MICROBIOLOGICAL REVIEWS, Dec. 1987, p. 381–395
0146-0749/87/040381-15$02.00/0

Vol. 51, No. 4

Plasmid Incompatibility

RICHARD P. NOVICK

Department of Plasmid Biology, The Public Health Research Institute of the City of New York, Inc., New York, New York, 10016

INTRODUCTION

Plasmid Replicon

A bacterial plasmid is a species of nonessential extrachromosomal deoxyribonucleic acid (DNA) that replicates autonomously as a stable component of the cell's genome. Naturally occurring plasmids range in size from one to several hundred kilobases and in copy number from one to several hundred per cell.

Copy number is a fixed characteristic of any plasmid under constant conditions and is controlled by a plasmid-coded system that determines the rate of initiation of replication. Because self-replication is potentially autocatalytic, plasmid replication control mechanisms must utilize inhibition as their primary regulatory strategy (67). As is true of any self-regulatory system, plasmid replication control systems must be capable of sensing and correcting stochastic fluctuations. This is accomplished by ensuring an inverse proportionality between copy number and replication rate in individual cells.

Naturally occurring plasmids are almost always inherited with high fidelity despite their non-essentiality. An explicit partitioning mechanism is thus an important element in the total hereditary biology of unit- or oligocopy plasmids. Active partitioning may or may not be required for multicopy plasmids; its possible importance in their hereditary biology is presently uncertain.

Stable plasmid inheritance depends not only upon the precisely coordinated functioning of a complex set of plasmid-coded regulatory elements that govern replication and partitioning, but also upon effective synchrony with certain host functions, not yet well characterized, that provide the requisite cellular environment. It is presumed that plasmid instability is caused by defective functioning of any of these elements.

Plasmid Instability and Incompatibility

Plasmid incompatibility is generally defined as the failure of two coresident plasmids to be stably inherited in the absence of external selection (60). Put another way, if the introduction of a second plasmid destabilizes the inheritance of the first, the two are said to be incompatible. This paper will attempt to establish that (i) incompatibility is due to the sharing of one or more elements of the plasmid replication or partitioning systems, and (ii) plasmid loss due to incompatibility is often a consequence of interference with the ability of the plasmid to correct stochastic fluctuations in its copy number. It is suggested that plasmid instability is also frequently due to inadequacy of the self-correction mechanism.

Elements of the plasmid replicon that express incompatibility should not be regarded as incompatibility genes; just as prophage immunity is an automatic consequence of the activities of regulatory elements that maintain the prophage state, rather than the function of any specific "*imm*" gene, so plasmid incompatibility is an automatic consequence of the normal activities of certain plasmid maintenance and

replication functions rather than the province of any specific "*inc*" gene.

Incompatibility may be symmetric (either coresident plasmid is lost with equal probability) or vectorial (one plasmid is lost exclusively or with higher probability than the other). Although certain plasmid elements can cause either type, as discussed below, it is suggested that the statistical mechanisms are slightly different: symmetric incompatibility is seen with coresident single replicons that share essential replication and maintenance functions and is due to inability to correct fluctuations arising as a consequence of the random selection of individual copies for replication and partitioning events within the plasmid pool (61). Vectorial incompatibility is usually due to interference with replication by cloned plasmid fragments containing elements of the replication control or maintenance systems or by certain copy control mutations of directly regulated plasmids (see below). Sometimes the replication of the affected plasmid is completely blocked; more often the block is partial, or even minimal, and it is proposed that in such cases loss of the plasmid is due to inability to correct fluctuations arising as a consequence of the random temporal distribution of replication events. Also, vectorial incompatibility may result from interference with partitioning (56, 61) and has been observed with cloned fragments of unknown function (20, 53). It is noted parenthetically that, with unit copy plasmids such as F and P1, it is impossible to analyze coresident incompatible plasmids simply because the copy numbers are too low to permit the construction of heteroplasmid strains.

In this paper, the random selection mode of plasmid replication and partitioning is first examined from a theoretical viewpoint, and the concept of incompatibility (and other forms of instability) as a consequence of self-correction failure is developed. These theoretical considerations are followed by an exploration of how replication/maintenance functions cause incompatibility for the better known plasmid systems, in the light of self-correction failure and other possible effects. The presentation is more analytical and theoretical than encyclopedic; readers are referred for details to a recent comprehensive and fully referenced review by Scott (75) of plasmid replication and its control and to reviews of Kline (26), Nordstrom et al. (58), and Chattoraj et al. (10) which deal with the F, R1, and P1 systems, respectively. A glossary of terms used is provided as Table 1. Some of the defined usages therein may refer only to this paper.

INCOMPATIBILITY AS A PROBABLISTIC PHENOMENON

Random Selection and Its Basis

It has been shown for oligocopy plasmids such as those of the IncFII group (21, 73) and for multicopy plasmids such as ColE1 (5) that individual plasmid molecules are selected randomly from a common pool for replication, largely without regard to previous replication history. The behavior of other plasmids, such as pT181 and pC221, is consistent with this (69), and as there is no published exception, it is assumed to be true for all oligo- and multicopy bacterial plasmids. The replication control system defines the rate-limiting step in initiation, which is presumed to be replicon specific. It is assumed that this step determines membership in the plasmid-specific replication pool and is the object of random selection. Similarly, copies are assumed to be drawn at random from a common pool for partitioning, prior to cell division, and it is also assumed that the specificity of the *par* function determines membership in the hypothetical partitioning pool.

For replicons whose Rep protein is rate limiting and *trans* active, individual plasmid molecules compete for the free pool of this protein; success in this competition is a matter of chance: thus, it is argued, the randomization step must be selection of the plasmid replication origin for initiation. The randomization step is less obvious for plasmids whose regulated step is *cis* specific, since there is no free pool of any rate-limiting product. Here, it is suggested, the replication inhibitor permits entry into the replicating pool of only those individual molecules whose replication is, by chance, not inhibited at any particular point in time. The randomization step would thus be selection by nonexclusion.

Incompatibility and Self-Correction Failure in Isologous Heteroplasmids

Replicons that have identical nucleotide sequences for all regions involved in replication and maintenance are referred to as isologous. Heteroplasmid strains containing coresident isologous plasmids can generally be established and maintained by growth in a medium that is selective for the presence of both. A copy pool is established that is the same size as that of either alone, and in the absence of differential selection, this pool is necessarily split between the two. Random selection for replication from such a pool generates inequalities between the two plasmids in individual cells because the selection mechanism cannot distinguish between them and the replication control system cannot respond to them: it sees only the overall pool. These inequalities are then amplified because a numerical advantage for one plasmid over the other in any individual cell confers a proportionate replication advantage: the probability that a plasmid of one type will be chosen for any replication event is directly proportional to the fraction of the total pool occupied by that plasmid. These simple rules have been formulated as a branched Markov chain (12, 25, 61), and this formulation predicts that homoplasmid segregants will appear at a constant rate during growth of the heteroplasmid strain and that their rate of appearance will be solely a function of the size of the aggregate copy pool. The manner of replication and of its regulation are irrelevant to this process.

Partitioning inequalities will occur because the individual copies are probably randomly assorted, even if there is an explicit partitioning mechanism (61). These inequalities will contribute to the segregation of isologous heteroplasmids. A simple illustration of these principles is presented in Fig. 1; in this illustration, baby cells have 4 plasmid copies and old cells have 8 (i.e., the plasmid replicates according to a 4-8-4 cycle). With equipartitioning (at top), the eight plasmids can be assorted randomly in five possible ways (the probabilities of each are given above in the figure). These probabilities were calculated according to a modified binomial formula, where P_a is the probability of a copies of plasmid A, and N is the normal homoplasmid copy number in a newborn cell.

$$P_a = \frac{\left[\frac{N!}{(N-a)!a!}\right]^2}{\sum_{n=0}^{N}\left[\frac{N!}{(N-n)!n!}\right]^2}$$

Here, the probability of homoplasmid segregants is consid-

TABLE 1. Glossary of terms[a]

Term	Definition
Binomial (random) partitioning	Distribution of plasmid copies during cell division such that each copy is equally likely to go to either daughter cell
Equipartitioning	Distribution of precisely half the copies of a plasmid to each daughter cell
Contralateral	Affecting other different plasmids in the same cell
Ipsilateral	Affecting the same plasmid or other copies of the same plasmid in the same cell
Copy number	No. of plasmids per avg cell
Unit copy plasmid	Plasmid maintained at 1 copy per cell or per chromosome
Oligocopy plasmid	Plasmid maintained at 2–6 copies per cell
Multicopy plasmid	Plasmid maintained at >6 copies per cell[b]
Countertranscript	Inhibitory RNA molecule transcribed in the antisense direction from its target RNA molecule with which it interacts by complementary base pairing
Democratic replication	Replication of each plasmid molecule once in every cell cycle
Random replication	Replication of any plasmid copy with equal probability at all times
Homoplasmid strain	Containing only plasmids of a single genotype
Heteroplasmid strain	Containing two (or more) different plasmids
Isologous heteroplasmid strain	Containing two or more differentially marked plasmids with isologous replicons
Heterologous heteroplasmid strain	Containing two or more plasmids with nonisologous replicons
Hyperbolic function	Regulatory function in which there is an inverse proportionality between inhibitor concentration and target activity
Step function	Regulatory function in which a small change in inhibitor concentration brings about an all-or-none response in target activity
Incompatibility	Inability (of two or more plasmids) to be comaintained without external selection
Symmetric	Equal segregation probability of two incompatible co-resident plasmids
Vectorial	Unequal segregation probability of two incompatible co-resident plasmids (If only one of the plasmids ever segregates, the incompatibility is unilateral.)
Inhibitor-target regulation	Type of regulation involving a diffusible inhibitory substance that acts by binding to a specific receptor or target
Iteron-binding regulation	Type of regulation involving binding of initiator protein to iterons
Iterons	Repeated oligonucleotide sequences (usually involved in plasmid replication control and incompatibility)
Plasmid diploidy	Mode of plasmid inheritance characterized by the ability to maintain balanced heterozygosity within the plasmid pool
Plasmid haploidy	Mode of plasmid inheritance characterized by inability to maintain balanced heterozygosity within the plasmid pool
Regulatory isolation	Total indifference of a mutant plasmid to the regulatory system of its progenitor
Tsr	Thermosensitive for replication

[a] Some of these usages may refer only to this paper.

