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Replication of Plasmids in Gram-Negative Bacteria

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INTRODUCTION

Many procaryotes contain plasmids in addition to their chromosomes (10, 283). Such plasmids are normally circular (exceptions are the linear forms found in *Streptomyces rochei*, *Borrelia* species, and *Thiobacillus versutus* [15, 119, 353]) and range between a few and several hundred kilobases (e.g., p15A from *Escherichia coli* is 2.2 kilobases [kb] long [39], pZA2 from *Zymomonas anaerobia* is 1.7 kb long [362], and megaplasmids of lithoautotrophic bacteria can be about 700 kb long [120]). Most plasmids are cryptic, but often they provide their host with new phenotypic characteristics (for reviews, see references 283 and 328).

Plasmids represent an important factor in bacterial evolution: they enable rapid short-term adaption of bacteria to changing environmental conditions; they confer gene amplification; and they can be transferred within one or between many species. Normally, plasmids are nonessential to their hosts, conferring only an energy burden that can slow cell growth (42, 369). However, plasmids can be stably maintained in a bacterial population even under nonselective conditions (54, 210, 289). Strategies such as overreplication (33), partition modes (5, 176), killing of plasmid-free segregants (98, 118, 235), infectious conjugal transfer (165, 175), and surface (entry) exclusion against conjugative entrance of additional, related deoxyribonucleic acid (DNA) molecules (334, 335) contribute to the maintenance of plasmids in a bacterial population.

Some plasmids occur in only one or a few copies per cell; others occur in several copies (for examples, see references 214 and 306). The maintenance of low-copy-number plasmids requires a tighter regulation of replication and of segregation than does maintenance of multicopy plasmids (176, 257). The existence of related plasmids in the same cell line can be prevented by both types of regulation; however, replication-based incompatibility is usually more stringent than segregation-based incompatibility (47, 214, 257, 314). The control mechanisms determining copy number and replication-based incompatibility are usually plasmid inherited (for an exception, see reference 361) and involve the initiation of replication (144, 209, 214, 257, 304). In contrast to elongation, the initiation of replication is molecule specific, and this step is therefore of great importance for the propagation of a plasmid in a specific host (257).

In addition to plasmid-inherited determinants, replication initiation is dependent on various host-encoded enzymes

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(257). Many plasmids can therefore replicate only in one or a few closely related hosts (examples are given in reference 297). In contrast, promiscuous plasmids are adapted to a wide range of bacteria and can be stably inherited in distantly related hosts (152, 230, 311).

In this review we describe the initiation of plasmid replication in gram-negative bacteria, with particular emphasis on factors determining host range. Possible reasons for host range limitations are discussed.

REPLICATION OF NARROW-HOST-RANGE PLASMIDS

Information regarding the replication of plasmids with a restricted or narrow host range has been obtained mainly by the examination of plasmids from members of the family *Enterobacteriaceae* (for reviews, see references 40, 53, 81, 138, 139, 144, 211, and 257). Depending on the absence or presence of a plasmid-encoded protein for replication initiation (Rep protein) narrow-host-range plasmids can be divided into two classes, example of which are ColE1 and pSC101.

ColE1 and Related Plasmids

ColE1 is a 6.6-kb *E. coli* plasmid with a copy number of nearly 20 (6, 38). Related multicopy plasmids of members of the family *Enterobacteriaceae* include p15A, pMB1, RSF1010 (NTP1), CloDF13 (259), NTP16 (158), and other coligenic plasmids (116, 196, 370, 371). Several multicopy cloning vectors such as the well-known plasmids pACYC184 and pBR322 are derived from p15A and pMB1 (13, 39).

Requirements for replication. The replication of ColE1 is initiated in a 0.6-kb region, the origin (*ori*), and progresses unidirectionally in the θ-shaped manner of Cairns-type replication (6, 125, 220, 324–326). For the whole process, ColE1 requires only proteins from its host bacterium *E. coli* (64, 324). For initiation at the origin, a DNA-dependent ribonucleic acid (RNA) polymerase, ribonuclease H (RNase H), and DNA polymerase I (Pol I), as well as DNA gyrase and topoisomerase I, participate (117, 126, 127, 193). Gyrase participates in the opening of the DNA double strand and may provide the topological driving force for movement of the replication fork (193, 221). By modulation of plasmid superhelicity, topoisomerase I may favor the recognition of the primer promoter by RNA polymerase to make the primer transcript (193). Normally, by the combined action of RNA polymerase and RNase H, a processed primer transcript is formed which is used by Pol I for the synthesis of the leading strand over a length of about 400 nucleotides (127, 247) (see below). Replication in vivo (140, 141, 204, 218, 219) and in vitro (52, 182) can also start in the absence of RNase H and Pol I by using alternative modes (see below).

Essential for extension of the leading strand and also for discontinuous synthesis of the lagging strand are DNA polymerase III (Pol III), single-strand binding proteins, and the proteins of the primosome complex (86, 180, 193, 194, 284, 285), i.e., DnaB helicase (12, 163), DnaB-complexed DnaC protein (23), DnaG primase, and the preprimosome organizer i, n, n′, n″ (269). Efficient lagging-strand synthesis can be initiated by primosome formation on an n′ recognition site 150 nucleotides downstream of the origin (*rriA pas-B1*) (24, 179, 208, 368) or, alternatively, by formation of primosomelike DnaA-dependent complexes of DnaB, DnaC, and DnaG proteins formed at a DnaA recognition box adjacent to the origin (90, 261, 262). In vivo, both the DnaA box and the n′ site are dispensable (6, 182, 219).

Mechanisms of primer formation and replication initiation. At present, the only detailed model of replication initiation and regulation that is supported by genetic and biochemical data is that for ColE1-type plasmids (36, 53, 183, 321). They are the only ones with a well-studied primer for leading-strand synthesis (127, 319). The replication models for ColE1-type plasmids, proposed mostly by Tomizawa and co-workers, are depicted in Fig. 1 and Fig. 2.

Beginning 555 base pairs (bp) upstream of the ColE1 origin (or at an appropriate position in the *ori* region of other ColE1-type plasmids [196, 260]) and terminating heterogeneously downstream from the origin, RNA polymerase transcribes a primer precursor nearly 700 bp long, called RNA II (127, 322, 323) (Fig. 1 and 2). The 3′ ends of approximately half of these nascent RNA II transcripts form a persistent hybrid with their DNA template near the origin (127, 260) (Fig. 1). This process, named coupling (321), is dependent on the secondary structure of the 5′ end of RNA II (35, 183, 184, 355). If the specific conformation for hybridization does not form (i.e., RNA II folds in another way [Fig. 2]), coupling does not occur and priming aborts (183, 184) (Fig. 1). Determined by structure X (Fig. 2), which is the origin-proximal stem-loop of secondary-structured DNA-hybridized precursor transcripts (183, 219), RNase H recognizes and cleaves the coupled RNA II-DNA hybrids at the origin within a sequence of five A's and generates mature primer molecules. These processed transcripts are the target for Pol I, which adds deoxynucleotides to their 3′ OH ends (127, 260) (Fig. 1, mechanism type I). Precursor RNA still hybridized to DNA is then cleaved at secondary sites by RNase H (Fig. 1, arrows) and digested by the 5′-3′-exonuclease activity of Pol I during DNA elongation. Further cleavage by RNase H removes the RNA primer from the newly synthesized DNA strand (260). The type I mechanism of replication initiation seems to be the normal one, because Pol I is essential for plasmid maintenance in bacteria that contain RNase H (52, 137, 219) and in vitro initiation is inhibited by RNase H in the absence of Pol I (52). In addition to the main type I mechanism, there are two alternative RNase H-independent pathways of unidirectional replication (52, 182, 219) (Fig. 1, type II and type III) which act at the same region as the RNase H-dependent replication initiation (140). In both alternative pathways, transcription of RNA II and its hybridization to the template are necessary for replication initiation both in vivo and in vitro (52, 204, 219). In the absence of RNase H, RNA II may act as a transcriptional activator which unwinds double-stranded DNA by forming a DNA-RNA hybrid (52, 182, 219). Normally, during transcription by RNA polymerase, about 17 bp of the DNA-helix is opened and only a few nucleotides of the nascent transcripts are hybridized to the DNA template (342). This short single-stranded stretch is protected by the RNA polymerase. Thus, enzymes like DnaG primase are unable to initiate replication and helicases like DnaB are unable to extend the single-stranded area to a length adequate for assembly of proteins that mediate lagging-strand synthesis. In contrast, in ColE1, the extensive elongation of the stable RNA II-DNA hybrid allows a displacement loop to form downstream from the origin (127, 182, 260), which is sufficient for initiation of lagging-strand synthesis on the nontranscribed single strand of DNA (182) (Fig. 1, type II mechanism). Replication initiation by lagging-strand synthesis within the origin region requires an RNA II-DNA hybrid having a corresponding single strand of at least 40 nucleotides; the latter may be extended by the action of a helicase (182). At the single strand, a replisome (i.e., a macromolec-

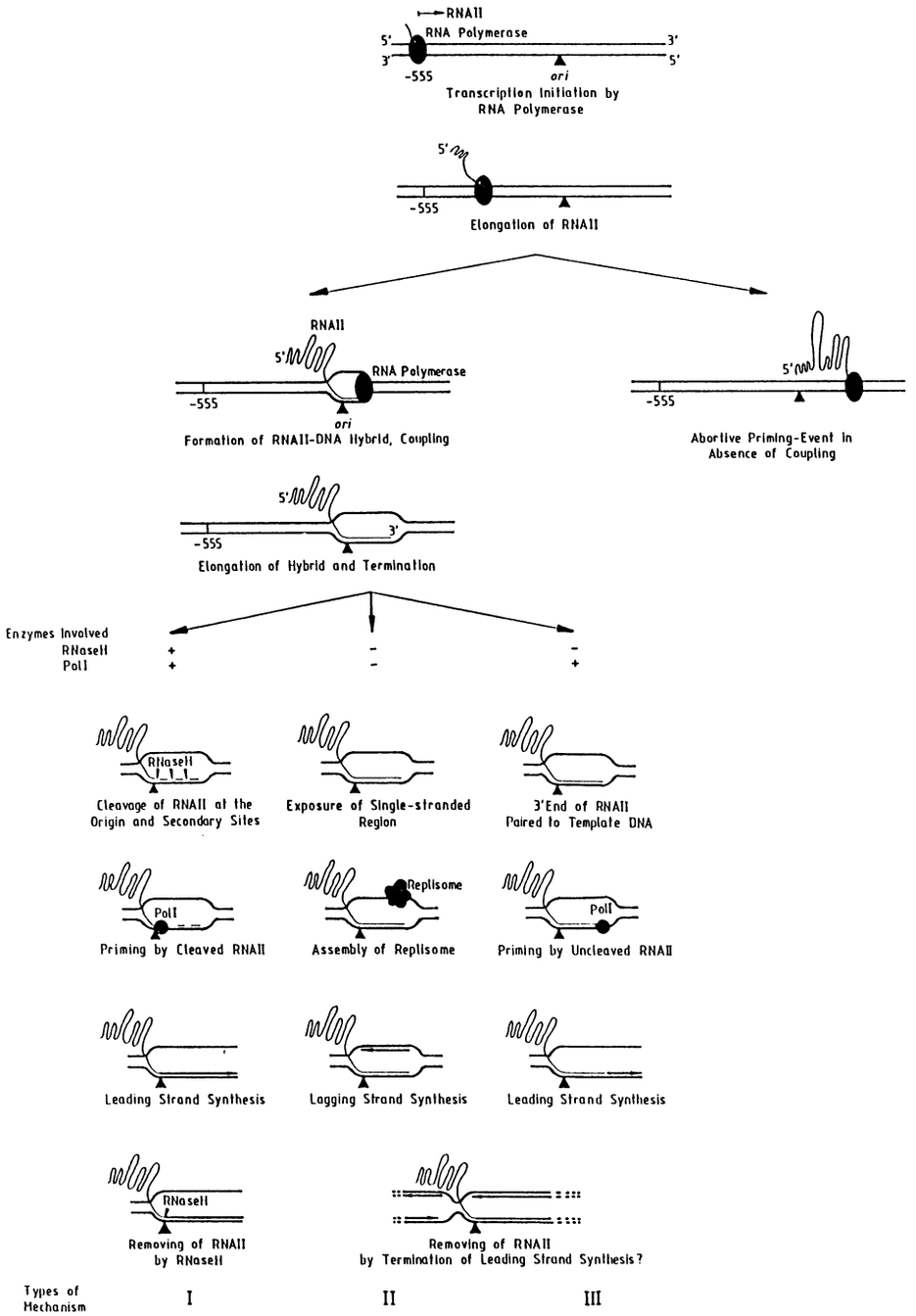


FIG. 1. Schematic illustration of multiple mechanisms for the initiation of ColE1 replication (adapted from references 52, 182, and 183). The type I mechanism is proposed for wild-type *E. coli*, the type II mechanism is proposed for RNase H-negative and DNA polymerase I-positive or -negative strains (*rnh polA* and *rnh polA*$^+$ strains, respectively), and the type III mechanism is proposed for RNase H-negative and Pol I-positive strains (*rnh polA*$^+$ strains). Position −555 corresponds to the start of the RNA II transcript. The triangle (▲) marks the origin (*ori*), where RNA II processing by RNase H takes place and where the target site of Pol I (◍) is normally situated. The ellipse indicates the RNA polymerase. The DNA (▬) and RNA strands (——) are marked. The different folds of RNAs symbolize the secondary structure of active and inactive primer transcripts (see Fig. 2). The direction of DNA synthesis is shown by arrows (further explanations are given in the figure and the text).

ular protein-DNA complex which starts DNA synthesis [150]) could be formed without the need for a primosome- or DnaA-binding site (182, 219). Initiation of replication can occur at various sites within the region extending approximately 500 bases downstream from the origin; however, owing to the presence of the unhybridized part of RNA II, replication always terminates at a site termed *terH*, 17 nucleotides upstream from the origin. Termination at *terH* seems to account for the unidirectionality of ColE1 replication (52).

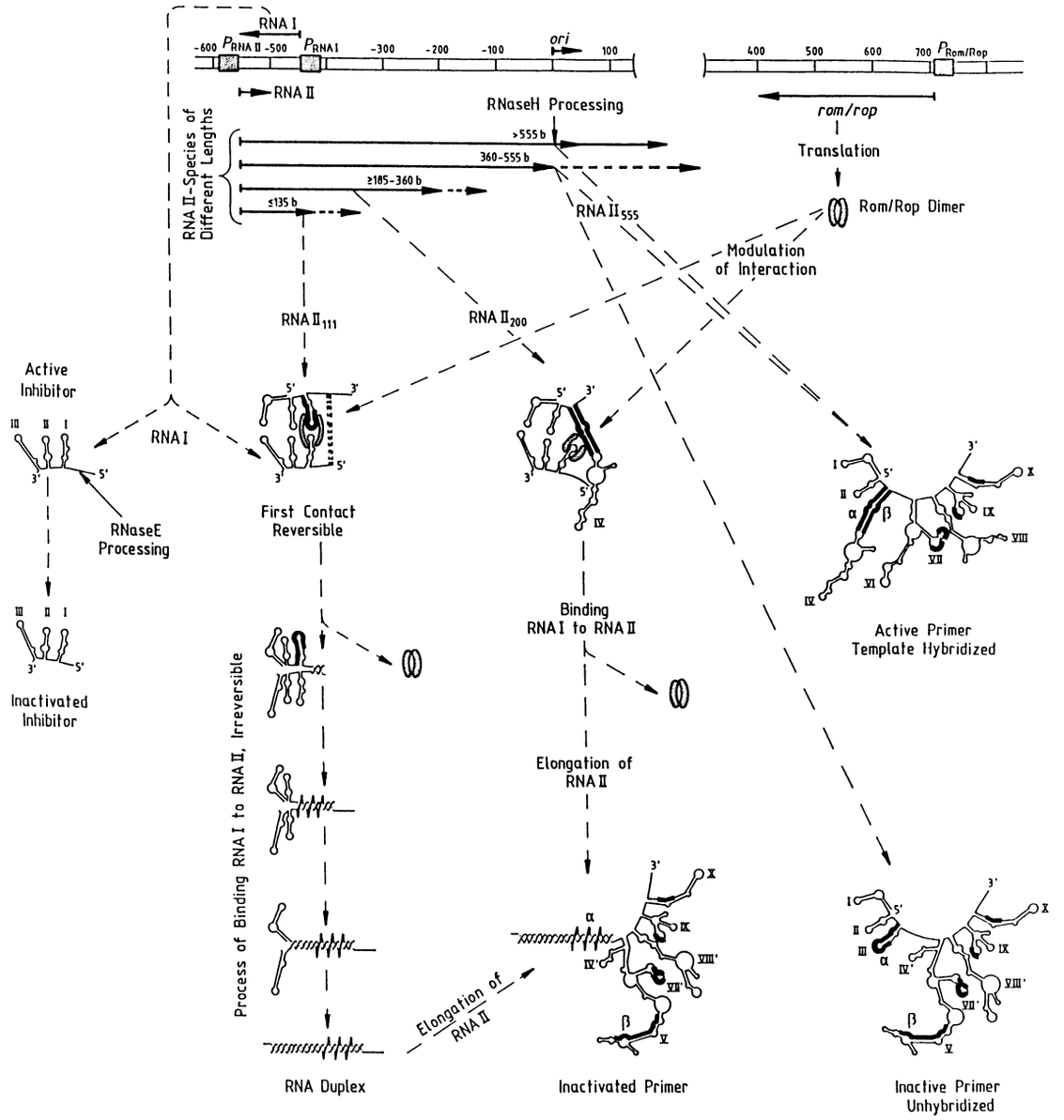


FIG. 2. Schematic illustration of the regulation of replication initiation in ColE1-type plasmids (compiled from data and figures in references 36, 115, 184, and 316–319; for further information, see the text). At the top, the DNA double strand of ColE1-type plasmids with the replication origin and the gene for the Rom (Rop) protein is shown. The numbers refer to the distance in nucleotides from the origin. Promoters for the RNA I ($P_{RNA\ I}$), the RNA II ($P_{RNA\ II}$), and the Rom (Rop) transcript ($P_{Rom/Rop}$) are shown (□). Thick arrows below the double strand indicate the direction and length of transcription; numbers indicate the length of transcripts. The lower part illustrates possible reactions and putative secondary structures of the RNA I and RNA II transcripts. At the left, the inactivation of replication inhibitor RNA I by RNase E processing is shown. The position of specific RNase E cleavage leading to a removal of the 5′ end of RNA I is indicated by an arrow. Roman numerals mark the three stem-loops of RNA I. In the second column the stepwise process of the binding of inhibitor RNA I to the growing preprimer RNA II is illustrated. The first reversible kissing reaction between loops I, II, and III of the complementary RNAs (compare other structures in the figure) can be influenced positively or negatively by the Rom protein (●) depending on the length of the RNA II. Pairing starts at the 5′ end of RNA I (marked by several broken lines). Subsequently, the loop-to-loop contacts may be broken and the pairing proceeds by a zipping mechanism, which unfolds the three pairs of stem-loops. This pairing is irreversible, even when the RNA II transcript is elongated. In consequence, the downstream sequences of RNA II exhibit a secondary configuration unable to hybridize to its DNA template (inactivated primer, bottom of the figure in the middle). Owing to their location, the two segments termed α and β (the longer solid regions in the different RNA II structures) cannot pair to each other to form the hairpin IV (compare the Roman numerals and stem-loops, respectively, of the different RNA II structure at the right), which is a prerequisite for primer processing. RNA II transcripts longer than 185 bases possess stem-loop IV (as shown in the third column), but it can be destroyed by the RNA I inhibitor. In the scheme, the reaction of RNA I and RNA II at the stage of the greatest effect of Rom on the pairing of the countertranscripts is illustrated. Irrespective of the binding of RNA I, the RNA II transcripts longer than 360 bases may form the hairpins VI, VII, VIII, IX, and X at their 3′ end, as postulated for the active, template-hybridized primer in the absence of RNA I (shown in the right column). RNA II folded in such a structure and hybridized to the DNA can be processed by RNase H at the origin (marked by an arrow at the transcripts below the DNA double strand). In the right corner, the putative secondary structure of an RNA II mutant (*pri7* RNA II [184]) is shown, which is nonfunctional in template hybridization and priming of DNA synthesis in vitro. The shaded and black short boxes, situated at different positions in active and inactive primer transcripts, denote regions which may be involved in a tertiary pairing during coupling of RNA II to its template.

The type II initiation mechanism can occur in bacteria lacking both RNase H and Pol I (*rnh polA*). In cells that contain Pol I but lack functional RNase H (*rnh polA*$^+$) replication may start by the type III mechanism or by both type II and III mechanisms (52) (Fig. 1). In the type II mechanism, DNA synthesis is activated indirectly by the persistent RNA II-DNA hybrid; in the type III mechanism, the hybridized RNA II transcripts may be recognized as primers by Pol I without previous cleavage of RNA II at the origin (52). Normally, the type III mechanism using RNA polymerase and Pol I is inefficient in vitro (127), because the 3′ ends of the usual RNA II transcripts of nearly 700 bases are not used as primers by Pol I (52). Only short transcripts with a hybridized section less than 50 nucleotides are efficiently used, as has been demonstrated by the insertion of a transcription terminator 9 bases downstream from the origin (323) and by trimming the 3′ ends of RNA II transcripts with nuclease (182). However, the type III mechanism may occur in vivo in a group of ColE1 mutants that are defective for replication in the *E. coli* wild type (204). This class of mutants, having base alterations changing the structure VIII of folded RNA II (Fig. 2) (183, 203), allows RNA II synthesis, hybrid formation, and RNase H cleavage, but the RNase H-cleaved RNA II cannot serve as a primer by Pol I (183). Such mutant plasmids can replicate in RNase H-negative strains (182, 202, 204), but not in RNase H-positive cells unless hyperactivated by Pol I (204). Overproduced Pol I or Pol I with altered properties might initiate DNA synthesis at the DNA-hybridized RNA II before RNase H can act (204).

In contrast to the type I mechanism, in which RNase H participates in the removal of primers (260), it is probably removed in the type II and type III mechanisms by the termination of leading-strand synthesis (52) (Fig. 1).

