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ADVANCES IN PLASMID REPLICATION MECHANISM: A REVIEW

Franco G. Teves

Introduction

The replication of duplex helical DNA involves a complex, coordinated series of reactions in which new DNA chains are initiated and elongated on each strand of the duplex. Genetic studies of *E. coli* and its phage have identified a large number of gene products that participate in DNA replication and in some cases have identified the position of the replication origins. These genetic studies have provided the framework necessary for the development of multienzyme replication systems used to determine the sequences, proteins and enzymatic reactions required for DNA replication.

The steps used to replicate duplex DNA are indicated schematically in Figure 1. The figure emphasizes the similarity between replication on duplex eyes and on rolling circle intermediates. The figure is a combination of fragmented information from many systems which may not totally hold true for certain individual systems. Replication eyes are thought to be formed by the recognition and activation (unwinding) of a chromosomal origin site by specific proteins and the synthesis of an RNA primer for the leading strand (1A, Figure 1). Elongation by DNA synthesis of the leading strand proceeds with simultaneous unwinding of the duplex ahead of the replication fork (IB). Alternatively, an initiation site for leading strand synthesis can be created by a site-specific nick in one strand of the duplex (IIA). If the duplex DNA is circular, elongation from such a nick leads to a rolling circle intermediate, in which the 5' end of the displaced strand is at least sometimes linked to the growing 3' terminus (IIB). Elongation of the leading strand can be facilitated actively by a helicase such as the rep protein that unwinds in the 3' to 5' direction (IIA and IIB). Helicase direction is indicated by convention, as the direction of movement of the helicase on the strand that is not displaced. On the lagging strand, synthesis proceeds by the synthesis (I and IIB) and elongation (I and IIC) of RNA primers to form discontinuous fragments which, following removal of the primer and repair of the gap, are ligated to form a continuous strand. The priming systems that synthesize these short RNA fragments range from the single bacteriophage T7 gene 4 protein to at least 7 proteins in the *E. coli* primosome. In those systems in which it has been tested, the priming enzymes move 5' to 3' along the lagging strand template and have a nucleoside triphosphate-dependent 5' to 3' helicase activity to unwind the duplex DNA template ahead of the leading strand (I and IIC).

This paper is limited only to bacterial plasmid replication in species so far studied, with emphasis on regulatory mechanisms for initiation and elongation. Only the most recent articles on the subject were reviewed. However, older publications that shed light on certain issues were also cited.

Furthermore, this review aims to present a comparative analysis of plasmid replication regulation in specific groups of bacteria and to enumerate certain plasmids which may be used for replication and mapping studies.

