plasmids have apparently retained their own site-specific recombination and partitioning sys-

tems which seem to provide an efficient strategy for the stable propagation of these benign parasites. They are benign because they do not overtax the host metabolic machinery, still parasitic because they rely on the host for their replication and propagation.

Why does a multicopy element (approximately 60 per cell) equipped with an amplification device resort to active partitioning? Why not random segregation, since the probability of plasmid loss at any cell division event would be extremely low? Furthermore, a drop in copy number could be readily reversed via amplification. Fluorescence tagging reveals the plasmids to be organized as a tight-knit cluster in the nucleus and to segregate as a cluster (Scott-Drew and Murray 1998; Scott-Drew *et al* 2002; Velmurugan *et al* 2000). This effective reduction in copy

number to unity justifies the need for an active partitioning mechanism. The amplification system is tightly regulated under steady state growth, reducing the potential hazard of runaway increase in copy number that would be deleterious to the host cell and indirectly so to the plasmid.

4.1 The plasmid partitioning system

The partitioning system consists of the *trans*-acting proteins Rep1p and Rep2p together with the *cis*-acting locus *STB*, which is made up of a tandem array of five to six copies of a 65 bp consensus sequence. Interactions among these protein and DNA components appear to be essential for normal plasmid segregation. In the yeast nucleus, the plasmid cluster (figure 5A) exists in tight association with