Regulation of replication initiation. The frequency of the replication initiation at the origin depends in part on the frequency of RNA II formation by RNA polymerase (34, 35, 96, 223, 244, 336); however, the main regulation of initiation is exerted during the synthesis of RNA II by a 108-nucleotide antisense transcript termed RNA I (319). By hybridizing to the primer precursor, RNA I inhibits hybrid formation between RNA II and its DNA template, leading to abortive primers (156, 184, 320, 322). Because the synthesis of the primer precursor takes place in 12 s, inhibition by RNA I in vivo can occur only within a few seconds. This requires the presence of relatively high concentrations of RNA I, which is guaranteed because transcription of RNA I occurs five times more often than transcription of RNA II. Hence, on average, 1 in 20 preprimer transcriptions leads to a replication event (171). Inasmuch as the pairing of RNA I and RNA II depends on the presence of the 5′ end of RNA I (66, 84, 295, 317) (see below), inactivation of RNA I occurs when its 5′ end is specifically removed by RNase E (316). This cleavage of RNA I by RNase E seems to stimulate replication in vivo by reducing the number of active inhibitor molecules (115, 316). Furthermore, depending on the physiological state of the cells, regulation of the level of active RNA I molecules might also occur by interactions of RNA I with transfer RNAs (tRNAs) with complementary sequences to RNA I (361). This theory may be supported by the finding that when certain amino acids are limited, plasmid amplification in some *E. coli* strains (*relA*) results (112–114), probably because the concentration of uncharged tRNAs is increased (361). In addition, RNA I may be affected in an unknown fashion by growth conditions, as occurs with conditional high-copy-number pBR322 derivatives (130).

Transcription of RNA I is initiated 445 bp upstream from the origin, proceeds in the opposite direction to RNA II, and is terminated a few bases before the RNA II start (Fig. 2) (127, 195). The RNA I transcript assumes a tRNA-like conformation (cloverleaf structure) composed of three stem-loops and a 5′ single-stranded tail of 9 nucleotides (195, 294, 295, 361). Similar foldings (palindromes I, II, and III) occur at the 5′-proximal region of the primer transcript shortly after the start of the RNA II synthesis (319, 355). The folded RNA I and RNA II molecules interact in an initial reversible contact by a "kissing" between specific bases of the homologous single-stranded loops (157, 317, 318). This interaction brings together the single-stranded 5′ end of RNA I and its complementary RNA II sequence, causing a progressive pairing between both RNAs by a zipping mechanism accompanied by the unfolding of their secondary structures (317, 318) (Fig. 2).

Palindrome III of the primer transcript partly overlaps structure IV in RNA II (259) (Fig. 2, segments α and β), which is characteristic for RNA II-DNA hybrids and determines the secondary structure of the downstream RNA II region, i.e., the formation of stem-loops VI to X (184) (Fig. 2). As a result of RNA I pairing, palindrome IV of RNA II cannot be developed, and therefore, far downstream of the RNA I-binding region, the secondary structure of RNA II is altered in the palindromes V, VII′, and probably VIII′, preventing hybridization between the primer precursor and its DNA template. Thus, for stem-loops VI and VII of RNA II, an interaction with the DNA template is assumed which probably acts as an signal for the downstream hybridization of RNA II. Structure IX, in which tertiary base pairings with a complementary loop of structure VII or possibly with the DNA template occur within the RNA II-DNA hybridization, also seems to be crucial for hybrid formation (184). Evidence from point mutations shows that two G stretches, one in stem-loop VII and the other in palindrome X, probably also participate in hybrid formation. Alternatively, or in turn, the G stretches may interact with a third region, e.g., with the C-rich stretch opposite the poly(G) sequence in the stem of structure X (183, 219). Finally, structure VIII may play only a small part in the DNA-RNA interaction because (i) partial digestion with RNases of DNA-hybridized and unhybridized transcripts reveals no differences in the configuration of VIII and VIII′ (184); (ii) in contrast to the other stem-loops, structures VIII in different ColE1-type plasmids show only a slight sequence homology (184, 196, 259); and (iii) even with base changes that influence the folding of VIII, RNA II is capable of hybridizing to the DNA template (183). In contrast to this minor role in DNA-RNA interaction, structures VIII and VIII′ may influence the subsequent reaction of Pol I as a result of their steric configuration (183).

The binding and the inhibitory effect of RNA I are influenced by conformational variations dependent on the length of the nascent primer transcript (319, 355) (Fig. 2). Growing RNA II transcripts of between 110 and 360 nucleotides are sensitive to the inhibitory action of RNA I (319). Longer transcripts also interact with RNA I but are immune to the inhibitory effect on primer formation. Even if stem-loop IV of RNA II is destroyed through the action of RNA I, the configuration of the downstream region of RNA II, which is essential for the hybridization to the DNA, becomes stable (319). The period of inhibitory action of RNA I may be extended by transcriptional pausing at or near the loop of structure IV, as is suggested by RNA II mutants which are resistant to RNA I inhibition (85).

On the other hand, the pairing of the complementary RNAs is modified by a small protein dimer of 63 amino acids

which is called Rom (RNA one inhibition modulator) or Rop (repressor of primer) (4, 37, 155, 318, 319, 327). The Rom protein is encoded downstream from the origin of some ColE1-type plasmids (280, 332) (Fig. 2); in others it is missing (196, 207). The deletion of the nonessential *rom* gene as well as overproduction of Rom affects plasmid copy number and incompatibility (215, 288, 335); both phenomena are based on the RNA I-RNA II interaction (66, 84). Rom enhances RNA I-RNA II binding in vitro about twofold (65, 327); the actual value depends on the length of the RNA II transcript; frequently, it even inhibits binding (319). RNA I chains longer than 135 nucleotides consistently bind more strongly to RNA II in the presence of Rom. The binding of smaller transcripts can be enhanced, reduced, or completely abolished by Rom, depending on length differences of only a few bases (319). As yet, the molecular basis of the Rom interference with the RNA I-RNA II interaction is not understood. Point mutations in RNA I suggest that the target site of Rom is the stem-loop I of RNA I, which is the counterpart of stem-loop III in RNA II (37, 65, 197) (Fig. 2). New biochemical evidence shows that Rom specifically interacts with all three stem sequences in RNA I, but interaction with stem I is sufficient to ensure binding of RNA I-RNA II. Rom was also shown to interact with structures I, II, and IV of the RNA II transcript. The affinities of RNA I and RNA II with Rom are similar, possibly indicating that the protein interacts symmetrically with two complementary RNAs (115) (Fig. 2). The monomer of Rom consists of two α helices connected by a sharp bend, and the dimer forms an α-helix dipole by using a coiled-coil protein architecture (14). Owing to its symmetric structure, the dimer may function as an adaptor between the two RNA types: each of its subunits should bind to the stem(s) of one RNA and direct the complementary loops to the correct position for the kissing interaction. During this reaction, the twofold axis of symmetry of the Rom dimer and both the RNAs should form (36, 37, 115). This simple model of Rom reaction with complementary stem-loop configurations of RNA I and RNA II may not explain all results; e.g., Rom causes the greatest increase in the RNA I-RNA II binding when the RNA II transcripts are larger than 135 bases (319) and therefore lack stem-loop III (319, 355), which is complementary to stem-loop I of RNA I. Thus, for the Rom reaction, a requirement for the correct folding of complementary loops may not be necessary in some steric configurations of RNA II. In contrast, other transient configurations of RNA II may act as steric barriers to the formation of the correct Rom-RNA complexes, thereby suppressing RNA I-RNA II pairing.

pSC101

Regardless of which incompatibility group they belong to, enterobacterial plasmids not related to ColE1 share similar structures with respect to the replicon. They have a gene encoding an essential replication initiation protein (*rep* gene), a cluster(s) of direct repeats (iterons), binding sites for the DnaA protein, and A+T-rich sequences (81, 173, 257). One such plasmid is pSC101, originally isolated from *Salmonella panama* and used as a cloning vector in the first recombinant DNA experiment (45, 46). In contrast to the ColE1-type plasmids, pSC101, as well as other plasmids with a *rep* gene (159, 257), does not require Pol I for replication initiation (32).

Structure of the basic replicon. pSC101 is a low-copy-number (oligo-copy-number) plasmid, producing five copies per *E. coli* chromosome (108). Replication of this plasmid, which is 9.26 kb long (21), is unidirectional, starting at a unique origin (32, 359). The basic replicon, which has a maximum size of 1.3 kb, consists of a 250-bp *cis*-acting segment (the origin) and a sequence of about 1 kb encoding the 37.5-kilodalton (kDa) initiation protein RepA (3, 44, 172, 339, 358, 359) (Fig. 3A). The chromosomal replication origin (*oriC*) of *E. coli* and other members of the family *Enterobacteriaceae* show limited but significant homologies to the *cis*-acting fragment of pSC101 (3, 358). The homologous region consists of a 13-bp repeat together with the binding site for the DnaA protein (27, 90) (Fig. 3A). The 13-mer repeat is part of an 82-bp 84% A+T-rich region which carries a binding site for the integration host factor (IHF) (94) (Fig. 3A). Three 18-bp iterons (Fig. 3A, numbers 1, 2, and 3), which act as binding sites for the plasmid-encoded RepA protein (339, 340), are located immediately adjacent to the A+T-rich region (3, 339, 358). Additional binding sites for the RepA protein are found outside the origin in front of the *repA* gene (Fig. 3A, numbers 4, 5′, and 5) (173, 341, 357).

Replication initiation. The replication initiator protein, RepA, functions in replication as a positive factor by binding to the three direct repeats in the origin (340, 341, 357). This binding may be the beginning of a replisome formation (compare the events of replisome formation at *oriC* [27, 93, 258]). To form a correct protein-protein and protein-DNA complex, RepA may interact with host proteins, which are bounded to the origin (e.g., DnaA [90]). Alternatively or in addition, RepA could direct such essential host proteins, which are per se not able to interact with the DNA, to the start site of replication.

For replication initiation, the host DnaA protein is essential not only for the *E. coli* chromosome but also for plasmid pSC101 (27, 74, 87, 90, 109). Specific binding of DnaA to its recognition site (DnaA box: TTATA/CCAA/CA [90]) has been demonstrated for the chromosomal origin and for the origin of pSC101 (90), but the function of DnaA in the replication initiation in both cases is only insufficiently clarified (258, 286). With respect to *oriC*, the DnaA protein seems to be involved with the correct RNA-primer formation by RNA polymerase and/or DnaG primase (187, 239, 263); however, there are no clues about the mode of primer generation and the action of DnaA in the initiation of pSC101 replication.

At *oriC* the binding of DnaA causes structural changes (258) and mediates the formation of DNA-protein complexes with DnaB, DnaC, and other proteins (93). The pSC101 origin already contains naturally bent DNA in the A+T-rich region (286), which may be destined to melt rapidly (343) and to form an anomalous DNA bend (146, 296) owing to its high A+T-content and its poly(dA) · poly(dT) stretches (3, 94, 358). This natural bending in the pSC101 origin is enhanced by binding of the IHF (286), which is an essential host protein in pSC101 replication (94). The natural bending in the pSC101 origin may not span the distance between DnaA and RepA proteins, which are bounded to their recognition sites at the flanks of the A+T-rich region (see schematic model in Fig. 3B). As a consequence of the strong bending by IHF, the binding sites for DnaA and RepA can be brought close together and proper protein contacts between DnaA and RepA may be established (286). A replisome may thus be formed, which consists of a macromolecular complex of DNA and the proteins participating in the replication initiation such as DnaA, the DnaB-DnaC complex, and the RepA protein (286) (see the model in Fig. 3B). This model may be supported by several observations regarding pSC101 replication and/or host proteins: (i) DnaA and the DnaB helicase

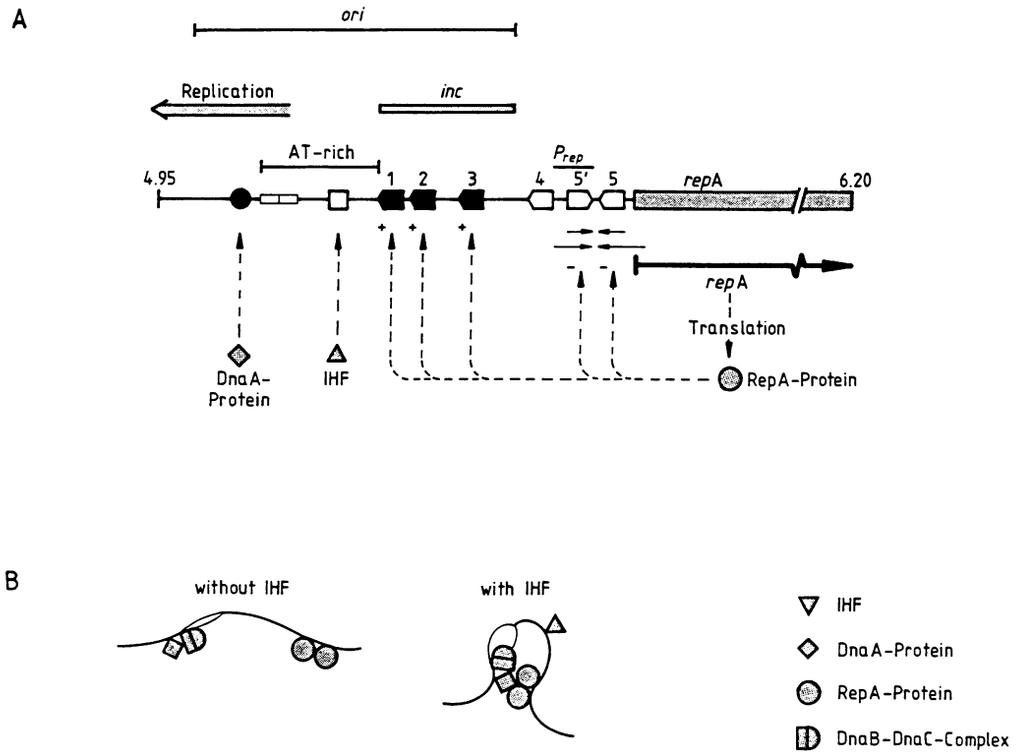


FIG. 3. (A) Schematic illustration of the 1.3-kb basic replicon of plasmid pSC101 (complied from reference 356 with data from references 27, 90, 92, 94, 286, 340, and 341). Structural features of the basic replicon are shown: a DnaA box (●) two 13-mer repeats (▭▭), an IHF-binding site (□), three direct repeats with the origin (◀ numbered 1, 2, and 3), and three similar repeats (▭ numbered 4, 5', and 5) in dyad symmetry in the promoter region (P_{rep}) of the *repA* gene (▨). Numbers at the end of the map refer to coordinates (in kilobases) of the pSC101 standard map (21, 358). Above the map, the extents of the origin, of an A+T-rich sequence, and of a fragment responsible for incompatibility and the direction of replication are shown. Below the map, the small arrows show the extent of sequences of dyad symmetry in the *repA* promoter region (the thick arrow marks the direction of *repA* transcription). The DnaA protein, IHF, and the RepA protein are shown. Broken lines point to the sites of their DNA-protein interaction. + and − mark the positive effect of RepA on replication and its negative effect on expression of the *repA* gene, respectively. (B) Schematic model (compiled from reference 286 with data from reference 27) showing the postulated role of IHF in the DNA bending and replisome formation by proteins encoded by the plasmid (RepA) and the host (e.g., DnaA, DnaB, and DnaC). ▬, DNA double strand; ——, single strands, corresponding to the position of the 13-mer repeats in panel A and the putative site of the initial opening of the double strand. At the left side, the natural bending of the origin is illustrated by the curve. Without binding of IHF, this curving may be not strong enough to bring together DnaA and RepA molecules if they are bound to their recognition sites at the origin. Bending of the origin is strengthened by the binding of IHF to the DNA, as illustrated in the right-hand diagram. In consequence, DnaA and RepA proteins could initially interact with each other, and then the DnaB-DnaC complex (bound to DnaA before or after IHF-DNA interaction) may be directed to the position of the 13-mer repeats for opening the DNA double strand.

seem to interact in the replication initiation of pSC101 (88), (ii) DnaB and DnaC are both essential proteins for the pSC101 replication (109), and (iii) DnaB and DnaC have been shown to operate together (23).

Bends or folds at replication origins and formation of macromolecular protein complexes are associated in other systems (e.g., *oriC*, bacteriophage λ, and plasmid R6K) with an opening of the DNA double strand (27, 60, 70, 200, 367). The three tandem repeats of the 13-mer located in the *oriC* of *E. coli* near a DnaA box are the sites where the opening of the duplex is initiated (27). Starting from the protein-DNA complex organized at the DnaA box, the DnaB helicase probably recognizes the initial single-stranded DNA structure (27) and travels in both directions along the DNA helix, during which time the complementary DNA strands are separated (11). At the pSC101 origin, which has a similar arrangement including the same 13-mer repeat near a DnaA box as in *oriC* (27) (Fig. 3A), the initial opening of the DNA strands may also start at these 13-mer sequences by the DnaB helicase (Fig. 3B) as a prerequisite for primer formation. Apart from the *E. coli oriC* (187, 239, 241, 248), a replication initiation of the pSC101 leading strand mediated by RNA polymerase is unlikely for two reasons: first, a promoter was found only with the opposite orientation to replication, and second, transcription occurs only in the opposite direction to replication (44, 94, 172). The function of this antisense transcription in pSC101 replication is unknown, but it may assist in activation of the replication origin via an opening the DNA double strand (44). Such a process of transcriptional activation is involved in replication initiation of phage λ (330) and probably also of the *E. coli* chromosome (187). In the origin region of pSC101, homologous sequences to the DnaG-binding site of phage G4 are found in both DNA strands (44, 358). Hence, primer formation by DnaG primase, which is an essential protein in pSC101 replication (71), might be needed not only for the lagging strand but also for the leading strand. As yet, the possibility of such a replication initiation by DnaG primase has not been examined for pSC101.

Regulation of initiation by RepA. The RepA protein has a positive role in the initiation of DNA synthesis by binding to the iterons of the origin (341, 357), which is likely to be an

essential primary step for replisome generation. Apart from this, the RepA protein negatively regulates the initiation of pSC101 replication by its intracellular concentration (173, 341, 357). The promoter region (putative promoters [see Fig. 6]) of the *repA* gene is overlapped by a palindromic arrangement of three repeats (341, 357, 358) (Fig. 3, numbers 4, 5, and 5'), which are similar to the sequence of the iterons in the origin (3, 339, 358). By binding to the inverted repeats, RepA competes with RNA polymerase for the *repA* promoter sequence and inhibits the *repA* transcription by autoregulation (173, 341, 357). Since the binding of RepA to the promoter region has priority over binding to the origin (340), the concentration of RepA can be maintained under a critical level when the correct plasmid copy number is reached (341).

Apart from the regulation of the copy number (3), the RepA protein determines the incompatibility between pSC101 and related plasmids by binding to the three iterons of the origin (44, 172, 358). Such an incompatibility mechanism could be explained by a passive adsorption of replication initiator proteins according to the titration model of Tsutsui et al. (331).

REPLICATION OF BROAD-HOST-RANGE PLASMIDS

Some plasmids belonging to the *E. coli* incompatibility groups IncC, IncJ, IncN, IncP, IncQ, and IncW are capable of replication and remain more or less stable in diverse unrelated gram-negative bacteria (152, 230). In particular, the plasmids of the IncP and IncC groups (equivalent to the IncP1 and the IncP4 groups, respectively, in *Pseudomonas* spp.) display a very extensive host range (7, 8, 152, 311), providing that they possess the complete IncP or IncQ replication system in addition to incompatibility determinants (246, 279). As far as is known, only in strains of *Myxoccocus xanthus* (131), *Bradyrhizobium japonicum* (63), and *Bacteroides* spp. (105, 270, 271) are IncP, IncQ, and both groups of plasmids, respectively, incapable of autonomous propagation.

The plasmid-encoded replication functions of IncP and IncQ plasmids and their control are far more complex than those in narrow-host-range plasmids. They also differ in being distributed in several regions of the plasmid genome (9, 152, 230, 249, 300, 311, 364).

IncP Plasmids

Depending on the homology of their replication and transfer functions, IncP plasmids are divided into the two subgroups, IncPα and IncPβ (43, 278, 356). Extensive investigations have been made with the IncPα plasmids RK2 and RP4. These plasmids cannot be distinguished from each other or from RP1, R18, or R68 (31, 51, 287, 338).

Properties of the replicon. RK2 is 60 kb long (161, 224) and has a copy number of 4 to 7 in *E. coli* (76, 102) and 3 in *Pseudomonas aeruginosa* (129). Three major areas participate in replication and initiation regulation. They are distributed in a region of 20 kb on the RK2 genome (16, 309, 312) (Fig. 4) and are separated by a copy of the transposon Tn*1* and by determinants of tellurite and tetracycline resistance (26, 224). One of these three regions contains the replication origin (188, 282); the others, designated *trfA* and *trfB* (*trf* represents *trans*-acting replication and maintenance function [300]), act in *trans* on replication initiation (*trfA*) (75, 231, 307) and its regulation (*trfA* and *trfB*) (255, 298, 307, 365). Replication in various bacteria requires the replication origin (*oriV*) and the *trfA** operon (250, 251), which is derived from the *trfA* operon by deletion of a regulator determinant (*trfA* Δ*kilD* operon [251, 301]; see below). Nevertheless, for stable maintenance of RK2 in *Rhizobium meliloti*, *Agrobacterium tumefaciens*, *Caulobacter crescentus*, *Acinetobacter calcoaceticus*, and *Rhodopseudomonas sphaeroides*, regulator genes are necessary to a certain extent, whereas such genes in *E. coli*, *P. aeruginosa*, *P. putida*, *Azotobacter vinelandii*, and *Alcaligenes eutrophus* are not essential (251, 256).

Origin structure: requirements in different hosts. The θ mode of replication starts in RK2 unidirectionally at *oriV* between the coordinates at kb 12.2 and 13 on the plasmid standard map (82, 188, 305). The molecular organization of RK2 *oriV* shows no fundamental differences from those of many narrow-host-range *E. coli* plasmids (81, 173, 257, 282, 305) (also compare Fig. 3 with Fig. 4, left side). The *oriV* region consists of (i) eight 17-bp iterons in two clusters (of three and five), (ii) a putative promoter surrounded by DnaA-binding sites and a putative IHF-binding site (Table 1), (iii) a 49-bp A+T-rich sequence (74% A+T) with a further DnaA box, and (iv) a 67-bp G+C-rich sequence (79% G+C) (97, 277, 282). An additional copy of the 17-bp iteron overlapping a putative promoter is situated upstream from *oriV* (305), and two degenerated repeats in an inverted orientation are found downstream (311). In the *oriV* region three open reading frames are found (305); two of these are associated with obviously *trans*-acting elements concerned with copy number and incompatibility (Fig. 4, *copA*/*incA* and *copB*/*incB*) (305).