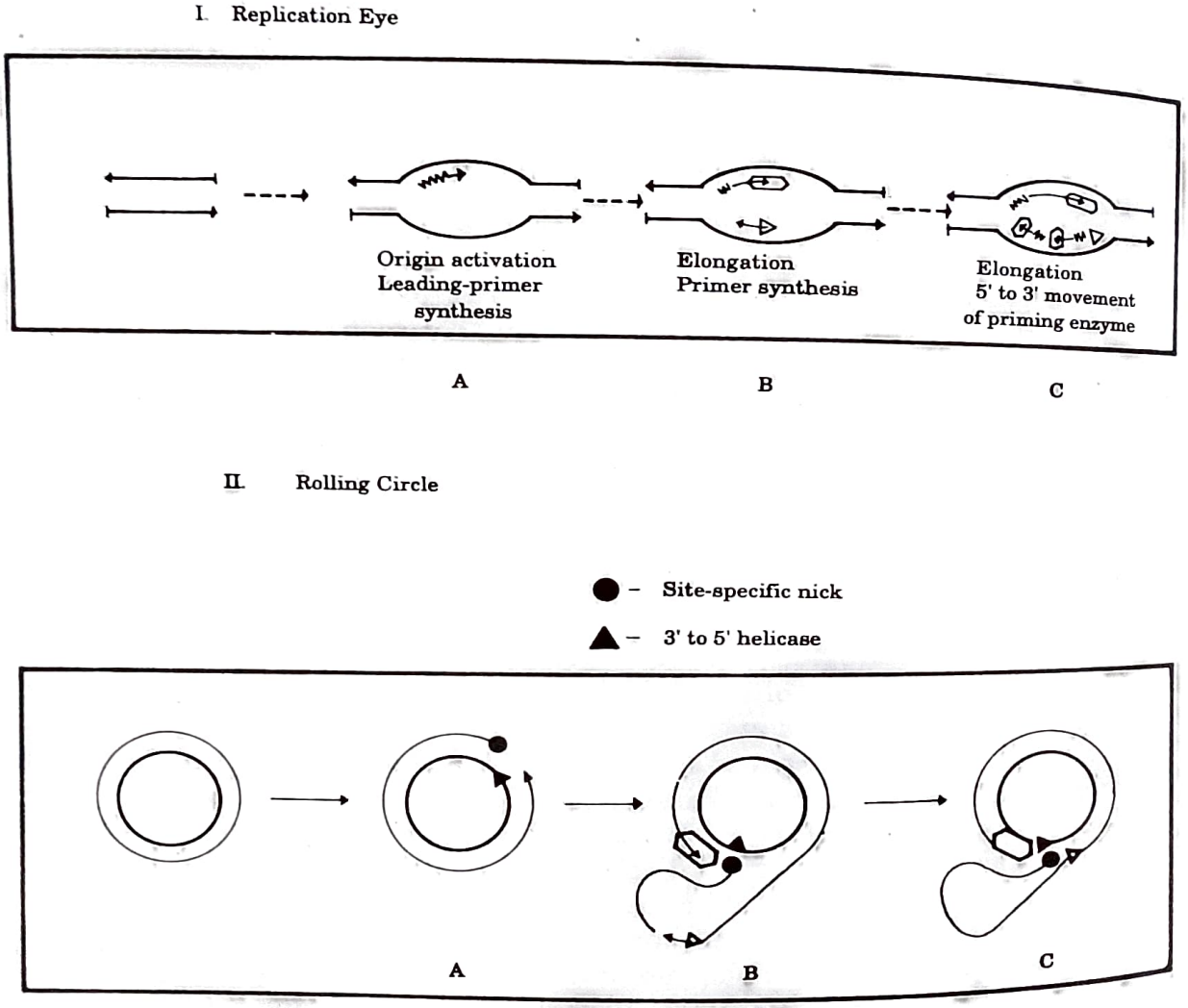

Figure 1. Duplex DNA replication via replication eye, or rolling circle intermediates (Nossal, 1983).

PLASMID INITIATION AND REPLICATION SYSTEMS

Co1E1 Initiation and Replication

Initiation

Nossal (1983) mentioned that while duplex circular DNA of the Co1E1 plasmid can be replicated in cell-free extracts of *E. coli* that do not carry the plasmid-encoded proteins, are not required, genetic studies have shown that RNA polymerase I and III, as well as the primosome proteins (dnaB, dnaC and dnaG) are required.

Initiation of replication starts at the H-strand (lagging strand) leading to the D-loop intermediate containing the newly synthesized L-strand (leading strand of about 6S (400 bases) according to Tomizawa (1975). Itoh and Tomizawa (1978 and 1982) reported that initiation on the H-strand occurs in vitro with RNA polymerase, DNA polymerase I and RNase H with DNA origin (RNA-DNA junction) occuring at the same 3 contiguous nucleotides used int he crude cell extract. RNase H increases the number of DNA chains initiated and specifically stimulates synthesis at the normal RNA-DNA junction. Gyrase enzyme is not required with supercoiled templates but increases the length of DNA products without increasing the number of chains initiated (Itoh and Tomozawa, 1978).

More recently, Hillenbrand and Staudenbauer (1982) developed a system for the initiation of Co1E1 DNA replication consisting of the following:

1. DNA polymerase I
2. "priming fraction" which co-purifies with RNA polymerase and DNA gyrase
3. "discriminating fraction" containing RNase H
4. other polypeptides whose size is about 20,000 D.

They found out that certain plasmids behave differently in the presence or absence of the discriminatory fraction. There is origin-specific synthesis of the amplifiable plasmids Co1E1, pBR322, RSF 1030 and C1oDF13 as judged by the position of the D-loops in the products. However, there is little synthesis of the non-amplifiable plasmids pSC101, pKN182, pSEO14 and pSC138. In the absence of the discriminatory fraction, there is random initiation of both sets of plasmids.

This system is almost completely inhibited by novobiocin. In extracts, Co1E1 replication is sensitive to gyrase inhibitors and mutations in gyrB (Gellert et al., 1976; Orr and Staudenbauer, 1981).