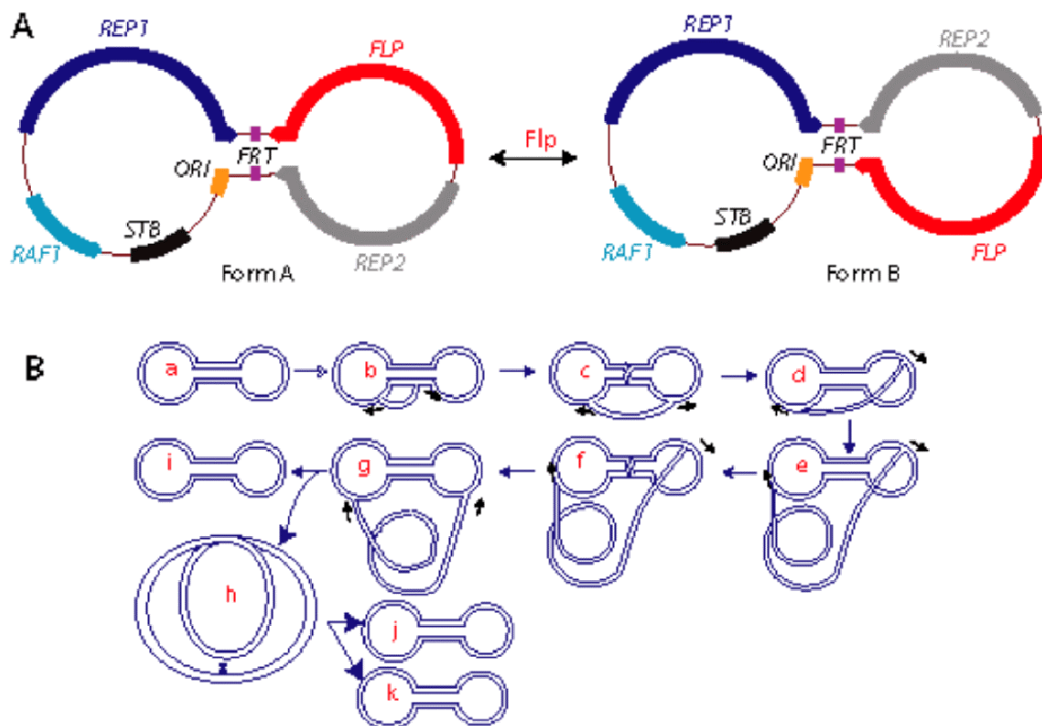


Figure 4. (A) The double stranded circular genome of the 2 μ m plasmid is normally represented in the form of a dumbbell. The parallel lines indicate a 599 bp inverted repeat, each copy of the repeat elements harbouring one *FRT* site (the target for the Flp site-specific recombinase). The relative locations of the plasmid replication origin (*ORI*) and the partitioning locus *STB* with respect to *FRT* are shown. The open reading frames in the plasmid are highlighted, and the arrows denote their transcriptional orientation. The partitioning system is constituted by *REP1*, *REP2* and *STB*, and the amplification system by *FLP* and the *FRTs*. *RAF1* encodes a protein that positively regulates amplification. The two plasmid forms A and B result from Flp mediated site-specific recombination. (B) The model for plasmid amplification proposed by Futcher (1986) is illustrated. During bidirectional replication of the plasmid (b), the *FRT* site proximal to the plasmid replication origin is duplicated first. A recombination event between one of the replicated *FRTs* and the unreplicated distal *FRT* (c) results in the inversion of one replication fork with respect to the other (d). The unidirectional forks travelling around the circular template spin out tandem multiple copies of the plasmid (e). A second recombination event may reestablish bidirectional replication (f–g). Termination of replication results in the amplified product (h) plus the unit copy template (i). The plasmid multimer can be resolved into monomers via homologous recombination or via Flp mediated recombination.

the Rep proteins (and perhaps other host encoded proteins) to form a high-order partitioning complex (figure 5B).

There is a superficial similarity between the yeast plasmid and bacterial plasmid partitioning systems in that both utilize two proteins in conjunction with a *cis*-acting DNA site. However, the two partitioning systems appear to be quite disparate in their functional elements. Neither Rep1p nor Rep2p has been shown either to bind ATP or possess ATP hydrolyzing activity. They do bind the

STB DNA, but this interaction in all likelihood is mediated through a host factor (Hadfield *et al* 1995). Recently, the carboxyl-terminal region of Rep2p has been shown to possess non-specific DNA binding activity (Sengupta *et al* 2001). How does an apparently rudimentary partitioning system confer chromosome-like stability on the plasmid, with a loss rate that is only 10^{-5} to 10^{-4} per cell per generation? The answer to this puzzle is just beginning to emerge. In brief, the plasmid appears to utilize the