Generally, the same origin fragment is used for replication in different bacteria; however, depending on the host, some fine structures of *oriV* are not as important or can be totally omitted. For replication initiation in *E. coli*, a 393-base-pair *Hpa*II fragment (Fig. 4, *oriV**) carrying the DnaA boxes, five of the iterons, and the A+T- and G+C-rich sequences is sufficient (249, 282, 313). In contrast to the A+T-rich sequence, the G+C-rich area is not needed for replication (50, 152), but by creating secondary intrastrand folding (282) and/or by using poly(dG-dC) stretches (143), it may cause a dissociation of the A+T-rich region, which is possibly bent by dA clusters (146, 282, 367).

Unlike *E. coli*, replication in *Pseudomonas* species requires the presence of the whole *oriV* segment of 617 bp (250), whereas for replication initiation only certain parts of it are absolutely necessary. In *E. coli*, the ability to replicate is lost if the last iteron located in the cluster of five in the wild-type plasmids is destroyed; however, this is not the case in *P. aeruginosa*, *P. putida*, or *P. stutzeri* (152, 153, 205). Minimal replicons consisting of *oriV* and the *trfA* gene with a similar destruction of the fifth iteron or an interruption in the sequence near the fifth repeat are unable to replicate in *P. putida* and show a complete or partial loss of replication ability in *P. aeruginosa* (50). Another host range mutant of the wild-type plasmid which is able to replicate in *E. coli*, *P. aeruginosa*, and *P. putida*, but not in *P. stutzeri*, interferes with the iteron that overlaps the promoter outside of the *oriV* region. It is therefore suggested that a gene product made from this promoter is required by *P. stutzeri* but not by the other bacteria (152, 205). Furthermore, hosts may differ with respect to the involvement of the DnaA protein in replication initiation, because insertions between the DnaA boxes and the cluster of five iterons inactivate the *oriV* in *E. coli* and *P. putida*, but not in *P. aeruginosa* (50, 152). This effect of unnatural spacing may indicate either that there are different specificities of DnaA proteins, which are, as far as is known, highly conservative (216, 272) or that replication does not

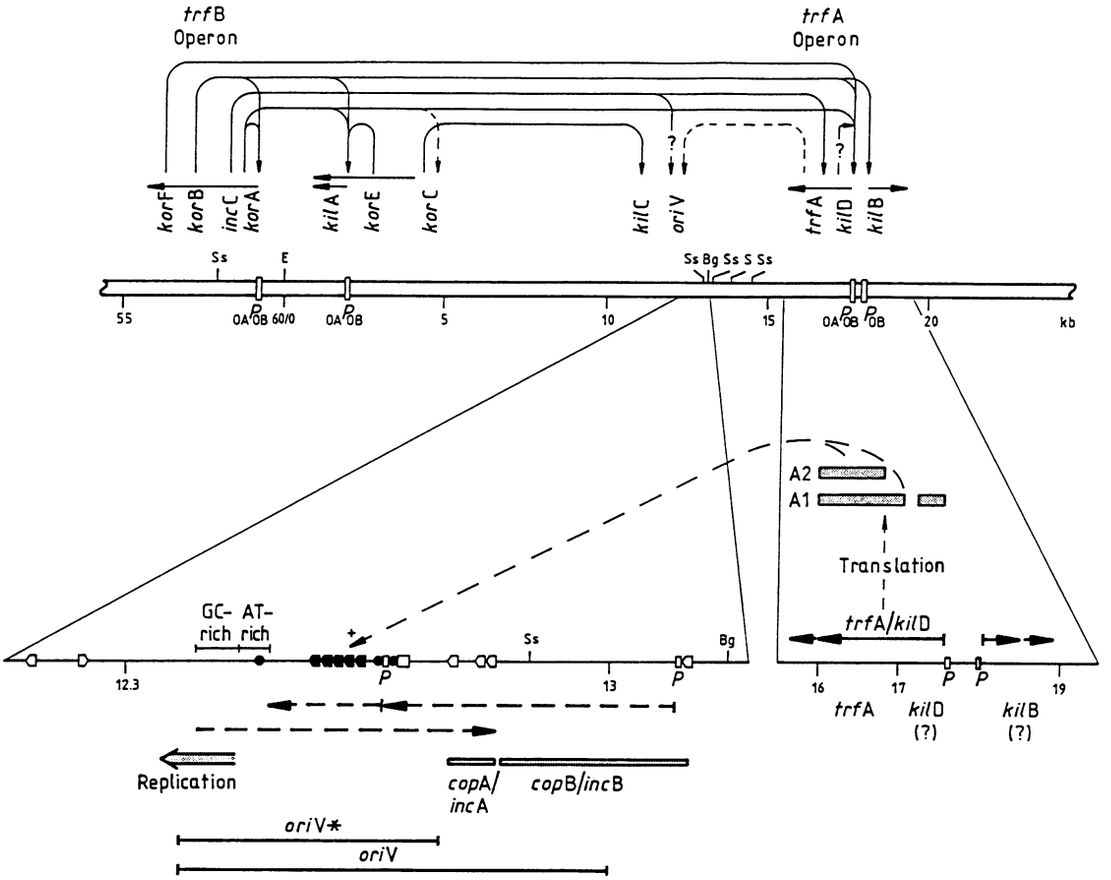


FIG. 4. Regions of broad-host range IncP plasmid RK2 involved in replication initiation and its regulation (compiled from figures and data in references 97, 148, 152, 188, 277, 282, 298, 304–306, 310, and 364; for further information, see the text). At the top of the figure, the loci of the origin and genes involved in the initiation of replication and its regulation are shown according to the standard map (224) (*trf*A and *trf*B, *trans*-acting replication functions; *inc*C, incompatibility determinant; *kil* and *kor* genes, complex gene system of replication control). $_{OA}P_{OB}$ indicate promoters with a KorA operator (OA) and a KorB operator (OB); P_{OB} marks a promoter having only a KorB operator. Horizontal arrows show the direction of gene or operon transcription. The sites of interaction of gene products are indicated by curved arrows. Positive interactions are depicted by dashed lines; negative interactions are depicted by solid lines. Question marks signify proposed interactions of unknown mechanisms. Some significant restriction sites (Bg, *Bgl*II; E, *Eco*RI; S, *Sal*I; Ss, *Sst*II) and the coordinates in kilobases are given as reference points. At the bottom, at the left, a schematic illustration of the *oriV* region is shown. Arrows (◀, ▶) mark direct and inverse repeats of 17 bp, which are the putative binding sites for the *trfA* proteins A1 and A2; ●, DnaA boxes; □, putative IHF-binding site; □ (*P*), putative promoters. In addition, the extension of the minimal origin in most bacteria (*oriV*) and in *E. coli* (*oriV** with five 17-bp repeats), the length of a G+C-rich and an A+T-rich segment, the direction of replication, and the extension of elements involved in copy number control and incompatibility are given. Dashed horizontal arrows show putative transcripts. At the bottom, at the right, the region coding for the replication initiation proteins A1 and A2 is illustrated. Horizontal arrows indicate transcripts with their direction of transcription, and the boxes mark the extension of the putative *kilD* reading frame and of the reading frames for the *trfA* products A1 and A2. The dashed curved arrow represents the positive (+) action of proteins A1 and A2 at the origin.

depend on DnaA in all hosts. It has recently been shown that RK2 replication in *E. coli* is dependent on DnaA in vivo and in vitro as well as that the DnaA-binding site in the A+T-rich region is necessary (97, 228).

Replication initiation proteins of the *trfA* operon. The different requirements of hosts concerning the fine structure of *oriV* and the different effects of the destruction of the fifth iteron in the wild type or in the minimal replicon (showing the dependence of the presence or absence of regulator genes) could be interpreted as an indication that in distinct hosts, different *trfA* gene products are required and/or that a differential amount of the *trfA* gene expression is necessary. Similar conclusions can be drawn from the observation that an interruption of the *trfA* gene and its accompanying promoter region by transposon integration prevents RK2 replication in *E. coli* but not in *Acinetobacter calcoaceticus*, *R. meliloti*, or various *Pseudomonas* species (48, 152, 154). Further support for this conclusion is given by *trfA* mutants, which are temperature sensitive for replication in *E. coli* but not in *P. aeruginosa* or *R. meliloti* (123, 311, 329).

The *trfA* operon codes for three different proteins: one is a regulator determinant called KilD, and the other two are the replication initiation proteins A1 and A2 (Fig. 4). The proteins A1 (43 kDa) and A2 (32 kDa) are products of the same reading frame, but the synthesis of A2 starts 291 bases downstream from the A1 start codon (69, 149, 267, 268, 275). A2 is an essential and efficient protein for RK2 replication initiation in *E. coli* (149, 268) as well as in *P. putida*, *R. meliloti*, *Agrobacterium tumefaciens*, and *Azotobacter vinelandii* (69). In contrast, RK2 replication in *P. aeruginosa* requires the gene for the larger protein, A1 (and probably A2 as well) (69). A1 and A2 possess homologies to double-

TABLE 1. Putative IHF-binding sites in the basic replicons of plasmids coding for a Rep protein[a]

Plasmid[b]	Position[c]	Sequence[d]	Characteristics of surrounding sequences	Reference
pSC101	547–559	**TAA**ccca**TTGAA**aT	Located between a DnaA box, two 13-mer tandem repeats, and RepA-binding sites, surrounded by long poly(T) and poly(A) stretches	94
R6K γ	61–73	**TAA**gttg**cTGATT**	Adjacent to five interons in a 113-bp A+T-rich region (79% A+T)	280
	76–88	**TA**ttaat**TTtATT**		
	262–258	**CAA**cctg**TTGATA**	Flanked directly by five iterons and two putative Dam methylation sites	
P1	557–569	**Tg**Acggg**TTGcTA**	Overlapping the third iteron in a cluster of five between two Dam methylation sites, near two DnaA boxes	2
	432–420	**TA**tgacc**TGATT**		
	1831–1843	**Tc**Accag**TTGATA**	Adjacent to a cluster of nine iterons	
R1	1528–1540	**ac**Acctg**TTtATA**	Adjacent to RepA-binding sites, separated by three TTTAAA repeats in two turns of the helix	178
	1549–1561	**aA**Actac**TTaATT**		
	1558–1570	**aAt**tacaT**TcATT**		
R751	171–183	**TA**tgtcc**TTGATc**	Located between a group of three iterons and a DnaA box	276
RK2	260–272	**aAA**acgc**cTGATT**	Located between a group of three iterons and a DnaA box, flanked by A and T stretches in turn of the helix	281
pBE-2	648–660	**TAA**aacg**TTaAaT**	Adjacent to a DnaA box and a cluster of four iterons of 19 bp, surrounded by three direct 13-bp repeats	Kües et al., in preparation
	663–675	**gAA**ggcg**TTaAaA**		
	672–660	**TAA**cgcc**TTctTA**		

[a] Not complete. In addition, sites had been found by the authors, e.g., in the plasmids, F, P4, pUC1, R485, and Rts1. R751 and RK2 are broad-host-range plasmids (277); pBE-2 is a narrow-host-range plasmid of *Methylomonas clara* (177); all others are *E. coli* plasmids.

[b] Dependence in replication and binding of IHF is shown for pSC101 and R6K γ (78, 94, 286).

[c] Coordinates of nucleotides according to the references.

[d] Boldface letters mark the positions conserved in IHF-binding sites; capital letters show nucleotides which agree with the IHF consensus sequence **YAA----TTGATW** (95, 164).

stranded DNA-binding proteins and may form the secondary structure characteristic of these protein types (275). They are presumably analogous in their function and may bind to the 17-bp iterons in the *oriV* region (152, 278). As yet, there is no experimental evidence for this assumption, although both proteins are detected together with others in replicating DNA-membrane-protein complexes from *E. coli* minicells (148). Analogous to the procedures in the *oriC* of *E. coli* and to the model of pSC101 replication initiation, proteins A1 and A2 bound to the *oriV* region and also *oriV*-linked host proteins (e.g., DnaA [97]) may associate with a protein-DNA complex under deformation of the DNA. If the proteins are correctly assembled and DNA curvature is correct, this complex may act as a replisome. It is not yet known whether the same replisome must form in all hosts. Different replisomes seem more likely because, for example in *P. aeruginosa*, the DnaA protein appears to be superfluous and this bacterium is the only one with a detectable requirement for A1 (see above). Further arguments for variations in the replisome structure are supported by the differences in sequence requirements for *oriV* function in different bacterial species, as discussed above. One might also speculate that in the same host some variations in the replisome, e.g., fluctuations in the use of A1 and A2, may occur, because in *P. aeruginosa* containing only A2 some residual replication takes place (69).

Replication initiation. At present, only limited knowledge relating to the replication initiation process in the RK2 origin and the host enzymes involved is available. In *E. coli* Pol I is not required (300, 309), whereas the DnaA protein, DnaB helicase, DnaG primase, gyrase, and Pol III are (97, 228). This situation is similar to the replication initiation of narrow-host-range plasmids coding for their own initiator protein (for examples, see references 106, 142, 178, 201, 222, and 350 and references therein). Owing to the presence and position of an IHF recognition site in the RK2 *oriV* (Fig. 4; Table 1), participation of the IHF in replication in *E. coli* is suggested; binding of the *E. coli* DnaA protein to the origin has been shown to occur (97).

Both biochemical and ultrastructural evidence from using DNA-membrane complexes isolated from *E. coli* supports the involvement of RNA polymerase in RK2 replication initiation in vitro (82, 83, 148). Since replication also occurs in soluble extracts of *E. coli* under RNA polymerase-inhibiting conditions, a primer formation independent of RNA polymerase is plausible (229, 311). However, this observation cannot exclude the possibility of an RNA polymerase-dependent priming, since, similar to the *E. coli oriC*, alternative pathways via primases or RNA polymerase could exist (187, 372).

In the membrane-dependent in vitro system, in addition to primer formation, the requirement for the RNA polymerase can be explained by transcription and translation of the *trfA* and the *trfB* proteins, which occur at the same time as replication initiation (83, 148). Proteins associated with the DNA-membrane complex and synthesized de novo are the essential Rep proteins, A1 and A2 (69, 148, 149, 267), and the 38- and 30-kDa *trfB* proteins B1 and B2, which are responsible for the *trans* incompatibility effect of the *incC* determinant (20, 148, 189, 303, 310) (Fig. 4, top). Possible functions of B1 and B2 could be (i) a fine regulation of the replication initiation events within the origin, (ii) a transcription or translation modulation of *trfA* operon expression, or (iii) an effect on the stability of *trfA* proteins A1 and A2 (303).

Regulation of replication by the *kil-kor* genes. In addition to the proposed regulation of the *trfA* protein level by the *incC* determinant (303) (see above), the expression of *trfA* operon is negatively influenced by the products of *korA*, *korB*, and *korF* (20, 297, 303, 363, 364), which are located together with *incC* in the *trfB* operon (147, 275, 299, 304, 310).

korA (also called *korD* or *trfB* [268, 298]), *korB*, and *korF* are part of the complex regulation network organizing the adaptation of the RK2 basic replicon to the prevailing host conditions and to autonomous maintenance in the different gram-negative bacteria (251, 255, 308). The regulation network (Fig. 4) consists of various *kil* determinants potentially lethal to the host cells and of *kor* products (*kor* genes are *kil*-override genes) preventing the lethal action of the *kil* determinants (20, 363, 364). Altogether, five *kor* determinants (*korA*, *korB*, *korC*, *korE*, and *korF*) and four *kil* determinants (*kilA* and *kilB* [synonym to *kilB2*] [19, 273]; *kilC*, and *kilD* [synonym to *kilB1*] [233, 274]) are known (304, 364).

One key step of the *kil-kor* regulation is exerted by the 101-amino-acid basic KorA polypeptide (19). KorA has a negative influence on the *trfA-kilD* expression and *kilA* expression and inhibits *trfB* operon expression by autorepression (298, 365). KorA acts positively on the expression of the *korC* product (363), which, in turn, negatively controls the *kilC* gene (77, 363). The second regulator protein, KorB, with a molecular mass of 39 kDa (147, 299) (former estimations were 49 to 52 kDa [20, 276]), represses the *trfA* operon (255, 268, 307), the *trfB* operon, and the expression of *kilB* (77, 232, 268). The *kilA* gene is influenced by KorB only in the presence of KorA (17, 364). KorB can be replaced by KorE in its function as a corepressor of the *kilA* gene (364). KorF has similar effects as KorB on the expression of the *trfA* and *trfB* operons (304). KilD, which is probably encoded by the 116-amino-acid open reading frame of the *trfA* operon (267, 275), can counteract the *korA-korB* repression of the *trfA* operon, although at present this mechanism is not established (255, 298). Such regulation effects have not been reported for other *kil* genes whose molecular mechanism of action(s) are also unknown.

The regulation system, with the multiple repression of the *trfA* operon essential for replication, is significant for the broad-host-range character of RK2. Reductions of the host range or instabilities can be induced by changes in the natural arrangement of the regulator genes or by deletions of singular components (16, 17, 251, 255, 298, 302, 308).

Promoters of genes involved in replication and its regulation. The genes underlying both KorA and KorB repression are characterized by strong homologous promoters with two operatorlike palindromes (19, 275, 298, 365). One palindrome (called OA [Fig. 4]), which overlaps the −10 sequence of the promoters, causes the KorA sensitivity (298, 365); the other (called OB [Fig. 4]) is located near the −35 region and seems to be the KorB target site (273). The putative *kilB* promoter is somehow an exception, since it is regulated only by KorB with an operator which overlaps the −10 region in a palindrome (267, 268, 273).

The promoter structures of the *trfA* operon and of regulator determinants are important not only in gene regulation but also for the replication capability or RK2 in different bacteria. The promoter intensity of the *trfA* operon is quite similar in *E. coli*, *P. aeruginosa*, and *P. putida* (229). It is therefore not unlikely that the promoters of the regulatory genes, which show considerable homology to the *trfA* promoter (see Fig. 6), direct similar levels of transcription in these three and probably other strains, too (229, 230).

IncQ Plasmids

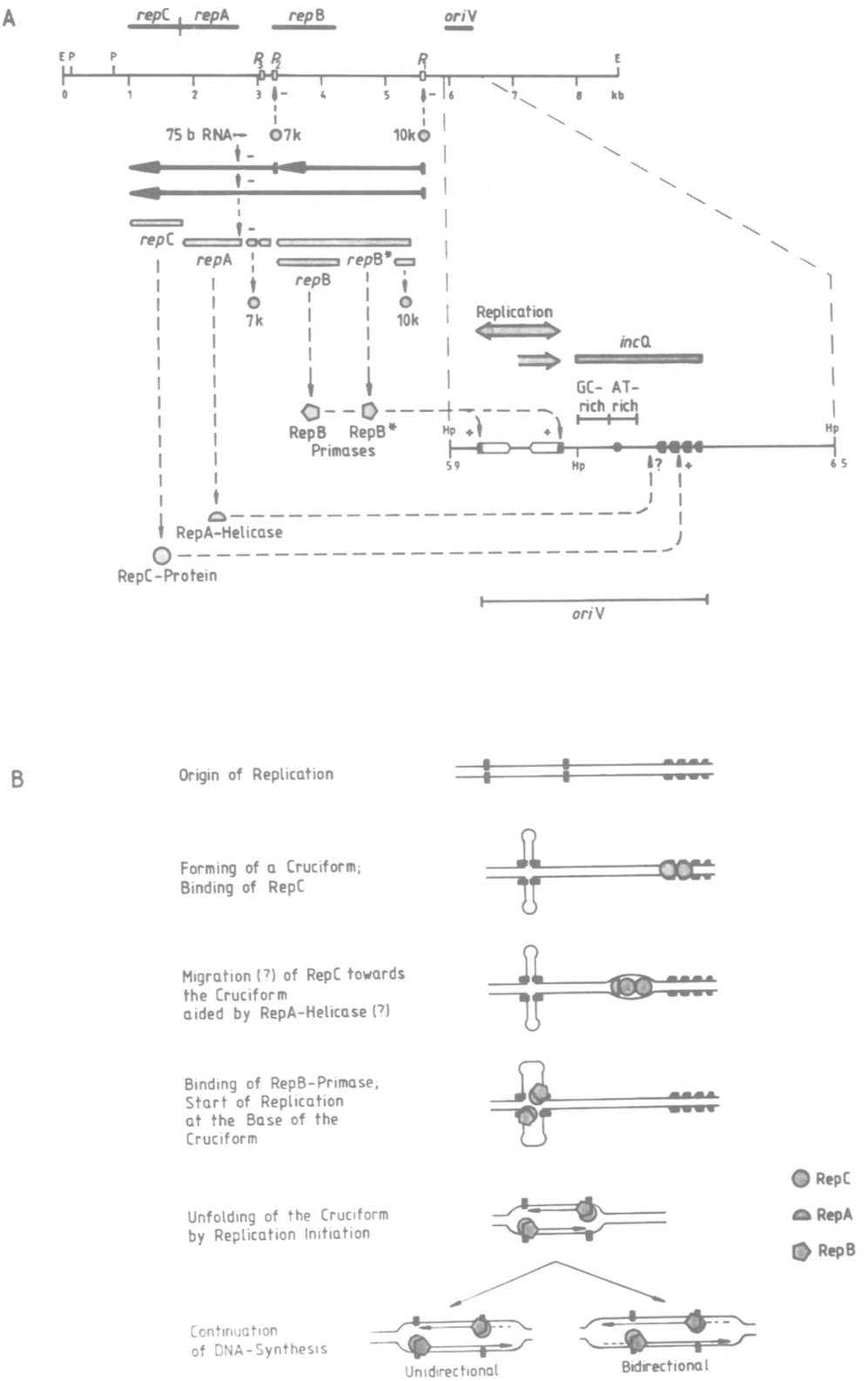
The best-known representatives of IncQ plasmids are the 8.7-kb multicopy plasmids RSF1010, R1162, and R300B, which are obviously identical but were independently isolated from different hosts (18, 103, 104). RSF1010 and R1162 are used mainly for replication studies in vivo and in vitro. These plasmids have a copy number of 10 to 12 per chromosome in *E. coli* and 30 to 60 in *P. aeruginosa* (18, 166); other reports give values of 10 to 50 (103), 9 to 28 (253), and 40 to 60 (190) per *E. coli* chromosome. Replication in *E. coli* starts uni- or bidirectional with the same frequency from an origin 2.9 kb from the unique *Eco*RI site (55) (Fig. 5). In *P. aeruginosa*, replication starts preferentially from the same region (253).