[b] Numerical distinction between oligo- and multicopy plasmids is arbitrary; however, there seems to be a class of plasmids, such as R1, with regulatory properties intermediate between those of unit and multicopy plasmids.

erable; it diminishes rapidly as a function of increasing copy number. In the next generation, there will be four randomly determined replication events. For each of the heteroplasmid cells, there are five different possible outcomes, each having a different probability; again, most of these can be partitioned in several ways with different probabilities. Starting from a single 4/4 cell, the population distribution rapidly becomes heterogeneous but establishes an equilibrium state in which the proportions of each class are constant, except for the homoplasmids, which steadily increase at a constant rate. A pair of compatible plasmids, however, replicates and is partitioned with a high degree of regularity. A recent test of this scheme (69) involved analyzing the consequences of imposing differential selection on pairs of coresident incompatible and compatible plasmids. As one would predict, the composition of the copy pool could be manipulated at will by this means for the incompatible pair but not for the compatible pair. An experiment illustrating this effect is presented in Fig. 2. The incompatible plasmids used were pT181 and pSA5000, isologous multicopy plasmids differing only in their resistance markers (Tc^r and Cm^r, respectively). The compatible pair were pT181 and pC194, an unrelated plasmid also carrying the Cm^r marker. The cells were pregrown in a selective medium containing tetracycline (Tc) at 1 μg/ml and chloramphenical (Cm) at 20 μg/ml. Drug concentrations were then reversed (tetracycline, 20 μg/ml; chloramphenicol, 1 μg/ml), and the plasmid copy numbers were monitored for 20 generations. The plasmid population was initially skewed about 10:1 in favor of pSA5000, the Cm^r plasmid, and this asymmetry was reversed during the course of the experiment. Throughout the experiment, over 95% of the cells contained both plasmids, and the sum of their

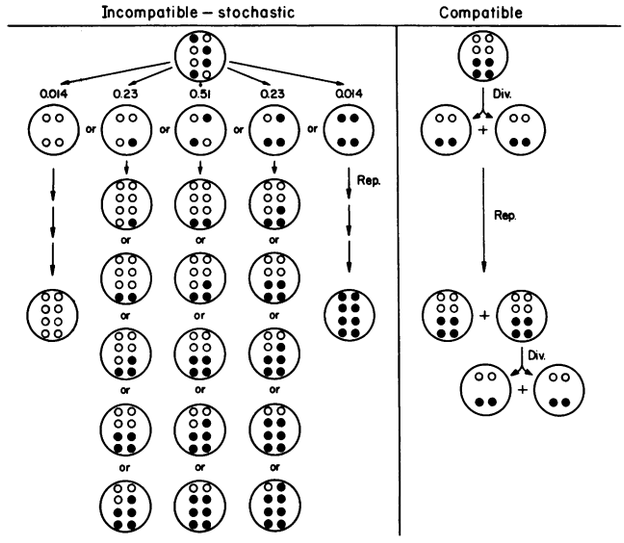

FIG. 1. Segregational incompatibility. Black and white symbols represent differentially marked plasmids that replicate according to a 4-8-4 cycle. In the left half, the two plasmids have isologous replicons and show segregational incompatibility. At the top the various combinations that can arise via random assortment with equipartitioning are modeled; numbers represent the binomial probabilities for each combination. Beneath the partitioning diagram are given all of the possible combinations that can result from random selection for replication. In the right half, the two plasmids have heterologous replicons and are compatible. These two patterns represent the haploid and diploid modes of inheritance as applied to multicopy plasmids.

average copy numbers remained constant. With the compatible pair (not shown), each plasmid maintained its homoplasmid copy number regardless of external selection conditions. In a formal sense, a cell with a pair of compatible plasmids can be regarded as a plasmid diploid, whereas a pair of incompatible plasmids shows a haploid type of inheritance.

It is evident that segregation of isologous heteroplasmids will inevitably occur regardless of external (antibiotic) selection. Although differential selection may modify the proportions of the two types of homoplasmid segregants by distorting the average copy pools, and will probably affect the fate of the homoplasmids once they have appeared, it does not alter the basic stochastic processes by which individual copies are chosen for replication and partitioning. A practical consequence of this statistical mechanism is that it is impossible to obtain a pure population of heteroplasmids unless the selective agents used rapidly kill (not merely inhibit) both classes of homoplasmid segregants. It cannot be overemphasized that incompatibility between isologous replicons is a fundamental property of the (haploid) mode of plasmid inheritance and it cannot be eliminated by mutation.

Self-Correction Failure and Exclusion in Heterologous Heteroplasmids

Replication of oligo- and multicopy plasmids such as R1 and ColE1, respectively, occurs throughout the cell cycle and is indifferent to cell division (21, 34); however, it is generally assumed that individual replication events are not evenly spaced in time but are probably Poisson distributed; although total plasmid mass increases coordinately with cell growth, a Poisson distribution of replication events will cause time-dependent fluctuations in copy number in individual cells which must be correctible if inheritance is to be stable. The regulation of plasmid replication thus requires a control mechanism that not only determines the overall initiation rate but also responds to upward, and especially to downward, fluctuations in copy number in a time frame that is relatively short in comparison to the host cell's generation time; fluctuations that persist will broaden the frequency distribution of plasmid copies even if the overall average remains constant. This will lead to an increased frequency of plasmid loss (instability) because cells at the low end of the distribution are prone to segregate plasmid negatives (87), even if the plasmid has an active partitioning mechanism (see below). It is proposed, therefore, that in certain cases the loss of a plasmid due to vectorial incompatibility is caused by interference with self-correction rather than by simple blockage of replication.

As delineated by Nordstrom et al. (58), one may imagine that replication rate could respond to inhibitor concentration according to either a hyperbolic function (replication rate is inversely proportional to inhibitor concentration) or a "step" function (a small change in inhibitor concentration has an all-or-none effect on replication) (58). With plasmids such as F, in which replication rate is evidently regulated by a step function (see below), the cloned inhibitor determinant totally blocks replication of the plasmid. With plasmids such as ColE1, R1, and pT181, in which replication rate is a hyperbolic function of inhibitor concentration (see below), the cloned inhibitor determinant causes a decrease in the replication rate of a test plasmid in *trans* in proportion to its dosage: in other words, the replication rate approaches zero asymptotically as inhibitor concentration increases. One would not expect detectable segregation of such a test plasmid unless its copy number fell below the level required for accurate partitioning. This would be much lower for a plasmid with equipartitioning than for one with binomial partitioning, but it would have to average less than about 5 per cell even for the latter (57). In fact, we have observed that a plasmid with a copy number of 35 is greatly destabi-

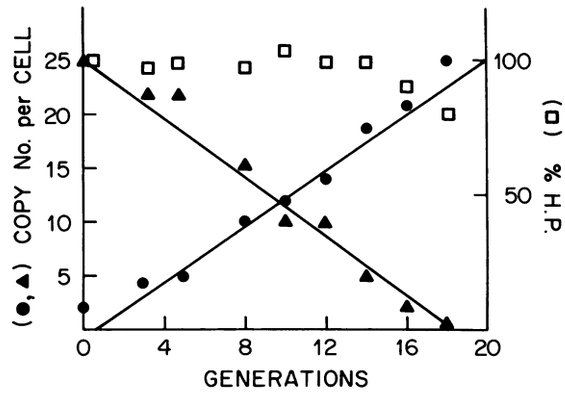

FIG. 2. Reciprocal intrapool variation (69). A staphylococcal strain containing two isologous but differentially marked plasmids (pT181-Tc^r [●] and pSA5000-Cm^r [▲]) was pregrown in a medium containing 1 μg of tetracycline and 20 μg of chloramphenicol per ml and then transferred to fresh medium with the antibiotic concentrations reversed and maintained by dilution in exponential growth for 20 generations. Samples were removed periodically and scored for plasmid content by agarose gel electrophoresis and for the heteroplasmid (H.P.) fraction (□) by differential plating. Reprinted from *Plasmid* with the permission of the publisher.

TABLE 2. Prototypical plasmids[a]

Plasmid	Size (kilobases)	Copy no. per cell	Regulation	*par*	*ccd*
F	93	1–2	Iteron binding	+	+
P1	90	1–2	Iteron binding	+	+
pSC101	6.5	15–30	Iteron binding	+	–
R6K	39	15–30	Iteron binding	U[b]	U
R1	102	3–4	Inhibitor-target	+	+
ColE1	7.2	15–30	Inhibitor-target	U	–
pT181	4.4	20–25	Inhibitor-target	+	–

[a] *par*, Partitioning; *ccd*, inhibition of cell division if plasmid has not replicated (64).
[b] U, Unknown.

lized by a cloned copy of the inhibitor gene that is maintained at approximately half this number (A. Gruss, J. Kornblum, and R. Novick, unpublished data). A suggested explanation of this instability is that the cloned inhibitor gene provides a fixed source of inhibitor extrinsic to the target plasmid which prevents the latter from increasing its replication rate in response to time-dependent down fluctuations in its own copy number. This would cause the broadening of the frequency distribution that has been suggested to cause instability, while the average copy number remains constant.

It is suggested, therefore, that in segregational incompatibility and in certain types of vectorial incompatibility, plasmid loss can be caused by inability to correct stochastic fluctuations in copy number, in the face of normal or nearly normal replication rate.

INCOMPATIBILITY-CAUSING PLASMID FUNCTIONS AND THEIR MECHANISMS

Replication Control Elements

The known plasmid-determined negative regulation systems utilize either of two strategies, referred to here as inhibitor-target and iteron-binding strategies. In inhibitor-target systems, the plasmid encodes a diffusible replication inhibitor that acts by binding to a specific, plasmid-determined target, and this binding either inhibits replication directly or inhibits the synthesis of a required product. In iteron-binding systems, the plasmid encodes a diffusible initiator protein and contains one or more sets of tandemly arrayed directly repeated oligonucleotides, known as iterons, in or near the replication origin. The regulatory mechanism involves both autoregulation of the initiator protein and binding of the protein to the iterons. This binding may have a direct inhibitory effect on replication or it may regulate the initiator concentration by titration, or both. Plasmid systems discussed most extensively in this paper are listed in Table 2.

Direct Versus Indirect Regulation (Fig. 3)

As suggested by Grindley et al. (18), a negative regulator may act directly to inhibit a particular process or it may act to inhibit the synthesis of a product required for that process. The former is referred to as direct regulation and the latter is referred to as indirect, a distinction with important consequences for plasmid incompatibility (discussed below). Among the inhibitor-target plasmids, those of the ColE1 type, in which the inhibitor blocks synthesis of the replication primer (95), exemplify direct regulation; those of the pT181 type, in which the inhibitor blocks synthesis of the *trans*-active replication protein, RepC (30), exemplify indirect regulation. For plasmids of the IncFII group, the inhibitor also blocks synthesis of a replication protein, RepA (36). However, this protein is *cis* active (28, 39) so that the inhibition of its synthesis affects only the inhibited plasmid and cannot be relieved by another plasmid in *trans*. This means that the regulation is effectively direct.

Among the iteron-regulated systems (see below), plasmids such as F and P1 are also indirectly regulated since the primary regulatory target is the Rep protein, which is *trans* active. Although the π protein of R6K is also *trans* active, it is not rate limiting for replication and presumably cannot relieve iteron-specific incompatibility. This would seem to place R6K in the directly regulated category, but this placement seems dissatisfyingly arbitrary and will probably require modification when the regulatory mechanism of the plasmid is better understood.

Inhibitor-Target Regulation

Regulatory circuits. As noted, the groups of plasmids that use a regulatory system of the inhibitor-target type are the ColE1 and IncFII groups of *Escherichia coli* and the pT181 group of *Staphylococcus aureus*. In Fig. 3 is a diagram of the major components of the three best-known inhibitor-target plasmid regulons, illustrating the principle of direct versus indirect regulation, and in Fig. 4 is a more detailed presentation of the regulatory elements, including mutants and predicted secondary structures of the interacting ribonucleic acid (RNA) species. The target in all three systems is a major transcript (wavy arrow rightward in Fig. 3) whose function is required for replication. For ColE1, the target transcript is the replication preprimer (95); for the others, it is the messenger RNA for the rate-limiting plasmid initiator protein: RepA for the IncFII (36) and RepC for the pT181 (30) plasmids. The RepA and RepC messenger RNAs are each initiated from two tandem promoters and each has an untranslated leader of some 200 or more nucleotides (Fig. 4).