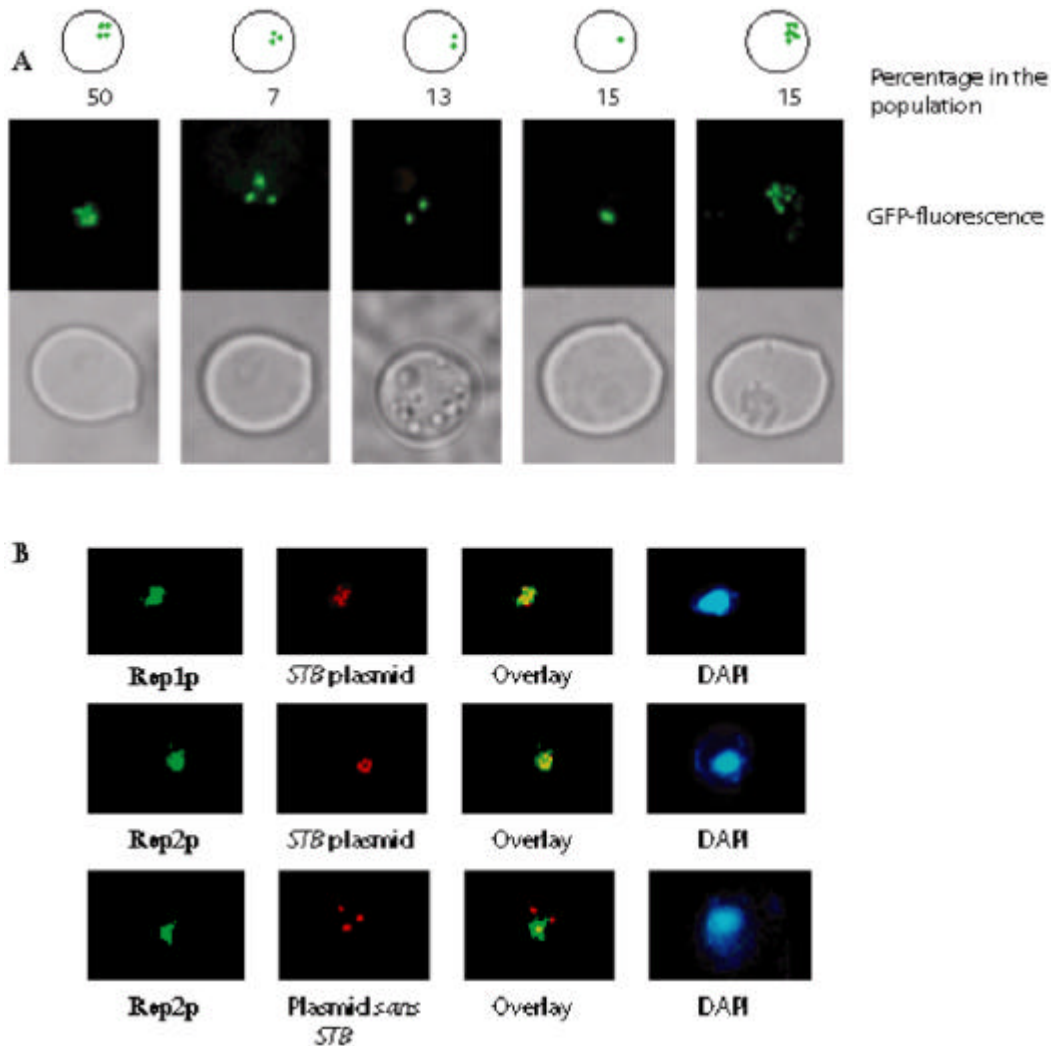


Figure 5. Organization of the 2 μ m plasmid within the yeast nucleus. **(A)** A 2 μ m circle derived reporter plasmid containing multiple copies of the Lac operator can be visualized in live yeast cells expressing GFP fused to the Lac repressor. The operator-repressor interaction tags the plasmid with fluorescence. Resident in the nucleus, the plasmid has a clustered organization. In a majority of cases, the cluster has a four lobed pattern. However, clusters with fewer or greater than four lobes are also seen. **(B)** The Rep1 and Rep2 proteins colocalize with an *STB*-containing plasmid in the yeast nucleus in the form of an apparently high order protein-DNA complex (top and middle rows). When the plasmid lacks *STB*, the tight association between the Rep proteins and the plasmid is lost (bottom row). Note the red plasmid dots that are not coincident with the Rep2 protein. The Rep proteins and the plasmids are visualized by indirect immunofluorescence using fluorescein or Texas red conjugated secondary antibodies. The plasmids are targeted by primary antibodies to the Lac repressor, which remains bound to the operator sequences.

Rep-*STB* system to exploit the chromosomal segregation machine to ensure its stable propagation.

4.2 Coupling between plasmid and chromosome segregation in yeast

Mutations which impair the fidelity of chromosome segregation in yeast also affect plasmid segregation in a similar manner (Velmurugan *et al* 2000; Mehta *et al* 2002; figure 6). Strikingly, the plasmid cluster shows a strong tendency to missegregate in tandem with the bulk of the chromosomes. This finding suggests that the plasmid shares at least some of the steps of the chromosome segregation pathway or utilizes a surveillance mechanism to escape entry into a cell with an incomplete chromosome content. Avoiding a cell that is destined for elimination would be a logical strategy for a selfish DNA element.

The recent finding that the partitioning system recruits the yeast cohesin complex, essential for the fidelity of chromosome segregation, to the plasmid breaks new ground in our thinking on plasmid segregation mechani-

