Published in final edited form as:
Mol Microbiol. 1998 August ; 29(3): 671–675.

Are minichromosomes valid model systems for DNA replication control? Lessons learned from *Escherichia coli*

Tsuneaki Asai[1,3,*], **David B. Bates**[2,3], **Erik Boye**[4], and **Tokio Kogoma**[1,2,3,#]

[1] Department of Cell Biology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131

[2] Department of Biology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131

[3] Cancer Center, University of New Mexico Health Sciences Center, Albuquerque, NM 87131

[4] Department of Cell Biology, Institute for Cancer Research, Montebello, 0310 Oslo, Norway

Summary

Initiation of chromosome replication is a key event in the life cycle of any organism. Little is known, however, about the regulatory mechanisms of this vital process. Conventionally, the initiation mechanism of chromosome replication in microorganisms has been studied using plasmids in which an origin of chromosome replication has been cloned, rather than using the chromosome itself. The reason for this is that even bacterial chromosomes are so large that biochemical and genetic manipulations become difficult and cumbersome. Recently, the combination of flow cytometry and genetic methods, where modifications of the replication origin are systematically introduced onto the chromosome, has made possible detailed studies of the molecular events involved in the control of replication initiation in *Escherichia coli*. The results indicate that requirements for initiation at the chromosomal origin, *oriC*, are drastically different from those for initiation at cloned *oriC*.

Keywords

DnaA box; DNA replication; Histone-like proteins; Initiation; *oriC*; Transcriptional activation

Initiation at Cloned *oriC*

An *oriC*-containing fragment was first isolated from the chromosome as an autonomously replicating sequence (ARS) and the fragment was subsequently subcloned into a plasmid carrying the Col E1-type replication origin. Since the ColE1 origin, but not *oriC*, requires the *polA* gene product (DNA polymerase I) for initiation, the ARS activity of an *oriC* fragment cloned in such a hybrid plasmid (*oriC* plasmid) can be determined by assaying the replication ability of the plasmid in *polA* mutant cells. This hybrid plasmid made it possible to analyze the effects of many mutations, including lethal ones, introduced into *oriC* and proved its usefulness in defining the minimal *oriC* fragment required for ARS activity (245 bp, Figure 1). Until recently, all mutational analyses of *oriC* have been carried out with plasmids, allowing identification of a number of cis-acting regulatory elements within and near *oriC*. This led to the widely accepted concept that regulation of initiation at *oriC* is complex and involves a

Corresponding Author: Erik Boye, Department of Cell Biology, Institute for Cancer Research, Montebello, 0310 Oslo, Norway, Phone, 47-22934256; Fax, 47-22934580, erik.boye@labmed.uio.no.
*Present address: Department of Molecular Biology, Massachusetts General Hospital, Boston, MA02114
#Dr. Kogoma passed away on Oct. 9, 1997, and the other authors dedicate this paper to him and his scientific achievements.

number of different elements in the *oriC* region (reviewed in Messer and Weigel, 1996). Cloning of *oriC* was essential for Kornberg and collaborators to develop a reconstituted replication system containing only purified proteins (see Kornberg and Baker, 1992). With this system, the functions of many cis- and trans-acting components were characterized in detail. Therefore, *oriC* plasmid replication has served as a leading model not only for bacterial chromosomes but also for other replicons including eukaryotic viruses and chromosomes.

The first and critical step of initiation at *oriC* is DNA strand separation, which is performed by the initiator protein, DnaA (see Kornberg and Baker, 1992; Skarstad and Boye, 1994; Messer and Weigel, 1996). After binding to its five cognate 9 bp sequences (DnaA boxes, Figure 1) within *oriC* DnaA forms a nucleoprotein complex around which *oriC* DNA is wrapped. Under conditions in which DNA is negatively supercoiled and ATP is bound to DnaA, the DNA duplex becomes unwound at the three AT-rich 13mer sequences (Figure 1). Next, the DnaB helicase is loaded onto the unwound region by the aid of DnaA and DnaC, forming a prepriming complex. The helicase can unwind the DNA duplex in both directions and priming of new strands and bidirectional chain elongation follow. It should be emphasized that these are the minimal factors and reactions required to make initiation occur *in vitro*. Several other factors have been shown to affect initiation efficiency, such as topoisomerases, histone-like proteins and transcription (see below).