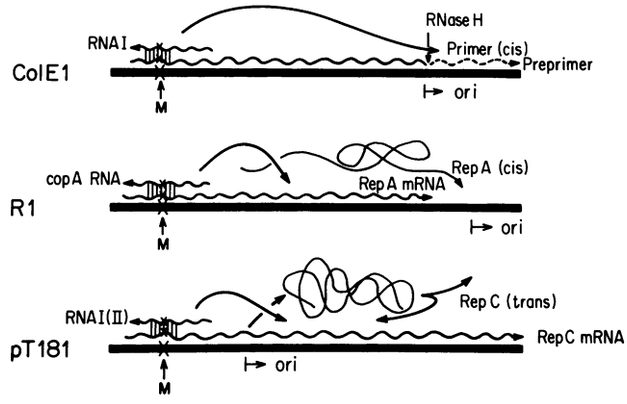

FIG. 3. Direct versus indirect regulation of inhibitor-target plasmids. Each of the three prototypical inhibitor-target plasmid systems has a major rightward transcript (wavy line) that is the target of the inhibition (the ColE1 preprimer, the RepA and RepC messenger RNAs) and a minor leftward transcript, the inhibitor (ColE1 RNAI, R1 *copA* RNA, and pT181 RNAI and -II). The product of the regulated step (the ColE1 primer, the R1 RepA protein, and the pT181 RepC protein) is *cis* specific for the two *E. coli* plasmids but *trans* active for the pT181 system. "M" represents mutations in the region where the inhibitor and target overlap; *ori* indicates the location of the replication origin, and the arrow gives the direction of replication.

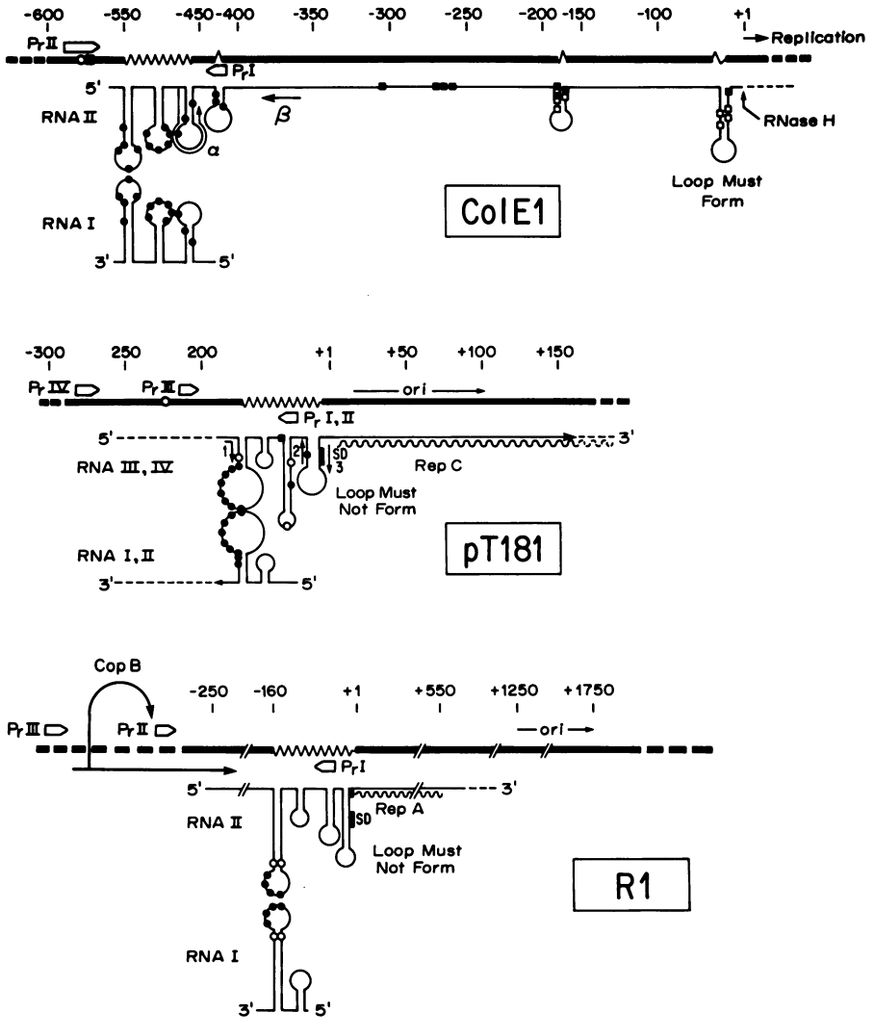

FIG. 4. Mutational analysis of countertranscript regulons. The same three prototypical plasmid systems are depicted as in Fig. 3, indicating the types and locations of regulatory mutations that have been isolated. Circles represent sites of dominant (filled circles) and recessive (open circles) copy control mutations. See Muesing et al. (50), Wong et al. (102), Stuitje et al. (85), Tomizawa and Itoh (93), Tomizawa and Itoh (94), Yarranton et al. (104), Lacatena and Cesareni (31), Givskov and Molin (17), Brady et al. (7), and Carleton et al. (8) for properties and sequence analysis of copy mutants in the three systems. Solid squares are mutations causing replication defects; open squares represent suppressors of some of these replication-defective mutations. See Moser et al. (49), Naito and Uchida (52), Naito et al. (51), Oka et al. (65), and Masukata and Tomizawa (40) for properties and sequence analysis of replication mutations in the ColE1 family. The inhibitor and target transcripts are shown as secondary structures according to computer predictions and (for ColE1 and R1) biochemical determinations (88, 91, 15). Numbers refer to nucleotide positions along the respective plasmid genomes; these are gapped (jagged portions) to condense distances and to allow for the RNA folding. For plasmids in the ColE1 family, it is thought that pairing of preprimer sequences, arrows labeled α and β, is required for the formation of the downstream hairpin necessary for preprimer processing (40) and that the inhibitor-target interaction prevents this. For pT181-like plasmids, pairing of sequences 1 and 2 (labeled arrows) prevents the formation of the 2–3 stem, which is thought to interfere with translation of the initiator protein *repC* (wavy line), and it is the 1–2 pairing that is blocked by the inhibitor-target interaction (62). Similar considerations apply to the R1-like plasmids (74). Pr, Promoter; SD, Shine-Dalgarno site; ori, replication origin; CopB, secondary copy control substance which represses one of the two *repA* promoters of plasmid R2 (47).

For the IncFII plasmids, the downstream Rep promoter is controlled transcriptionally (see below) and the upstream one is constitutive (35); for pT181, there is no evidence for or against transcriptional control of either. For all three plasmid types the inhibitors are RNA molecules of 80 to 150 nucleotides that are transcribed from the region encoding the 5′ end of the target transcript and in the opposite direction (wavy leftward arrows, Fig. 3). We have therefore proposed to term these inhibitory RNAs 5′ countertranscripts (ctRNAs) (30). Two countertranscripts have been identified for pT181 (30) and R1 (84). In both cases, they have a common 5′ start and different 3′ termination points. In all three systems, mutations (M, Fig. 3) in the overlap region have been isolated and found to affect copy number and incompatibility. These mutations have shown that the inhibitory interaction involves complementary base pairing between the inhibitor and target transcripts (31) which modifies the secondary structure of the target transcript (41, 94, 103) so as to interfere with its function. Some of the secondary structures important for regulation are illustrated in Fig. 4.

For ColE1, there is an important palindromic region just 3′ to the replication start point; it is likely that this region must fold into a stem-loop, as shown, for processing of the preprimer to occur (40) and that the countertranscript somehow prevents this stem-loop from forming. For pT181, pairing of sequences 2 and 3 are presumed to block translation of the initiator gene, *repC*, by sequestering the Shine-Dalgarno site; pairing of 1 and 2 preempts this, permitting translation to occur: the countertranscript would thus act by blocking sequence 1. A similar mechanism probably operates for R1. It has been shown for ColE1 that the inhibitor-target interaction occurs in two separable stages: a reversible interaction between complementary single-stranded loops in the inhibitor and target transcripts followed by the formation of a complete RNA-RNA duplex initiated by the free 5′ end of the inhibitory RNA (91). The reversible stage is subject to competitive interference by a cognate inhibitor (that of RSF1030) that lacks homology with the preprimer at its 5′ end; this interference elevates the copy number of the test plasmid (92) and may be viewed as an anti-incompatibility activity.

This type of regulation is characterized by hyperbolic inhibitor kinetics. The inhibitory RNAs are synthesized constitutively, and their concentrations, normally much higher than that of the target, are proportional to the plasmid copy number (48, 62). Since initiation events are random, the inhibitor concentration must determine replication rate by fixing the probability that any target molecule will achieve a functional configuration. In an exponentially growing steady-state culture, the rate of replication initiation, averaged over the cell, is, of course, equivalent to the copy number. Since its concentration is high, the inhibitor is not appreciably titrated by binding to the target. The self-corrective feature of such a system is clear: for example, an increase in the number of plasmid copies is shortly followed by a corresponding increase in the inhibitor concentration; this reduces the probability of target function which slows the replication rate until the correct copy number is restored, and vice-versa. It follows that the inhibitor level at which replication rate is stable is determined automatically by the relative promoter strengths of inhibitor and target and by the efficiency of the inhibitor-target interaction.

ColE1 and IncFII each encode a secondary regulatory factor that meets a unique requirement of the replicon. For ColE1, the primary inhibitor-target interaction is evidently inefficient; the secondary factor, Rop, is a protein (9, 79, 101) that facilitates the interaction between the ctRNA and preprimer transcripts (96). In the absence of this protein, the copy number is elevated about fivefold and the plasmid is unstably inherited (86). This instability is probably an example of the consequences of a self-correction deficit: the response of the plasmid copy pool to temporal fluctuations must be too sluggish to accomplish self-correction within a single cell division cycle so that a broad frequency distribution develops despite the overall high average copy number.

The IncFII plasmids have a special need for rapid upward self-correction: because of their low inherent copy numbers, any down fluctuation can be disastrous. Accordingly, the Rep gene of these plasmids is transcribed from two tandem promoters (72), one of which is repressed at normal copy numbers by the plasmid-coded CopB protein (36). Relief of this repression, which occurs only when the copy number of the plasmid falls below normal (36), enables rapid self-correction through increased transcription of *repA*. CopB$^-$ mutants of R1 have an eightfold elevation in copy number (47), but in this case the plasmid is not detectably unstable (54). This is presumably because CopB is not involved in self-correction by the primary inhibitor-target interaction.

For pT181, deletion of the ctRNA promoter results in a greatly elevated but stable copy number (59), suggesting the existence of either a secondary rate-limiting factor or a secondary control mechanism.