Itoh and Tomizawa (1980) constructed a small plasmid, pNT7, containing 812 bp surrounding the Co1E1 origin. Two transcripts were made from sites on opposite strands of this plasmid in a region 450-455 nucleotides upstream from the origin. This region is required for both replication and maintenance of the plasmid.

These two transcripts are:

1. transcript from the H-strand (RNA II) which the RNase cuts at the replication origin to make a 555-nucleotide primer and
2. second transcript, a 108 base RNA (RNA 1) which is read from the L-strand; this inhibits the synthesis of pNT7 DNA and prevents RNase H from cutting the primer transcript by interfering with the hybridization of the primer transcript to the DNA.

These authors proposed that the secondary structure of RNA II in regions upstream from the DNA origin (palindromic region of the H-strand transcript) influences primer formation by RNase H. RNA 1 from one plasmid inhibits the synthesis of that plasmid and other incompatible plasmids but does not inhibit the synthesis of closely related compatible plasmids.

A mechanism of replication similar to that of Co1E1 has been shown for the plasmids RSF1030, C1oDF13, pBR322 and p15A. Although there are differences in the sequences of these plasmids in the region determining the primer transcript, in each plasmid there is a potential set of three neighboring palindromes in the region specifying RNA I (Som and Tomizawa, 1982; Selzer et al., 1983).

Elongation

Elongation of the 6S L-strand in extracts is dependent on dnaE and dnaZ gene products. These defects can be complemented by pol III holoenzyme. L-strand elongation is blocked by antisera to dnaB but not by mutations in the dnaG primase.

It has been proposed by Staudenbauer et al. (1979) that dnaB may facilitate leading strand synthesis in addition to its role in the synthesis of primers, analogous to the helicase function of the phage T7 gene 4 and T4 gene 41 priming proteins.

Meanwhile, discontinuous synthesis of the new H-strand is dependent on dnaB, dnaC and dnaG proteins (Conrad and Campbell, 1979). The elongation of new L-strand on duplex super-coiled Co1E1 templates does not require dnaG protein but would require dnaB, dnaC and dnaG protein if copied on isolated H single strand (Boldicke et al., 1981).

The following sites have been identified on each strand of Co1E1 (Nomura and Ray, 1981; Boldicke et al., 1981):

1. rifampicin-resistant initiation site (rri site) and
2. protein n' (Y) ATPase.

The L-strand rri site acts to assemble the primosome for discontinuous synthesis of the lagging H-strand by the reactions described for OX174SS-- RF synthesis. It has been proposed that the H-strand rri site is where continuous L-strand synthesis by pol 1 stops and discontinuous synthesis of primosome-initiated synthesis by pol III begins or that it is required for the initiation of L-strand synthesis on the transferred H-strand during mobilization of the plasmid DNA (Marians et al., 1982).

There is conflicting evidence on the role of RNase III in the replication of plasmids related to Co1E1. Conrad and Campbell (1979) reported that plasmid RSF1030 synthesis was defective in preparations from *E. coli* with mutations in RNase III. However, Tomizawa and Itoh (1981) and Hillenbrand and Staudenbauer (1982) reported otherwise. In vivo Co1E1 replication is not affected by RNase III mutations (Ely and Staudenbauer, 1981).

When all conditions are not limiting, termination of replication will proceed to completion.

Host Gene-F Plasmid Replication Interactions in *Escherichia coli*

A more recent, specific control of plasmid replication via host gene interaction was reported by Wada and Yura (1984). It has been shown previously that replication of F, mini-F and some related plasmids is specifically inhibited in mafA mutants of *E. coli* K-12. However, mini-F mutants have been shown to overcome this replication inhibition. Such mutant plasmids (pom plasmids for permissive on maf) are obtained spontaneously or after hydroxylamine or Tn3 insertion mutagenesis.

Outstanding characteristics of these pom mutants are the following:

1. ability to replicate in maf A mutant bacteria
2. increased copy number
3. increased resistance to curing by acridine dye in the maf⁺host.

If Tn3 is inserted into the incC region of mini-F DNA (region involved in incompatibility, control of copy number and sensitivity to acridine dye), the above characteristics will show while other plasmids that carry the Tn3 outside this tandem repeat (incC region), remain wild type.