oriC plasmids share many important features of replication initiation with the chromosome (see Messer and Weigel, 1996); both of them replicate bidirectionally, require transcription and *de novo* protein synthesis, and they respond similarly to many host mutations. When the cells contain multiple copies of *oriC* all of them fire in synchrony, whether they are located on the chromosome or on a plasmid (Skarstad et al., 1986; Helmstetter and Leonard, 1987). Based on these and other observations, *oriC* plasmids have long been thought to be faithful models for the chromosome and results obtained with the plasmids have been taken to reflect what is occurring at a chromosomal *oriC*. However, on some important points the results are contradictory and controversial. For example, the ARS activity of cloned *oriC* changes depending on various factors such as the type of cloning vector, the cloning sites, the size of the cloned *oriC* fragment, the orientation of *oriC* with respect to the vector, and the host strain. More importantly, recent direct measurements of the activity of chromosomal *oriC* are at variance with analogous plasmid experiments, compelling a reassessment of the validity of using cloned ARS elements as models of chromosomal origins.

The Sequence Required for Chromosome Replication

The smallest element having ARS activity, as determined by deletion analysis of *oriC* plasmids, the minimal *oriC* (Figure 1), was considered the smallest origin sequence required for chromosome replication. However, the minimal *oriC* is unable to support plasmid replication when cloned into certain plasmids (Asai et al., 1990). It turned out that an additional AT-rich sequence (Figure 1), immediately to the left of the minimal *oriC*, is necessary for the ARS activity in these plasmids and that the sequence can be functionally replaced by a promoter directed away from *oriC*. These results imply that the boundaries of the minimal *oriC* are conditional rather than absolute, and suggest that the minimal origin sequence necessary for chromosome replication may be different from the smallest ARS.

The importance of the different DnaA boxes at cloned *oriC* has been demonstrated by mutational analyses. In particular, the significance of DnaA box R4 (Figure 1) was emphasized in experiments where the distance between R4 and the rest of the minimal *oriC* was varied. The ARS activity of *oriC* was abolished unless the distance was changed by one helical turn (Woelker and Messer, 1993), suggesting that a specific phasing of R4 with respect to other element(s) within *oriC* is critical for formation of the initiation complex at cloned *oriC*.

As the first attempt to address directly the significance of the different *oriC* elements for chromosome initiation, Bates et al. (1995) recently evaluated the requirement for the DnaA box R4. In contrast to the above results, a chromosomal *oriC* was shown to be initiated in the absence of R4. Cells carrying an R4-deletion mutation on the chromosome are viable and chromosome replication still depends on DnaA and presence of the mutant *oriC*, giving the first demonstration that the sequence requirement for the minimal *oriC* differs dramatically between chromosomal and cloned *oriC* sites. Flow cytometric analyses show that initiation at *oriC* lacking R4 is inefficient and that initiation synchrony is lost, suggesting that the initiation complex is formed more effectively on the chromosome in the presence of R4.

The more stringent requirements of cloned *oriC* for R4 could be explained by competition for DnaA protein between wild type and mutant *oriC* sites. A strain replicating its chromosome from an integrated R1 origin, inserted into and thereby inactivating *oriC*, allows limited replication of an *oriC* plasmid carrying an R4 deletion, but only when DnaA protein is overproduced (Langer et al., 1996). In this strain, other *oriC* mutations on plasmids are also suppressed, at least partially, and the suppression is more pronounced by overproduction of DnaA, suggesting that the mutant origins require higher concentrations of DnaA for initiation than does the chromosomal wild type *oriC*. Thus, plasmids replicating from such mutant *oriC* sites may be only partially able to maintain a replication efficiency compatible with stable plasmid maintenance. A prediction of this model is that when an R4-deleted *oriC* is placed on the chromosome, it should support chromosome replication, because there is no competition for DnaA. This has indeed been observed (Bates et al., 1995).

