Extended Function of Plasmid Partition Genes: the Sop System of Linear Phage-Plasmid N15 Facilitates Late Gene Expression

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Received 21 December 2007/Accepted 10 March 2008

The mitotic stability of the linear plasmid-prophage N15 of Escherichia coli depends on a partition system closely related to that of the F plasmid SopABC. The two Sop systems are distinguished mainly by the arrangement of their centromeric SopB-binding sites, clustered in F (sopC) and dispersed in N15 (IR1 to IR4). Because two of the N15 inverted repeat (IR) sites are located close to elements presumed (by analogy with phage λ) to regulate late gene expression during the lytic growth of N15, we asked whether Sop partition functions play a role in this process. In N15, a putative Q antiterminator gene is located 6 kb upstream of the probable major late promoter and two intrinsic terminator-like sequences, in contrast to λ, where the Q gene is adjacent to the late promoter. Northern hybridization and lacZ reporter activity confirmed the identity of the N15 late promoter (p52), demonstrated antiterminator activity of the Q analogue, and located terminator sequences between p52 and the first open reading frame. Following prophage induction, N15 mutated in IR2 (downstream from gene Q) or IR3 (upstream of p52) showed a pronounced delay in lysis relative to that for wild-type N15. Expression of ir3⁻p52: lacZ during N15 wild-type lytic growth was strongly reduced relative to the equivalent ir3⁺ fusion. The provision of Q protein and the IR2 and SopAB proteins in trans to ir3⁻p52: lacZ increased expression beyond that seen in the absence of any one of these factors. These results indicate that the N15 Sop system has a dual role: partition and regulation of late gene transcription during lytic growth.

The genetic determinants that govern the active partition of low-copy-number plasmids are generally devoted to that function alone. The deletion of sopABC, parABS, or parMRC from F, P1, and R1 miniplasmids (to cite well-studied cases) results in no immediate phenotype except the instability of their plasmids (1, 11, 26). We have found that the partition functions of phage N15, which are closely related to the well-known SopABC system of plasmid F (14, 28), exercise a distinct regulatory activity unrelated to plasmid maintenance. The activity is the subject of this paper.

N15 is a temperate coliphage with sequence, structural, and physiological similarities to lambdoid phages. But unlike λ, which integrates, the N15 prophage is a linear plasmid with covalently closed ends (33, 38; for a review, see reference 29), a property it shares with two more recently described plasmids, pY54 of Yersinia enterocolitica (16) and ϕKO2 of Klebsiella oxytoca (7), as well as with the linear replicons of Borrelia burgdorferi (17) and one of the two chromosomes of Agrobacterium tumefaciens (12). The prophage state is maintained by a lambda-like primary immunity system (24). The repressor gene, cB (Fig. 1), is flanked by operators (not shown), both leftward, upstream of the replication gene (repA), and rightward, within a promoter region that controls the expression of gene 39, homologous to cro, and gene 40, homologous to that of the antiterminator of late gene transcription, Q. Further rightward is a putative late promoter upstream of an operon encoding lambda phage-like lysis and virion proteins. Sequences resembling Rho-independent terminators are located between the promoter and the first gene, an arrangement characteristic of late promoters regulated by Q-mediated antitermination (34). Like λ, N15 prophage is induced as part of the SOS response, although the mechanism, inactivation by an induced antirepressor rather than by RecA-mediated auto-cleavage, appears to differ (25, 31).

Mitotic stability (partition) of N15 prophage depends on multiple inverted repeats (IR) similar in sequence to the sopC centromere sites of F, a SopB protein which binds to them to create a partition complex, and a Walker-box ATPase, SopA, needed for segregating N15 molecules carrying the partition complexes into the daughter cells-to-be (28). The N15 proteins also resemble those of F in acting as autoregulators of their own sopAB operon, with SopA as the primary repressor and SopB as the corepressor (30). However, the N15 centromere elements (IR1 to IR4) differ from those of circular bacterial replicons in that rather than constituting a tandem array adjacent to their operon, like sopC, they occupy distinct sites close to elements which potentially regulate lytic development (14, 28). In particular, IR2 is situated 193 bp downstream of the putative Q gene, and IR3 is 23 bp upstream of a probable late promoter, p52, from which the lysis gene operon would be transcribed (Fig. 1). The location of the IRs suggests that the N15 Sop system could do double duty as a regulator of lytic development and as an executor of plasmid partition.