Regulatory mutations. Mutations affecting the copy control system in countertranscript regulons have been highly informative with respect to both the mechanism of incompatibility and the regulatory system itself. These mutations are most often located in the region of overlap between inhibitor and target transcripts (7, 31, 59, 93). In all three systems there is a remarkable cluster of mutations in a region of the inhibitory RNA that remains single stranded in the optimal computer-predicted secondary structure of the inhibitor and target molecules (Fig. 3). For ColE1 and R1, the computer prediction for the ctRNA has been verified biochemically (15, 88, 91). The corresponding single-stranded loops of the ctRNA and target transcripts are referred to as the inhibitor and target loops, respectively, and the base-paired regions flanking these are called the inhibitor and target stems. The overlap mutations involve changes in the complementary sequences of the inhibitor and target loops or stems, affecting the efficiency of the pairing interaction, the structure of the loop, or the structure of the stem. Mutations that affect the loop only are presumed to act by modifying the energetics of the loop-loop interaction (7). Mutations that decrease the size of the loop usually reduce the effectiveness of the interaction; mutations that increase the size of the loop have effects that are not readily predictable. Mutations that destabilize the stem decrease the effectiveness of the interaction; mutations that stabilize the stem have not been described. For pT181, the inhibitor stem doubles as the terminator for one of the two countertranscripts (68) so that the effects of stem mutations may be complex. For the three systems taken together, all but one of 38 mutations isolated on the basis of increased copy number and affecting the sequence but not the structure of the target loop have guanine-cytosine (GC) to adenine-thymine (AT) changes in this region (7, 8, 47, 93), which is consistent with the critical nature of the energetics of the loop-loop interaction (32, 93). Mutations have also been isolated by selection for resistance to a cloned copy of the inhibitor gene (31); some of these have AT to GC changes in the target loop and actually cause a slight decrease in copy number. These observations indicate that the inhibitor-target interaction is initiated by complementary base pairing between the single-stranded regions (31, 32) and that the efficiency of this initial pairing is a critical determinant of regulatory activity. For ColE1, mutations affecting the 5′ end of the ctRNA eliminate its inhibitory activity versus wild type (66, 91), consistent with the requirement of 5′ homology for formation of the RNA duplex and with the essentiality of the duplex for regulation.

Mutations in the overlap region sometimes affect the inhibitory activity of the ctRNA more than the sensitivity of the target and vice versa (7, 17, 93); some of these can be understood on the basis of the specific nucleotide substitution involved. For example, a G-to-A transition in the target corresponds to a C-to-U transition in the inhibitor. In the heterologous interaction between mutant target and wild-type inhibitor, a G-C pair is replaced by an A-C pair, whereas in the interaction between the wild-type target and mutant inhibitor the G-C pair is replaced with a G-U pair. Since a G-U pair is less destabilizing than a C-A pair, the wild-type target is more sensitive to the mutant inhibitor

than vice versa. All of these mutations have important consequences for ColE1 incompatibility that can largely be predicted on the basis of their effects on copy number, as discussed below.

Mutations that affect countertranscript promoters or terminators have been isolated for several plasmids (8, 50, 62, 74); as these mutations often involve complementary base changes in the target transcript, their effects on plasmid copy number may be due to their effects either on ctRNA synthesis or on the target transcript, or both. In some cases, they have high copy numbers and remain fully sensitive to the inhibitory activity of the countertranscripts; these are referred to as recessive.

Most of the control mutations result in a new regulatory state in which the elevated copy number is stable and is a reflection of residual inhibitor activity (58, 62). Mutations that eliminate the inhibitory RNAs have been isolated for pT181 but not for either ColE1 or IncFII plasmids; for the two latter plasmids such mutations may cause lethal runaway replication.

Mutations decreasing the sensitivity of the plasmid to the wild-type inhibitor (referred to as dominant) occur outside of the overlap region as well as in the target loop. These evidently modify the downstream secondary structure of the target transcript in such a manner as to reduce or eliminate the effect of the upstream inhibitor-target interaction. For example, the inhibitor-target interaction for pT181 is thought to facilitate the formation of a downstream hairpin that sequestors the Shine-Dalgarno sequence of the *repC* gene and so blocks translation of the protein (30, 62) (Fig. 3). A mutation, *cop-623*, in the segment that pairs with the Shine-Dalgarno sequence grossly destabilizes this hairpin and renders the plasmid indifferent to the inhibitor (8). Mutations in the region of the preprimer immediately 5′ to the countertranscript start in ColE1 and related plasmids cause a high-copy phenotype that, in some cases, leads to autocatalytic runaway replication at elevated temperature (49, 102). A similar type of mutation has been isolated for R1 (45). As these mutants continue to synthesize wild-type ctRNAs at greatly elevated concentrations, the defect must be due to insensitivity to the inhibitor. Presumably, at the restrictive temperature, the mutation effectively uncouples the upstream inhibitor-target interaction from the consequent modification of the downstream folding sequence in the target transcript by which the inhibition is achieved. Mutations have been isolated in the regulatory regions of ColE1 and pT181 that affect the ability of the plasmid to replicate, and, for ColE1, suppressors of these have been isolated secondarily. These are shown as squares in Fig. 4. Their effects on incompatibility have not been determined.

Incompatibility in inhibitor-target systems. It has been demonstrated for ColE1 (93) and pT181 (70) that any two plasmids that are isogenic for the countertranscript coding region and use it to regulate replication are always incompatible whether they have any other region in common or not; this incompatibility is due to occupancy of a common replication pool, as discussed above. Predictably, the cloned ctRNA determinants express strong vectorial incompatibility (46, 59, 88); as any sensitive plasmid is displaced by such clones, the strength and gene-dosage dependence of this incompatibility can be demonstrated only by means of a vector with conditional copy number, such as a Tsr mutant plasmid. At an elevated temperature, when the vector is maintained at a low copy number, an inhibitor-sensitive plasmid can be comaintained with the cloned ctRNA; during a transition to the high-copy condition, following a temperature shift-down, the replication rate of the sensitive plasmid falls as the copy number of the clone increases (S. K. Highlander, Ph.D. thesis, New York University, New York, N.Y., 1985). Once the cloned inhibitor gene has reached a stable copy number at the permissive temperature, the concentration of inhibitor becomes constant, representing the sum of that produced under the control of cloned gene and that produced by the target plasmid. The average copy number of the latter is then determined by this total concentration according to an inverse proportionality relationship. Given any appreciable contribution of the cloned inhibitor to the total pool, the test plasmid is always destabilized, regardless of its copy number (Highlander, Ph.D. thesis). The proposed explanation for this, as noted above, is that the test plasmid cannot effectively correct fluctuations in its own copy number. In the face of a fixed external source of inhibitor, the inhibitor concentration does not vary appreciably in response to random fluctuations in the copy number, and so replication rate cannot be adjusted to compensate.

For the directly regulated inhibitor-target plasmids, namely, ColE1 and R1, the countertranscript region is the major incompatibility determinant. The secondary regulatory elements, Rop and CopB, respectively, do not ordinarily cause incompatibility by themselves, although they do play a role. As Rop acts to facilitate the inhibitor-target interaction, it predictably enhances the incompatibility activity of the cloned inhibitor (96) and, by lowering the copy number of a heteroplasmid pool, increases the segregation rate of isologous coresident plasmids. Since the CopB-sensitive promoter is fully repressed when R1 replicates at its normal copy number, elevation of the CopB concentration does not inhibit replication of the wild-type plasmid (47). Predictably, if the CopB-insensitive promoter is deleted so that all *repA* transcripts originate from the sensitive promoter, CopB then becomes a determinant of strong vectorial incompatibility (54). For indirectly regulated inhibitor-target plasmids such as pT181, the replication origin is a second major incompatibility determinant (see below). A weak incompatibility associated with partitioning systems (56, 61) is discussed below.

Effect of mutations on incompatibility. Directly and indirectly regulated plasmids differ strikingly in the effects of many regulatory mutations on incompatibility behavior. For directly regulated plasmids, these mutations usually result in regulatory asymmetry: one plasmid is less sensitive to the other's inhibitor. This causes a corresponding asymmetry in incompatibility behavior: the more sensitive plasmid is preferentially or exclusively eliminated from heteroplasmids under nonselective conditions (93). Differential inhibitor sensitivity causes differential interference with self-correction, as outlined above. The simplest case is a pure target mutant, one that synthesizes the wild-type inhibitor and is insensitive to its action. In strains containing such mutant plasmids, there is an elevation of inhibitor concentration commensurate with the plasmid's elevation in copy number. This creates an intracellular environment that reduces the replication rate of the wild type or other inhibitor-sensitive plasmids and results in their exclusion or rapid displacement. This type of dominant mutant is generally rare for countertranscript regulons; an example for ColE1 is pMM1 (102). The mutation maps 5′ to the countertranscript start and prevents the countertranscript from interfering with primer formation, presumably by disrupting the secondary structure of the preprimer. Less simple to interpret are mutations in the overlap region, which rarely affect inhibitor

and target function equally. These mutations create disparities between ipsilateral and contralateral inhibitor-target interactions. These disparities cause asymmetrical segregation of heteroplasmids which can be understood in terms of the in vitro activities of inhibitors and targets. For example, if a mutant inhibitor is more active against a wild-type target than against its own, the heteroplasmid will segregate asymmetrically in favor of the mutant. If the mutant inhibitor is less active than the wild-type inhibitor, the mutant will be lost preferentially. If both inhibitors are less active contralaterally than ipsilaterally, the result will be a degree of regulatory independence that leads, in the extreme case, to complete regulatory isolation (93). Mutations that cause regulatory isolation result in independently self-correcting copy pools and thus establish new incompatibility specificities. The occurrence of mutations that cause regulatory isolation is a unique property of countertranscript regulons; if inhibitor and target were any molecular species other than complementary polynucleotides, the probability that any single-step mutation could change their specificities in a complementary manner would be vanishingly small.

Most copy mutations in the overlap region eliminate or greatly reduce the inhibitory activity of the cloned countertranscript and so diminish or eliminate its incompatibility activity. Such mutations also eliminate or greatly diminish the sensitivity of the mutant plasmid to the cloned wild-type inhibitor (31, 93; Highlander, Ph.D. thesis), making the plasmid incompatibility insensitive. In this connection it is noted that the mutated countertranscripts of plasmids with regulatory mutations of this type are often only partially active against heterologous (e.g., wild-type) targets so that the observed residual incompatibility has both vectorial and segregational components. In other words, symmetric incompatibility and unilateral vectorial incompatibility should be regarded as the ends of a continuum: overlap mutations of this type superimpose partial unilateral inhibition upon the mutual interference with self-correction seen with isologous replicons.

Indirectly regulated plasmids differ strikingly from directly regulated ones in that mutations affecting the countertranscript or its target or both do not generally cause any regulatory asymmetry between the mutant and wild type or between two different mutant plasmids (62). In contrast to the above-mentioned ColE1 mutant pMM1, pT181 mutants such as *cop-623*, which elaborate the wild-type inhibitor at a greatly elevated concentration, do not show any segregational asymmetry: heteroplasmids containing such mutant plasmid and any inhibitor-sensitive plasmid can readily be established and maintained on selective media, where they exist in approximately equal copy numbers and segregate symmetrically (62). This is because any inhibitor-sensitive pT181 replicon can always replicate, no matter how high the inhibitor concentration, so long as any RepC-producing plasmid is present in the cell. This type of result predicts that, with indirectly regulated plasmids, changes in inhibitor-target specificity can never generate new incompatibility types. We have confirmed this prediction by showing that pT181 copy control mutations that make one plasmid completely indifferent to the other's inhibitor and vice versa are still incompatible; similarly, a pT181 derivative in which the copy control region has been replaced by that of a related compatible plasmid, pC221, is incompatible with pT181 even though the two copy control systems are completely independent (70). These pairs of plasmids remain incompatible because utilization of a common Rep protein precludes the establishment of independently self-correcting copy pools.