Most of the genes or regions directly involved in mini-F DNA replication have been localized in a short segment of about 2 kb. This segment contains:

1. ori-2 = origin of replication
2. repA = its product (a 29 kilodalton protein) is essential for DNA replication
3. copA = controls copy number
4. 9 tandem repeat sequences of 19 to 22 bases present in two clusters of opposite orientations (incB and incC) flanking the repA gene; these sequences are specifically involved in incompatibility and control of plasmid DNA replication.

Other host-specific functions involved in F replication (DNA polymerase III, RNA polymerase, dnaC gene product) are quite similar to those reported in part A of this paper.

Strict control of F plasmid of *E. coli* K-12 is exerted on the replication and partitioning of the plasmid into the two daughter cells so as to maintain basically a single copy per host chromosome.

Studies on the Regulation of Replication of Plasmids By Plasmid-encoded Products

As stated by Nossal (1983, see part A), plasmid-encoded proteins are not necessary in plasmid replication control in Co1E1. Dong et al. (1985) described how the gene dosage of the repA2 transcription repressor protein (plasmid-encoded) regulates transcription of the repA1 gene, a gene whose product is required for the initiation of DNA replication at the plasmid origin.

Transcription of the repA1 gene of the plasmid NR1 of the IncFII group is initiated at two promoters in the 1.1 kb Pst1 fragment (Figure 2) (Easton et al., 1981; Rownd et al., 1984). Transcription from the upstream promoter for RNA-CX is constitutive at a low level, while that from the downstream promoter for RNA-A is regulated by rep A2 repressor protein encoded by the 5' and of RNA-CX (Womble et al., 1985). The copB protein of plasmid R1, although substantially different in amino acid sequence from the rep A2 protein of NR1 (Ryder et al., 1981), appears to play a similar role in the regulation of R1 transcription (Light and Molin, 1982; Molin et al., 1981). RepA3 which lies between repA2 and rep A1 genes (Rosen et al., 1980) and is transcribed by RNA-CX and RNA-A has been identified but no function has been ascribed to it yet.

At the wild type copy number of NR1, transcription from the regulated promoter (RNA-A) is 96% repressed but substantial derepression occurs when the copy number falls below the normal value. At or above normal plasmid copy number, the basal level of repA1 mRNA is provided by transcription from the

constitutive upstream promoter (RNA-CX). An excess of repA2 protein can completely repress transcription from the RNA-A promoter but has very little effect on copy number or incompatibility.

Dong et al. (1985) concluded that the level of repressor protein in the cells is gene-dosage dependent and that the relationship between the rate of transcription from the regulated promoter and the repressor protein concentration is a function of gene dosage. The authors have shown a quantitative relationship between repA2 protein concentration in the cell and the amount of transcription from the RNA-A promoter.