An alternative explanation for the discrepancy in the requirement for box R4 is that the minimal origin sequence necessary for initiation, in any given case, is determined in part by the superhelical structure of *oriC* DNA. In support of this, initiation at a chromosomal *oriC* carrying a deletion of R4 requires transcription near *oriC* (Bates et al., 1997) and transcription is known to alter the local superhelicity. The following evidence suggests that chromosomal and cloned *oriC* sites likely exist in different topological states. (i) The two sites show differences in the requirements for histone-like proteins, which change the structure of *oriC* DNA (see below). (ii) Certain *oriC* plasmids are unable to transform mutant cells with decreased negative DNA supercoiling (Leonard et al., 1985). The latter observation indicates that cloned *oriC* is more sensitive to DNA relaxation than chromosomal *oriC* and suggests that R4 may become more important when template supercoiling is lowered. This idea is consistent with the *in vitro* observation that deletion of R4 severely inhibits initiation in a reconstituted replication system where the number of free supercoils has been reduced by high amounts of the histone-like protein HU (Bramhill and Kornberg, 1988).

The Role of Histone-like Proteins in Chromosome Replication

The ability of DnaA to induce duplex opening at the 13mers *in vitro* is modified by several factors, such as temperature, the density of free supercoils (those not constrained by DNA binding proteins), and the histone-like proteins HU, IHF, and Fis (Baker and Kornberg, 1988; see below). Binding of these proteins induces DNA bending and helps form higher order DNA structures in many different physiological processes (Schmid, 1990). At low concentrations, HU or IHF assists DnaA in duplex opening by facilitating formation of the *oriC*-DnaA complex (Skarstad et al, 1990). DNA bending may also promote initiation directly by reducing the energy necessary for strand separation. Cells deficient in both HU and IHF are viable (Kano et al., 1991), demonstrating that neither of them is essential for initiation from chromosomal *oriC*, although flow cytometric analyses indicate that they are necessary for synchronous initiation (Boye et al., 1992; Jaffé et al., 1997). On the other hand, *oriC* plasmids are unable to replicate in cells lacking both HU and IHF (Kano et al., 1991), showing that the

requirements of transacting factors as well are different between chromosomal and cloned *oriC* sites.

Fis binds strongly to *oriC* between DnaA boxes R2 and R3 (Figure 1), where it induces DNA bending (Gille et al., 1991). The data on the function of Fis in initiating chromosomal and plasmid copies of *oriC* are also contradictory. Several lines of evidence suggest that *oriC* plasmid replication requires Fis *in vivo*. (i) *oriC* plasmids transform *fis* mutants with significantly reduced frequencies at 37 and 42°C (Gille et al., 1991;Filutowicz et al., 1992). (ii) *oriC* plasmids are poorly maintained in *fis* mutants even at low temperatures (Filutowicz et al., 1992), suggesting that Fis is a component of the replication machinery at all temperatures. (iii) Base changes in the Fis binding site inactivate cloned *oriC* (Roth et al., 1994). In contrast, Fis is dispensable for initiation of chromosome replication. *fis* mutants grow reasonably well under standard laboratory conditions (Filutowicz et al., 1992) and flow cytometric analyses show that the DNA/mass ratio is only slightly reduced (Bates et al., 1997). In *fis* null mutants, DNA synthesis stops upon a shift from 32 to 44°C (Filutowicz et al., 1992), suggesting that, as is the case for *oriC* plasmid replication, chromosome replication requires Fis at high temperatures. To our knowledge, Fis is essential for chromosome replication under normal growth conditions only in a mutant strain that lacks the DnaA box R4 on the chromosome (Bates et al., 1995). However, Fis is required to maintain initiation synchrony (Boye et al., 1992). This regulatory role of Fis may depend on its ability to prevent duplex opening at the 13mers, an ability recently demonstrated *in vitro* (Hiasa and Marians, 1994;Wold et al., 1996).

Transcriptional Activation of Chromosomal *oriC*

The idea that one or more transcriptional events independent of any protein synthesis are required for initiation of chromosome replication arose from early experiments demonstrating that rifampicin, an inhibitor of RNA polymerase, prevents initiations at a time when protein synthesis is no longer required (see Messer and Weigel, 1996). On the chromosome, *oriC* is located between two transcriptional units, the *gidAB* operon and the *mioC* gene (Figure 1). Based on the findings that *oriC* plasmids require both of these transcriptions for efficient replication (see below), a great deal of attention has been directed towards elucidating the roles of *gid* and *mioC* transcription in initiation. Given that the protein products of these genes are dispensable for initiation, two possible roles have been postulated for these transcriptions. First, the RNA synthesized may serve as primers for DNA synthesis. Second, the RNA transcript may activate an otherwise inert *oriC*, for which several different mechanisms have been proposed. It should be noted that these two possibilities are not mutually exclusive.