Basic replicon and structure of the origin. The basic replicon of RSF1010 is composed of two *trans*-acting regions and one *cis*-acting region, which are separated by mobilization genes and a transfer origin (28, 191, 244) (Fig. 5). The *cis* sequence (430 bp between positions 5.9 and 6.3 kb [Fig. 5]) containing the origin comprises two regions, of 210 and 220 bp. Their relative orientation and distance can be altered without the loss of replication ability in vivo, but in vitro a reduction of replication has been reported (134, 169). Nevertheless, measurable in vivo instabilities and copy number reduction occur when the two elements are more than 2 kb apart (134, 169).

The larger of the *cis*-acting fragments regulates replication and determines incompatibility (167–169, 192, 226). It is composed of three and one-half direct repeats of 21 bp, a 40-bp A+T-rich (68% A+T) sequence, a 60-bp G+C-rich (68% G+C) sequence (168, 169), and a putative nonfunctional DnaA box within the A+T-rich region (unpublished work of E. Scherzinger, cited in reference 230). The structure of this region is similar to regions in the origin of IncP plasmids (Fig. 5 and Fig. 4, bottom), and there are strong homologies in the iteron sequences of both groups of plasmids (168, 192). For the broad-host-range characteristic, these similarities seem to be insignificant because sequence similarities are also found in the direct repeats in IncP plasmids and in *E. coli* plasmid of six different incompatibility groups (277). In addition, similarities in the iteron sequences could be detected among IncQ and IncP plasmids, the *E. coli* plasmid F, and the *Methylomonas clara* plasmid pBE-2 (U. Kües et al., manuscript in preparation).

The second fragment (210 bp) in the RSF1010 origin has a large inverted repeat which may be joined to make a stem of 46 or 60 bp (including some mispairs) with an intervening loop of 37 bp (167), producing a cruciform structure. The formation of such a cruciform structure may be supported by the presence of dA clusters in-turn to the helix, which are interrupted by GC sequences (an arrangement predetermined for DNA bending [146, 367]) and/or by destabilization of homologous strand pairing through Pur · Pyr stretches (348), both found in the palindromic sequence (170). The inverted repeat and probably its secondary structures are important for replication initiation, because on deletion of one palindrome half, the origin DNA is disabled or only a rest activity of replication remains (170; P. Scholz, Ph.D. thesis, Free University of Berlin, 1985). Replication starts at two points within a conserved 10-bp sequence at the base of the 60-bp stem (170), where two plasmid-specific single-strand DNA initiation signals (designated *ssiA* and *ssiB* or *oriL* and *oriR*) are located in opposing directions (122, 254).

Replication initiation. Replication initiation in vitro and in vivo depends on the three proteins RepA, RepB, and RepC, which are encoded in the two *trans* regions of the basic replicon (56, 136, 249) (Fig. 5). RepC recognizes the origin and positively regulates the replication initiation by binding to the iterons of the larger *cis* region (107, 136). On the basis of sequence homologies between gene 12 of phage P22, the



dnaB gene of *E. coli*, and the *repA* gene of RSF1010 and on the basis of structural similarities between the product of gene 12, the DnaB protein, and the RepA protein, it has been postulated that *repA* codes for a helicase (9; P. Scholz, Ph.D. thesis). Recently, an adenosine triphosphatase activity, an adenosine triphosphate-dependent single-stranded DNA-binding activity, and a helicase activity were demonstrated for purified RepA protein (254). The RepB protein (38 kDa),

like its related larger protein RepB* (70 kDa), has an RSF1010-specific primase activity in vitro (9). If single-stranded DNA is used, primase activity can be observed; in this case, the proteins RepA and RepC are not required (254).

The replication of RSF1010 is dependent on host DNA Pol III and gyrase but is independent of DnaA; the primosome proteins DnaB, DnaC, and DnaG; and RNA polymerase (56, 107, 230, 253). As the primosome proteins functions are taken over by the plasmid proteins, it is assumed that RepA, RepB, and RepC form a primosomelike replisome, which operates in replication initiation (9, 56, 107). As suggested by the model in Fig. 5, after specific binding to the iterons, RepC may be directed to the inverted repeat of the second *cis* domain of the origin (169), perhaps assisted by the action of the RepA helicase. RepB primase could then be added to the protein complex as soon as RepA and RepC reach the palindromic sequence. This protein replication complex might recognize the cruciform structure of the inverted repeats or promote and stabilize its formation. Simultaneously, the replisome could initiate DNA synthesis at both strands at the conserved 10-bp sequence at the base of the 60-bp stem (Fig. 5B). Alternatively, by the action of the protein complex, the two initiation points could be brought together by looping of the intervening 47-bp palindrome before it starts replication at both points by the action of the RepB primase (170). After the initial stages of initiation are mediated by RepB, the decision about uni- or bidirectional replication may occur (170).

In contrast to the idea of initiation by a cooperation between both start points, the function of the single-strand initiation signals of *oriV*, *ssiA*, and *ssiB* have been observed to be independent of each other (122). However, another mechanism of replication initiation is conceivable concerning observations at the *E. coli oriC*, or the origin of pSC101, λ, and R6K γ (27, 59, 60, 70, 199, 258, 286, 367). By this mechanism, the binding of RepC to the one region of the RSF1010 origin may alter the DNA structure of the other *cis* element, offering RepA helicase a chance for entry into the DNA helix. In consequence, double-stranded DNA may be opened and one or both single-stranded initiation signals may be used by the RepB primase for replication initiation.

In all the models, bidirectional replication of IncQ plasmids can be explained simply by the existence of the two inverted initiation points, whereas unidirectional replication may be determined by the relative positions of the two *cis* regions. The priming of DNA synthesis, or chain elongation at the start point nearer to the RepC-binding sites, i.e., *ssiA*, is a prediction of the models (122, 170), consistent with the preferred direction of undirectional replication found in vivo (55, 253). In contrast, it was recently shown by subcloning the initiation points separately in an M13 mutant that *ssiA* is more efficient than *ssiB*, irrespective of its position relative to the RepC-binding site (122).

Expression of the Rep proteins. Both the Rep proteins, which make primer formation independent of host enzymes, and precise regulation of the *rep* gene expression are responsible for the broad-host-range character of IncQ plasmids (9). The RepA and RepC proteins are encoded by the same operon, i.e., one of the *trans* regions, sometimes called *repI*. The *repB* and *repB** genes are localized in another regulator unit, i.e., in the second *trans* region (also called *repII*) (9, 135, 249) (Fig. 5A). Alternatively, starting at the promoter of the *repII* operon (Fig. 5, promoter P_1), all the *rep* genes could be transcribed simultaneously, giving a polycistronic messenger RNA (9).

Each transcription unit is autoregulated by small gene products, whose reading frames are located directly behind their operon promoters and in front of the coding sequences of the *rep* genes (Fig. 5A). The promoter of the *repII* operon, P_1, is probably regulated by a 10-kDa protein. A 7-kDa protein specifically binds to the promoter of the *repI* operon, P_2. The regulation of P_1 seems to influence replication in *P. putida*, whereas P_2 seems to be fundamental for gene regulation in *E. coli* (9).

As well as regulation of the expression of RepA on the transcriptional level, another negative regulation factor is known on the translational level. This is a 75-base RNA which is antisense to the 5′ end of the *repA-repC* transcript and prevents the synthesis of the RepA protein (and probably of RepC) (134). In addition, expression of *repC*, which is rate limiting in replication (107), may be hampered by a thermodynamically stable structure overlapping the ribosome-binding site in front of the *repC* gene (254).

REASONS FOR HOST RANGE SPECIFICATIONS

Plasmid replication in gram-negative bacteria depends on host enzymes and on plasmid-encoded and plasmid-controlled *cis* and *trans* determinants. Some plasmids have determinants that are recognized in almost all gram-negative bacteria and act correctly in each host during replication initiation and regulation. Other plasmids possess this ability only in some bacteria. The reasons for these host-dependent properties of plasmids are at present only partly understood.

Replication Initiation at the Origins

The sequence and structure of plasmid origins and basic replicons and, in addition, some regulatory mechanisms of replication initiation are reasonably well known. However, with the exception of ColE1-type plasmids and, to a certain degree, RFS1010, the sequence of events in replication initiation at the origins is not well understood. Little is known about the role of the host in plasmid replication.

FIG. 5. (A) Basic replicon of broad-host-range IncQ plasmid RSF1010 (compiled from data and figures in references 9, 107, 122, 134, 135, 167, 169, 170, and 249; for further information, see the text). The bold lines above the map mark the extension of the essential *rep* genes (the *repI* and the *repII* operon) and of the origin (*oriV*). P_1, P_2, P_3 and the small boxes designate promoters for the *rep* operons. Horizontal arrows show different transcripts and the direction of their transcription. Symbols: ◀, reading frames of different proteins. The proteins are shown as follows: ● (the 7-kDa repressor, the 10-kDa repressor, and the RepC protein), ◗ (RepA protein), and ◗ (primases). Dashed arrows mark sites of interactions (positive [+], negative [−], or putative [?]). E, Hp, and P (*Eco*RI, *Pst*I, and *Hpa*II, respectively) are restriction sites used as reference points to the map of RSF1010 (161, 170, 254). In the expanded segment, the structural features of the origin are shown: three and one-half repeats of 21 bp (▭), a nonfunctional DnaA box (●), and an inverted repeat of 46 bp (▭) or 60 bp (▭), respectively. The borders correspond to the start sites of replication. Horizontal arrows indicate directions of replication. The extensions of the origin, of the incompatibility determinant, and of a G+C-rich and of an A+T-rich fragment are shown. (B) Model of uni- and bidirectional replication initiation of RSF1010 (by using data from reference 170). The DNA double strand is schematically shown by two lines. Symbols: ▮, 21-bp repeats; ▬, start sites of replication; ●, ◗, ◗, proteins; ◀, leading-strand synthesis; – – –, lagging-strand synthesis (further information can be found in the figure and the text).

For ColE1-type plasmids, there are indications that host range restriction depends on initiation of replication. For example, ColE1 cannot replicate in cell-free *Pseudomonas* extracts, but it shows a partial replication ability when purified *E. coli* gyrase and DNA polymerase I are added. Therefore, in gram-negative bacteria, differences in these enzymes (and possibly other factors) seem to be responsible for the restriction of ColE1 replication to members of the family *Enterobacteriaceae* (56) and, somewhat surprisingly, members of the unrelated family *Legionellaceae* (73). For plasmids such as pSC101, which require the DnaA protein for initiation, the restriction may be due to other host proteins, e.g., DnaB, which cannot be replaced by a DnaB-like helicase from *P. aeruginosa* in the replication of *E. coli* phage ϕX174 in vitro (67).

In contrast, the broad-host-range IncQ plasmid RSF1010 seems to have gained extensive independence from host enzymes; this plasmid carries out the first priming step in the absence of significant host proteins (9). At present, there is no evidence for similar host independence for primer formation at the origins of the broad-host-range IncP plasmids. Indeed, RK2 codes for its own primase, which can act during vegetative replication (160, 162); however, the encoding gene is located outside the region that is essential for vegetative broad-host-range replication (161); the primase is required for conjugative plasmid transfer (152, 186, 206).

The basis for the broad-host-range replication initiation of RK2 is not clear, because the structure of the minimal replicon does not differ significantly from that of the enterobacterial plasmid pSC101 and those of other narrow-host-range *E. coli* plasmids. In addition, with regard to host range, it should be mentioned that only a few plasmid-encoded proteins are functionally characterized: (i) the RSF1010 Rep proteins (see above); (ii) the initiation protein O of phage λ and the π protein of the *E. coli* plasmid R6K, which, by binding to their corresponding origins, induce conformational alterations and helix destabilization (199, 200, 252, 366, 367); and (iii) protein P of phage λ, which, in association with protein O, directs the assembly of host proteins such as DnaB and DnaG to the origin and leads to the formation of a replisome (60, 89, 351). It is not known whether the function(s) of the *trfA* proteins A1 and A2 at the origin of RK2 is comparable to that of the initiator proteins of narrow-host-range plasmids, but this can be assumed. However, with two types of initiator proteins, RK2 may have a more extended flexibility for adaptation to different hosts. Depending on the specificities of the host enzymes, both proteins may alternatively act in the same way as an inductor and/or a part of the replisome (69, 267). Using the strategy of two protein types, a better adaptability to different hosts may also be gained for RK2 replication by the two IncC proteins, the putative replication fine regulators B1 and B2 (303), and for RSF1010 by the two different RepB primases (9). Even though the RSF1010-encoded Rep proteins seem to be sufficient for the replication initiation, some unknown host factors, e.g., for modifying the origin DNA structure, may exist which, depending on the host, influence the use of the two RepB species.

Factors involved in replication initiation in *E. coli* which strengthen DNA curvatures (30, 57, 234, 243, 286) and destabilize the helix (72, 237) could be DNA-binding histone-like proteins such as IHF (27, 94) and protein HU (27, 58, 217) or methylases such as Dam (1, 244, 360). HU and most of the histonelike proteins interact nonspecifically with DNA (68). Specific recognition sites for IHF (YAA – – – – TTGATW [95, 164]), as for Dam (GATC [110]), are found in different amounts among *E. coli* plasmids and broad-host-range plasmids (Table 1). Since these factors do not generally modify the DNA conformation (57, 95), the necessity of the recognition sites for IHF and Dam and their function in changing the DNA structure remain to be determined for most of the origins. However, one should except IHF also to be involved in replication initiation of plasmids other than pSC101 (94, 286) and R6K (78), e.g., because the putative IHF-binding sites are located (often in clusters) adjacent to sequences important for replication (e.g., DnaA boxes, Dam methylation sites, binding sites for Rep proteins, and tandem repeats in A+T-rich sequences). In addition, the IHF-binding sites are often found in or near sequences prone to natural bending [e.g., by poly(dA) stretches in-turn to the helix]. Similar sequences to the *E. coli* IHF-binding sites are located adjacent to a DnaA box and a group of iterons in the origin fragment of the *Methylomonas clara* narrow-host-range plasmid pBE-2 (Table 2). At present, nothing is known about the involvement of factors such as IHF and other host proteins such as DnaA in replisome formation and replication initiation of pBE-2 in *Methylomonas clara*. Differences in the structure or the use of those proteins in *E. coli* and *Methylomonas clara* could disturb replisome formation at the pBE-2 origin in *E. coli* and may account for the inability of pBE-2 to replicate in *E. coli* (177).

In general, the ability to form a replisome is an important step for the replication capacity of a plasmid in a bacterial host. In the precise recognition of a replication origin and the first steps of replisome formation, at least five elements are involved (compare the synopsis in reference 68): (i) the nucleotide sequence of the origin itself, (ii) proteins recognizing specific sites within this sequence (e.g., DnaA, replication initiation proteins such as pSC101 RepA, RK2 A1 and A2, and RSF1010 RepC), (iii) proteins modifying the DNA structure of the origin (IHF, HU, and Dam), (iv) DNA supercoiling (27, 91, 92, 252), and (v) bends in the DNA. Further proteins, e.g., helicases and primases, should bind to the prereplisome formed in the first steps, and if the macromolecular DNA-protein complex is correctly formed, replication should begin. Since a lot of factors participate in correct prereplisome and replisome composition, it could be assumed that essential factors are missing or mistakes in their action and interaction occur if plasmids invade bacteria beyond their usual host range.

In this context, the interesting observation should be mentioned that the π protein of the narrow-host-range multicopy IncX plasmid R6K of *E. coli* (13 to 38 copies per cell [145]) can complement *repA* gene-deficient derivatives of the broad-host-range low-copy-number IncW plasmid pSa (two or three copies per cell [292]) in *E. coli* (293). The initiator proteins of both plasmids seem to recognize the same palindromic arrangement of binding sites in the pSa origin (293). Since the R6K π protein interacts well with the heterologous origin of pSa, one wonders why these plasmids are so different in their host ranges. For the variation found in the host range extension of R6K and pSa, three explanations (not mutually exclusive) are presented, as follows. (i) R6K has three different origins (49), all dependent on the π protein (81). Origins α and β are preferentially used in vivo with approximately equal frequencies (49), but the activities of both depend on the existence of the γ origin in *cis* (81). Probably by binding to two different origins, dimers of the π protein mediate a tertiary interaction between them as shown in vitro for the γ and β origins (199). Hence, a replisome formed at R6K origins may have a more complex structure and may be more susceptible to incorrect protein-

TABLE 2. Codon usage of genes coding for replication initiation proteins and comparison of the average usage of *E. coli* and *P. aeruginosa*[a]

Amino acid	Codon	Avg usage[b]		RK2 *trfA*[c]		RSF1010 *repC*[c]	R401 *repA*[c]	pSC101 *repA*[c]	Amino acid	Codon	Avg usage[b]		RK2 *trfA*[c]		RSF1010 *repC*[c]	R401 *repA*[c]	pSC101 *repA*[c]
		E. coli	*P. aeruginosa*	A1	A2						*E. coli*	*P. aeruginosa*	A1	A2			
Phe	UUU	10	1	7.9	3.5	3.5	34.7	22.2	Tyr	UAU	7	5	5.2	7.0	7.1	20.8	25.3
Phe	UUC	20	31	31.4	38.9	21.3	20.8	28.5	Tyr	UAC	15	29	20.9	24.6	17.7	17.4	19.0
Leu	UUA	5	1	0	0	0	17.4	38.0									
Leu	UUG	6	8	13.1	14.0	7.1	6.9	19.0									
Leu	CUU	5	2	5.2	7.0	10.6	27.8	15.8	His	CAU	7	6	2.6	3.5	14.2	10.4	12.7
Leu	CUC	5	23	10.5	10.5	14.2	6.9	15.8	His	CAC	13	20	18.3	24.6	17.7	3.5	6.3
Leu	CUA	**1**	1	2.6	3.5	**7.1**	**20.8**	**28.5**	Gln	CAA	9	6	10.5	3.5	14.2	24.3	25.3
Leu	CUG	62	61	52.3	59.6	63.8	27.8	9.5	Gln	CAG	31	37	36.6	38.6	28.4	6.9	15.8
Ile	AUU	17	2	5.2	7.0	0	17.4	28.5	Asn	AAU	6	3	7.9	7.0	7.1	17.4	28.5
Ile	AUC	41	39	31.4	28.1	31.9	41.7	9.5	Asn	AAC	31	36	10.5	14.0	14.2	10.4	41.1
Ile	AUA	**0**	0	2.6	3.5	3.5	**24.3**	**22.2**	Lys	AAA	52	4	18.3	24.6	3.5	66.0	69.6
Met	AUG	23	21	28.8	24.6	10.6	17.4	34.8	Lys	AAG	20	35	52.3	59.6	53.2	31.3	25.3
Val	GUU	32	3	2.6	3.5	0	24.3	25.3	Asp	GAU	21	8	15.7	14.0	14.2	38.2	28.5
Val	GUC	9	33	31.4	31.6	24.8	6.9	0	Asp	GAC	31	52	44.5	45.6	35.5	24.3	9.5
Val	GUA	22	5	0	0	10.6	3.5	9.5	Glu	GAA	50	23	34.0	31.6	17.7	52.1	53.8
Val	GUG	18	30	26.2	28.1	35.5	20.8	9.5	Glu	GAG	18	37	44.5	45.6	39.0	17.4	31.6
Ser	UCU	17	1	2.6	3.5	0	27.8	9.5	Cys	UGU	3	1	0	0	7.1	3.5	3.2
Ser	UCC	14	16	15.7	14.0	3.5	0	3.2	Cys	UGC	4	13	15.7	21.1	7.1	0	3.2
Ser	UCA	3	1	0	0	0	17.4	9.5									
Ser	UCG	**3**	16	**28.8**	**24.6**	3.5	**10.4**	6.3	Trp	UGG	7	12	15.7	21.1	17.7	6.9	12.7
Pro	CCU	3	1	5.2	3.5	10.6	10.4	9.5	Arg	CGU	38	6	15.7	7.0	3.5	17.4	0
Pro	CCC	**2**	16	**10.5**	0	**21.3**	3.5	3.2	Arg	CGC	19	42	39.3	45.6	28.4	10.4	6.3
Pro	CCA	7	1	5.2	3.5	7.1	17.4	6.3	Arg	CGA	**1**	2	**13.1**	**14.0**	**10.6**	**13.9**	**6.3**
Pro	CCG	25	30	18.3	24.6	24.8	6.9	3.2	Arg	CGG	**1**	9	**15.7**	**14.0**	**42.6**	0	3.2
Thr	ACU	20	2	2.6	0	0	17.4	19.0	Ser	AGU	4	2	0	0	3.5	20.8	22.2
Thr	ACC	25	39	34.0	31.6	14.2	10.4	19.0	Ser	AGC	10	24	13.1	10.5	24.8	10.4	9.5
Thr	ACA	4	1	0	0	0	10.4	22.2	Arg	AGA	**1**	1	0	0	**7.1**	**6.9**	**12.7**
Thr	ACG	5	6	39.9	52.6	7.1	3.5	19.0	Arg	AGG	**0**	2	2.6	0	0	**10.4**	**19.0**
Ala	GCU	46	8	5.2	3.5	21.3	6.9	15.8	Gly	GGU	41	10	2.6	0	14.2	13.9	6.3
Ala	GCC	14	62	44.5	35.1	31.9	17.4	3.2	Gly	GGC	31	71	31.4	38.6	63.8	17.4	6.3
Ala	GCA	28	3	10.5	3.5	14.2	24.3	19.0	Gly	GGA	**3**	3	5.2	3.5	7.1	3.5	6.3
Ala	GCG	28	32	28.8	24.6	56.7	10.4	3.2	Gly	GGG	**4**	5	5.2	3.5	**21.3**	**10.4**	0

[a] Numbers refer to the number of times a specific codon occurs per 1,000 codons.
[b] Data for *E. coli* and *P. aeruginosa* were obtained from references 337 and 349; bold numbers indicate rare codons in *E. coli* (264), and underlined numbers indicate rare codons in *P. aeruginosa* (349).
[c] Data for *rep* genes were determined from sequences in references 254, 275, 291, and 339; bold numbers indicate codons which are highly used in a *rep* gene but are rare in *E. coli*, and underlined numbers indicate codons which are often present in *rep* genes but are rare in *P. aeruginosa*.