Before pursuing this idea, we considered it necessary to settle the uncertain status of the putative late gene expression control elements, until now assigned only by analogy and homology. N15 and lambdoid phages appear to differ significantly with respect to late gene regulation. First, N15 has no analogue of the λ N antitermination system. Second, the putative Q gene...
of N15 overlaps the putative cro gene, while in lambdoid phages the Q gene is typically 5 to 10 kb downstream of cro, at the distal end of the early right operon; this position plays a role in the timing of late gene expression. Third, although gene 40 mutants behave like λQ mutants, lysogenizing but failing to grow lytically (35), the transcription of gene 40 appears incompatible with the role of late gene regulator. It is almost as strong from the prophage as during lytic growth, even though transcription of the late prophage operon(s) from gene 52 on is weak or undetectable (32). Thus, the functional equivalence of N15 gene 40 and the Q genes of lambdoid phages was unclear.

We addressed this issue as well as the question of whether the IR sites and the complexes formed on them by the binding of SopB intervene in late gene expression and so influence lytic development. In this paper, we present evidence that the gene 40 product and elements upstream of gene 52 do indeed act as expected for late gene regulators and that the Sop partition system facilitates their action. The N15 Sop system is thus bifunctional.

**MATERIALS AND METHODS**

**Bacterial strains, media, and growth conditions.** The ΔlacZ strain of *Escherichia coli* K12, MC1061 (6), was the host for reporter gene experiments and for phage N15 propagation, and strain DH10B (13) was the recipient for cloning experiments. Bacteria were grown in LB broth (22) or on LB agar at 37°C, supplemented as appropriate with ampicillin (final concentration, 100 μg/ml), chloramphenicol (20 μg/ml), kanamycin (50 μg/ml), and arabinose (0.1%).

**Bacteriophages and plasmids.** Bacteriophage N15 was described by Ravin and Shulga (33). N15 coordinates are based on the complete sequence (GenBank accession number, AF064539). Bacteriophage N15ir3 was obtained by cleaving circularized N15 prophage DNA at its unique NcoI site in IR3, filling it with Klenow polymerase, and recircularizing it with DNA ligase. N15ir2 was obtained by recombineering (9). MC1061 N15ir2 cells carrying pKD46 (8) were induced to produce λ-red recombinase by growth in LB with 0.2% arabinose, washed, electroporated with oligonucleotide IR2mut (complementary to the lagging strand at IR2), diluted, and plated on LB agar. Eleven of 230 colonies screened by PCR using primers IR2mutscr and Q15-1 tested positive; two of these were sequenced and one was confirmed to be the expected N15ir2 mutant. The mutation does not alter the coding properties of a hypothetical open reading frame, g40.1, in which IR2 lies.