sms (Mehta *et al* 2002). Cohesin is a multi-protein complex that provides eukaryotic cells with a mechanism for remembering a DNA replication event as well as appropriately counting the products resulting from it (Uhlmann and Nasmyth 1998; Skibbens *et al* 1999; Toth *et al* 1999; Uhlmann *et al* 1999, 2000; Nasmyth *et al* 2000; Carson and Christman 2001; Cohen-Fix 2001; Nasmyth 2001). Cohesin is loaded on to specific chromosomal sites concomitant with DNA duplication, and bridges sister chromatids at the kinetochores and along the chromosome arms (Blat and Kleckner 1999; Tanaka *et al* 1999; Carson and Christman 2001). By virtue of this pairing, a diploid cell is able to distinguish chromosome sisters from chromosome homologs. During metaphase to anaphase transition, a protease cleaves one of the cohesin components, causing the bridge to disassemble and the sister chromatids to be pulled apart to opposite cell poles by the mitotic spindle. The cohesin complex associates with the *STB* locus specifically in a Rep protein dependent manner. The timing of this association during the cell cycle is the same as that of cohesin-chromosome association (figure 7A). Similarly, cohesin is disassembled from *STB*

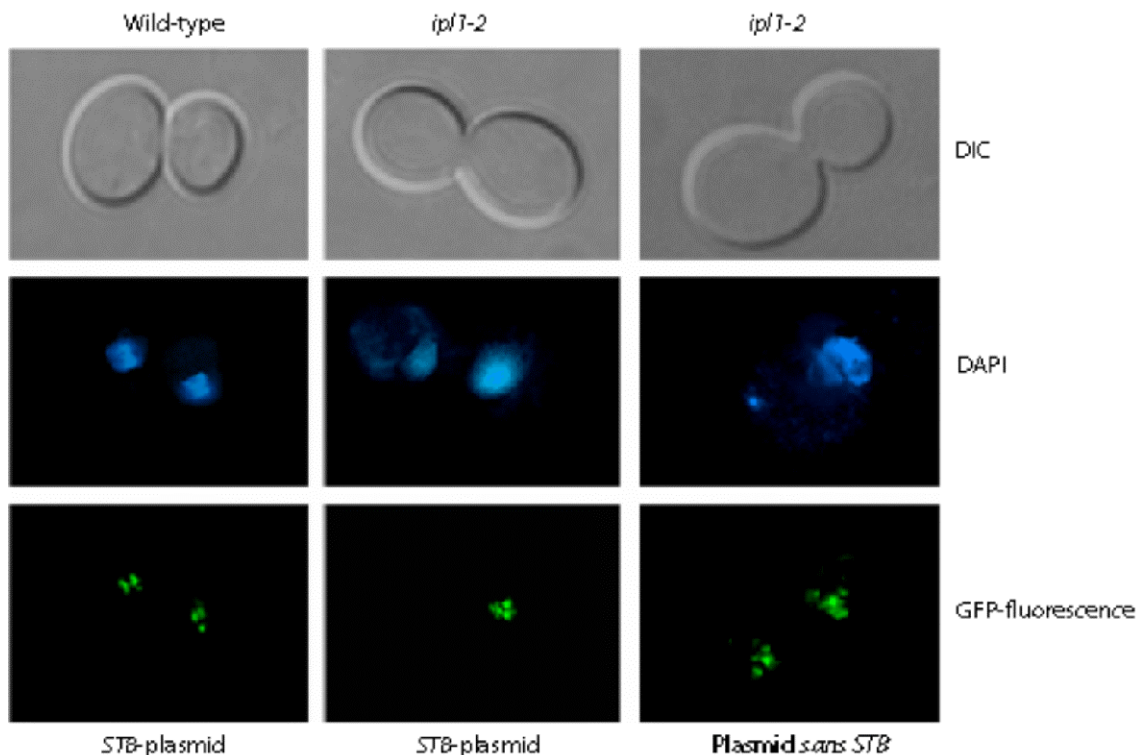


Figure 6. Chromosome segregation and 2 μ m plasmid segregation in yeast. In wild type cells, the 2 μ m circle reporter plasmid segregates roughly equally to the daughter cells (left). The equivalent DAPI staining in the two daughter cells denotes normal chromosome segregation. When a conditional mutant strain (*ipl1-2*) defective for chromosome segregation is shifted to the non-permissive temperature, the plasmid tends to missegregate with the bulk of the chromosomes in the vast majority of the cells (middle). Only in 20% or fewer cells can one see the plasmid cluster occupying the cell compartment with little or no DAPI staining. When the plasmid lacks the *STB* locus, the proclivity to segregate in tandem with the chromosomes is lost (right).