Of course, the behavior of the cloned inhibitor gene and the consequences of mutations affecting it or its target are the same for indirectly regulated and directly regulated plasmids, since there is no source of Rep protein extrinsic to the test plasmid.

Iteron Regulation

Regulatory circuits. Plasmids such as R6K, F, P1, Rts1, λdv, pSC101, and RK2 encode a diffusible initiator protein which binds to sets of directly repeated oligonucleotides (iterons) in or near their replication origins. This discussion deals with two of these systems, those of R6K and F/P1, that have been studied in considerable detail and serve to illustrate the contrasting adaptations involving this type of regulation. F and P1 have essentially the same functional organization and regulatory strategy so that observations from each separately can be combined to give a more complete picture than would be obtained from either alone. The R6K system, however, is unique among plasmids studied to date. In both of these systems, the initiator protein has three distinct activities: in addition to being required for replication (at low concentration), it inhibits replication directly at high concentration (11, 76) and is an autorepressor at the level of transcription (11, 76, 78, 97). These three activities have been adapted in different ways to the two systems, providing an extremely tight regulatory mechanism for the unit copy F and P1 plasmids and much looser type of regulation for the multicopy "relaxed" R6K. R6K regulation seems formally analogous to that of the countertranscript regulons in that its replication rate is determined probabilistically by steady-state concentrations of the interacting substances. F/P1 replication events seem to be governed by a temporally activated on-off microswitch, which causes a fixed time delay between individual events (10). Consistent with this view is the observation that supernumerary copies of the F replicon cause an absolute switch-off of replication (99), i.e., F and P1 are regulated by a step function rather than by a hyperbolic function. The regulatory kinetics of R6K are presently unknown.

Although F and P1 plasmid replication events are not coordinated with any specific event in the cell cycle (1, 82), the very low copy numbers require that they be much more democratic (every plasmid must replicate once in every cell cycle) than the replication events in multicopy pools, which are random.

The regulatory circuit of R6K involves a set of seven iterons that are colinear with the central replication origin, γ, and an eighth copy that is within the promoter for the initiator protein, π (81). π binds to all of these (16); binding to the promoter iteron inhibits transcription of the initiator (*pir*) gene (autoregulation), and binding to the *ori*-γ iterons is required for the initiation of replication but is inhibitory at elevated concentrations (13). Under normal conditions, π concentration is greatly in excess of that required for replication (76); also, the replication rate is virtually insensitive to changes in π concentration over at least a 95-fold range. It thus appears that neither π titration by iteron binding nor autorepression is directly involved in the regulation of copy number under normal conditions. One might suppose that the replication rate is determined by a balance between the negative and positive activities of the protein, a supposition that is consistent with the isolation of *pir* missense mutations that cause an increase in copy number (80). If the ratio of positive to negative activities is inversely proportional to π concentration, the balance could easily give rise to a con-

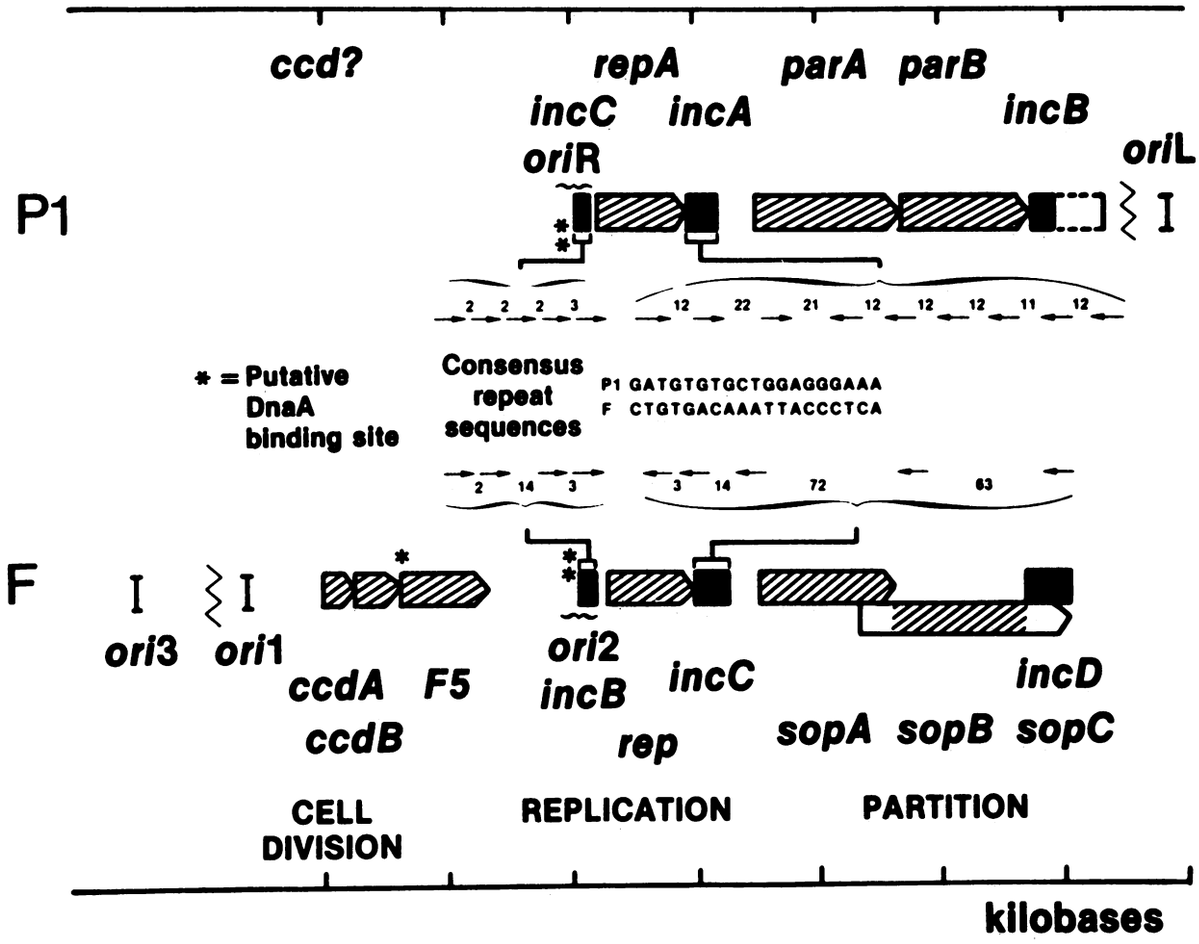

FIG. 5. Comparison of mini-F and P1 replicon organization. The shaded arrows represent open reading frames for proteins that have been identified; black boxes represent segments that can exert incompatibility. Small arrows indicate arrangements of the 19-base pair repeat sequences; small numbers represent numbers of nucleotides between repeats. The distance between *oriR* and *oriL* is 10.2 kilobases (83). The P1 coordinates of the replication region are from Abeles et al. (2), and those of the partition region are from Austin and Abeles (3). The F coordinates of the cell division region are from Bex et al. (6) and Ogura and Hiraga (64), those of the replication region are from Komai et al. (29) and Lane and Gardner (33), and those of the partition region are from Ogura and Hiraga (63). There is evidence that *ccdA* and *ccdB* may each produce two small proteins (6). The pair of asterisks represent the sequence TTATCCACATTATCCAC in *oriR* of P1 and GTTATCCACTTATCCACG in *ori2* of F; the single asterisk just within the putative open reading frame of the F5 protein represents the sequence GTTATACACAG (44). *ccd*, control of cell division; *inc*, incompatibility determinants (P1-*incA*, P1-*incC*, F-*incB*, and F-*incC* represent iteron-specified incompatibility; P1-*incB* and F-*incD* represent partitioning incompatibilities); *ori*, replication origins, of which each plasmid has several: P1-*oriR* and F-*ori2* are the origins that have been studied with respect to regulation, and P1-*par* (partitioning) and F-*sop* (stability of plasmid) are the determinants of partitioning. *sopC*, thought to be the F partitioning site, corresponds to F-*incD* and to P1-*incB*. P1-*repA* and F-*rep* are the initiator protein coding sequences. F5 is an open reading frame of unknown function. Reproduced from Chattoraj et al. (10) by kind permission of the authors and Plenum Publishing Corp.

centration-independent replication rate. This balance could be readily achieved by the existence of two forms of the protein, one active and the other inhibitory, whose interconversion is effectively irreversible. Self-correction in such a system could be ensured by maintaining a very low (rate-limiting) concentration of the active form of the π protein such that an increase in copy number would result in a reduced replication probability per copy and a decrease would result in an increased probability.

In F (reviewed by Kline [26]) and P1 (reviewed by Chattoraj et al. [10]), the initiator coding sequence is flanked by two sets of iterons, referred to here as L and R (Fig. 5). The L set is probably colinear with the replication origin and is required for autonomous replication (note that in F there is another origin a short distance away that is used exclusively when present); only when this origin, *ori*1, is deleted, can the other, *ori*2, be utilized. All of the detailed regulatory studies have been done with plasmids deleted for *ori*1; *ori*2 is the organizational analog of P1 *oriR*. For both plasmids, the Rep protein is produced in very small quantities and is probably rate limiting for replication (11, 71, 97). In contrast to R6K, Rep protein concentration is regulated by titration (binding to the R iterons) and by transcriptional autorepression (binding to a partial copy of the iteron sequence in the region of its promoter); copy number is affected inversely by deletions or additions to the R iteron set, and these effects are independent of orientation or location (10, 90, 98). Note that in F and P1 the *ori* and regulatory functions are performed by separate sets of iterons whereas in RK6 the two functions are performed by a single set. F or P1 copy

number is also increased by certain Rep protein mutations (4, 97) which are hypothesized to reduce the autorepressive activity of the protein (97). It is paradoxical in these systems that the replication rate is jointly regulated by autorepresson and titration of the RepC protein. As with R6K, it is possible to resolve this paradox by the postulation of two forms of the protein, one active as autorepressor and the other active as initiator (97). The primary gene product would be the autorepressor, which sets the transcription rate and is irreversibly converted at a constant rate to the initiator species. The latter gradually fills up the iterons, eventually triggering replication and creating a new set of empty iterons. In an alternative solution, initiator proteins bound to the iterons would remain active as an autorepressor, contacting both regions simultaneously through a looped configuration of the DNA (D. Chattoraj, personal communication). Perhaps the initial binding would be required to activate the autorepressor function of the protein, a possibility that would in effect combine the two models. Note that in F (71), and probably also in P1 (4), mutations have been obtained that eliminate or greatly reduce the autorepressor activity of the Rep protein without affecting its initiation function, suggesting that the proteins possess independent binding sites that subserve these two functions. Note also that in the P1 system, although the initiator protein is inhibitory at high concentration, it never reaches an inhibitory level under normal conditions (11). This inhibitory activity, then, is not ordinarily involved in copy control and may rather be regarded as a fail-safe mechanism to prevent autocatalytic replication. One is struck here by the adaptation of the same basic regulatory elements to two very different ends in the F/P1 and R6K systems.