**Plasmids for expression of N15 genes.** sopAB was constitutively expressed from pDAG216 and pDAG242, and sopB from pDAG434 (28, 30). CB repressor and AntA antirepressor were produced from pCA15 and pCA12, respectively.
A Q antitermination production plasmid, pBAD-Q, was constructed by inserting N15 g60 DNA, amplified using primers Q-L and Q-R, between the EcoRI and HindIII sites of expression vector pBAD24 (15). Expression of β-galactosidase was carried out using DNA polymerase (amplification grade; Sigma) and RNase-free DNase (Promega, New England BioLabs, Fermentas, SibEnzyme), and T4 polynucleotide kinase (New England BioLabs), restriction enzymes (Promega, New England BioLabs, Fermentas, SibEnzyme), and RNase-free DNase (Promega, New England BioLabs). A Q promoter-β-galactosidase reporter plasmid was constructed by cloning downstream oligonucleotides IR3L-1, IR3L-2, IR3L-3, and IR3L-4. The 3′ ends were cleaved with EcoRI and BamHI and inserted to the downstream corresponding sites upstream of lacZ in the transcription reporter vector pRS551 (36) to yield plasmids pNR251 (N15 nucleotides [nt] 41084 to 41290; no terminator), pNR253 (nt 41084 to 41265; T1), and pNR252 (nt 41084 to 41410; T1, T2, and g52 codons 1 to 3). pNR254 is the equivalent of pNR252 derived from N15r3. The p52::lacZ fusions were transferred by recombination and lysogenization to the att site of MC1061, using λ88 (36), to yield strains NR427, NR429, NR428, and NR430. From pRK551, these pNR plasmids inherit a terminator, trpt, just upstream of lacZ. Derivatives of NR251, NR252, and NR253 with an R7a3 III cleavage site in place of trpt were made by replacing their BamHI-SacI (trp-lac-Z) fragments with the equivalent fragment of P7611/1/2 (23) to form pNR325, pNR326, and pNR327, respectively. The corresponding λ88 lysogens are DLT2450, DLT2451, and DLT2452.

Enzymes and oligonucleotides. DNA polymerase I Klenow fragment T4 DNA ligase, T4 polynucleotide kinase (New England BioLabs), restriction enzymes (Promega, New England BioLabs, Fermentas, SibEnzyme), and RNase-free DNase (amplification grade; Sigma) were used according to the manufacturers’ recommendations. PCR amplification was carried out using DNA polymerase Pfu (Promega) or Phusion (Finzymes) and oligonucleotides (5′ to 3′) as follows: IR3-L, CGGAGATCTCATATTACATTCCATCGTCTCGGCTGCGGAACACCCCTTGTGTTTCCAGATAAGCTGGTGGACGCA; IR2mutscr, GGAGTATTCTTCATATTCCATCGTCTCGGCTGCGGAACACCCCTTGTGTTTCCAGATAAGCTGGTGGACGCA; IR2mutscr, GGAGTATTCTTCATATTCCATCGTCTCGGCTGCGGAACACCCCTTGTGTTTCCAGATAAGCTGGTGGACGCA; and GALE1, ATGAGAGTTCTGGTTACCGGT. Amplification was performed using PCR random priming kit (MegaPrime; New England BioLabs). Fragments of N15 comprising IR3 and the downstream oligonucleotides IR3L-2, IR3L-4, and IR3L-3. The fragment ends were cleaved with EcoRI and BamHI and inserted between the corresponding sites upstream of lacZ in the transcription reporter vector pRS551 (36) to yield plasmids pNR251 (N15 nucleotides [nt] 41084 to 41290; no terminator), pNR253 (nt 41084 to 41265; T1), and pNR252 (nt 41084 to 41410; T1, T2, and g52 codons 1 to 3). pNR254 is the equivalent of pNR252 derived from N15r3. The p52::lacZ fusions were transferred by recombination and lysogenization to the att site of MC1061, using λ88 (36), to yield strains NR427, NR429, NR428, and NR430. From pRK551, these pNR plasmids inherit a terminator, trpt, just upstream of lacZ. Derivatives of NR251, NR252, and NR253 with an R7a3 III cleavage site in place of trpt were made by replacing their BamHI-SacI (trp-lac-Z) fragments with the equivalent fragment of P7611/1/2 (23) to form pNR325, pNR326, and pNR327, respectively. The corresponding λ88 lysogens are DLT2450, DLT2451, and DLT2452.