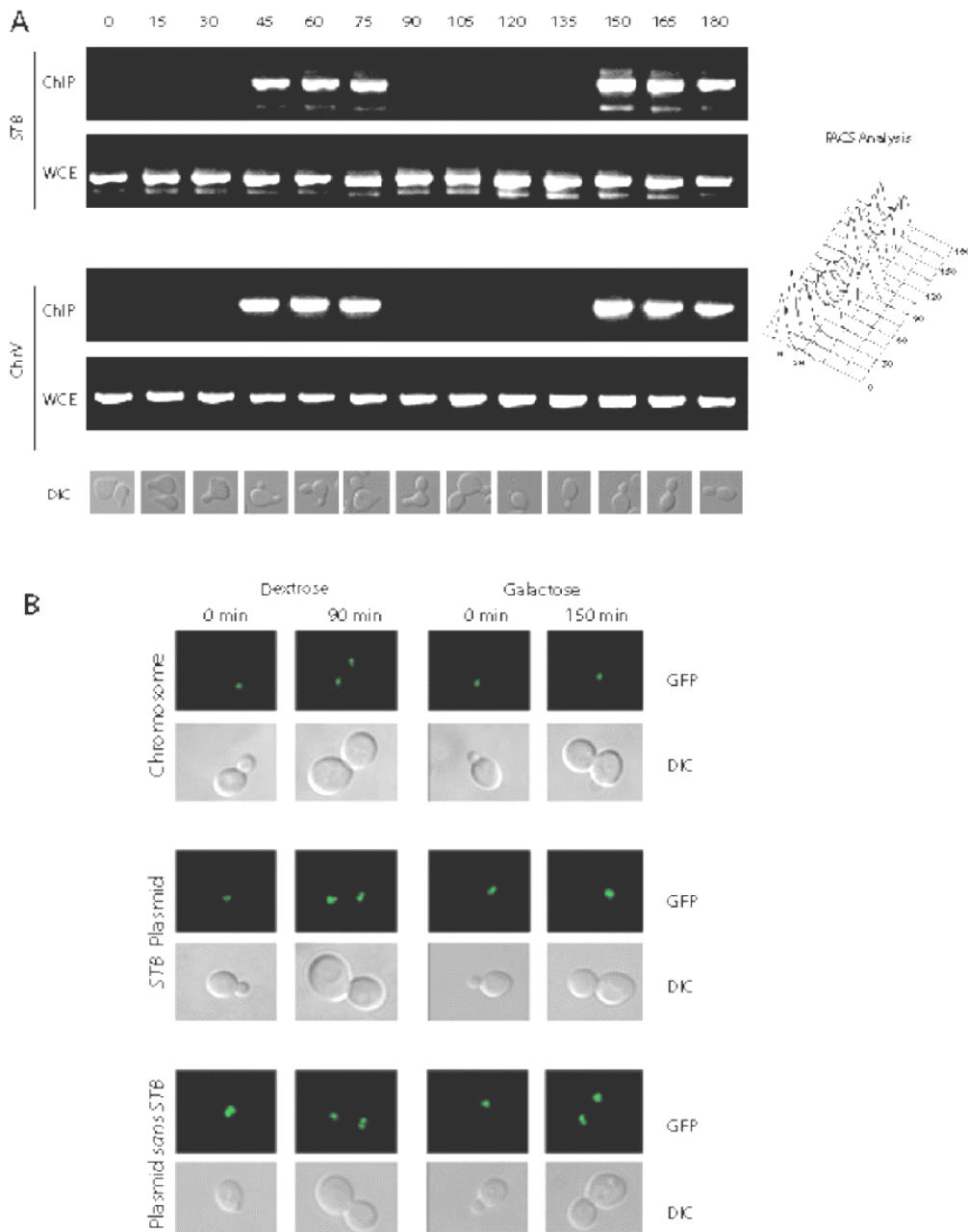


Figure 7. The yeast cohesin complex is recruited to the *STB* locus in a cell cycle dependent manner; lack of cohesin disassembly results in failure of replicated plasmid clusters to separate. **(A)** The association of the cohesin complex with the *STB* locus or an authentic cohesin binding site on chromosome V is followed by chromatin immunoprecipitation (ChIP) using antibodies to the cohesin component Mcd1p. Cells synchronized in G1 with alpha factor are released into pheromone-free medium at time zero. The progression of cell cycle is followed by examining cells by light microscopy and by estimation of DNA content by FACS analysis. Cohesin associates with *STB* and the chromosomal locus synchronously during S phase and also dissociates from both sites synchronously during late G2/M. The rows marked WCE refer to whole cell extracts used as positive controls in the PCR reactions. **(B)** The host strains harbouring a tagged chromosome or a reporter plasmid contain a normal copy of the *MCD1* gene plus a galactose inducible copy of *MCD1-nc* (encoding a non-cleavable version of the Mcd1 protein). Dextrose grown synchronized cells are released from G1 arrest into dextrose or galactose medium, and followed by time lapse fluorescence microscopy. The end points of the assay are 90 min for dextrose grown cells and 150 min. for galactose grown cells. Representative fluorescence patterns for the initial and final time points are shown. For each experiment, the number of cells that show a given pattern is expressed as a fraction of the total number of cells assayed. Note that in the galactose induced cells, there is predominantly one fluorescent chromosome dot or one fluorescent plasmid cluster. The results are consistent with the failure of sister chromatids and replicated plasmid clusters to separate. For a plasmid lacking *STB*, two separated plasmid clusters are seen even in galactose induced cells.