Since any deficiency in the replication of a unit copy plasmid would cause loss of the plasmid, a replicative mechanism for self-correction of deficiencies in copy number would serve no useful purpose. Instead, these plasmids have developed the clever strategem of either preventing cell division until after they have replicated (64) or ensuring the death of any plasmid negatives that arise (14a). The ability of F (and, presumably, P1) to switch off its own replication if its copy number is elevated (99) indicates a highly effective mechanism for preventing (or correcting) overreplication. Since an autorepressor maintains its own concentration independent of the number of copies of its gene, overreplication would result in an excess of iteron sequences for which there would be insufficient initiator. This would delay replication, presumably until after the cell had divided, reducing the number of plasmids.

Incompatibility due to iterons. For all of the known plasmids that contain iterons to which the plasmid-specific initiator protein binds, the primary determinants of incompatibility, as analyzed with cloned segments, are the iteron-bearing regions. The F iterons express incompatibility in direct proportion to their gene dosage (90, 98), which complements nicely the finding that mini-F deletions affecting the iterons cause an increase in copy number that is inversely proportional to the number of iterons remaining (27). Since the rate of production of initiator is proposed to be constant, an excess of iteron sequences would delay or prevent the activation of the origin while a deficiency of the regulatory iterons would decrease the time between successive replications. The possibility of bifunctional binding of the initiator would mean that the initiator bound to cloned iterons might be able to act as an autorepressor in *trans*, increasing the incompatibility activity of the repeated sequence. Mutations affecting the autorepressor activity of the protein would obviously cause an increase in copy number; if such a mutation were to abolish autorepressor activity, autocatalytic runaway replication would be prevented by the direct inhibitory activity of excess initiator. Predictably, overproduction of the Rep protein relieves iteron-specific incompatibility (97).

A paradoxical feature of the P1 system (and one would assume the same to be true of F) is that the presence of several hundred copies of the cloned iterons does not cause any detectable increase in the total amount of Rep protein detectable immunologically in the cell (Chattoraj, personal communication). This observation is difficult to reconcile with an autorepressor function. A necessary feature of these systems is that the incompatibility expressed by the cloned iterons must involve reduction of replication rate and interference with self-correction; however, incompatibility activity has been evaluated exclusively by displacement or segregation tests (38, 77, 90) which cannot be meaningfully interpreted in such terms.

A similar explanation may be applicable to iteron-specific incompatibility for R6K; however, such an explanation would be tenable only if the above hypothesis, that the active form of π is rate limiting for replication, is correct and only if the active form cannot be rapidly generated from the inhibitory form. This requirement seems inconsistent with the view that the two forms are in an equilibrium that must respond quickly to changes in π concentration to maintain a constant replication rate. A further complication of iteron-specific incompatibility in R6K is the observation that its strength is enhanced by transcription across the cloned iterons in either direction (77). Conceivably, the transcripts could also bind π, which would mean that the protein can bind to both double-stranded DNA and single-stranded RNA. Thus the mechanism of iteron-specific incompatibility for R6K must be regarded as still a mystery and one that will not become clear until the plasmid's regulatory mechanism is fully understood.

Replication Origins and Incompatibility

As noted above, indirectly regulated plasmids with isologous origins are incompatible, even if they share no other replication or maintenance function. Similarly, the cloned *ori* of an indirectly regulated plasmid, but not that of a directly regulated plasmid, is a determinant of vectorial incompatibility. Again, this type of incompatibility can be interpreted in terms of interference with self-correction. In this case, the interference would be due to titration of the *trans*-active initiator. Thus the cloned *ori* of any of the iteron-bearing plasmids expresses incompatibility (2, 77, 98), as does that of the pT181 plasmids (59), whereas those of the directly regulated ColE1 and IncFII plasmids do not (22, 89). This incompatibility has been demonstrated as a destabilization of the parental plasmid by cloned fragments containing the origin. In the case of pT181, the cloned origin maintained at a copy number of 2.5-times normal caused a 60% reduction in copy number (Highlander, Ph.D. thesis); because RepC concentration is rate limiting for replication (37), it is likely that binding of RepC to any supernumerary copies of the origin present in the cell titrates the protein and reduces the copy number of the plasmid. As this titration effect makes unavailable a corresponding fraction of the RepC molecules synthesized by the plasmid in an attempt to elevate its copy number, self-correction becomes very sluggish, resulting in a broad frequency distribution, which causes instability as discussed above.

It is argued that the origin-specific incompatibility of pT181 does not represent a component of the actual copy control mechanism: it is, rather, an inevitable consequence of the system's functional organization. Similarly, the F and P1 origins should probably not be regarded as elements of the negative regulatory system even though they contain iterons that are homologous to the regulatory set. Rather, they should be regarded as the initiator-binding component of the origin itself.

The iteron-specific incompatibility of R6K is, in a sense, origin specific since the iterons define the γ origin of the plasmid. As these iterons are also involved in copy control, it is presently impossible to differentiate between the replicational and regulatory consequences of π binding to this region. Any attempt to explain the basis of iteron-specific incompatibility for R6K is complicated by the fact that π is normally present at a concentration greatly in excess of that required for replication and by the observation that transcription of the cloned iterons enhances the incompatibility (77). The titrational explanation requires the ad hoc assumption that a small subset of π molecules existing in an alternative configuration is rate limiting for replication and is subject to titration. It is possible also that a product encoded in the origin region inhibits the replication of R6K.

Incompatibility Due to Partitioning Determinants

Do all *inc* determinants act by interfering with self-correction? One exception would be partitioning incompatibility (56, 61). The occurrence of segregational incompatibility between isologous multicopy replicons implies that random assortment occurs during partitioning even if the overall plasmid pool is equipartitioned. Perfect equipartitioning, however, can probably not be distinguished experimentally from a model in which any two copies are explicitly partitioned and the rest are partitioned randomly (55). In either case, homoplasmid segregants will appear whether partitioning is active or passive, and it will do so even if the two plasmids can independently self-correct their copy pools. If there is independent self-correction that can restore normal copy number within one cell cycle, the segregation rate due to partitioning incompatibility will be determined solely by the frequency of monoplasmid segregants produced by random assortment during cell division. This frequency is given by the solution for $a = 0$ of the formula given earlier; it will be very small for any high-copy plasmid. Heteroplasmid segregation due purely to partitioning incompatibility should be distinguishable from any type of incompatibility with a replication component since reciprocal intrapool variation (see above) should not occur with the former but would always be demonstrable with the latter, given favorable selective markers. In practice, incompatibility caused by cloned *par* determinants could be readily demonstrated for R1 (56), which has a copy number of 3 to 4 per cell, and for the unit copy F (*incD* and *incG* [26]) and P1 plasmids (*incB* [3]) but not for pSC101 (42), which has an average copy number of about 20 (23). The latter result implies that pSC101 can self-correct any fluctuations in its copy pool within one cell cycle so that interference with partitioning does not cause an abnormally wide frequency distribution. Plasmid loss in the presence of a cloned *par* gene would occur at the vanishingly low frequency predicted for binomial partitioning when the predivision copy number is 30.

If the plasmids are isologous (do not self-correct independently), then the random assortment associated with partitioning will contribute to the intrapool fluctuations caused by random selection for replication and the contribution of the two components will be approximately equal (56, 61).

Competition (*cmp*) Phenomenon

Deletions that do not affect the integrity of the replicon have been isolated that profoundly affect the ability of the plasmid to replicate in the presence of a cognate wild-type replicon. These Cmp$^-$ plasmids are either disadvantaged in establishment (100) or show a gross decrease in copy number in the presence of a Cmp$^+$ plasmid (14, 24). Formally, the Cmp effect is a type of incompatibility; however, the cloned pT181 *cmp* region does not express either vectorial or symmetrical incompatibility (19), nor does deletion of *cmp* cause any obvious defect in plasmid replication. In the case of pSC101, the Cmp$^-$ phenotype has been observed with deletions in the *par* locus that do not necessarily cause any detectable partitioning defect; with pT181, this phenotype occurs with deletions in a region that is not involved in any known replicon function. In the latter case, it is clear that the Cmp effect involves competition for the rate-limiting initiator protein: *cmp*$^-$ deletions cause the plasmid to become hypersensitive to *ori*-specific incompatibility and this hypersensitivity is relieved by mutations affecting the cloned replication origin. It seems probable that the Cmp effect seen with pSC101 will be found to have the same basis, and it is suggested that as the *cmp* determinant increases the efficiency of Rep protein utilization, it can be viewed as a replication enhancer.

A number of instances have been described of vectorial incompatibility associated with cloned plasmid regions of unknown function. See, e.g., Nijkamp et al. (53), Gryczan et al. (20), and Meyer et al. (43). These have not been characterized and could involve Cmp-like phenomena (20, 53).

CONCLUDING REMARKS: INCOMPATIBILITY AS A STABILITY DEFECT

In summary, I hope to have demonstrated that incompatibility is basically a stochastic phenomenon. A heteroplasmid cell will give rise to homoplasmid segregants as a direct consequence of random assortment during cell division, as in partitioning incompatibility, or to interference with self-correction, which gives rise to a broad frequency distribution of plasmid copies and so causes hereditary instability. Vectorial incompatibility is seen with fragments cloned to an unrelated vector, particularly when the latter is maintained at a higher copy number than the target plasmid. It is also seen with mutations of directly regulated plasmids, which cause regulatory asymmetry, but not with mutations of indirectly regulated plasmids because any intrinsic regulatory asymmetry is relieved by the *trans*-active product of the regulated step. Examples of incompatibility have been described that are too strong to permit the establishment of any heteroplasmid system. In such cases, one plasmid may totally block the replication of the other; however, this has been satisfactorily demonstrated in only a single case, that of F, as noted. Three different incompatibility-causing plasmid elements have been identified, corresponding to the three known elements of the typical plasmid replicon, namely, *cop*, *rep*/*ori*, and *par*; in most cases, a plausible mechanistic hypothesis can be advanced. The most notable exception to this is the iteron-specific incompatibility of plasmid R6K, and it is likely that its investigation will reveal the mechanism of replication control for this plasmid as well as that of the incompatibility.

EPILOG: COUNTERSELECTION AND DISLODGEMENT

The technology of investigating plasmid incompatibility is fraught with pitfalls for the unwary and I should like to close by noting two of the most troublesome. Segregation rates of unstable plasmids should properly be measured with exponential cultures: true segregation kinetics are always exponential no matter what the cause; the observed kinetics of plasmid loss are often not purely exponential but rather show acceleration. Such acceleration is due to a growth advantage for cells that have "unburdened" themselves of the plasmid, which I like to refer to as counterselection against the plasmid-carrying cells. This phenomenon can grossly distort segregation rate measurements and must be carefully corrected (57).

In displacement tests, it is often observed that a certain fraction of transcipients lose the resident plasmid even though it is compatible with and not detectably related to the incoming one. This phenomenon is referred to as dislodgment and it has remained a total mystery during the 20 or so years since its discovery. The problem with dislodgment is that it can occur with disconcertingly high frequencies and can be mistaken for incompatibility unless one carefully rules out the occurrence of stable heteroplasmids.