**FIG. 2.** Termination of transcription initiated at p52. (A) Hybridization of a 32P-labeled oligodeoxynucleotide representing the 5′ end of p52-initiated mRNA to a Northern blot of total RNA extracted at the times (min) shown after induction of MC1061 (N15) pCA12 and from exponentially growing cultures of MC1061 carrying plasmids pRS551 (−) and pNR251, 252, and 253. The markers are 32P-labeled HindIII fragments of φX174 DNA. (B) Specific activity of β-galactosidase in exponentially growing cultures of MC1061 derivatives that carry the p52-terminator-lacZ fusions of pNR251, 252, 253, and 254 (open bars), and of pNR325, 326, and 327 (shaded bars) integrated at att. The values shown are normalized to those of NR427 (1,740 Miller units) and DLT2450 (7,920 units), which do not include the putative terminator sequences.

**Northern analysis.** RNA was extracted from culture samples taken into ethanol-phenol (37) by the hot-phenol method (2) and further purified by digestion with RNase-free DNase (Sigma; amplification grade). Twenty micrograms of each RNA were fractionated by denaturing 6% polyacrylamide gel electrophoresis and electroblotted to Hybond N+ membranes (Amersham). Blotted RNAs were prehybridized in 0.5 M Na-phosphate (pH 7.0), 7% sodium dodecyl sulfate, 1 mM Na3-EDTA, and 100 µg/ml denatured herring sperm DNA prior to the addition of 20 pmol of 5′-32P-labeled oligodeoxynucleotide 52-RNA5′ (3′ × 106 dpm/pmol) and further incubation for 4 h. After being washed at room temperature (final solution, 0.03 M Na-phosphate-0.1% sodium dodecyl sulfate), the membranes were exposed to phosphorimagery screens, and the images were processed with MultiGauge software (Fujiﬁlm).

**Q mRNA slot blot hybridization.** RNAs prepared as above were denatured and applied to Hybond N+ membranes by standard methods, using a slot blot apparatus (5 µg per slot). The membranes were incubated and treated essentially as above, with hybridization probes consisting of DNA fragments amplified from pBAD-Q and E. coli chromosomal DNA, using Q15-1/2 and GALE1/2, respectively, and 32P-labeled using a random priming kit (Megaprime; New England BioLabs).

**Western blot analysis.** SopA protein was detected as described previously (28), except that the transfer buffer was Tris-glycine and the antibody was a polyclonal antiserum (kindly provided by S. Hiraga) to allow normalization using cross-reacting E. coli proteins.

**N15 one-step growth curves.** Exponential cultures of MC1061 N15 lysogens carrying pCA12 were treated with arabinose (final concentration, 0.4%) to induce prophage (time zero). At intervals, one 10-µl sample was added to 1 ml iced-cold 20 mM MgSO4, and plated directly (after appropriate dilution) to estimate the number of PFUs, and a second was added to 1 ml 20 mM MgSO4, 0.1 mg/ml lysozyme, and 5 mM KCN, incubated 5 min at 37°C, vortexed briefly with 50 µl CHCl3, and plated to estimate the number of intracellular PFU. Plating was done in 0.6% agar-LB on LB agar, using MC1061 as the indicator.

**RESULTS**

Initiation and premature termination of transcription upstream of gene 52. To ascertain whether the putative promoter and terminators shown in Fig. 1 act as such in vivo we ﬁrst performed Northern blot hybridization of total RNA puriﬁed from cultures of an induced N15 lysogen. Hybridization with a 32P-labeled oligodeoxynucleotide complementary to the ﬁrst 30 nt of the presumed transcript (Fig. 2A) revealed two small RNA...
species. They were first detected 20 min after induction and increased to a constant level by 30 min. The shorter species, at \(~53\) nt, is just the length expected for initiation at the presumed promoter and terminator within the \(U\) run that follows the \(T1\) inverted repeat. The longer one, at \(~100\) nt, was shorter than the \(~165\)-nt species expected for termination at \(T2\). It could indicate termination at an imperfect inverted repeat sequence, which we dubbed \(T_{app}\) (Fig. 1), although this repeat does not precede a \(U\) run characteristic of intrinsic terminators; processing of a longer transcript has not been ruled out as the source of the \(~100\) nt species.