at the same time that it dissociates from chromosomal sites. When the cohesin complex is made resistant to proteolytic cleavage and dissociation by appropriate mutations, the duplicated plasmid clusters fail to separate in a manner which is reminiscent of the block in the sister chromatid separation under similar conditions (Mehta *et al* 2002) (figure 7B).

The temporally regulated and Rep-*STB*-specified association between cohesin and the 2 μ m plasmid and the life time of this complex during the cell cycle suggest that duplicated plasmid clusters are held together by the cohesin bridge until the onset of anaphase, when the collapse of the bridge triggers their separation and movement away from each other. In principle, the plasmid may follow this pathway by tethering to chromosome(s) (hitchhiking) or utilizing cohesin-mediated segregation independently of the chromosomes (figure 8). Mammalian viruses such as the Epstein-Barr virus (Harris *et al* 1985; Kanda *et al* 2001) and bovine papilloma virus (Lehman and Botchan 1998; Ilves *et al* 1999), which exist predominantly as extrachromosomal episomes, have been

shown to utilize chromosome tethering as a means for stable segregation. The tethering mechanism provides an advantage for these viruses by preventing their exclusion into the cytoplasm during the breakdown of the nuclear envelope and subsequent reassembly. For the 2 μ m plasmid, though, the benefits of chromosomal attachment are less obvious, since the nuclei remain intact during yeast mitosis. From the partitioning standpoint, the 2 μ m circle cluster may perhaps be considered as a non-essential yeast chromosome – the ‘plasmosome’.

5. The optimized molecular selfishness of the 2 μ m circle

The genetic organization of the yeast plasmid consisting of a yeast replication origin together with a partitioning machinery and an amplification machinery is a highly economical design for a selfish DNA element. With the assistance of the plasmid replication origin, each plasmid molecule ensures its duplication, once per S phase, by the