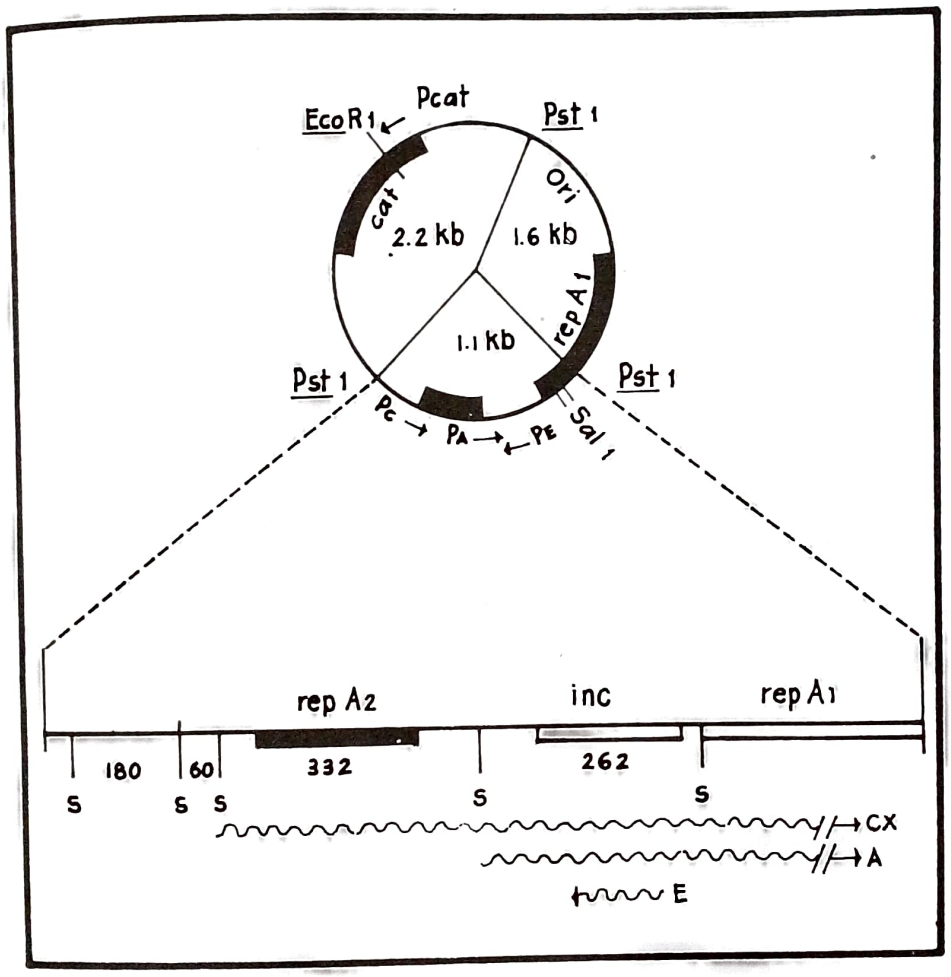

Figure 2. Structure of NR1 minireplicator plasmids composed of 3 Pst1 restriction fragments (See text).

Another plausible work was reported by Schreiner et al. (1985) on the interaction between kil and kor genes (plasmid genes) and their effects on the expression of the essential replication gene trfA in the RK2 plasmid (Inc group). The broad host-range plasmid RK2 encodes multiple host-lethal kil determinants (kilA, kilB1, kilB2, kilC) which are controlled by RK2-specified kor functions (korA, korB, korC).

Kil and kor are determinants with significant effects on RK2 replication control. KorA and korB inhibit the replication of certain RK2 derivatives unless plasmid replication is made independent of the RK2 gene trfA. On the other hand, kilB 1 exerts a strong positive effect on this interaction. If the target plasmid is defective in kilB1, sensitivity to korA and korB is enhanced at least a hundred-fold. A mutant RK2 derivative resistant to korA and korB was found by Schreiner et al. (1985) to have fused a new promoter to trfA indicating that the targets for korA and korB are at the 5' end of trfA gene. They constructed a trfA-lacZ fusion and found that synthesis B-galactosidase is inhibited by korA and korB. Therefore, korA, korB and kilB1 influence RK2 replication by regulating trfA expression. Previously, other workers identified two plasmid loci that are essential for RK2 replication (Meyer and Helsinki, 1977; Figurski and Helsinki, 1979; Thomas et al., 1980; Thomas et al., 1981; and Stalker et al., 1981). These loci are:

1. oriV = origin of unidirectional replication which is activated by a diffusible function encoded elsewhere on the plasmid.
2. trfA = encodes 3 polypeptides, at least one of which is functional in replication.

It was suggested by Schreiner et al. (1985) that the complex network of kil and kor determinants is part of a replication control system for RK2.

Why should replication control be so complex for IncP plasmids (Figure 3)? The reason given by them is in relation to the plasmids' extraordinary host range among Gram negative bacteria. The network may provide a level of adaptability to allow the plasmids to respond to differences in the various hosts and thus ensure appropriate levels of trfA product for stable maintenance.