To facilitate further analysis, we made transcriptional reporters consisting of the IR3 centromere site, the presumed \(p52\) \(~35\) and \(~10\) motifs, and various stretches of downstream N15 DNA fused to \(lacZ\) on a pBR322-based vector (Fig. 1). RNA extracted from cells carrying these plasmids contained a short species that comigrated with the \(~53\)-nt transcript seen in the induced lysogen when the \(T1\) element was present on the plasmid (pNR252, pNR253) but not when it was absent (pNR251), consistent with the promoter and terminator functions assigned above. Termination further downstream was barely perceptible, whether \(T_{app}\) was present (pNR252) or not (pNR253). Termination at \(T1\) might have reduced the level of longer transcripts below the detection threshold. The strong band in the pNR251 sample, migrating just ahead of the \(~100\)-nt species, is probably an artifact: it is of just the size/\(H11011\) band in the pNR251 sample, migrating just ahead of the longer transcripts below the detection threshold. The strong (pNR253).

Termination at \(T1\) might have reduced the level of upstream of the source of the tors; processing of a longer transcript has not been ruled out as any contribution of variable stability and translation efficiency renders the 5'-ends of all transcripts identical, thus minimizing differences in \(p52: lacZ\) expression. The relative \(\beta\)-galactosidase activities were essentially unchanged (Fig. 2B), although the absolute levels rose four- to fivefold, presumably due to removal of the \(trpt\) element.

The data shown in Fig. 2B thus confirm the activity of the promoter and strongly suggest that the main factor limiting late gene transcription is termination at \(T1\) and \(T_{app}\).

The product of N15 gene 40 is the transcription antiterminator. The action of the N15 putative gene 40 product, a \(\lambda\) gene homologue, as an antiterminator of late gene transcription had been suggested by the inability of N15 40 mutants to grow lytically (35). A corollary of this observation would be failure of N15 to lysogenize when gene 40 expression is forced. We placed gene 40 under control of the arabinose-inducible \(F_{BAD}\) promoter as plasmid pBAD-Q and analyzed the effect of its expression on N15 propagation. N15 formed clear plaques on MC1061 carrying pBAD-Q in the presence of arabinose, indicating that gene 40 expression suppresses lysogenization. When arabinose was added to an exponentially growing culture of an N15 lysogen of MC1061/pBAD-Q to activate gene 40, but the prophage was left uninduced, the culture lysed 3 h later, presumably reflecting production of the holin, lysozyme, and Rz protein products of late operon genes 53 to 55.1 (32) without significant phage production (data not shown). A reverse transcription-PCR analysis by Ravin et al. (32) had revealed low-level transcription of these genes from N15 prophage, which presumably provides enough antiterminator substrate for accumulation of the lytic enzymes. These results are consistent with the gene 40 product being the antiterminator of late gene transcription.

To confirm the antiterminator activity of gene 40 protein, we introduced pBAD-Q into the \(p52: lacZ\) reporter strains (above) and measured the influence of gene 40 expression on \(\beta\)-galactosidase activity (Table 1). Induction of gene 40 resulted in

### Table 1. \(\beta\)-galactosidase activity expressed by \(p52: lacZ\) transcriptional fusions

<table>
<thead>
<tr>
<th>Regulatory factor</th>
<th>Plasmida</th>
<th>(\beta)-galactosidase activity relative to that of the indicated strainbd</th>
<th>NR427 (1740 MU)</th>
<th>NR429</th>
<th>NR428</th>
<th>NR430</th>
<th>DLT2450 (7980 MU)</th>
<th>DLT2451</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noneb</td>
<td>None</td>
<td>(1)</td>
<td>0.14</td>
<td>0.024</td>
<td>0.026</td>
<td>(1)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>CB</td>
<td>pCA15</td>
<td>0.90</td>
<td>ND</td>
<td>0.025</td>
<td>ND</td>
<td>ND</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>Q</td>
<td>pBAD-Q</td>
<td>1.41</td>
<td>0.93</td>
<td>0.61</td>
<td>ND</td>
<td>0.91</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>SopB</td>
<td>pDAG434</td>
<td>0.47</td>
<td>ND</td>
<td>0.014</td>
<td>0.021</td>
<td>ND</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>SopB and SopAe</td>
<td>pDAG242</td>
<td>0.23</td>
<td>0.031</td>
<td>0.008</td>
<td>0.022</td>
<td>ND</td>
<td>0.0038</td>
<td></td>
</tr>
</tbody>
</table>