ACKNOWLEDGMENTS

I thank M. Gennaro, A. Gruss, B. Kline, D. Helinski, K. Nordstrom, B. Polisky, S. Projan, and D. Sherratt for stimulating discussions; A. Gruss, M. Gennaro, and S. Projan for critical reading of the manuscript; and V. Ricciardi for expert secretarial assistance.

LITERATURE CITED

1. **Abe, M.** 1974. The replication of prophage P1 DNA. Mol. Gen. Genet. **132**:63–72.
2. **Abeles, A. L., K. M. Snyder, and D. K. Chattoraj.** 1984. P1 plasmid replication: replicon structure. J. Mol. Biol. **173**: 307–324.
3. **Austin, S., and A. Abeles.** 1985. The partition functions of P1, P7 and F miniplasmids, p. 215–226. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
4. **Baumstark, B. R., K. Lowery, and J. R. Scott.** 1984. Location by DNA sequence analysis of cop mutations affecting the number of plasmid copies of prophage P1. Mol. Gen. Genet. **194**:513–516.
5. **Bazaral, M., and D. R. Helinski.** 1970. Replication of a bacterial plasmid and an episome in *Escherichia coli*. Biochemistry **9**:399–406.
6. **Bex, F., L. Rokeach, P. Dreze, L. Garcia, and M. Couturier.** 1983. Mini-F encoded proteins: identification of a new 10.5 kilodalton species. EMBO J. **2**:1853–1861.
7. **Brady, G., J. Frey, H. Danbara, and K. N. Timmis.** 1983. Replication control mutations of plasmid R6-5 and their effects on interactions of the RNA-I control element with its target. J. Bacteriol. **154**:429–436.
8. **Carleton, S., S. J. Projan, S. K. Highlander, S. Moghazeh, and R. P. Novick.** 1984. Control of pT181 replication. II. Mutational analysis. EMBO J. **3**:2407–2414.
9. **Cesareni, G., M. A. Muesing, and B. Polisky.** 1982. Control of ColE1 DNA replication: the *rop* gene product negatively affects transcription from the replication primer promoter. Proc. Natl. Acad. Sci. USA **79**:6313–6317.
10. **Chattoraj, D. K., A. L. Abeles, and M. B. Yarmolinsky.** 1985. P1 plasmid maintenance: a paradigm of precise control, p. 355–381. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
11. **Chattoraj, K. K., K. M. Snyder, and A. L. Abeles.** 1985. P1 plasmid replication: multiple functions of RepA protein at the origin. Proc. Natl. Acad. Sci. USA **82**:2588–2592.
12. **Cullum, J., and P. Broda.** 1979. Rate of segregation due to plasmid incompatibility. Genet. Res. **33**:61–79.
13. **Filutowicz, M., M. McEachern, A. Greener, P. Mukhopadhyay, E. Uhlenhopp, R. Durland, and D. R. Helinski.** 1985. Role of the π initiation protein and direct nucleotide sequence repeats in the regulation of plasmid R6K replication, p. 125–140. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
14. **Gennaro, M. L., and R. P. Novick.** 1986. *cmp*, a *cis*-acting plasmid locus that increases the interaction between replication origin and initiator protein. J. Bacteriol. **168**:160–166.

14a. **Gerdes, K., L. Boe, P. Anderson, and S. Molin.** 1987. Plasmid stabilization in populations of bacterial cells, p. 275–282. *In* R. P. Novick and S. B. Levy (ed.), Antibiotic resistance genes: ecology, transfer and expression. Banbury Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

14b. **Gerhart, E., H. Wagner, J. Heijne, and K. Nordstrom.** 1986. Replication control in plasmid R1: CopA/CopT interaction and the role of the 7K protein, p. 165–175. *In* R. P. Novick and S. B. Levy (ed.), Antibiotic resistance genes: ecology, transfer and expression. Banbury Center, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

15. **Gerhart, E., H. Wagner, and K. Nordstrom.** 1986. Structural analysis of an RNA molecule involved in replication control of plasmid R1. Nucleic Acids Res. **14**:2523–2538.
16. **Germino, J., and D. Bastia.** 1983. Interaction of the plasmid R6K-encoded replication initiator protein with its binding sites on DNA. Cell **34**:125–134.
17. **Givskov, M., and S. Molin.** 1984. Copy mutants of plasmid R1: effects of base pair substitutions in the copA gene on the replication control system. Mol. Gen. Genet. **194**:286–292.
18. **Grindley, N. D. F., J. N. Grindley, and W. S. Kelley.** 1978. Mutant plasmid with altered replication control, p. 71. *In* D. Schlessinger (ed.), Microbiology—1978. American Society for Microbiology, Washington, D.C.
19. **Gruss, A., R. P. Novick, and H. F. Ross.** 1986. Functional analysis of a palindromic sequence required for normal replication of several staphylococcal plasmids. Proc. Natl. Acad. Sci. USA **84**:2165–2169.
20. **Gryczan, T., J. Hahn, S. Contente, and D. Dubnau.** 1982. Replication and incompatibility properties of plasmid pE194 in *Bacillus subtilis*. J. Bacteriol. **152**:722–735.
21. **Gustafsson, P., K. Nordstrom, and J. W. Perram.** 1978. Selection and timing of replication of plasmids R1*drd-119* and F'*lac* in *Escherichia coli*. Plasmid **1**:187–203.
22. **Hashimoto-Gotoh, T., and J. Inselburg.** 1979. ColE1 plasmid incompatibility: localization and analysis of mutations affecting incompatibility. J. Bacteriol. **139**:608–619.
23. **Hashimoto-Gotoh, T., and M. Sekiguchi.** 1977. Mutations to temperature sensitivity in R plasmid pSC101. J. Bacteriol. **131**: 405–412.
24. **Iordanescu, S.** 1986. Effect of the deletion of a fragment dispensable for the autonomous maintenance of plasmid pT181 on the competition between incompatible plasmids. Plasmid **15**:191–198.
25. **Ishii, K., T. Hashimoto-Gotoh, and K. Matsubara.** 1978. Random replication and random assortment model for plasmid incompatibility. Plasmid **1**:435–446.
26. **Kline, B. C.** 1985. A review of Mini-F plasmid maintenance. Plasmid **14**:1–16.
27. **Kline, B. C., and J. Trawick.** 1983. Identification and characterization of a second copy number control gene in mini-F plasmids. Mol. Gen. Genet. **192**:408–415.
28. **Kollek, R., M. Oertel, and W. Goebel.** 1978. Isolation and characterization of the minimal fragment required for autonomous replication ('Basic Replicon') of a copy mutant (pKN102) of the antibiotic resistance factor R1. Mol. Gen. Genet. **162**:51–57.
29. **Komai, N., T. Nishizawa, Y. Hayakawa, T. Murotsu, and K.**

Matsubara. 1982. Detection and mapping of six mini F-encoded proteins by cloning analysis of dissected mini-F segments. Mol. Gen. Genet. **186**:193–203.
30. **Kumar, C., and R. P. Novick.** 1985. Plasmid pT181 replication is regulated by two countertranscripts. Proc. Natl. Acad. Sci. USA **82**:638–642.
31. **Lacatena, R. M., and G. Cesareni.** 1981. Base pairing of RNA I with its complementary sequence in the primer precursor inhibits ColE1 replication. Nature (London) **294**:623–626.
32. **Lacatena, R. M., and G. Cesareni.** 1983. The interaction between RNA I and the primer precursor in the regulation of ColE1 replication. J. Mol. Biol. **170**:635–650.
33. **Lane, D., and R. C. Gardner.** 1979. Second *Eco*RI fragment of F capable of self-replication. J. Bacteriol. **139**:141–151.
34. **Leonard, A. C., and C. E. Helmstetter.** 1986. Cell cycle-specific replication of *Escherichia coli* minichromosomes. Proc. Natl. Acad. Sci. USA **83**:5101–5105.
35. **Light, J., and S. Molin.** 1982. Expression of a copy number control gene (*copB*) of plasmid R1 is constitutive and growth rate dependent. J. Bacteriol. **151**:1129–1135.
36. **Light, J., and S. Molin.** 1982. The sites of action of the two copy number control functions of plasmid R1. Mol. Gen. Genet. **187**:486–493.
37. **Manch-Citron, J., M. L. Gennaro, S. Majumder, and R. P. Novick.** 1986. RepC is rate-limiting for pT181 plasmid replication. Plasmid **16**:108–115.
38. **Manis, J. J., and B. C. Kline.** 1978. F plasmid incompatibility and copy number genes: their map locations and interactions. Plasmid **1**:492–507.
39. **Masai, H., Y. Kaziro, and K. Arai.** 1983. Definition of oriR, the minimum DNA segment essential for initiation of R1 plasmid replication in vitro. Proc. Natl. Acad. Sci. USA **80**:6814–6818.
40. **Masukata, H., and J. Tomizawa.** 1984. Effects of point mutations on formation and structure of the RNA primer for ColE1 DNA replication. Cell **36**:513–521.
41. **Masukata, H., and J. Tomizawa.** 1986. Control of primer formation for ColE1 plasmid replication: conformational change of the primer transcript. Cell **44**:125–136.
42. **Meacock, P., and S. N. Cohen.** 1980. Partitioning of bacterial plasmids during cell division. A *cis*-acting locus that accomplishes stable plasmid inheritance in cell populations. Cell **20**:529–542.
43. **Meyer, R. J., L. Lin, K. Kim, and M. A. Brasch.** 1985. Broad host-range plasmid R1162: replication, incompatibility, and copy-number control, p. 173–188. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
44. **Miki, T., K. Yoshioka, and T. Horiuchi.** 1984. Control of cell division by sex factor F in *E. coli*. The 42.84–43.6 F segment couples cell division of the host bacteria with replication of plasmid DNA. J. Mol. Biol. **174**:605–625.
45. **Molin, S., R. Diaz, B. E. Uhlin, and K. Nordstrom.** 1980. Runaway replication of plasmid R1 is not caused by loss of replication inhibitor activity of gene *cop*. J. Bacteriol. **143**:1046–1048.
46. **Molin, S., and K. Nordstrom.** 1980. Control of replication of plasmid R1. Functions involved in replication, copy number control, incompatibility, and switch-off of replication. J. Bacteriol. **141**:111–120.
47. **Molin, S., P. Stougaard, J. Light, M. Nordstrom, and K. Nordstrom.** 1981. Isolation and characterization of new copy mutants of plasmid R1 and identification of a polypeptide involved in copy number control. Mol. Gen. Genet. **181**: 123–130.
48. **Moser, D. R., and J. L. Campbell.** 1983. Characterization of a *trans*-complementable pTM1 copy number mutant: effect of RNA I gene dosage on plasmid copy number and incompatibility. J. Bacteriol. **154**:809–818.
49. **Moser, D. R., C. D. Moser, E. Sinn, and J. L. Campbell.** 1983. Suppressors of a temperature sensitive copy number mutation in plasmid NTP1. Mol. Gen. Genet. **192**:95–103.
50. **Muesing, M., J. Tamm, H. M. Shepard, and B. Polisky.** 1981. A single base-pair alteration is responsible for the DNA overproduction phenotype of a plasmid copy number mutant. Cell **24**:235–242.
51. **Naito, S., T. Kitani, T. Ogawa, T. Okazaki, and H. Uchida.** 1984. *Escherichia coli* mutants suppressing replication-defective mutations of the ColE1 plasmid. Proc. Natl. Acad. Sci. USA **81**:550–554.
52. **Naito, S., and H. Uchida.** 1980. Initiation of DNA replication in a ColE1-type plasmid: isolation of mutations in the ori region. Proc. Natl. Acad. Sci. USA **77**:6744–6748.
53. **Nijkamp, H. J. J., B. van Gemen, M. J. J. Hakaart, A. J. van Putten, and E. Veltkamp.** 1985. Stable maintenance of plasmid CLO DF13: structural relationships between replication control, partitioning, and incompatibility, p. 283–298. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
54. **Nordstrom, M., and K. Nordstrom.** 1985. Control of replication of FII plasmids: comparison of the basic replicons and of the small *copB* systems of plasmids R100 and R1. Plasmid **31**:81–87.
55. **Nordstrom, K., and H. Aagaard-Hansen.** 1984. Maintenance of bacterial plasmids: comparison of theoretical calculations and experiments with plasmid R1 in *Escherichia coli*. Mol. Gen. Genet. **197**:1–7.
56. **Nordstrom, K., S. Molin, and H. Aagaard-Hansen.** 1980. Partitioning of plasmid R1 in *Escherichia coli*. II. Incompatibility properties of the partitioning system. Plasmid **4**:332–349.
57. **Nordstrom, K., S. Molin, and H. Aagaard-Hansen.** 1980. Partitioning of plasmid R1 in *Escherichia coli*. I. Kinetics of loss of plasmid derivatives deleted for the *par* region. Plasmid **4**:215–227.
58. **Nordstrom, K., S. Molin, and J. Light.** 1984. Control of replication of bacterial plasmids: genetics, molecular biology and physiology of the plasmid R1 system. Plasmid **12**:71–90.
59. **Novick, R. P., G. K. Adler, S. J. Projan, S. Carleton, S. Highlander, A. Gruss, S. A. Khan, and S. Iordanescu.** 1984. Control of pT181 replication. I. The pT181 copy control function acts by inhibiting the synthesis of a replication protein. EMBO J. **3**:2399–2405.
60. **Novick, R. P., R. C. Clowes, S. N. Cohen, R. Curtiss III, N. Datta, and S. Falkow.** 1976. Uniform nomenclature for bacterial plasmids: a proposal. Bacteriol. Rev. **40**:168–189.
61. **Novick, R. P., and F. C. Hoppensteadt.** 1978. On plasmid incompatibility. Plasmid **1**:421–434.
62. **Novick, R. P., S. J. Projan, C. C. Kumar, S. Carleton, A. Gruss, S. K. Highlander, and J. Kornblum.** 1985. Replication control for pT181, an indirectly regulated plasmid, p. 299–320. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
63. **Ogura, T., and S. Hiraga.** 1983. Partition mechanism of F plasmid: two plasmid gene-encoded products and a *cis*-acting region are involved in partition. Cell **32**:351–360.
64. **Ogura, T., and S. Hiraga.** 1983. Mini-F plasmid genes that couple host cell division to plasmid proliferation. Proc. Natl. Acad. Sci. USA **80**:4784–4788.
65. **Oka, A., N. Nomura, M. Morita, H. Sugisaki, and K. Sugimoto.** 1979. Nucleotide sequence of small ColE1 derivatives: structure of the regions essential for autonomous replication and colicin E1 immunity. Mol. Gen. Genet. **172**:151–159.
66. **Polisky, B., J. Tamm, and T. Fitzwater.** 1985. Construction of *E. coli* RNA1 mutants and analysis of their function *in vivo*, p. 321–334. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
67. **Pritchard, R. H., P. T. Barth, and J. Collins.** 1969. Control of DNA synthesis in bacteria. Symp. Soc. Gen. Microbiol. **19**:263–297.
68. **Projan, S. J., J. Kornblum, S. L. Moghazeh, I. Edelman, M. L. Gennaro, and R. P. Novick.** 1985. Comparative sequence and functional analysis of pT181 and pC221, cognate plasmid replicons from *S. aureus*. Mol. Gen. Genet. **199**:452–464.
69. **Projan, S. J., and R. P. Novick.** 1984. Reciprocal intrapool