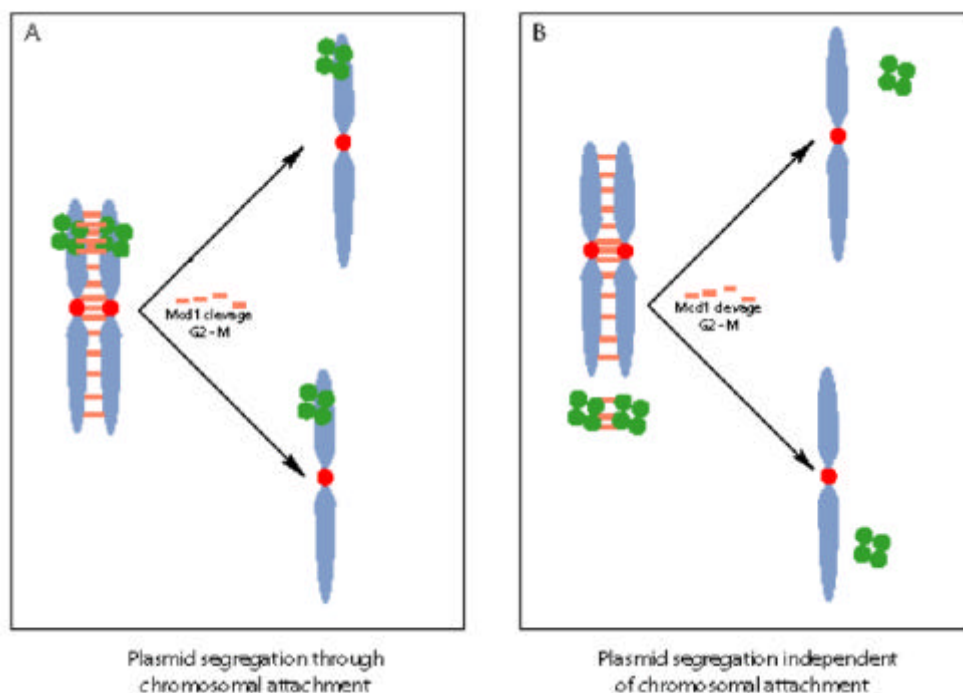


Figure 8. Models for cohesin assisted segregation of 2 μ m plasmid clusters. (A) In one model, the plasmid cluster is tethered to one of the chromosomes. Concomitant with DNA replication, the cohesin complex bridges the sister chromatids and the duplicated plasmid clusters. Each cluster is now tethered to each of the two chromosome sisters. Following cohesin disassembly due to cleavage of the Mcd1 protein in anaphase, the plasmid clusters move to opposite cell poles by hitchhiking on the chromosomes. (B) In the second model, the replicated plasmid clusters are paired by cohesin, but there is no plasmid-chromosome tethering. Dissolution of the cohesin bridge dispatches the plasmid clusters to the daughter cells by an unknown mechanism that is independent of chromosomes.

cellular replication machinery. By limiting its abundance to a fairly high but carefully optimized value within the context of the equipartitioning and amplification mechanisms, the plasmid persists stably as an extrachromosomal element that apparently does neither good nor harm to its host. The amplification system is kept quiescent under steady state conditions to minimize the possibility of runaway increase in plasmid copy number (Som *et al* 1988). At the same time, mechanisms are in place to rapidly commission it when a drop in copy number occurs.

The yeast plasmid provides a paradigm for how a simple DNA-protein system might raise its level of functional sophistication by feeding into the elaborate and highly regulated molecular processes established for the chromosomes of its host. Understanding the finer details of this hitherto unsuspected molecular poaching must await further work.

6. Summary

We have reviewed here some of the molecular strategies employed by extrachromosomal DNA elements for their successful persistence. Low copy number bacterial plasmids achieve stable inheritance by daughter cells through the action of two partitioning proteins, one of which is an ATPase, together with a centromere-like locus with which these proteins associate. This partitioning complex is localized to a defined position within the bacterial cell, where the plasmid DNA is replicated. The product molecules are then moved away from each other to occupy equivalent positions in the daughter cells. One of the surprises from very recent studies is the finding that an elementary cytoskeletal apparatus may mediate plasmid segregation in bacteria. Like their bacterial counterparts, circular plasmids in yeast strains also utilize a partitioning system consisting of two proteins and a DNA locus crudely equivalent to a centromere. Note, though, that the *STB* DNA of the plasmid is quite distinct in sequence and apparent mode of action from the centromeres of yeast chromosomes. The mechanisms of bacterial plasmid and yeast plasmid partitioning are very different. As illustrated in this review with the example of the cohesion complex, the yeast plasmids appear to utilize the partitioning system to appropriate components of the highly efficient segregation pathway established for the host chromosomes.

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