a In the presence of empty expression vectors or pBAD-Q without arabinose, \(\beta\)-galactosidase activity levels remained within 10% of those in strains without plasmids.
b This line recapitulates the data shown in Fig. 2B to aid comparison.
c The effect of SopA might be underestimated because the copy number of pDAG242 (pSC101 low-copy mutant vector) is about one-fifth that of pDAG434 (p15A vector).
d ND, not determined.
a 25-fold increase in lacZ transcription in NR428 and DLT2451, to nearly half the level of transcription in strains NR427 and DLT2450, which lack terminators. It also raised the level of β-galactosidase 1.4-fold in NR427 itself. This modest increase was abolished by removal of tpt (DLT2450), implying that the product of gene 40 enables limited bypass of the vector terminator and that about 30% of the stimulation in the NR strains is due to this factor. The upstream regulatory system, consisting of the major N15 repressor CB, had essentially no effect on p52-initiated transcription, in accordance with the absence from this region of any sequence resembling the known CB-binding sites (24). These data, taken together with the circumstantial evidence cited above, are best explained as antitermination by gene 40 protein, which is henceforth named Q.

**Influence of the IR3 centromere on p52 activity and phage growth.** The presence of IR3 20 bp upstream of p52 raised the possibility that it affects promoter activity. The data in Table 1 show this to be the case. Production of SopB reduced transcription to about half that seen without it (NR427 and 428), unless the IR3 was mutated (NR430), in which case transcription was not affected. This modest repression appeared to be unrelated to termination of transcription, since it was seen in NR427 lacking terminators as well as in NR428. It could indicate diminished initiation of transcription, since N15 SopB is known to silence promoters near to its binding sites (14). The presence of SopA reduced transcription still further, possibly by enhancing SopB-mediated silencing, as was also seen with SopA and SopB of the F plasmid (D. Lane, unpublished results).

To evaluate the significance of SopB-IR3 interactions, we examined the propagation of an N15 mutant carrying an inactive IR3. The mutation had no major consequences at a gross level; N15ir3– forms lysogens and achieves productive lysis, forming turbid plaques on a lawn of strain MC1061. However, the level of spontaneous phage production by MC1061 N15ir3– lysogens was half that of the wild-type lysogen (data not shown). Therefore, we examined the lytic development of N15ir3+ and ir3–, adding arabinose to induce the corresponding prophages as described above. Whole-lysis following induction took about 15 min longer for the ir3– mutant than for the N15 wild-type (Fig. 4A). This delay was due less to the rate of mature phage production, which trailed only slightly behind that of the wild-type (Fig. 4C), than to slower phage release, which began about 5 min later than that of the wild-type and rose more slowly (Fig. 4B). This difference implies that lysis operon (genes 52 to 58) expression is the primary focus of IR3-mediated regulation.

These data concur with the reduced expression of lacZ from p52 in the ir3 mutant reporter strain NR430 observed under identical conditions (Fig. 3). Taken together, these results imply that SopB-IR3 complexes play a significant role in optimizing late gene expression through antitermination. Note that this activity is distinct from, and contrary to, the moderate, terminator-independent silencing of p52 exerted by SopB (and SopA) seen in the data of Table 1.