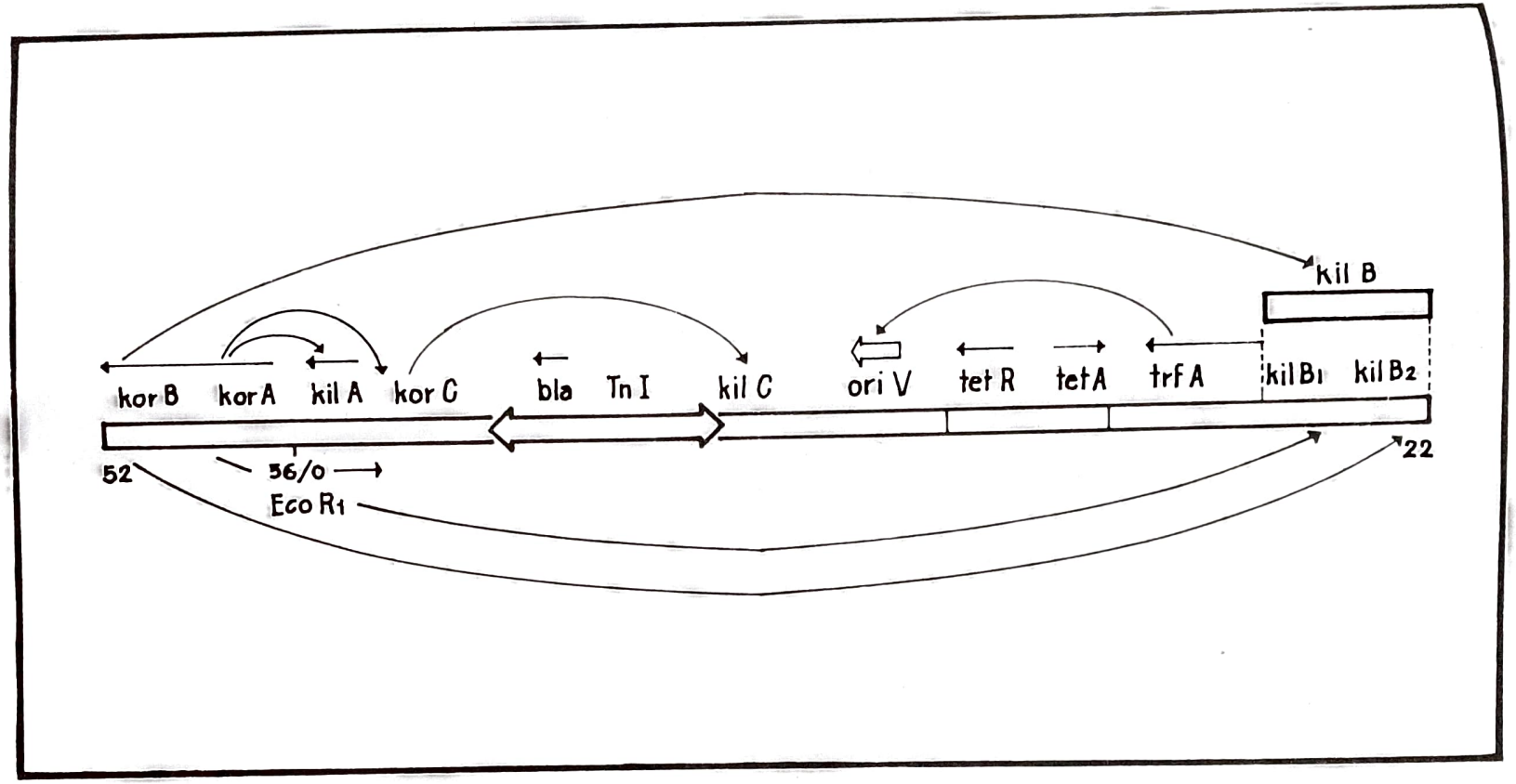

Figure 3. Genetic interactions of plasmid Rk2. A 26-kb segment of RK2 is displayed with coordinates representing distance in Kb clockwise from the unique EcoR1 site on the RK2 circular map. Positive interactions are depicted by red arcs and negative interactions with blue arcs. Positions of the tet genes are from Waters et al. (1983).

Conclusion

The prokaryotic DNA replication systems have, in common, a DNA polymerase that is higly processive either as a holoenzyme or when assisted by accessory proteins, single-stranded DNA-binding proteins, helicases and enzymes making short RNA primers to remove single-stranded regions and unwind the duplex ahead of the fork, and to initiate discontinuous synthesis on the lagging strand.

Helicase activity in the priming enzyme helps coordinate leading and lagging-strand synthesis. These systems meet the varying needs of replication of bacteriophage, plasmid and stable chromosomes. The system that can be most finely regulated is used for chromosomal replication in bacteria coupled tightly to cell division.

A very complex and puzzling replication control mechanism is exemplified by the interactions of kil and kor genes in IncP plasmids as previously shown. This complex control mechanism may be related to the extraordinary host-range among gram-negative bacteria for IncP plasmids. This could provide a level of adaptability to respond to differences in various hosts ensuring stable maintenance in host cells.

Interactions between plasmid genes and plasmid-encoded products are among the most recent significant findings in microbial genetics. Such discoveries will surely lead to the development of new genetic models to solve equally puzzling problems in the genetics of eukaryotic organisms, especially man.

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