DNA interactions than a replisome formed at the relatively simple pSa origin; pSa has 3 13-bp repeats in its minimal replicon, whereas the R6K γ origin exclusively has 14 of these 13-bp repeats (293) or, including this 13-mer, eight plus two half copies of a 22-bp repeat (281). These additional sequences of the R6K γ origin, which are strong binding sites for the π protein (81, 99), may frequently sequester the R6K origins in nonproductive DNA-protein-DNA complexes. This may be a normal mechanism for copy number control of plasmids (304), but in nonpermissive hosts it could suppress replication initiation totally. (ii) Compared with those of R6K, RepA and/or the origin of pSa may have a more extended flexibility concerning the affinity of replisome-involved host enzymes of different bacteria. (iii) In contrast to pSa RepA, the expression of R6K π protein may be deregulated or may not occur in hosts unrelated to *E. coli* (see below).

Replication Control

It is important for replication control systems to maintain the frequency of initiation at a relatively low level, because overreplication or runaway replication of plasmids will kill the bacterial host (100, 197, 198, 333, 336). It has also been shown that overproduction of initiator proteins can reduce or inhibit plasmid replication (41, 80, 81). Failure of replication initiation control determinants may be another explanation for the inability of plasmids to replicate in all bacteria.

Expression of replication initiation proteins. In plasmid P1, which has a copy number equal to that of the *E. coli* chromosome (233), 20 RepA dimers are found per plasmid copy in vivo (290); for the F factor, having a copy number of 1 or 2, the upper limit of the concentration of the RepE protein is estimated to be 100 ± 50 molecules per cell (139). The π protein of R6K is estimated to be present at between

3,500 and 10,000 dimers per cell (80), i.e., about 90 to 770 dimers per plasmid copy, which might be an overestimation (225). The intracellular concentrations of Rep proteins for other plasmids are not as yet known, but it is generally accepted that initiation proteins are present in the cell in small and replication rate-limiting amounts.

Most replication control systems are closely connected with the transcriptional regulation of expression of plasmid *rep* genes either by autoregulation, as in the case of pSC101 (see above) and F, P1, or R6K (22, 41, 79, 133, 181, 240, 315), or by the action of (small) repressors, as in the case of IncP and IncQ plasmids (see above) and also IncFII plasmids (61, 212). In bacteria that are not hosts of a particular plasmid, an inadequate masking of promoters by (auto)repressors for RNA polymerases may be one reason for the incorrect amount of *rep* genes being transcribed. Indeed, a promoter itself may not be recognized by RNA polymerases per se in different species (229). The promoter of the RK2 *trfA* operon, postulated to be a broad-host-range promoter, functions efficiently in diverse species (229). The same is proposed for other RK2 and RSF1010 promoters (229). If one compares these "broad host-range promoters" with those involved in the replication of narrow-host-range plasmids of *E. coli* (such as ColE1 or pSC101), no striking differences can be found with respect to the homology of the promoter consensus sequences in *E. coli* and *P. putida* (Fig. 6). It is therefore doubtful whether the proposed broad-host-range property is really specific for the RK2 and RSF1010 promoters. Nevertheless, these broad-host-range promoters may have a better buffer capacity in the correct regulation of gene expression than those of narrow-host-range plasmids. This buffer capacity may be improved by the complex system of transcription regulator genes, as in RK2 (see above) and other IncP plasmids (311) and probably also in the IncN plasmid pKM101 (R46) (352). In contrast, for RSF1010, a buffer capacity could exist through the possibility of two different transcription starts for the *rep* genes, which are preferentially selected in different organisms (9).

In the posttranscriptional stage, the intracellular concentration of Rep protein could be regulated by the messenger RNA (mRNA) half-life (29) or by protein stability (101). Both may vary among different bacteria and may be decisive for the ability of a plasmid to replicate. However, the amount of translation of a *rep* mRNA depends not only on its relative stability, but also on the further transcript properties; e.g., the distance of the Shine-Dalgarno sequence from the start codon causes impaired translation if it is shorter than 5 or longer than 9 nucleotides (151). In contrast, the homology between a *rep* transcript and the 16S ribosomal RNA (rRNA) may have a minor influence on the translation efficiency in *E. coli* as long as three bases of the favored CUCC sequence of the 16S rRNA are present (151). According to an analysis of the Shine-Dalgarno sequences based on spacer length and homology to the 16S rRNA (Fig. 7), the translation of *rep* transcripts may be poor in *E. coli* (and perhaps in other bacteria), which is consistent with a low and rate-limiting intracellular level of replication initiation proteins. An exception seems to be *repC* of RSF1010, which should be highly expressed according to the criteria stipulated in Fig. 7, but a thermodynamic stable secondary structure may mask the Shine-Dalgarno sequence in the *repC* mRNA (254), and this would negatively interfere with its translation (151). In contrast, regulator proteins or nonlimiting products of *rep* genes such as RepA of RSF1010 could be highly expressed (Fig. 7).

Translation could also be influenced by the start codon of

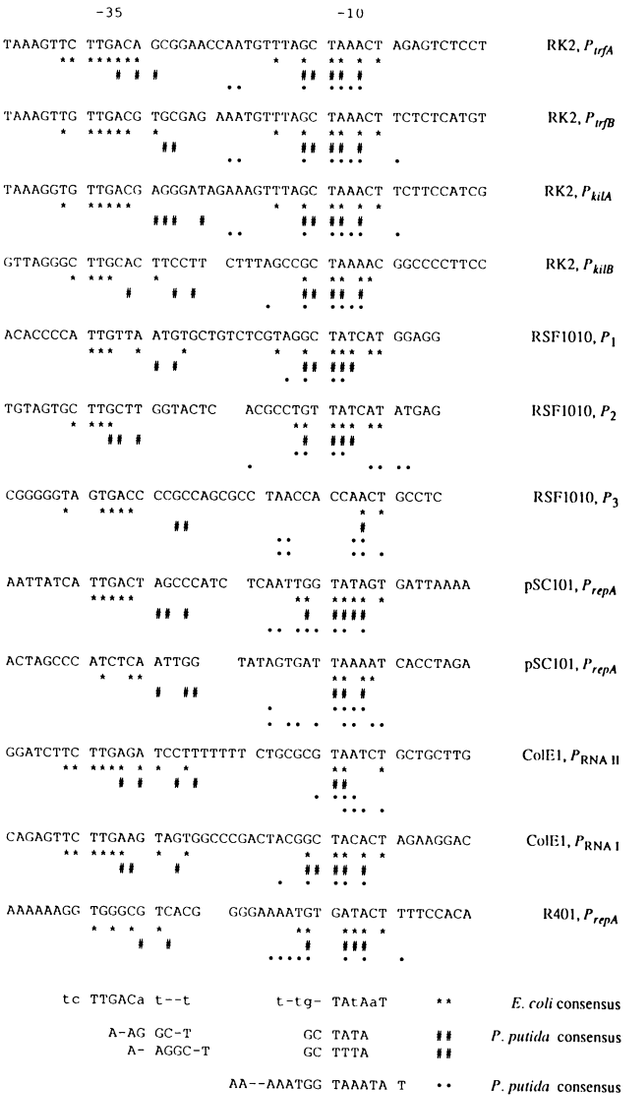


FIG. 6. Homologies of broad-host-range promoters (224, 225) and of (putative) promoters of some *E. coli* plasmids (44, 230, 259, 291, 357) to the proposed promoter consensus sequences of *E. coli* (111) and *P. putida* (124, 185). Symbols: ★, homologous nucleotides to the *E. coli* consensus sequence; #, •, homologous nucleotides to the different *P. putida* consensus sequences.

the gene. Some *rep* genes start with GUG (RepC of RSF1010 [254], RepA1 of NR1 [354] and R100 [242], RepA1 of P307 [245], and RepA of ColV2-K94 [347]) or UUG (RepA of Rts1 [132]), which both have a lower translation efficiency than the most common start codon, AUG (174, 236).

Another possibility of posttranscriptional regulation could be mediated by codon usage. Codons used in the *rep* genes of *E. coli* narrow-host-range plasmids, in contrast to those of the broad-host-range plasmids, are mostly not common in *P. aeruginosa* (Table 2). The influence on the translational rate in *P. aeruginosa* is unknown, since, e.g., the tRNA content of *P. aeruginosa* has yet not been determined (349). Rare codons of *E. coli* interfering with a minor tRNA level, which are found more frequently in genes having a low expression level rather than in those having a high expression level (264), are found to a high degree in genes of Rep proteins

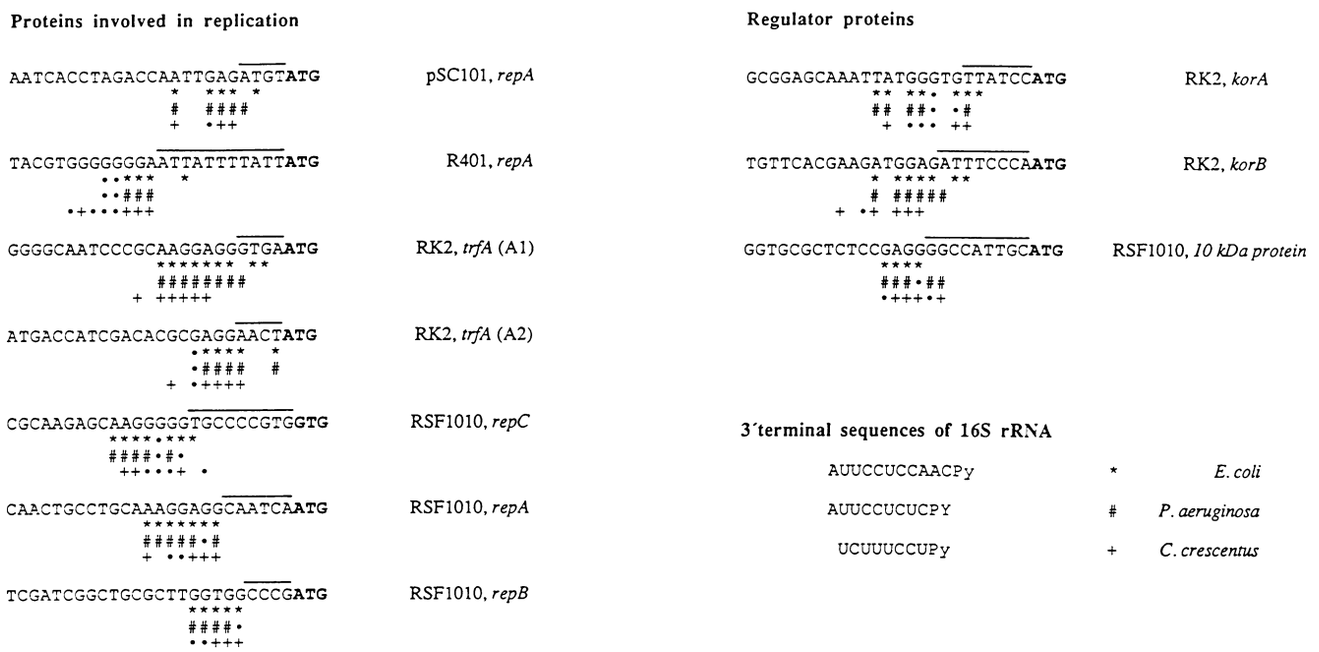


FIG. 7. Ribosome-binding sequences of different genes coding for proteins involved in replication of plasmids or its regulation. Sequences are from references 9, 19, 135, 254, 275, 291, 310, and 357. The signs +, #, and ★ show nucleotides complementary to the 3'-terminal sequences of 16S rRNAs of *E. coli* (+), *P. aeruginosa* (#), and *C. crescentus* (★) (266). The point (•) marks G · U pairing. The spacer length between Shine-Dalgarno sequences and start codons (boldface letters) is marked by a horizontal line above the sequence.

(Table 2). For example, the rare codon, AGG, which may negatively influence translation in *E. coli* (25, 238), occurs at an unusually high level in the *rep* genes of pSC101 or R401 (Table 2). However, there is no clear opinion for or against a regulation of gene expression by codon usage (see, e.g., references 25, 121, 238, and 265). On the basis of high variations in the G+C content among gram-negative bacteria (213), it is an obvious suggestion that codon usage in *rep* genes can influence the maintenance of plasmids in bacteria.

However, pre- and posttranscriptional regulation mechanisms of Rep proteins are necessary to maintain a low-level translation of replication initiation genes. There are various strategies which stringently control the expression of *rep* genes in *E. coli*. In addition to those mentioned above, they include transcriptional pausing (62, 354), a dependence of a minor class of RNA polymerase involving the σ32-factor (344, 345), or a counteraction of gene translation to an RNA-RNA annealing (227, 346). It may be possible to resolve some aspects of host control in plasmid replication if it is clarified whether and at what stage differences in pre- and posttranslational regulation occur in specific hosts.

CONCLUDING REMARKS

At present, the knowledge concerning events in replication initiation of plasmids in gram-negative bacteria and reasons for variation in host ranges is very incomplete. The examples discussed in this review (ColE1, pSC101, RK2, and RSF1010) account for most of the progress that has been made in understanding replication and its control. It can be assumed that more than one of the reasons discussed above or factors which are at present undiscovered could be responsible for the propagation of plasmids in gram-negative bacteria. The ability to replicate in a specific host may depend on the stage of initiation and/or regulation of plasmid replication. Further investigations of replication initiation of narrow- and broad-host-range plasmids and their regulation mechanism in different bacteria should bring further clarification. To examine the relationship between plasmid replication and host range, replication initiation of narrow-host-range plasmids in hosts unrelated to *E. coli* (e.g., *Methylomonas clara* [U. Kües et al., submitted]) should help to further clarify the situation.

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LITERATURE CITED

1. **Abeles, A. L., and S. J. Austin.** 1987. P1 plasmid replication requires methylated DNA. EMBO J. **6:**3185–3189.
2. **Abeles, A. L., K. M. Snyder, and D. K. Chattoraj.** 1984. P1 plasmid replication: replicon structure. J. Mol. Biol. **173:** 307–324.
3. **Armstrong, K. A., R. Acosta, E. Ledner, Y. Machida, M. Pancetto, M. McCormick, H. Ohtsubo, and E. Ohtsubo.** 1984. A 37×10^3 molecular weight plasmid-encoded protein is required for replication and copy number control in the plasmid pSC101 and its temperature-sensitive derivative pHS1. J. Mol. Biol. **175:**331–347.
4. **Ataai, M. M., and M. L. Shuler.** 1986. Mathematical model for the control of ColE1 type plasmid replication. Plasmid **16:** 204–212.
5. **Austin, S. J.** 1988. Plasmid partition. Plasmid **20:**1–9.
6. **Backman, K., M. Betlach, H. W. Boyer, and S. Yanofsky.** 1978. Genetic and physical studies on the replication of ColE1-type plasmids. Cold Spring Harbor Symp. Quant. Biol. **43:**69–76.
7. **Bagdasarian, M., R. Lurz, B. Rückert, F. C. H. Franklin, M. M. Bagdasarian, J. Frey, and K. N. Timmis.** 1981. Specific-purpose plasmid cloning vectors. II. Broad host range, high copy number, RSF1010-derived vectors, and a host-vector system for gene cloning in *Pseudomonas*. Gene **16:**237–247.

8. **Bagdasarian, M., and K. N. Timmis.** 1982. Host:vector systems for gene cloning in *Pseudomonas*. Curr. Top. Microbiol. Immunol. **96**:47–67.
9. **Bagdasarian, M. M., P. Scholz, J. Frey, and M. Bagdasarian.** 1986. Regulation of the *rep* operon expression in the broad host range. Banbury Rep. **24**:209–224.
10. **Bainbridge, B. W., and M. A. Typas.** 1984. Miscellaneous bacteria, p. 93–114. *In* C. Ball (ed.), Genetics and breeding of industrial microorganisms. CRC Press, Inc., Boca Raton, Fla.
11. **Baker, T. A., B. E. Funnell, and A. Kornberg.** 1987. Helicase action of dnaB protein during replication from the *Escherichia coli* chromosomal origin *in vitro*. J. Biol. Chem. **262**:6877–6885.
12. **Baker, T. A., K. Sekimizu, B. E. Funnell, and A. Kornberg.** 1986. Extensive unwinding of the plasmid template during staged enzymatic initiation of DNA replication from the origin of the *Escherichia coli* chromosome. Cell **45**:53–64.
13. **Balbas, P., X. Soberon, E. Merino, M. Zurita, H. Lomeli, F. Valle, N. Flores, and F. Bolivar.** 1986. Plasmid vector pBR322 and its special-purpose derivatives—a review. Gene **50**:3–40.
14. **Banner, D. W., M. Kokkinidis, and D. Tsernoglou.** 1987. Structure of the ColE1 Rop protein at 1.7 Å resolution. J. Mol. Biol. **196**:657–675.
15. **Barbour, A. G., and C. F. Garon.** 1987. Linear plasmids of the bacterium *Borrelia burgdorferi* have covalently closed ends. Science **237**:409–411.
16. **Barth, P. T.** 1979. RP4 and R300B as wide host-range plasmid cloning vehicles, p. 399–410. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
17. **Barth, P. T., K. Ellis, D. H. Bechhofer, and D. H. Figurski.** 1984. Involvement of *kil* and *kor* genes in the phenotype of a host range mutant of RP4. Mol. Gen. Genet. **197**:236–243.
18. **Barth, P. T., and N. J. Grinter.** 1974. Comparison of the deoxyribonucleic acid molecular weights and homologies of the plasmids conferring linked resistance to streptomycin and sulfonamides. J. Bacteriol. **120**:618–630.
19. **Bechhofer, D. H., and D. H. Figurski.** 1983. Map location and nucleotide sequence of *korA*, a key regulatory gene of promiscuous plasmid RK2. Nucleic Acids Res. **11**:7453–7469.
20. **Bechhofer, D. H., J. A. Kornacki, W. Firshein, and D. H. Figurski.** 1986. Gene control in broad host range plasmid RK2: expression, polypeptide product, and multiple regulatory functions of *korB*. Proc. Natl. Acad. Sci. USA **83**:394–398.
21. **Bernardi, A., and F. Bernardi.** 1984. Complete sequence of pSC101. Nucleic Acids Res. **12**:9415–9426.
22. **Bex, F., P. Pierard, A. Desmyter, P. Dreze, M. Colet, and M. Couturier.** 1986. Mini-F E protein: the carboxy-terminal end is essential for E gene repression and mini-F copy number control. J. Mol. Biol. **189**:293–303.
23. **Biswas, S. B., and E. E. Biswas.** 1987. Regulation of *dnaB* function in DNA replication in *Escherichia coli* by *dnaC* and λP gene products. J. Biol. Chem. **262**:7831–7838.
24. **Böldicke, T. W., G. Hillenbrand, E. Lanka, and W. L. Staudenbauer.** 1981. Rifampicin-resistant initiation of DNA synthesis on the isolated strands of ColE plasmid DNA. Nucleic Acids Res. **9**:5215–5231.
25. **Bonekamp, F., and K. J. Jensen.** 1988. The AGG codon is translated slowly in *E. coli* even at very low expression levels. Nucleic Acids Res. **16**:3013–3024.
26. **Bradley, D. E., and D. E. Taylor.** 1987. Transposition from RP4 to other replicons of a tellurite-resistance determinant not normally expressed by IncPα plasmids. FEMS Microbiol. Lett. **41**:237–240.
27. **Bramhill, D., and A. Kornberg.** 1988. Duplex opening by dnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell **52**:743–755.
28. **Brasch, M. A., and R. J. Meyer.** 1986. Genetic organization of plasmid R1162 DNA involved in conjugative mobilization. J. Bacteriol. **167**:703–710.
29. **Brawerman, G.** 1987. Determinants of messenger stability. Cell **48**:5–6.
30. **Broyles, S., and D. E. Pettijohn.** 1986. Interaction of the *E. coli* HU protein with DNA: evidence for formation of nucleosome-like structures with altered DNA helical pitch. J. Mol. Biol. **187**:47–60.
31. **Burkardt, H. J., G. Riess, and A. Pühler.** 1979. Relationship of group P1 plasmids revealed by heteroduplex experiments: RP1, RP4, R68, and RK2 are identical. J. Gen. Microbiol. **114**:341–348.
32. **Cabello, F., K. Timmis, and S. N. Cohen.** 1976. Replication control in a composite plasmid constructed by *in vitro* linkage of two distinct replicons. Nature (London) **259**:285–290.
33. **Campbell, A.** 1981. Evolutionary significance of accessory DNA elements in bacteria. Annu. Rev. Microbiol. **35**:55–83.
34. **Castagnoli, L.** 1987. Characterization of a promoter up mutation in the −35 region of the promoter of the primer for ColE1 replication. Mol. Gen. Genet. **206**:178–180.
35. **Castagnoli, L., R. M. Lacatena, and G. Cesareni.** 1985. Analysis of dominant copy number mutants of the plasmid pMB1. Nucleic Acids Res. **13**:5353–5367.
36. **Cesareni, G., and D. W. Banner.** 1985. Regulation of plasmid copy number by complementary RNAs. Trends Biochem. Sci. **10**:303–306.
37. **Cesareni, G., M. Cornelissen, R. M. Lacatena, and L. Castagnoli.** 1984. Control of pMB1 replication: inhibition of primer formation by Rop requires RNA1. EMBO J. **3**:1365–1369.
38. **Chan, P. T., H. Ohmori, J. Tomizawa, and J. Lebowitz.** 1985. Nucleotide sequence and gene organization of ColE1 DNA. J. Biol. Chem. **260**:8925–8935.
39. **Chang, A. C. Y., and S. N. Cohen.** 1978. Construction and characterization of amplifiable multicopy DNA cloning vehicles derived from the P15A cryptic miniplasmid. J. Bacteriol. **134**:1141–1156.
40. **Chattoraj, D. K., A. L. Abeles, and M. B. Yarmeolinsky.** 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.
41. **Chattoraj, D. 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.
42. **Cheah, U. E., W. A. Weigand, and B. C. Stark.** 1987. Effects of recombinant plasmid size on cellular processes in *Escherichia coli*. Plasmid **18**:127–134.
43. **Chikami, G. K., D. G. Guiney, T. J. Schmidhauser, and D. R. Helinski.** 1985. Comparison of 10 IncP plasmids: homology in the regions involved in plasmid replication. J. Bacteriol. **162**:656–660.
44. **Churchward, G., P. Linder, and L. Caro.** 1983. The nucleotide sequence of replication and maintenance functions encoded by plasmid pSC101. Nucleic Acids Res. **11**:5645–5659.
45. **Cohen, S. N., and A. C. Y. Chang.** 1977. Revised interpretation of the origin of the pSC101 plasmid. J. Bacteriol. **132**:734–737.
46. **Cohen, S. N., A. C. Y. Chang, H. W. Boyer, and R. B. Helling.** 1973. Construction of biologically functional bacterial plasmids *in vitro*. Proc. Natl. Acad. Sci. USA **70**:3240–3244.
47. **Couturier, M., F. Bex, P. L. Bergquist, and W. K. Maas.** 1988. Identification and classification of bacterial plasmids. Microbiol. Rev. **52**:375–395.
48. **Cowan, P., and V. Krishnapillai.** 1982. Tn7 insertion mutations affecting the host range of the promiscuous IncP-1 plasmid R18. Plasmid **8**:164–174.
49. **Crosa, J. H.** 1980. Three origins of replication are active *in vivo* in the R plasmid RSF1040. J. Biol. Chem. **255**:11075–11077.
50. **Cross, M. A., S. R. Warne, and C. M. Thomas.** 1986. Analysis of the vegetative replication origin of broad-host-range plasmid RK2 by transposon mutagenesis. Plasmid **15**:132–146.
51. **Currier, T. C., and M. K. Morgan.** 1981. Restriction endonuclease analysis of the incompatibility group P-1 plasmids RK2, RP1, RP4, R68, and R68.45. Curr. Microbiol. **5**:323–327.
52. **Dasgupta, S., H. Masukata, and J. Tomizawa.** 1987. Multiple mechanisms for initiation of ColE1 DNA replication: DNA synthesis in the presence and absence of ribonuclease H. Cell **51**:1113–1122.