In reconstituted replication systems, transcription by RNA polymerase is not essential for initiation and RNA primer synthesis can be efficiently carried out by DnaG primase alone. Transcription is necessary to activate initiation only under unfavourable conditions for origin unwinding, including reduced levels of supercoiling, low temperature, or high levels of HU protein (Baker and Kornberg, 1988). Transcriptional activation has been shown to occur *in vitro* through formation of an R-loop. The position and orientation of promoters with respect to *oriC* is rather irrelevant for the activation. Thus, both *gid* and *mioC* transcriptions could activate *oriC* via the same mechanism. A GC-rich clamp between an R-loop and the 13mers inhibits transcriptional activation, suggesting that helix instability generated by an R-loop is propagated to the 13mers, thereby stimulating strand opening (see Skarstad et al., 1990; and references therein).

Unlike *in vitro* systems, transcription is essential *in vivo* for initiation of *oriC* plasmid replication. However, a specific transcriptional event required for initiation has not yet been identified; both *gid* and *mioC* transcriptions are dispensable for *oriC* plasmid replication. Even

though these transcriptions are not essential, they greatly increase the copy number, and thereby stability, of *oriC* plasmids. This phenomenon has been observed with most *oriC* plasmids so far analyzed, including an *oriC* plasmid that contains relatively long (more than 2 kb) chromosomal sequences at both sides of *oriC* (Bates et al., 1997). The precise mechanism(s) of the activation of initiation by these transcriptions is yet to be elucidated.

Surprisingly, when on the chromosome, *oriC* seems to have no need for *gid* or *mioC* transcription for efficient initiation. This was first shown in experiments where several *mioC* mutations that had previously been shown to affect *oriC* plasmid replication, were introduced onto the chromosome and the effects of the mutations on chromosome replication were analyzed by flow cytometry (Løbner-Olesen and Boye, 1992). Under a variety of growth conditions, all parameters measured (cell mass, DNA/mass, number of origins per cell, and timing of initiation) were the same for wild type and *mioC* mutant cells. This work was recently extended by showing that deletion of both *mioC* and *gid* promoters has little, if any, effect on chromosome replication (Bates et al., 1997). Even in cells deficient in IHF and/or Fis, deletion of these promoters showed only subtle effects on the DNA/mass ratio. This is in stark contrast to the observation that *mioC* transcription is essential for *oriC* plasmid replication in cells lacking IHF (Kano et al., 1991). Only when the chromosomal *oriC* was severely impaired by deletion of the DnaA box R4, was transcription from either *mioC* or *gid* required to activate initiation. Again, a marked difference between the requirements of cloned and chromosomal *oriC* has been demonstrated.

The above experiments demonstrated that transcription of *gid* or *mioC* do not represent the rifampicin sensitive step in initiation. We suggest three reasonable explanations for the effect of rifampicin on initiation: First, it is possible that transcription is not actually required for chromosome replication but that rifampicin blocks initiation directly. Conceivably, rifampicin could arrest initiation by forming an inhibitory complex with RNA polymerase that is fixed within or near *oriC*. This possibility is suggested by the finding that rifampicin blocks DNA synthesis in a reconstituted system utilizing DnaG primase, but only in the co-presence of RNA polymerase holoenzyme (Ogawa et al., 1985). Second, it is possible that transcription originating within *oriC* is responsible for activating initiation. Unfortunately, these promoters are embedded in the complex sequence of *oriC*, making mutational analysis extremely difficult. On the basis of genetic data, DnaA protein has been suggested to modify a fraction of the RNA polymerases by direct interaction, which might activate *oriC* by initiating transcription within *oriC* (Hansen, 1995). Third, the rifampicin effect may not be specific to a single promoter, but a result of the global shut down of all transcription on the chromosome. Studies have shown that the sedimentation rates of *E. coli* nucleoids are greatly decreased upon treatment with rifampicin (Pettijohn and Hecht, 1973). This suggests that rifampicin treatment results in drastic changes in the overall topology of the chromosome, which might render *oriC* incapable of duplex melting and/or binding the required initiation proteins.