**Involvement of IR2 and Sop proteins in p52 activity.** The position of another centromere site, IR2, 190 bp downstream from the Q gene (Fig. 1), suggests that it also might influence Q antiterminator function. This suggestion was strengthened by the observation that the mutation of IR2 resulted in a delay in lysis following prophage induction identical to that observed for the IR3 mutant (Fig. 4D). We again measured the effect on p52 activity of Q protein produced from pBAD-Q, this time in parallel with that from the equivalent plasmid carrying the downstream IR2 site as well (pBAD-Qir2). The results are
The Western blot of Fig. 5C showed that in cells carrying pDAG216, SopA was present at 0.64- and 0.37-fold its concentration at 30 and 40 min, respectively, after N15 prophage induction. It should also be noted that in these experiments, IR2 is in \textit{trans} to the p52:***lacZ*** fusion, where it might be less effective than in its \textit{cis} position in N15. SopB and IR2, even when provided less than optimally, appear able to stimulate transcription from p52 and thus to contribute to late gene expression and lytic development.

**DISCUSSION**

The results presented here show that in contrast to the unique role of the F plasmid Sop system, the closely related Sop system of N15 not only assures stability of the prophage but also optimizes lytic development of vegetative phage. In N15, centromeric sites (IRs) are not fixed at a single location, as in F, but are dispersed, which has presumably provided the opportunity for involvement in other processes. In this respect the N15 Sop system is like many of its homologues in bacterial chromosomes, which are often seen to serve other purposes through dispersed centromeric (\textit{parS}) sites. The ParA/ParB protein duo of \textit{Streptomyces coelicolor} regulates sporulation (19), Soj/Spo0J of \textit{Bacillus subtilis} regulates both sporulation and initiation of replication (18, 27), ParA/B of \textit{Caulobacter crescentus} is essential for cell division control (10), and ParA/B of \textit{Pseudomonas aeruginosa} appears to play a role in cell physiology (3). This functional versatility and centromere dispersal is rare among plasmids so far studied but is a notable feature of the IncP-1 group. KorB, the SopB/ParB analogue of the IncP-1 archetype, RK2, not only forms partition complexes on scattered centromere-like sites but also binds to these sites to repress the promoters of genes involved in plasmid transfer, vegetative replication, and stable maintenance (20). Moreover, binding of KorB to these sites is modulated through interaction with other RK2 regulatory proteins. KorB and its partition partner, IncC2, are taxonomically more closely related to chromosomal analogues than to plasmid systems (4), and it will be interesting to see if the chromosomal Par proteins share this regulatory finesse. The obvious candidate for modulator of N15 SopB regulatory action is SopA, and enhancement of SopB-IR3 mediated silencing by SopA (Table 1) is preliminary evidence for such a role.

The participation of the Sop system in N15 propagation appears not to be limited to regulation of late gene antitermination. In unpublished work, we have found that the activity of a weak late promoter (of virion gene transcription) close to the IR4 centromere can be reduced in the presence of Sop proteins but is unaffected by Q protein and that the IR1 centromere in the \textit{repA} gene mediates a stimulatory effect of the Sop proteins on N15 prophage replication. We do not yet understand either the mechanism or the purpose of these regulatory interventions of the Sop system. Involvement in replication complicates attempts to directly analyze SopB’s role in antitermination. Hence, in the work reported here, the role of SopB in the stimulation of late transcription was inferred rather than demonstrated.

Although partition and transcriptional regulation appear to be very different processes, it is likely that the same properties of the N15 partition functions are involved in both, and it is interesting to consider how N15 exploits them. Late gene expression during lytic development of N15 is superficially simi-
lar to that of λ. A late operon comprising two major gene clusters, which encode the proteins needed for lysis and for virion formation, are controlled by intrinsic transcription terminators and are activated by a mechanism that suppresses termination. But the details differ. In λ, synthesis of the late antiterminator Q is itself dependent on antitermination by N protein and hence does not occur in the lysogenic state.

Nevertheless, late gene transcription from the N15 prophage is negligible, suggesting that an additional control limits the amount or activity of N15 Q protein to below that needed to suppress termination. One limiting factor could be the 6-kb distance between gene 40 and the target of its product, the p52 late promoter. Unlike λ Q, whose gene is immediately upstream of its site of action at the late promoter, N15 Q would leave the cotranslating ribosome relatively distant from the late promoter and might be diluted beyond its effective concentration. If so, some mechanism must compensate for this separation when late gene expression is needed during lytic growth.