53. **Davison, J.** 1984. Mechanism of control of DNA replication and incompatibility in ColE1-type plasmids—a review. Gene **28**:1–15.
54. **de Bernandez, E. R., and P. S. Dhurjati.** 1987. Effect of a broad-host range plasmid on growth dynamics of *Escherichia coli* and *Pseudomonas putida*. Biotechnol. Bioeng. **29**:558–565.
55. **de Graaff, J., J. H. Crosa, F. Heffron, and S. Falkow.** 1978. Replication of the nonconjugative plasmid RSF1010 in *Escherichia coli* K-12. J. Bacteriol. **134**:1117–1122.
56. **Diaz, R., and W. L. Staudenbauer.** 1982. Replication of the broad host range plasmid RSF1010 in cell-free extracts of *Escherichia coli* and *Pseudomonas aeruginosa*. Nucleic Acids Res. **10**:4687–4702.
57. **Dieckmann, S.** 1987. DNA methylation can enhance or induce DNA curvature. EMBO J. **6**:4213–4217.
58. **Dixon, N., and A. Kornberg.** 1984. Protein HU in the enzymatic replication of the chromosomal origin of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **81**:424–428.
59. **Dodson, M., H. Echols, S. Wickner, C. Alfano, K. Mensa-Wilmot, B. Gomes, J. Lebowitz, J. D. Roberts, and R. McMacken.** 1986. Specialized nucleoprotein structures at the origin of replication of bacteriophage λ: localized unwinding of duplex DNA by a six-protein reaction. Proc. Natl. Acad. Sci. USA **83**:7638–7642.
60. **Dodson, M., J. Roberts, R. M. McMacken, and H. Echols.** 1985. Specialized nucleoprotein structures at the origin of replication of bacteriophage λ: complexes with λ O protein and with λ O, λ P, and *Escherichia coli* DnaB proteins. Proc. Natl. Acad. Sci. USA **82**:4678–4682.
61. **Dong, X., D. D. Womble, V. A. Luckow, and R. H. Rownd.** 1985. Regulation of transcription of the *repA1* gene in the replication control region of the IncFII plasmid NR1 by gene dosage of the *repA2* transcription repressor protein. J. Bacteriol. **161**:544–551.
62. **Dong, X., D. D. Womble, and R. H. Rownd.** 1987. Transcriptional pausing for plasmid NR1 replication control. J. Bacteriol. **169**:5353–5363.
63. **Donnelly, D. F., K. Birkenhead, and F. O'Gara.** 1987. Stability of IncQ and IncP-1 vector plasmids in *Rhizobium* spp. FEMS Microbiol. Lett. **42**:141–145.
64. **Donoghue, D. J., and P. A. Sharp.** 1978. Replication of colicin E1 plasmid DNA in vivo requires no plasmid-encoded proteins. J. Bacteriol. **133**:1287–1294.
65. **Dooley, T. P., and B. Polisky.** 1987. Suppression of ColE1 RNA-RNA mismatch mutations *in vivo* by the ColE1 Rop protein. Plasmid **18**:24–34.
66. **Dooley, T. P., J. Tamm, and B. Polisky.** 1985. Isolation and characterization of mutants affecting functional domains of ColE1 RNAI. J. Mol. Biol. **186**:87–96.
67. **Dreiseikelmann, B., H.-D. Riedel, and H. Schuster.** 1987. A dnaB-like protein of *Pseudomonas aeruginosa*. Nucleic Acids Res. **15**:385–416.
68. **Drlica, K., and J. Rouviere-Yaniv.** 1987. Histonelike proteins of bacteria. Microbiol. Rev. **51**:301–319.
69. **Durland, R. H., and D. R. Helinski.** 1987. The sequence encoding the 43-kilodalton trfA protein is required for efficient replication or maintenance of minimal RK2 replicons in *Pseudomonas aeruginosa*. Plasmid **18**:164–169.
70. **Echols, H.** 1986. Multiple DNA-protein interactions governing high-precision DNA transactions. Science **233**:1050–1056.
71. **Ely, S., and A. Wright.** 1985. Maintenance of plasmid pSC101 in *Escherichia coli* requires the host primase. J. Bacteriol. **164**:484–486.
72. **Engel, J. D., and P. H. von Hippel.** 1978. Effects of the methylation on the stability of nucleic acid conformations, studies at the polymer level. J. Biol. Chem. **253**:927–934.
73. **Engleberg, N. C., N. Cianciotto, J. Smith, and B. I. Eisenstein.** 1988. Transfer and maintenance of small, mobilizable plasmids with ColE1 replication origins in *Legionella pneumophila*. Plasmid **20**:83–91.
74. **Felton, J., and A. Wright.** 1979. Plasmid pSC101 replication in integratively suppressed cells requires *dnaA* function. Mol. Gen. Genet. **175**:231–233.
75. **Figurski, D. H., and D. R. Helinski.** 1979. Replication of an origin-containing derivative of plasmid RK2 dependent on a plasmid function provided *in trans*. Proc. Natl. Acad. Sci. USA **76**:1648–1652.
76. **Figurski, D. H., R. J. Meyer, and D. R. Helinski.** 1979. Suppression of ColE1 replication properties by the IncP-1 plasmid RK2 in hybrid plasmids constructed *in vitro*. J. Mol. Biol. **133**:295–318.
77. **Figurski, D. H., R. F. Pohlman, D. H. Bechhofer, A. S. Prince, and C. A. Kelton.** 1982. Broad host range plasmid RK2 encodes multiple *kil* genes potentially lethal to *Escherichia coli* host cells. Proc. Natl. Acad. Sci. USA **79**:1935–1939.
78. **Filutowicz, M., and K. Appelt.** 1988. The integration host factor of *Escherichia coli* binds to multiple sites at plasmid R6K γ origin and is essential for replication. Nucleic Acids Res. **16**:3829–3843.
79. **Filutowicz, M., M. J. McEachern, A. Greener, P. Mukhopadhyay, E. Uhlenhoop, 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.
80. **Filutowicz, M., M. J. McEachern, and D. R. Helinski.** 1986. Positive and negative roles of an initiator protein at the origin of replication. Proc. Natl. Acad. Sci. USA **83**:9645–9649.
81. **Filutowicz, M., M. J. McEachern, P. Mukhopadhyay, A. Greener, S. Yang, and D. R. Helinski.** 1987. DNA and protein interactions in the regulation of plasmid replication. J. Cell Sci. Suppl. **7**:15–31.
82. **Firshein, W., and L. Caro.** 1984. Detection of displacement ("D") loops with the properties of a replicating intermediate synthesized by a DNA/membrane complex derived from the low-copy-number plasmid RK2. Plasmid **12**:227–232.
83. **Firshein, W., P. Strumph, P. Benjamin, K. Burnstein, and J. Kornacki.** 1982. Replication of a low-copy-number plasmid by a plasmid DNA-membrane complex extracted from minicells of *Escherichia coli*. J. Bacteriol. **150**:1234–1243.
84. **Fitzwater, T., J. Tamm, and B. Polisky.** 1984. RNA1 is sufficient to mediate plasmid ColE1 incompatibility *in vivo*. J. Mol. Biol. **175**:409–417.
85. **Fitzwater, T., X.-Y. Zhang, R. Elble, and B. Polisky.** 1988. Conditional high copy number ColE1 mutants: resistance to RNA 1 inhibition *in vivo* and *in vitro*. EMBO. J. **7**:3289–3297.
86. **Fouser, L., and R. E. Bird.** 1983. Accumulation of ColE1 early replicative intermediates catalyzed by extracts of *Escherichia coli dnaG* mutant strains. J. Bacteriol. **154**:1174–1183.
87. **Frey, J., M. Chandler, and L. Caro.** 1979. The effects of an *E. coli* $dnaA_{ts}$ mutation on the plasmids ColE1, pSC101, R100.1, and RTF-Tc. Mol. Gen. Genet. **174**:117–126.
88. **Frey, J., M. Chandler, and L. Caro.** 1984. Overinitiation of chromosome and plasmid replication in a *dnaAcos* mutant of *Escherichia coli* K12. Evidence for *dnaA-dnaB* interactions. J. Mol. Biol. **179**:171–183.
89. **Friedman, D. I., E. R. Olson, C. P. Georgopoulos, K. Tilly, I. Herskowitz, and F. Banuett.** 1984. Interactions of bacteriophage and host macromolecules in the growth of bacteriophage λ. Microbiol. Rev. **48**:299–325.
90. **Fuller, R. S., B. E. Funnell, and A. Kornberg.** 1984. The dnaA protein complex with the *E. coli* chromosomal replication origin (*oriC*) and other DNA sites. Cell **38**:889–900.
91. **Fuller, R. S., and A. Kornberg.** 1983. Purified dnaA protein in initiation of replication at the *Escherichia coli* chromosomal origin of replication. Proc. Natl. Acad. Sci. USA **80**:5817–5821.
92. **Funnell, B. E., T. A. Baker, and A. Kornberg.** 1986. Complete enzymatic replication of plasmids containing the origin of the *Escherichia coli* chromosome. J. Biol. Chem. **261**:5616–5624.
93. **Funnell, B. E., T. A. Baker, and A. Kornberg.** 1987. *In vitro* assembly of a prepriming complex at the origin of the *Escherichia coli* chromosome. J. Biol. Chem. **262**:10327–10334.
94. **Gamas, P., A. C. Burger, G. Churchward, L. Caro, D. Galas,**

and M. Chandler. 1986. Replication of pSC101: effects of mutations in the *E. coli* DNA binding protein IHF. Mol. Gen. Genet. **204:**85–89.

95. **Gamas, P., M. G. Chandler, P. Prentki, and D. J. Galas.** 1987. *Escherichia coli* integration host factor binds specifically to the ends of the insertion sequence IS*1* and to its major insertion hot-spot in pBR322. J. Mol. Biol. **195:**261–272.
96. **Gayle, R. B., III, P. S. Vermersch, and G. N. Bennett.** 1986. Construction and characterization of pBR322-derived plasmids with deletions of the RNAI region. Gene **41:**281–288.
97. **Gaylo, P. J., N. Turjman, and D. Bastia.** 1987. DnaA protein is required for replication of the minimal replicon of the broad-host-range plasmid RK2 in *Escherichia coli*. J. Bacteriol. **169:**4703–4709.
98. **Gerdes, K., P. B. Rasmussen, and S. Molin.** 1986. Unique type of plasmid maintenance function: postsegregational killing of plasmid-free cells. Proc. Natl. Acad. Sci. USA **83:**3116–3120.
99. **Germino, J., and D. Bastia.** 1983. The replication initiator protein of plasmid R6K tagged with β-galactosidase shows sequence-specific DNA-binding. Cell **32:**131–140.
100. **Givskov, M., P. Stougaard, J. Light, and S. Molin.** 1987. Identification and characterization of mutations responsible for a runaway replication phenotype of plasmid R1. Gene **57:** 203–211.
101. **Goldman, A. L., and J. F. Dice.** 1974. Intracellular protein degradiation in mammalian and bacterial cells. Annu. Rev. Biochem. **43:**835–869.
102. **Grinter, N. J.** 1984. Replication control of IncP plasmids. Plasmid **11:**74–81.
103. **Grinter, N. J., and P. T. Barth.** 1976. Characterization of SmSu plasmids by restriction endonuclease cleavage and compatibility testing. J. Bacteriol. **128:**394–400.
104. **Guerry, P., J. van Embden, and S. Falkow.** 1974. Molecular nature of two nonconjugative plasmids carrying drug resistance genes. J. Bacteriol. **117:**619–630.
105. **Guiney, D. G., P. Hasegawa, and C. E. Davis.** 1984. Plasmid transfer from *Escherichia coli* to *Bacteroides fragilis*. Differential expression of antibiotic resistance genes. Proc. Natl. Acad. Sci. USA **81:**7203–7206.
106. **Hansen, E. B., and M. B. Yarmolinsky.** 1986. Host participation in plasmid maintenance: dependence upon *dnaA* of replicons derived from P1 and F. Proc. Natl. Acad. Sci. USA **83:**4423–4427.
107. **Haring, V., P. Scholz, E. Scherzinger, J. Frey, K. Derbyshire, G. Hatfull, N. S. Willetts, and M. Bagdasarian.** 1985. Protein repC is involved in copy number control of the broad host range plasmid RSF1010. Proc. Natl. Acad. Sci. USA **82:** 6090–6094.
108. **Hasunuma, K., and M. Sekiguchi.** 1977. Replication of plasmid pSC101 in *Escherichia coli* K-12: requirement for *dnaA* function. Mol. Gen. Genet. **154:**225–230.
109. **Hasunuma, K., and M. Sekiguchi.** 1979. Effect of *dna* mutations on the replication of plasmid pSC101 in *Escherichia coli*. J. Bacteriol. **137:**1095–1099.
110. **Hattman, S., J. E. Brooks, and M. Masurekar.** 1978. Sequence specifity of the P1-modification methylase (*M.Eco* PI) and DNA methylase (*M.Eco dam*) controlled by the *E. coli dam* gene. J. Mol. Biol. **126:**367–380.
111. **Hawley, D. K., and W. R. McClure.** 1983. Compilation and analysis of *Escherichia coli* promoter DNA sequences. Nucleic Acids Res. **11:**2237–2255.
112. **Hecker, M., A. Schroeter, and F. Mach.** 1983. Replication of pBR322 DNA in stringent and relaxed strains of *Escherichia coli*. Mol. Gen. Genet. **190:**355–357.
113. **Hecker, M., A. Schroeter, and F. Mach.** 1985. *Escherichia coli relA* strains as hosts for amplification of pBR322 plasmid DNA. FEMS Microbiol. Lett. **29:**331–334.
114. **Hecker, M., A. Schroeter, and F. Mach.** 1986. Physiological studies on pBR322 DNA amplification in an *Escherichia coli relA* strain. J. Basic Microbiol. **26:**329–333.
115. **Helmer-Citterich, M., M. M. Anceschi, D. W. Banner, and G. Cesareni.** 1988. Control of ColE1 replication: low affinity specific binding of Rop (Rom) to RNAI and RNAII. EMBO J. **7:**557–566.
116. **Higashi, M., M. Hata, T. Hase, K. Yamaguchi, and Y. Masamune.** 1986. The nucleotide sequence of *cea** and the region of plasmid pKY-1. J. Gen. Appl. Microbiol. **32:**433–442.
117. **Hillenbrand, G., and W. L. Staudenbauer.** 1982. Discriminatory function of ribonuclease H in the selective initiation of plasmid replication. Nucleic Acids Res. **10:**833–853.
118. **Hiraga, S., A. Jaffe, T. Ogura, H. Mori, and H. Takahashi.** 1986. F plasmid *ccd* mechanism in *Escherichia coli*. J. Bacteriol. **166:**100–104.
119. **Hirochika, H., K. Nakamura, and K. Sakaguchi.** 1984. A linear DNA plasmid from *Streptomyces rochei* with an inverted terminal repetition of 614 base pairs. EMBO J. **3:**761–766.
120. **Hogrefe, C., and B. Friedrich.** 1984. Isolation and characterization of megaplasmid DNA from lithoautotrophic bacteria. Plasmid **12:**161–169.
121. **Holm, L.** 1986. Codon usage and gene expression. Nucleic Acids Res. **14:**3075–3087.
122. **Honda, Y., H. Sakai, and T. Komano.** 1988. Two single-strand DNA initiation signals located in the *oriV* region of plasmid RSF1010. Gene **68:**221–228.
123. **Hooykaas, P. J. J., H. den Dulk-Ras, and R. A. Schilperoort.** 1982. Phenotypic expression of mutations in a wide-host-range R plasmid in *Escherichia coli* and *Rhizobium meliloti*. J. Bacteriol. **150:**395–397.
124. **Inoye, S., A. Yoshio, A. Nakazawa, and T. Nakazawa.** 1986. Nucleotide sequence of a DNA segment promoting transcription in *Pseudomonas putida*. J. Bacteriol. **166:**739–745.
125. **Inselburg, J.** 1974. Replication of colicin E1 plasmid DNA in minicells from a unique replication initiation site. Proc. Natl. Acad. Sci. USA **71:**2256–2259.
126. **Itoh, T., and J. Tomizawa.** 1978. Initiation of replication of plasmid ColE1 DNA by RNA polymerase, ribonuclease H and DNA polymerase I. Cold Spring Harbor Symp. Quant. Biol. **43:**409–418.
127. **Itoh, T., and J. Tomizawa.** 1980. Formation of an RNA primer for initiation of replication of ColE1 DNA by ribonuclease H. Proc. Natl. Acad. Sci. USA **77:**2450–2454.
128. **Itoh, T., and J. Tomizawa.** 1982. Purification of ribonuclease H as a factor required for *in vitro* ColE1 DNA replication. Nucleic Acids Res. **10:**5949–5965.
129. **Itoh, Y., J. M. Watson, D. Haas, and T. Leisinger.** 1984. Genetic and molecular characterization of the *Pseudomonas plasmid* pVS1. Plasmid **11:**206–220.
130. **Ivanov, I., L. Yavashev, L. Gigova, K. Alexciev, and C. Christov.** 1988. A conditional high-copy-number plasmid derivative of pBR322. Microbiologica **11:**95–99.
131. **Jaoua, S., J. F. Guespin-Michel, and A. M. Breton.** 1987. Mode of insertion of the broad-host-range plasmid RP4 and its derivatives into the chromosome of *Myxococcus xanthus*. Plasmid **18:**111–119.
132. **Kamio, Y., A. Tabuchi, Y. Itoh, H. Katagiri, and Y. Terawaki.** 1984. Complete nucleotide sequence of Mini-Rts1 and its copy mutant. J. Bacteriol. **158:**307–312.
133. **Kelley, W., and D. Bastia.** 1985. Replication initiator protein of plasmid R6K autoregulates its own synthesis at the transcriptional step. Proc. Natl. Acad. Sci. USA **82:**2574–2578.
134. **Kim, Y.-J., L.-S. Lin, and R. J. Meyer.** 1987. Two domains at the origin are required for replication and maintenance of broad-host-range plasmid R1162. J. Bacteriol. **169:**5870–5872.
135. **Kim, K., and R. Meyer.** 1986. Copy-number of broad host-range plasmid R1162 is regulated by small RNA. Nucleic Acids Res. **14:**8027–8046.
136. **Kim, K., and R. J. Meyer.** 1985. Copy number of the broad host-range plasmid R1162 is determined by the amounts of essential plasmid-encoded proteins. J. Mol. Biol. **185:**755–767.
137. **Kingsbury, D. T., and D. R. Helinski.** 1970. DNA polymerase as a requirement for the maintenance of the bacterial plasmid colicinogenic factor E1. Biochem. Biophys. Res. Commun. **41:**1538–1544.
138. **Kline, B. C.** 1985. A review of mini-F plasmid maintenance. Plasmid **14:**1–16.