Concluding Remarks

The evidence presented above indicates that many cis- and trans-acting components required for *oriC* plasmid replication are dispensable for chromosome replication. It is possible that the actual mechanism of initiation on the chromosome may be rather simple: As long as the chromosome contains sufficient negative supercoils, DnaA alone may be able to trigger origin unwinding without the aid of any auxiliary components such as histone-like proteins and specific transcriptional events. Some requirements for initiation at cloned *oriC* might be laboratory artifacts created by moving *oriC* into small plasmids which are apparently less capable, as compared to the chromosome, of adapting to changes in DNA supercoiling. The idea is consistent with the fact that most components that are required only for cloned *oriC* affect DNA topology. It is also possible, on the other hand, that these auxiliary components

may become essential for chromosome replication under certain environmental conditions. In fact, regulation of chromosome replication is disturbed, even under ideal laboratory growth conditions, by mutations in some of the auxiliary components. It has been demonstrated that various environmental signals such as temperature, osmolarity, nutrients, pH, and availability of oxygen change the density of DNA supercoiling *in vivo*. The environmentally induced changes are of a similar magnitude to those induced by gyrase inhibitors or mutant topoisomerases, which are known to have significant effects on DNA replication and other cellular processes. Therefore, it is not unlikely that initiation of chromosome replication is achieved without any auxiliary components only under conditions that provide proper DNA supercoiling of the chromosome. It is now clear that chromosomal and cloned *oriC* sites have different requirements for initiation in the same cell. Thus, the fundamental question of how *E. coli* controls DNA synthesis, and in particular how this process is adapted to environmental changes, can only be addressed by analyzing replication initiation with chromosomal *oriC*. However, data accumulated with the *oriC*-plasmid model system will undoubtedly be helpful in pursuing this question. In addition, a comparison of plasmid-based and chromosomal origins should be considered in studies of other bacteria and of yeasts, where the definition of ARS activity is heavily based on plasmid and minichromosome model systems.

Acknowledgments

This work was supported by Grant GM22092 from the National Institute of Health and by Grant BIR-9218818 from the National Science Foundation to T.K. and by a grant from the Norwegian Cancer Society and the Norwegian Research Council to E.B.

References

Asai T, Takanami M, Imai M. The AT richness and *gid* transcription determine the left border of the replication origin of the *E. coli* chromosome. EMBO J 1990;9:4065–4072. [PubMed: 2249664]

Baker TA, Kornberg A. Transcriptional activation of initiation of replication from the *E. coli* chromosomal origin: An RNA-DNA hybrid near *oriC*. Cell 1988;55:113–123. [PubMed: 2458841]

Bates DB, Asai T, Cao Y, Chambers MW, Cadwell GW, Boye E, Kogoma T. The DnaA box R4 in the minimal *oriC* is dispensable for initiation of *Escherichia coli* chromosome replication. Nucl Acids Res 1995;23:3119–3125. [PubMed: 7667087]

Bates DB, Boye E, Asai T, Kogoma T. The Absence of effect of *gid* or *mioC* transcription on the initiation of chromosomal replication in *Escherichia coli*. Proc Natl Acad Sci USA 1997;94:12497–12502. [PubMed: 9356478]

Boye, E.; Lyngstadaas, A.; Løbner-Olesen, A.; Skarstad, K.; Wold, S. Regulation of DNA Replication in *Escherichia coli*. In: Fanning, E.; Knippers, R.; Winnedler, EL., editors. DNA Replication and the Cell Cycle. Berlin: Springer-Verlag; 1992. p. 15-26.

Bramhill D, Kornberg A. Duplex opening by DnaA protein at novel sequences in initiation of replication at the origin of the *E. coli* chromosome. Cell 1988;52:743–755. [PubMed: 2830993]

Filutowicz M, Ross W, Wild J, Gourse RL. Involvement of FIS protein in replication of the *Escherichia coli* chromosome. J Bacteriol 1992;174:398–407. [PubMed: 1309527]

Gille H, Egan JB, Roth A, Messer W. The FIS protein binds and bends the origin of chromosomal DNA replication, *oriC*, of *Escherichia coli*. Nucleic Acids Res 1991;19:4167–4172. [PubMed: 1870971]

Hansen FG. Reinitiation kinetics in eight *dnaA*(Ts) mutants of *Escherichia coli*: rifampicin-resistant initiation of chromosome replication. Mol Microbiol 1995;15:133–140. [PubMed: 7752888]

Helmstetter CE, Leonard AC. Coordinate initiation of chromosome and minichromosome replication in *Escherichia coli*. J Bacteriol 1987;169:3489–3494. [PubMed: 3301802]

Hiasa H, Marians KJ. Fis cannot support *oriC* DNA replication *in vitro*. J Boil Chem 1994;269:24999–25003.