Sop protein levels begin to increase shortly after p52-initiated transcription is first detected (Fig. 2A and 5C), at just the time they would be needed to assist antitermination.

In apparent contradiction, the data of Table 1 show that the proximity of IR3 sensitizes p52 to the repressive effect of SopB, as could be expected from the known silencing activity of this protein (14). One approach to reconciling these observations is to propose that the IR3-SopB complex acts one way in the prophage and another way during lytic growth. In the prophage, its predominant function could be to trigger SopB spreading and so to silence p52, accounting for the inability of the N15 prophage to activate late transcription despite the presence of Q protein, whose action might in any case be diminished by the distance of its gene from its target. In contrast, during the latent period, its role would be to improve the efficiency with which Q finds its target (qut site) and interacts with RNA polymerase (40). An attractive mechanism for the latter activity is pairing between SopB molecules bound to IR2 and IR3 on the same N15 molecule (in cis), analogous to the pairing of complexes in trans widely thought to initiate plasmid segregation. Such pairing would effectively deliver Q to its qut site, so enhancing its effective concentration at p52. The exercise of both spreading and looping alternatives has also been invoked to explain regulatory activities of RK2 KorB (5).

We have observed internal pairing in vivo (B. Dorokhov, N. V. Ravin, and D. Lane, manuscript in preparation), which, together with the increase seen in antitermination,

![FIG. 5. Involvement of Sop proteins and centromere IR2 in antitermination. (A) lacZ expression from p52 in NR428 (open bars) and DLT2451 (shaded bars) carrying pBAD24 (lane 1), pBAD-Q (lanes 2 and 4), or pBAD-Qir2 (lanes 3 and 5) with (lanes 4 and 5) or without (lanes 2 and 3) the SopABN15-producing F miniplasmid, pDAG216. Specific activities are normalized to those of NR427 and DLT2450, without terminators (lane 6). “chr.” in the box at the top, illustrates the relevant elements; brackets around T2 signify lack of evidence for terminator function, and the filled hairpin denotes the trp terminator in the vector. ara, arabinose. (B) Q mRNA concentrations in the cells analyzed for panel A. Duplicate 5-μg aliquots of total RNA were applied in slots and hybridized with a radioactively labeled Q gene probe and a probe to galE taken as a constitutively expressed internal control. (C) Western blot of total proteins from MCI061 N15+ pCA12 induced at 0 min by the addition of arabinose and from MCI061 with or without (−) pDAG216. The cross-reacting host protein was used as an internal standard for quantification.](#/)
upon supplementing Q protein with IR2 and the Sop proteins (Fig. 5), implies that this is a plausible model. We are currently testing it.

The N15-like linear prophages δK02 and pYS4 carry different numbers of IR elements, but in both cases, IRs are present near their presumed Q genes and late promoters. Although we do not know whether the three prophages represent a succession or convergent evolution, they provide the raw material for addressing such intriguing questions as which function came first and was co-opted to the other, partition or regulation? A case for which this may have been answered is that of VirB, a positive regulator of virulence gene expression in Shigella flexneri. Although VirB is not a partition protein—it no longer has a ParA-like partner, for instance—its sequence and specific DNA-binding site show significant similarities with those of ParB of the P1 prophage-plasmid. Turner and Dorman (39) have recently suggested that the function of VirB has undergone an evolutionary shift, from partition to regulation. N15 SopB and its IR sites might be on the same path.

ACKNOWLEDGMENTS

We thank Thomas Linn for kindly providing pTL617, Kounthea Phok and Clouet-Doutor for assistance with Northern blotting, Taisia Strakhova for technical advice, and Jean-Yves Bouet for critical reading of the manuscript.

This work was supported by an INTEAS Young Scientists Fellowship (04-83-3332) awarded to N.V.R. and RFBR grant 07-04-01078 and Agence National de Recherche grant 06-BLAN-0280-01 to D.L.

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