139. **Kline, B. C.** 1988. Aspects of plasmid F maintenance in *Escherichia coli*. Can. J. Microbiol. **34**:526–535.
140. **Kogoma, T.** 1984. Absence of RNase H allows replication in pBR322 in *Escherichia coli* mutants lacking DNA polymerase I. Proc. Natl. Acad. Sci. USA **81**:7845–7849.
141. **Kogoma, T.** 1986. RNase H-defective mutants of *Escherichia coli*. J. Bacteriol. **166**:361–363.
142. **Kogoma, T., and B. C. Kline.** 1987. Integrative suppression of *dnaA*(Ts) mutations mediated by plasmid F in *Escherichia coli* is a DnaA-dependent process. Mol. Gen. Genet. **210**:262–269.
143. **Kohwi-Shigematsu, T., and Y. Kohwi.** 1985. Poly(dG)-poly(dC) sequences, under torsional stress, induce an altered DNA conformation upon neighboring DNA sequences. Cell **43**: 199–206.
144. **Kolter, R., and D. R. Helinski.** 1979. Regulation of initiation of DNA replication. Annu. Rev. Genet. **13**:355–391.
145. **Kontomichalou, P., M. Mitani, and R. C. Clowes.** 1970. Circular R-factors molecules controlling penicillinase synthesis, replicating in *Escherichia coli* under relaxed or stringent control. J. Bacteriol. **104**:34–44.
146. **Koo, H.-S., H.-M. Wu, and D. M. Crothers.** 1986. DNA bending at adenine · thymine tracts. Nature (London) **320**: 501–506.
147. **Kornacki, J. A., P. J. Balderes, and D. H. Figurski.** 1987. Nucleotide sequence of *korB*, a replication control gene of broad host-range plasmid RK2. J. Mol. Biol. **198**:211–222.
148. **Kornacki, J. A., and W. Firshein.** 1986. Replication of plasmid RK2 *in vitro* by a DNA-membrane complex: evidence for initiation of replication and its coupling to transcription and translation. J. Bacteriol. **167**:319–326.
149. **Kornacki, J. A., A. H. West, and W. Firshein.** 1984. Proteins encoded by the *trans*-acting replication and maintenance regions of broad host range plasmid RK2. Plasmid **11**:48–57.
150. **Kornberg, A.** 1982. DNA replication, 1982 supplement, p. 121–124. W. H. Freeman and Co., San Francisco.
151. **Kozak, M.** 1983. Comparison of initiation of protein synthesis in procaryotes, eucaryotes, and organelles. Microbiol. Rev. **47**:1–45.
152. **Krishnapillai, V.** 1986. Genetic analysis of bacterial plasmid promiscuity. J. Genet. **65**:103–120.
153. **Krishnapillai, V., J. Nash, and E. Lanka.** 1984. Insertion mutations in the promiscuous IncP-1 plasmid R18 which affect its host range between *Pseudomonas* species. Plasmid **12**: 170–180.
154. **Krishnapillai, V., M. Wexler, J. Nash, and D. H. Figurski.** 1987. Genetic basis of a Tn7 insertion mutation in the *trfA* region of the promiscuous IncP-1 plasmid R18 which affects its host range. Plasmid **17**:164–166.
155. **Lacatena, R. M., D. W. Banner, L. Castagnoli, and G. Cesareni.** 1984. Control of initiation of pMB1 replication: purified rop protein and RNA I affect primer formation *in vitro*. Cell **37**:1009–1014.
156. **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.
157. **Lacatena, R. M., and G. Cesareni.** 1983. Interaction between RNA1 and the primer precursors in the regulation of ColE1 replication. J. Mol. Biol. **170**:635–650.
158. **Lambert, C. M., C. J. Wrighton, and P. Strike.** 1987. Characterization of the drug resistance plasmid NTP16. Plasmid **17**:26–36.
159. **Lane, H. E. D.** 1981. Replication and incompatibility of F and plasmids in the IncFI group. Plasmid **5**:100–126.
160. **Lanka, E., and P. T. Barth.** 1981. Plasmid RP4 specifies a deoxyribonucleic acid primase involved in its conjugal transfer and maintenance. J. Bacteriol. **148**:769–781.
161. **Lanka, E., R. Lurz, and J. P. Fürste.** 1983. Molecular cloning and mapping of *Sph*I restriction fragments of plasmid RP4. Plasmid **10**:303–307.
162. **Lanka, E., R. Lurz, M. Kröger, and J. P. Fürste.** 1984. Plasmid RP4 encodes two forms of a DNA primase. Mol. Gen. Genet. **194**:65–72.
163. **LeBowitz, J. H., and R. McMacken.** 1986. The *Escherichia coli* dnaB replication protein is a DNA helicase. J. Biol. Chem. **261**:4738–4748.
164. **Leong, J. M., S. Nunes-Duby, C. F. Lesser, P. Youderian, M. M. Susskind, and A. Landy.** 1985. The ϕ80 and P22 attachment sites. Primary structure and interaction with *E. coli* integration host factor. J. Biol. Chem. **260**:4468–4477.
165. **Levin, B. R.** 1986. The maintenance of plasmids and transposons in natural populations of bacteria. Banbury Rep. **24**: 57–70.
166. **Lewington, J., and M. J. Day.** 1986. A rapid electrophoretic method for the measurement of plasmid copy number. Lett. Appl. Microbiol. **3**:109–112.
167. **Lin, L.-S., Y.-J. Kim, and R. J. Meyer.** 1987. The 20 bp, directly repeated DNA sequence of broad host range plasmid R1162 exerts incompatibility *in vivo* and inhibits R1162 replication *in vitro*. Mol. Gen. Genet. **208**:390–397.
168. **Lin, L.-S., and R. J. Meyer.** 1984. Nucleotide sequence and functional properties of DNA encoding incompatibility in the broad host-range plasmid R1162. Mol. Gen. Genet. **194**:423–431.
169. **Lin, L.-S., and R. J. Meyer.** 1986. Directly repeated, 20-bp sequence of plasmid R1162 DNA is required for replication, expression of incompatibility, and copy-number control. Plasmid **15**:35–47.
170. **Lin, L.-S., and R. J. Meyer.** 1987. DNA synthesis is initated within the origin of replication of plasmid R1162. Nucleic Acids Res. **15**:8319–8331.
171. **Lin-Chao, S., and H. Bremer.** 1987. Activities of the RNAI and RNAII promoters of plasmid pBR322. J. Bacteriol. **169**:1217–1222.
172. **Linder, P., G. Churchward, and L. Caro.** 1983. Plasmid pSC101 replication mutants generated by insertion of the transposon Tn*1000*. J. Mol. Biol. **170**:287–303.
173. **Linder, P., G. Churchward, X. Guixian, Y. Yi-Yi, and L. Caro.** 1985. An essential replication gene, *repA*, of plasmid pSC101 is autoregulated. J. Mol. Biol. **181**:383–393.
174. **Looman, A. C., and P. H. van Knippenberg.** 1986. Effects of GUG and AUG initiation codons on the expression of *lacZ* in *Escherichia coli*. FEBS Lett. **197**:315–320.
175. **Lundquist, P. D., and B. R. Levin.** 1986. Transitory derepression and the maintenance of conjugative plasmids. Genetics **113**:483–497.
176. **Mann, N. H.** 1985. Plasmid partitioning in *Escherichia coli*. Microbiol. Sci. **2**:299–302.
177. **Marquardt, R., G. Wöhner, and E.-L. Winnacker.** 1984. Properties of plasmids from *Methylomonas clara*. J. Biotechnol. **1**:317–330.
178. **Masai, H., and K.-I. Arai.** 1987. RepA and DnaA proteins are required for initiation of R1 plasmid replication *in vitro* and interact with the *oriR* sequence. Proc. Natl. Acad. Sci. USA **84**:4781–4785.
179. **Masai, H., and K.-I. Arai.** 1988. Initiation of lagging-strand synthesis of pBR322 plasmid DNA replication *in vitro* is dependent on primosomal protein i encoded by *dnaT*. J. Biol. Chem. **263**:15016–15023.
180. **Masai, H., M. W. Bond, and K. Arai.** 1986. Cloning of the *E. coli* gene for primosomal protein i: the relationship to *dnaT*, essential for chromosomal replication. Proc. Natl. Acad. Sci. USA **83**:1256–1260.
181. **Masson, L., and D. S. Ray.** 1986. Mechanism of autonomous control of the *Escherichia coli* F plasmid: different complexes of the initiator/repressor protein are bound to its operator and to an F plasmid replication origin. Nucleic Acids Res. **14**: 5693–5711.
182. **Masukata, H., S. Dasgupta, and J. Tomizawa.** 1987. Transcriptional activation of ColE1 DNA synthesis by displacement of the nontranscribed strand. Cell **51**:1123–1130.
183. **Masukata, H., and J. Tomizawa.** 1984. Effects of point mutations on formation and structure of the RNA primer for ColE1 replication. Cell **36**:513–522.
184. **Masukata, H., and J. Tomizawa.** 1986. Control of primer formation for ColE1 plasmid replication: conformational change of the primer transcript. Cell **44**:125–136.

185. **Mermod, N., P. R. Lehrbach, W. Reinecke, and K. N. Timmis.** 1984. Transcription of the TOL plasmid toluate catabolic pathway operon of *Pseudomonas putida* is determined by a pair of coordinately and positively regulated overlapping promoters. EMBO J. **3**:2461–2466.
186. **Merryweather, A., P. T. Barth, and B. M. Wilkins.** 1986. Role and specificity of plasmid RP4-encoded DNA primase in bacterial conjugation. J. Bacteriol. **167**:12–17.
187. **Messer, W.** 1987. Initiation of DNA replication in *Escherichia coli*. J. Bacteriol. **169**:3395–3399.
188. **Meyer, R., and D. R. Helinski.** 1977. Unidirectional replication of the P-group plasmid RK2. Biochim. Biophys. Acta **478**: 109–113.
189. **Meyer, R., and M. Hinds.** 1982. Multiple mechanisms for expression of incompatibility by broad-host-range plasmid RK2. J. Bacteriol. **152**:1078–1090.
190. **Meyer, R., M. Hinds, and M. Brasch.** 1982. Properties of R1162, a broad-host-range, high-copy-number plasmid. J. Bacteriol. **150**:552–562.
191. **Meyer, R. J., R. Laux, G. Boch, M. Hinds, R. Bayly, and J. A. Shapiro.** 1982. Broad-host-range IncP-4 plasmid R1162: effects of deletions and insertions on plasmid maintenance and host range. J. Bacteriol. **152**:140–150.
192. **Meyer, R. J., L.-S. 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.
193. **Minden, J. S., and K. J. Marians.** 1985. Replication of pBR322 DNA *in vitro* with purified proteins. Requirement for topoisomerase I in the maintenance of template specificity. J. Biol. Chem. **260**:9316–9325.
194. **Minden, J. S., and K. J. Marians.** 1986. *Escherichia coli* topoisomerase I can segregate replicating pBR322 daughter DNA molecules *in vitro*. J. Biol. Chem. **25**:11906–11917.
195. **Morita, M., and A. Oka.** 1979. The structure of a transcriptional unit on colicin E1 plasmid. Eur. J. Biochem. **97**:435–443.
196. **Morlon, J., M. Chartier, M. Bidaud, and C. Lazdunski.** 1988. The complete nucleotide sequence of the colicinogenic plasmid ColA. High extent of homology with ColE1. Mol. Gen. Genet. **211**:231–243.
197. **Moser, D. R., D. Ma, C. D. Moser, and J. L. Campbell.** 1984. *cis*-Acting mutations that affect rop protein control of plasmid copy number. Proc. Natl. Acad. Sci. USA **81**:4465–4469.
198. **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**:95–100.
199. **Mukherjee, S., H. Erickson, and D. Bastia.** 1988. Enhancer-origin interaction in plasmid R6K involves a DNA loop mediated by initiator-protein. Cell **52**:375–383.
200. **Mukherjee, S., I. Patel, and D. Bastia.** 1985. Conformational changes in a replication origin induced by an initiator protein. Cell **43**:189–197.
201. **Murakami, Y., H. Ohmori, T. Yura, and T. Nagata.** 1987. Requirement of the *Escherichia coli dnaA* gene function for *ori-2*-dependent mini-F plasmid replication. J. Bacteriol. **169**: 1724–1730.
202. **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.
203. **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.
204. **Naito, S., and H. Uchida.** 1986. RNase H and replication of ColE1 DNA in *Escherichia coli*. J. Bacteriol. **166**:143–147.
205. **Nash, J., and V. Krishnapillai.** 1987. DNA sequence analysis of host range mutants of the promiscous IncP-1 plasmids R18 and R68 with Tn7 insertions in *oriV*. Plasmid **18**:35–45.
206. **Nash, J., and V. Krishnapillai.** 1988. Role of IncP-1 plasmid primase in conjugation between *Pseudomonas* species. FEMS Microbiol. Lett. **49**:257–260.
207. **Nijkamp, H. J. J., R. de Lang, A. R. Stuitje, P. J. M. van den Elzen, E. Veltkamp, and A. J. van Putten.** 1986. The complete nucleotide sequence of the bacteriocinogenic plasmid CloDF13. Plasmid **16**:135–160.
208. **Nomura, N., R. L. Low, and D. S. Ray.** 1982. Identification of ColE1 DNA sequences that direct single strand to double strand conversion by ϕX174 type mechanism. Proc. Natl. Acad. Sci. USA **79**:3153–3157.
209. **Nordström, K.** 1985. Control of plasmid replication: theoretical considerations and practical solutions, p. 189–214. *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.
210. **Nordström, K., and H. Aagard-Hansen.** 1984. Maintenance of bacterial plasmids: comparison of theoretical calculations and experiments with plasmid R1 in *Escherichia coli*. Mol. Gen. Genet. **197**:1–7.
211. **Nordström, 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.
212. **Nordström, M., and K. Nordström.** 1985. Control of replication of FII plasmids: comparison of the basic replicons and of the *copB* systems of plasmids R100 and R1. Plasmid **13**:81–87.
213. **Normore, W. M.** 1973. Guanine-plus-cytosine (GC) composition of the DNA of bacteria, fungi, algae and protozoa, p. 585–740. *In* A. I. Laskin and H. A. Lechevalier (ed.), Handbook of microbiology, vol. II. CRC Press, Inc., Boca Raton, Fla.
214. **Novick, R. P.** 1987. Plasmid incompatibility. Microbiol. Rev. **51**:381–395.
215. **Nugent, M. E., T. J. Smith, and W. C. A. Tacon.** 1986. Characterization and incompatibility properties of Rom-derivates of pBR322-based plasmids. J. Gen. Microbiol. **132**: 1021–1026.
216. **Ogasawara, N. S., K. Moriya, F. G. von Meyenburg, F. G. Hansen, and H. Yoshikawa.** 1985. Conservation of genes and their organization in the chromosomal replication origin region of *Bacillus subtilis* and *Escherichia coli*. EMBO J. **4**:3345–3350.
217. **Ogawa, T., T. A. Baker, A. van der Ende, and A. Kornberg.** 1985. Initiation of enzymatic replication at the origin of the *Escherichia coli* chromosome: contributions of RNA polymerase and primase. Proc. Natl. Acad. Sci. USA **82**:3562–3566.
218. **Ogawa, T., and T. Okazaki.** 1984. Function of RNase H in DNA replication revealed by RNase H defective mutants of *Escherichia coli*. Mol. Gen. Genet. **193**:231–237.
219. **Ohmori, H., Y. Murakami, and T. Nagata.** 1987. Nucleotide sequences required for a ColE1-type plasmid to replicate in *Escherichia coli* cells with or without RNaseH. J. Mol. Biol. **198**:223–235.
220. **Oka, A., N. Nomura, M. Morita, H. Sugisaki, K. Sugimoto, and M. Takanami.** 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.
221. **Orr, E., and W. L. Staudenbauer.** 1981. An *Escherichia coli* mutant thermosensitive in the B subunit of DNA gyrase: effect on the structure and replication of the colicin E1 plasmid *in vitro*. Mol. Gen. Genet. **181**:52–56.
222. **Ortega, S., E. Lanka, and R. Diaz.** 1986. The involvement of host replication proteins and of specific origin sequences in the *in vitro* replication of miniplasmid R1 DNA. Nucleic Acids Res. **14**:4865–4879.
223. **Panayotatos, N.** 1984. DNA replication regulated by the priming promoter. Nucleic Acids Res. **12**:2541–2648.
224. **Pansegrau, W., and E. Lanka.** 1987. Conservation of a common 'backbone' in the genetic organization of the IncP plasmids RP4 and R751. Nucleic Acids Res. **15**:2385.
225. **Patel, I., and D. Bastia.** 1987. A replication initiator protein enhances the rate of hybrid formation between a silencer RNA and an activator RNA. Cell **51**:455–462.
226. **Persson, C., and K. Nordström.** 1986. Control of replication of the broad host range plasmid RSF1010: the incompatibility

determinant consists of directly repeated DNA sequences. Mol. Gen. Genet. **203**:189–192.
227. **Persson, C., E. G. H. Wagner, and K. Nordström.** 1988. Control of replication of plasmid R1: kinetics of *in vitro* interaction between the antisense RNA, CopA, and its target, CopT. EMBO J. **7**:3279–3288.
228. **Pinkney, M., R. Diaz, E. Lanka, and C. M. Thomas.** 1988. Replication of MiniRK2 plasmid in extracts of *Escherichia coli* requires plasmid-encoded protein TrfA and host-encoded proteins DnaA, B, G, DNA gyrase and DNA polymerase III. J. Mol. Biol. **203**:927–938.
229. **Pinkney, M., B. D. M. Theophilus, S. R. Warne, W. C. A. Tacon, and C. M. Thomas.** 1987. Analysis of transcription from the *trfA* promoter of broad host range plasmid RK2 in *Escherichia coli*, *Pseudomonas putida* and *Pseudomonas aeruginosa*. Plasmid **17**:222–232.
230. **Pinkney, M., and C. M. Thomas.** 1987. Replication and maintenance of promiscuous plasmids of gram-negative bacteria. Microbiol. Sci. **4**:186–191.
231. **Pohlman, R. F., and D. H. Figurski.** 1983. Conditional lethal mutants of the *kilB* determinant of broad host range plasmid RK2. Plasmid **10**:82–95.
232. **Pohlman, R. F., and D. H. Figurski.** 1983. Essential genes of plasmid RK2 in *Escherichia coli*: *trfB* controls a *kil* gene near *trfA*. J. Bacteriol. **156**:584–591.
233. **Prentki, P., M. Chandler, and L. Caro.** 1977. Replication of the prophage P1 during the cell cycle of *Escherichia coli*. Mol. Gen. Genet. **152**:71–76.
234. **Prentki, P., M. Chandler, and D. J. Galas.** 1987. *Escherichia coli* integration host factor bends the DNA at the ends of IS*1* and in an insertion hotspot with multiple IHF binding sites. EMBO J. **6**:2479–2487.
235. **Rasmussen, P. B., K. Gerdes, and S. Molin.** 1987. Genetic analysis of the $parB^+$ locus of plasmid R1. Mol. Gen. Genet. **209**:122–128.
236. **Reddy, P., A. Peterkofsky, and K. McKenney.** 1985. Translational efficiency of the *Escherichia coli* adenylate cyclase gene: mutating the UUG initiation codon to GUG or AUG results in increased gene expression. Proc. Natl. Acad. Sci. USA **82**: 5656–5660.
237. **Rinkel, L. J., G. A. Marel, J. H. Boom, and C. Altona.** 1987. Influence of N^6-methylation of residue A(5) on the conformational behaviour of d(C-C-G-A-A-T-T-C-G-G) in solution studied by ^{1}H-NMR spectroscopy. 2. The hairpin form. Eur. J. Biochem. **163**:287–296.
238. **Robinson, M., R. Lilley, S. Little, J. S. Emtage, G. Yarranton, P. Stephens, A. Millican, M. Eaton, and G. Humphreys.** 1984. Codon usage can affect efficiency of translation of genes in *Escherichia coli*. Nucleic Acids Res. **12**:6663–6671.
239. **Rokeach, L. A., G. A. Kassavetis, and J. W. Zyskind.** 1987. RNA polymerase pauses *in vitro* within the *Escherichia coli* origin of replication at the same sites where termination occurs *in vivo*. J. Biol. Chem. **262**:7264–7272.
240. **Rokeach, L. A., L. Sogaard-Anderson, 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.
241. **Rokeach, L. A., and J. W. Zyskind.** 1986. RNA terminating within the *E. coli* origin of replication: stringent regulation and control by dnaA protein. Cell **46**:763–771.
242. **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**:794–797.
243. **Rouviere-Yaniv, J., J. Germod, and M. Yaniv.** 1979. *E. coli* DNA binding protein HU forms nucleosome-like structure with circular double-stranded DNA. Cell **17**:265–274.
244. **Russell, D. W., and N. D. Zinder.** 1987. Hemimethylation prevents DNA replication in *E. coli*. Cell **50**:1071–1079.
245. **Saadi, S., W. K. Maas, D. F. Hill, and P. L. Bergquist.** 1987. Nucleotide sequence analysis of RepFIC, a basic replicon present in IncFI plasmids P307 and F, and its relation to the RepA replicon of IncFII plasmids. J. Bacteriol. **169**:1836–1846.
246. **Saano, A. K., and V. V. Zinchenko.** 1987. A new IncQ plasmid R89S: properties and genetic organization. Plasmid **17**:191–201.
247. **Sakabiara, Y., and J. Tomizawa.** 1974. Replication of colicin E1 plasmid in cell extracts. II. Selective synthesis of early replicative intermediates. Proc. Natl. Acad. Sci. USA **71**: 1403–1407.
248. **Schauzu, A. M., C. Kücherer, R. Kölling, W. Messer, and H. Lother.** 1987. Transcripts within the replication origin, *oriC*, of *Escherichia coli*. Nucleic Acids Res. **15**:2479–2497.
249. **Scherzinger, E., M. M. Bagdasarian, P. Scholz, R. Lurz, B. Rückert, and M. Bagdasarian.** 1984. Replication of the broad host range plasmid RSF1010: requirement for three plasmid-encoded proteins. Proc. Natl. Acad. Sci. USA **81**:654–658.
250. **Schmidthauser, T. J., M. Filutowicz, and D. R. Helinski.** 1983. Replication of derivatives of the broad host range plasmid RK2 in two distantly related bacteria. Plasmid **9**:325–330.
251. **Schmidthauser, T. J., and D. R. Helinski.** 1985. Regions of broad-host-range plasmid RK2 involved in replication and stable maintenance in nine species of gram-negative bacteria. J. Bacteriol. **164**:446–455.
252. **Schnos, M., K. Zahn, R. B. Inman, and F. R. Blattner.** 1988. Initiation protein induced helix destabilization at the λ origin: a prepriming step in DNA replication. Cell **52**:385–395.
253. **Scholz, P., V. Haring, E. Scherzinger, R. Lurz, M. M. Bagdasarian, H. Schuster, and M. Bagdasarian.** 1985. Replication determinants of the broad host range plasmid RSF1010, p. 243–260. *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.
254. **Scholz, P., V. Haring, B. Wittmann-Liebold, K. Ashman, M. Bagdasarian, and E. Scherzinger.** 1989. Complete nucleotide sequence and gene organization of the broad-host-range plasmid RSF1010. Gene **75**:271–288.
255. **Schreiner, H. C., D. H. Bechhofer, R. F. Pohlman, C. Young, P. A. Borden, and D. H. Figurski.** 1985. Replication control in promiscuous plasmid RK2: *kil* and *kor* functions affect expression of the essential replication gene *trfA*. J. Bacteriol. **163**: 228–237.
256. **Schwab, H., P. N. Saurugger, and R. M. Lafferty.** 1983. Occurrence of deletion plasmids at high rates after conjugative transfer of the plasmids RP4 and RK2 from *Escherichia coli* to *Alcaligenes eutrophus* H16. Arch. Microbiol. **136**:140–146.
257. **Scott, J. R.** 1984. Regulation of plasmid replication. Microbiol. Rev. **48**:1–23.
258. **Sekimizu, K., D. Bramhill, and A. Kornberg.** 1987. ATP activates dnaA protein in initiating replication of plasmids bearing the origin of the *E. coli* chromosome. Cell **50**:259–265.
259. **Selzer, G., T. Som, T. Itoh, and J. Tomizawa.** 1983. The origin of replication of plasmid p15A and comparative studies on the nucleotide sequences around the origin of related plasmids. Cell **32**:119–129.
260. **Selzer, G., and J. Tomizawa.** 1982. Specific cleavage of the p15A primer precursor by ribonuclease H at the origin of DNA replication. Proc. Natl. Acad. Sci. USA **79**:7082–7086.
261. **Seufert, W., B. Dobrinski, R. Lurz, and W. Messer.** 1988. Functionality of the dnaA protein binding site in DNA replication is orientation-dependent. J. Biol. Chem. **263**:2719–2723.
262. **Seufert, W., and W. Messer.** 1987. DnaA protein binding to the plasmid origin region can substitute for primosome assembly during replication of pBR322 *in vitro*. Cell **48**:73–78.
263. **Seufert, W., and W. Messer.** 1987. Start sites for bidirectional *in vitro* DNA replication inside the replication origin, *oriC*, of *Escherichia coli*. EMBO J. **6**:2469–2472.
264. **Sharp, P. M., and W.-H. Li.** 1986. An evolutionary perspective on synonymous codon usage in unicellular organisms. J. Mol. Evol. **24**:28–38.
265. **Sharp, P. M., and W.-H. Li.** 1986. Codon usage in regulatory genes in *Escherichia coli* does not reflect selection for 'rare' codons. Nucleic Acids Res. **14**:7737–7749.
266. **Shine, J., and L. Dalgarno.** 1975. Determinant of cistron specifity in bacterial ribosomes. Nature (London) **254**:34–38.
267. **Shingler, V., and C. M. Thomas.** 1984. Analysis of the *trfA* region of broad host-range plasmid RK2 by transposon muta-