Jaffé A, Vinella D, D'Ari R. The *Escherichia coli* histone-like protein HU affects DNA initiation, chromosome partitioning via MukB, and cell division via MinCDE. J Bacteriol 1997;179:3494–3499. [PubMed: 9171392]

Kano Y, Ogawa T, Ogura T, Hiraga S, Okazaki T, Imamoto F. Participation of the histone-like protein HU and of IHF in minichromosomal maintenance in *Escherichia coli*. Gene 1991;103:25–30. [PubMed: 1879696]

Kornberg, A.; Baker, TA. DNA Replication. New York: Freeman; 1992.

Langer U, Richter S, Roth A, Weigel C, Messer W. A comprehensive set of DnaA-box mutations in the replication origin, *oriC*, of *Escherichia coli*. Mol Microbiol 1996;21:301–311. [PubMed: 8858585]

Leonard AC, Whitford WG, Helmstetter CE. Involvement of DNA superhelicity in minichromosome maintenance in *Escherichia coli*. J Bacteriol 1985;161:687–695. [PubMed: 2981821]

Løbner-Olesen A, Boye E. Different effects of *mioC* transcription on initiation of chromosomal and minichromosomal replication in *Escherichia coli*. Nucleic Acids Res 1992;20:3029–3036. [PubMed: 1620598]

Messer, W.; Weigel, C. Initiation of chromosome replication. In: Neidhardt, FC.; Curtiss, R.; Ingraham, JL.; Lin, ECC.; Low, KB.; Magasanik, B.; Reznikoff, WS.; Riley, M.; Schaechter, M.; Umbarger, HE., editors. Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology. Washington, D.C.: American Society for Microbiology; 1996. p. 1579-1601.

Ogawa T, Baker TA, van der Ende A, Kornberg A. Initiation of enzymatic replication at the origin of the *Escherichia coli* chromosome: contributions of RNA polymerase and primase. Proc Natl Acad Sci USA 1985;82:3562–3566. [PubMed: 2987933]

Pettijohn DE, Hecht R. RNA molecules bound to the folded bacterial genome stabilize DNA folds and segregate domains of supercoiling. Cold Spring Harbor Symp Quant Biol 1973;38:31–41. [PubMed: 4598638]

Roth A, Urmoneit B, Messer W. Functions of histone-like proteins in the initiation of DNA replication at *oriC* of *Escherichia coli*. Biochimie 1994;76:917–923. [PubMed: 7748935]

Schmid MB. More than just “histone-like” proteins. Cell 1990;63:451–453. [PubMed: 2121364]

Skarstad K, Baker TA, Kornberg A. Strand separation required for initiation of replication at the chromosomal origin of *E. coli* is facilitated by a RNA-DNA hybrid. EMBO J 1990;9:2341–2348. [PubMed: 1694129]

Skarstad K, Boye E. The initiator protein DnaA: evolution, properties, and function. Biochim Biophys Acta 1994;1217:111–130. [PubMed: 8110826]

Skarstad K, Boye E, Steen HB. Timing of initiation of chromosomal replication in individual *Escherichia coli* cells. EMBO J 1986;5:1711–1717. [PubMed: 3527695]

Woelker B, Messer W. The structure of the initiation complex at the replication origin, *oriC*, of *Escherichia coli*. Nucleic Acids Res 1993;21:5025–5033. [PubMed: 8255756]

Wold S, Crooke E, Skarstad K. The *Escherichia coli* Fis protein prevents initiation of DNA replication from *oriC in vitro*. Nucleic Acids Res 1996;24:3527–3532. [PubMed: 8836178]

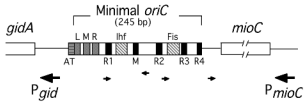

Figure 1.

The minimal *oriC and its flanking regions. The positions of the five DnaA boxes, R1-R4 and M, the 13-mer repeats L, M, and R, the AT-rich sequence (AT), and binding sites for IHF and Fis are indicated. Arrows represent the location and direction of promoters.*