variation in plasmid copy numbers: a characteristic of segregational incompatibility. Plasmid **12**:52.
70. **Projan, S. J., and R. P. Novick.** 1986. Incompatibility between plasmids with independent copy control. Mol. Gen. Genet. **204**:341–348.
71. **Rokeach, L. A., L. Sogaard-Andersen, and S. Molin.** 1985. Two functions of the E protein are key elements in the plasmid F replication control system. J. Bacteriol. **164**:1262–1270.
72. **Rosen, J., T. Ryder, H. Ohtsubo, and E. Ohtsubo.** 1981. Role of RNA transcripts in replication incompatibility and copy number control in antibiotic resistance plasmid derivatives. Nature (London) **290**:794–799.
73. **Rownd, R.** 1969. Replication of a bacterial episome under relaxed control. J. Mol. Biol. **44**:387–402.
74. **Rownd, R. H., D. D. Womble, X. Dong, V. A. Luckow, and R. P. Wu.** 1985. Incompatibility and IncFII plasmid replication control, p. 335–354. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
75. **Scott, J. R.** 1984. Regulation of plasmid replication. Microbiol. Rev. **48**:1–23.
76. **Shafferman, A., R. Kolter, D. Stalker, and D. R. Helinski.** 1982. Plasmid R6K DNA replication. III. Regulatory properties of the π initiation protein. J. Mol. Biol. **161**:57–76.
77. **Shafferman, A., D. M. Stalker, A. Tolun, R. Kolter, and D. R. Helinski.** 1981. Structure-function relationships in essential regions for plasmid replication, p. 259–270. *In* S. Levy, R. C. Clowes, and E. L. Konig (ed.), Molecular biology, pathogenicity and ecology of bacterial plasmids. Plenum Publishing Corp., New York.
78. **Sogaard-Andersen, L., L. A. Rokeach, and S. Molin.** 1984. Regulated expression of a gene important for replication of plasmid F in *E. coli*. EMBO **3**:257–262.
79. **Som, T., and J. Tomizawa.** 1983. Regulatory regions of ColE1 that are involved in determination of plasmid copy number. Proc. Natl. Acad. Sci. USA **80**:3232–3236.
80. **Stalker, D. M., M. Filutowicz, and D. R. Helinski.** 1983. Release of initiation control by a mutational alteration in the R6K π protein required for plasmid DNA replication. Proc. Natl. Acad. Sci. USA **80**:5500–5504.
81. **Stalker, D. M., R. Kolter, and D. R. Helinski.** 1982. Plasmid R6K DNA replication. I. Complete nucleotide sequence of an autonomously replicating segment. J. Mol. Biol. **161**:33–43.
82. **Steinberg, D. A., and C. E. Helmstetter.** 1981. F plasmid replication and the division cycle of *Escherichia coli* B/r. Plasmid **6**:342–353.
83. **Sternberg, N., and S. Austin.** 1983. Isolation and characterization of P1 minireplicons λ-P1:5R and λ-P1:5L. J. Bacteriol. **153**:800–812.
84. **Stougaard, P., S. Molin, and K. Nordstrom.** 1981. RNAs involved in copy number control and incompatibility of plasmid R1. Proc. Natl. Acad. Sci. USA **78**:6008–6012.
85. **Stuitje, A. R., C. E. Spelt, E. Veltkamp, and H. J. Nijkamp.** 1981. Identification of mutations affecting replication control of plasmid Clo DF13. Nature (London) **290**:264–267.
86. **Summers, D. K., and D. J. Sherratt.** 1984. Multimerization of high copy number plasmids causes instability: ColE1 encodes a determinant essential for plasmid monomerization and stability. Cell **36**:1097–1103.
87. **Summers, D. K., and D. J. Sherratt.** 1985. Stability functions of ColE1, p. 909. *In* D. R. Helinski, S. N. Cohen, D. B. Clewell, D. A. Jackson, and A. Hollaender (ed.), Plasmids in bacteria. Plenum Publishing Corp., New York.
88. **Tamm, J., and B. Polisky.** 1983. Structural analysis of RNA molecules involved in plasmid copy number control. Nucleic Acids Res. **11**:6381–97.
89. **Timmis, K., L. Andres, and P. Slocombe.** 1978. Plasmid incompatibility: cloning of an IncFII determinant of R6-5. Nature (London) **71**:4556–4560.
90. **Tolun, A., and D. R. Helinski.** 1981. Direct repeats of the plasmid *incC* region express F incompatibility. Cell **24**: 687–694.
91. **Tomizawa, J.** 1984. Control of ColE1 plasmid replication: the process of binding of RNA I to the primer transcript. Cell **38**:861–870.
92. **Tomizawa, J.** 1985. Control of ColE1 plasmid replication: initial interaction of RNA I and the primer transcript is reversible. Cell **40**:527–535.
93. **Tomizawa, J., and T. Itoh.** 1981. Plasmid ColE1 incompatibility determined by interaction of RNA I with primer transcript. Proc. Natl. Acad. Sci. USA **78**:6096–6100.
94. **Tomizawa, J., and T. Itoh.** 1982. The importance of RNA secondary structure in ColE1 primer formation. Cell **31**:575–583.
95. **Tomizawa, J., T. Itoh, G. Selzer, and T. Som.** 1981. Inhibition of ColE1 RNA primer formation by a plasmid-specified small RNA. Proc. Natl. Acad. Sci. USA **78**:1421–1425.
96. **Tomizawa, J., and T. Som.** 1984. Control of ColE1 plasmid replication: enhancement of binding of RNA I to the primer transcript by the Rom protein. Cell **38**:871–878.
97. **Trawick, J. D., and B. C. Kline.** 1985. A two-stage molecular model for control of mini-F replication. Plasmid **13**:59–69.
98. **Tsutsui, H., A. Fujiyama, T. Murotsu, and K. Matsubara.** 1983. Role of nine repeating sequences of the mini-F genome for expression of F-specific incompatibility phenotype and copy number control. J. Bacteriol. **155**:337–334.
99. **Tsutsui, H., and K. Matsubara.** 1981. Replication control and switch-off function as observed with a mini-F factor plasmid. J. Bacteriol. **147**:509–516.
100. **Tucker, W. T., C. A. Miller, and S. N. Cohen.** 1984. Structural and functional analysis of the *par* region of the pSC101 plasmid. Cell **38**:191–201.
101. **Twigg, A., and D. Sherratt.** 1980. Trans-complementable copy number mutants of plasmid ColE1. Nature (London) **283**:216–218.
102. **Wong, E. M., M. A. Muesing, and B. Polisky.** 1982. Temperature-sensitive copy number mutants of ColE1 are located in an untranslated region of the plasmid genome. Proc. Natl. Acad. Sci. USA **79**:3570–3574.
103. **Wong, E. M., and B. Polisky.** 1985. Alternative conformations of the ColE1 replication primer modulate its interaction with RNAI. Cell **42**:959–966.
104. **Yarranton, G. T., E. Wright, M. K. Robinson, and G. O. Humphreys.** 1984. Dual-origin plasmid vectors whose origin of replication is controlled by the coliphage lambda promoter pL. Gene **28**:298–300.