genesis and identification of polypeptide products. J. Mol. Biol. **175**:229–249.
268. **Shingler, V., and C. M. Thomas.** 1984. Transcription in the *trfA* region of broad host range plasmid RK2 is regulated by *trfB* and *korB*. Mol. Gen. Genet. **195**:523–529.
269. **Shlomai, J., and A. Kornberg.** 1980. An *E. coli* replication protein that recognize a unique sequence within a hairpin region in ΦX174 DNA. Proc. Natl. Acad. Sci. USA **77**: 799–803.
270. **Shoemaker, N. B., C. Getty, J. F. Gardner, and A. A. Salyers.** 1986. Tn*4351* transposes in *Bacteroides* spp. and mediates the integration of plasmid R751 into the *Bacteroides* chromosome. J. Bacteriol. **165**:929–936.
271. **Shoemaker, N. B., E. P. Guthrie, A. A. Salyers, and J. F. Gardner.** 1985. Evidence that the clindamycin-erythromycin resistance gene of *Bacteroides* plasmid pBF4 is on a transposable element. J. Bacteriol. **164**:230–236.
272. **Skovgaard, O., and F. G. Hansen.** 1987. Comparison of *dnaA* nucleotide sequence of *Escherichia coli*, *Salmonella typhimurium*, and *Serratia marcescens*. J. Bacteriol. **169**:3976–3981.
273. **Smith, C. A., V. Shingler, and C. M. Thomas.** 1984. The *trfA* and *trfB* promoter regions of broad host range plasmid RK2 share common potential regulatory sequences. Nucleic Acids Res. **12**:3619–3630.
274. **Smith, C. A., and C. M. Thomas.** 1983. Deletion mapping of *kil* and *kor* functions in the *trfA* and *trfB* regions of broad host range plasmid RK2. Mol. Gen. Genet. **190**:245–254.
275. **Smith, C. A., and C. M. Thomas.** 1984. Nucleotide sequence of the *trfA* gene of broad host-range plasmid RK2. J. Mol. Biol. **175**:251–262.
276. **Smith, C. A., and C. M. Thomas.** 1984. Molecular genetic analysis of the *trfB* and *korB* region of broad host range plasmid RK2. J. Gen. Microbiol. **130**:1651–1663.
277. **Smith, C. A., and C. M. Thomas.** 1985. Comparison of the nucleotide sequences of the vegetative replication origin of broad host range IncP plasmids R751 and RK2 reveals conserved features of probable functional importance. Nucleic Acids Res. **13**:557–572.
278. **Smith, C. A., and C. M. Thomas.** 1987. Comparison of the organization of the genomes of phenotypically diverse plasmids of incompatibility group P: members of the IncPβ subgroup are closely related. Mol. Gen. Genet. **206**:419–427.
279. **Smith, C. A., and C. M. Thomas.** 1987. Narrow-host-range IncP plasmid pHH502-1 lacks a complete IncP replication system. J. Gen. Microbiol. **133**:2247–2252.
280. **Som, T., and J.-I. Tomizawa.** 1983. Regulatory regions of ColE1 that are involved in determination of plasmid copy number. Proc. Natl. Acad. Sci. USA **80**:3232–3236.
281. **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.
282. **Stalker, D. M., C. M. Thomas, and D. R. Helinski.** 1981. Nucleotide sequence of the region of the origin of replication of the broad host range plasmid RK2. Mol. Gen. Genet. **181**:8–12.
283. **Stanisich, V. A.** 1988. Identification and analysis of plasmids at the genetic level. Methods Microbiol. **21**:11–48.
284. **Staudenbauer, W. L.** 1977. Replication of ampicillin resistance plasmid RSF1030 in extracts of *Escherichia coli*: separation of the replication cycle into early and late stages. Mol. Gen. Genet. **156**:27–34.
285. **Staudenbauer, W. L., E. Scherzinger, and E. Lanka.** 1979. Replication of colicin E1 plasmid in extracts of *E. coli*: uncoupling of leading strand from lagging strand synthesis. Mol. Gen. Genet. **177**:113–120.
286. **Stenzel, T. T., P. Patel, and D. Bastia.** 1987. The integration host factor of *Escherichia coli* binds to bent DNA at the origin of replication of the plasmid pSC101. Cell **49**:709–717.
287. **Stokes, H. W., R. J. Moore, and V. Krishnapillai.** 1981. Complementation analysis in *Pseudomonas aeruginosa* of the transfer genes of the wide host range R plasmid R18. Plasmid **5**:202–212.
288. **Stueber, D., and H. Bujard.** 1982. Transcription from efficient promoters can interfere with plasmid replication and diminish expression of plasmid specified genes. EMBO J. **1**:1399–1404.
289. **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.
290. **Swack, J., S. K. Pal, R. J. Mason, A. L. Abeles, and D. K. Chattoraj.** 1987. P1 plasmid replication: measurement of initiator protein concentration in vivo. J. Bacteriol. **169**:3737–3742.
291. **Tabuchi, A.** 1985. Nucleotide sequence of the replication region of plasmid R401 and its incompatibility function. Microbiol. Immunol. **29**:383–393.
292. **Tait, R. C., T. J. Close, R. L. Rodriguez, and C. I. Kado.** 1982. Isolation of the origin of replication of the IncW-group plasmid pSa. Gene **20**:39–49.
293. **Tait, R. C., C. I. Kado, and R. L. Rodriguez.** 1983. A comparison of the origin of replication of pSa with R6K. Mol. Gen. Genet. **192**:32–38.
294. **Tamm, J., and B. Polisky.** 1983. Structural analysis of RNA molecules involved in plasmid copy number control. Nucleic Acids Res. **11**:6381–6397.
295. **Tamm, J., and B. Polisky.** 1985. Characterization of the ColE1 primer-RNA1 complex: analysis of a domain of ColE1 RNA1 necessary for its interaction with primer RNA. Proc. Natl. Acad. Sci. USA **82**:2257–2261.
296. **Tan, R. K. Z., and S. C. Harvey.** 1987. A comparison of six DNA bending models. J. Biomol. Struct. Dyn. **5**:497–512.
297. **Tardif, G., and R. B. Grant.** 1983. Transfer of plasmids from *Escherichia coli* to *Pseudomonas aeruginosa*: characterization of a *Pseudomonas aeruginosa* mutant with enhanced recipient ability for enterobacterial plasmids. Antimicrob. Agents Chemother. **24**:201–208.
298. **Theophilus, B. D. M., M. A. Cross, C. A. Smith, and C. M. Thomas.** 1985. Regulation of the *trfA* and *trfB* promoters of broad host range plasmid RK2: identification of sequences essential for regulation by *trfB*/*korA*/*korD*. Nucleic Acids Res. **13**:8129–8142.
299. **Theophilus, B. D. M., and C. M. Thomas.** 1987. Nucleotide sequence of the transcriptional repressor gene *korB* which plays a key role in regulation of the copy number of broad host range plasmid RK2. Nucleic Acids Res. **15**:7443–7450.
300. **Thomas, C. M.** 1981. Molecular genetics of broad host range plasmid RK2. Plasmid **5**:10–19.
301. **Thomas, C. M.** 1981. Complementation analysis of replication and maintenance functions of broad host range plasmids RK2 and RP1. Plasmid **5**:277–291.
302. **Thomas, C. M.** 1983. Instability of a high-copy-number mutant of a miniplasmid derived from broad host range IncP plasmid RK2. Plasmid **10**:184–195.
303. **Thomas, C. M.** 1986. Evidence for the involvement of the *incC* locus of broad host range plasmid RK2 in plasmid maintenance. Plasmid **16**:15–29.
304. **Thomas, C. M.** 1988. Recent studies on the control of plasmid replication. Biochim. Biophys. Acta **949**:253–263.
305. **Thomas, C. M., M. A. Cross, A. A. K. Hussain, and C. A. Smith.** 1984. Analysis of copy number control elements in the region of the vegetative replication origin of the broad host range plasmid RK2. EMBO J. **3**:57–63.
306. **Thomas, C. M., and D. R. Helinski.** 1979. Plasmid DNA replication, p. 29–46. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
307. **Thomas, C. M., and A. A. K. Hussain.** 1984. The *korB* gene of broad host range plasmid RK2 is a major copy number control element which may act together with *trfB* by limiting *trfA* expression. EMBO J. **3**:1513–1519.
308. **Thomas, C. M., A. A. K. Hussain, and C. A. Smith.** 1982. Maintenance of broad host range plasmid RK2 replicons in *Pseudomonas aeruginosa*. Nature (London) **298**:674–676.
309. **Thomas, C. M., R. Meyer, and D. R. Helinski.** 1980. Regions of broad-host-range plasmid RK2 which are essential for replication and maintenance. J. Bacteriol. **141**:213–222.
310. **Thomas, C. M., and C. A. Smith.** 1986. The *trfB* region of broad host range plasmid RK2: the nucleotide sequence re-

veals *incC* and key regulatory gene *trfB/korA/korD* as overlapping genes. Nucleic Acids Res. **14**:4453–4468.
311. **Thomas, C. M., and C. A. Smith.** 1987. Incompatibility group P plasmids: genetics, evolution, and use in genetic manipulation. Annu. Rev. Microbiol. **41**:77–101.
312. **Thomas, C. M., D. Stalker, D. Guiney, and D. R. Helinski.** 1979. Essential regions for the replication and conjugal transfer of the broad host range plasmid RK2, p. 375–385. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
313. **Thomas, C. M., D. M. Stalker, and D. R. Helinski.** 1981. Replication and incompatibility properties of segments of the origin region of replication of the broad-host range plasmid RK2. Mol. Gen. Genet. **181**:1–7.
314. **Timmis, K. N.** 1979. Mechanisms of plasmid incompatibility, 13–22. *In* K. N. Timmis and A. Pühler (ed.), Plasmids of medical, environmental and commercial importance. Elsevier/North-Holland Biomedical Press, Amsterdam.
315. **Tokino, T., T. Murotsu, and K. Matsubara.** 1986. Purification and properties of the mini-F plasmid-encoded E protein needed for autonomous replication control of the plasmid. Proc. Natl. Acad. Sci. USA **83**:4109–4113.
316. **Tomcsanyi, T., and D. Apirion.** 1985. Processing enzyme ribonuclease E specifically cleaves RNAI an inhibitor of primer formation in plasmid DNA synthesis. J. Mol. Biol. **185**:713–720.
317. **Tomizawa, J.** 1984. Control of ColE1 plasmid replication: the process of binding of RNA I to the primer transcript. Cell **38**:861–870.
318. **Tomizawa, J.** 1985. Control of ColE1 plasmid replication: initial interaction of RNA I and the primer transcript is reversible. Cell **40**:527–535.
319. **Tomizawa, J.** 1986. Control of ColE1 plasmid replication: binding of RNA I to RNA II and inhibition of primer formation. Cell **47**:89–97.
320. **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.
321. **Tomizawa, J., and T. Itoh.** 1982. The importance of RNA secondary structure in ColE1 primer formation. Cell **31**:575–583.
322. **Tomizawa, J., T. Itoh, G. Selzer, and T. Som.** 1981. Inhibition of ColE1 RNA primer formation by plasmid-specified small RNA. Proc. Natl. Acad. Sci. USA **78**:1421–1425.
323. **Tomizawa, J., and H. Masukata.** 1987. Factor-independent termination of transcription in a stretch of a deoxyadenosine residues in the template DNA. Cell **51**:623–630.
324. **Tomizawa, J., M. Ohmori, and R. E. Bird.** 1977. Origin of replication of colicin E1 plasmid. Proc. Natl. Acad. Sci. USA **74**:1865–1869.
325. **Tomizawa, J., Y. Sakakibara, and T. Kakefuda.** 1974. Replication of colicin E1 plasmid DNA in cell extracts: origin and direction of replication. Proc. Natl. Acad. Sci. USA **71**: 2260–2264.
326. **Tomizawa, J., Y. Sakakibara, and T. Kakefuda.** 1975. Replication of colicin E1 plasmid DNA added to cell extract. Proc. Natl. Acad. Sci. USA **72**:1050–1054.
327. **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.
328. **Trevors, J. T., T. Barkay, and A. W. Bourquin.** 1987. Gene transfer among bacteria in soil and aquatic environments: a review. Can. J. Microbiol. **33**:191–198.
329. **Tsuda, M., S. Harayama, and T. Iino.** 1984. Tn*501* insertion mutagenesis in *Pseudomonas aeruginosa* PAO. Mol. Gen. Genet. **196**:494–500.
330. **Tsurimoto, T., and K. Matsubara.** 1983. Replication of bacteriophage λ DNA. Cold Spring Harbor Symp. Quant. Biol. **47**:681–691.
331. **Tsutsui, H., A. Fujiyama, T. Murotsu, and K. Matsubara.** 1983. Role of nine repeating sequences of mini-F genome for expression of F-specific incompatibility phenotype and copy number control. J. Bacteriol. **155**:337–344.
332. **Twigg, A. J., and D. Sherratt.** 1980. Trans-complementable copy-number mutants of plasmid ColE1. Nature (London) **283**:216–218.
333. **Uhlin, B. E., and K. Nordström.** 1978. A runaway-replication mutant of plasmid R1drd-19: temperature-dependent loss of copy number control. Mol. Gen. Genet. **165**:167–179.
334. **van der Hoeven, N.** 1985. Evolution of bacterial surface exclusion against incompatible plasmids. J. Theor. Biol. **117**:431–452.
335. **van der Hoeven, N.** 1986. Coexistence of incompatible plasmids in a bacterial population living under a feast and famine regime. J. Math. Biol. **24**:313–325.
336. **van Putten, A., R. de Lang, E. Veltkamp, H. J. J. Nijkamp, P. van Solingen, and J. van den Berg.** 1986. Methylation-dependent transcription controls plasmid replication of the CloDF13 *cop-1*(Ts) mutant. J. Bacteriol. **168**:728–733.
337. **Varenne, S., J. Buc, R. Lloubes, and C. Lazdunski.** 1984. Translation is a non-uniform process. Effect of tRNA availability on the rate of elongation of nascent polypeptide chains. J. Mol. Biol. **180**:549–576.
338. **Villaroel, R., R. W. Hegdes, R. Maenhaut, J. Leemans, G. Engler, M. van Montagu, and J. Schell.** 1983. Heteroduplex analysis of P-plasmid evolution: the role of insertion and deletion of transposable elements. Mol. Gen. Genet. **189**: 390–399.
339. **Vocke, C., and D. Bastia.** 1983. Primary structure of the essential replicon of the plasmid pSC101. Proc. Natl. Acad. Sci. USA **80**:6557–6561.
340. **Vocke, C., and D. Bastia.** 1983. DNA-protein interaction at the origin of DNA replication of the plasmid pSC101. Cell **35**: 495–502.
341. **Vocke, C., and D. Bastia.** 1985. The replication initiator protein of plasmid pSC101 is a transcriptional repressor of its own cistron. Proc. Natl. Acad. Sci. USA **82**:2252–2256.
342. **von Hippel, P. H., D. G. Blear, W. D. Morgan, and J. A. McSwiggen.** 1984. Protein-nucleic acid interactions in transcription: a molecular analysis. Annu. Rev. Biochem. **53**: 389–446.
343. **Wada, A., and A. Suyama.** 1986. Local stability of DNA and RNA secondary structure and its relation to biological functions. Prog. Biophys. Mol. Biol. **47**:113–157.
344. **Wada, C., Y. Akiyama, K. Ito, and T. Yura.** 1986. Inhibition of F plasmid replication in *htpR* mutants of *Escherichia coli* deficient in sigma 32 protein. Mol. Gen. Genet. **203**:208–213.
345. **Wada, C., M. Imai, and T. Yura.** 1987. Host control of plasmid replication: requirement for the factor 32 in transcription of mini-F replication initiator gene. Proc. Natl. Acad. Sci. USA **84**:8849–8853.
346. **Wagner, E. G., J. von Heijne, and K. Nordström.** 1987. Control of replication of plasmid R1: translation of the 7k reading frame in the RepA mRNA leader region counteracts the interaction between CopA RNA and CopT RNA. EMBO J. **6**:515–522.
347. **Weber, P. C., and S. Palchaudhuri.** 1986. Incompatibility repressor in a RepA-like replicon of the IncFI plasmid ColV2-K94. J. Bacteriol. **166**:1106–1112.
348. **Wells, R. D.** 1988. Unusual DNA structures. J. Biol. Chem. **263**:1095–1098.
349. **West, S. E. H., and B. H. Iglewski.** 1988. Codon usage in *Pseudomonas aeruginosa*. Nucleic Acids Res. **16**:9323–9335.
350. **Wickner, S. H., and D. K. Chattoraj.** 1987. Replication of mini-P1 plasmid DNA *in vitro* requires two initiation proteins, encoded by the *repA* gene of phage P1 and the *dnaA* gene of *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:3668–3672.
351. **Wickner, S. H., and K. Zahn.** 1986. Characterization of the DNA binding domain of bacteriophage λ O protein. J. Biol. Chem. **261**:7537–7543.
352. **Winans, S. C., and G. C. Walker.** 1985. Identification of pKM101-encoded loci specifying potentially lethal gene products. J. Bacteriol. **161**:417–424.
353. **Wlodarczyk, M., and B. Nowicka.** 1988. Preliminary evidence for the linear nature of *Thiobacillus versutus* pTAV2 plasmid. FEMS Microbiol. Lett. **55**:125–128.

354. **Womble, D. D., X. Dong, and R. H. Rownd.** 1987. Changes in the RNA secondary structure may mediate the regulation of IncFII plasmid gene expression and DNA replication, p. 225–247. *In* M. Inoye and B. S. Budock (ed.), Molecular biology of RNA: new perspectives. Academic Press, Inc., New York.
355. **Wong, E. M., and B. Polisky.** 1985. Alternative conformations of the ColE1 replication primer modulate its interaction with RNA I. Cell **42**:959–966.
356. **Yakobson, E., and G. Guiney.** 1983. Homology in the transfer origins of broad host range IncP plasmids: definition of two subgroups of P plasmids. Mol. Gen. Genet. **192**:436–438.
357. **Yamaguchi, K., and Y. Masamune.** 1985. Autogenous regulation of synthesis of the replication protein in plasmid pSC101. Mol. Gen. Genet. **200**:362–367.
358. **Yamaguchi, K., and M. Yamaguchi.** 1984. The replication origin of pSC101: the nucleotide sequence and replication functions of the *ori* region. Gene **29**:211–219.
359. **Yamaguchi, K., and M. Yamaguchi.** 1984. The replication origin of pSC101: replication properties of a segment capable of autonomous replication. J. Gen. Appl. Microbiol. **30**:347–358.
360. **Yamaki, H., E. Ohtsubo, K. Nagai, and Y. Maeda.** 1988. The *oriC* unwinding by *dam* methylation in *Escherichia coli*. Nucleic Acids Res. **16**:5067–5073.
361. **Yavachev, L., and I. Ivanov.** 1988. What does the homology between *E. coli* tRNAs and RNAs controlling ColE1 plasmid replication mean? J. Theor. Biol. **131**:235–241.
362. **Yoon, K. H., and M. Y. Pack.** 1987. Construction of a shuttle vector between *Escherichia coli* and *Zymomonas anaerobia*. Biotechnol. Lett. **9**:163–168.
363. **Young, C., D. H. Bechhofer, and D. H. Figurski.** 1984. Gene regulation in plasmid RK2: positive control by *korA* in the expression of *korC*. J. Bacteriol. **157**:247–252.
364. **Young, C., R. S. Burlage, and D. H. Figurski.** 1987. Control of the *kilA* gene of the broad-host-range plasmid RK2: involvement of *korA*, *korB*, and a new gene, *korE*. J. Bacteriol. **169**:1315–1320.
365. **Young, C., A. S. Prince, and D. H. Figurski.** 1985. *korA* function of promiscuous plasmid RK2: an autorepressor that inhibits expression of host-lethal gene *kilA* and replication gene *trfA*. Proc. Natl. Acad. Sci. USA **82**:7374–7378.
366. **Zahn, K., and F. R. Blattner.** 1985. Sequence induced curvature at the bacteriophage λ origin of replication. Nature (London) **317**:451–453.
367. **Zahn, K., and F. R. Blattner.** 1987. Direct evidence for DNA bending at the lambda replication origin. Science **236**:416–422.
368. **Zipurski, S. L., and K. J. Marians.** 1980. Identification of two *E. coli* factor Y effector sites near the origins of replication of the plasmids ColE1 and pBR322. Proc. Natl. Acad. Sci. USA **77**:6521–6525.
369. **Zünd, P., and G. Lebek.** 1980. Generation time-prolonging R plasmids: correlation between increases in the generation time of *Escherichia coli* caused by R-plasmids and their molecular size. Plasmid **3**:65–69.
370. **Zverev, V. V., and I. A. Khmel.** 1985. The nucleotide sequences of the replication origins of plasmids ColA and ColD. Plasmid **14**:192–199.
371. **Zverev, V. V., N. P. Kuzmin, L. A. Zuyeva, E. I. Burova, A. A. Alexandrov, and I. A. Khmel.** 1984. Regions of homology in small colicinogenic plasmids. Plasmid **12**:203–205.
372. **Zyskind, J. W., and D. W. Smith.** 1986. The bacterial origin of replication, *oriC*. Cell **46**:489–490.