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Overlapping genes at the DNA primase locus of the large plasmid ColI

Graham J.Boulnois*, Brian M.Wilkins* and Erich Lanka+

*Department of Genetics, University of Leicester LE1 7RH, UK, and +Max-Planck-Institut für Molekulare Genetik, Abteilung Schuster, D-1000 Berlin 33 (Dahlem), FRG

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ABSTRACT

The *sog* gene of the large plasmid ColI*drd-1* has previously been shown to encode a DNA primase and a smaller antigenically related polypeptide. Genesis of these two products has been examined using Sog+ recombinant plasmids. Effects of amber mutations, isolated after *in vitro* mutagenesis, and deletions into or within *sog* suggest that the smaller polypeptide is a separate translation product which is encoded by DNA specifying the C-terminal region of the larger protein. Under control of the *lac* promoter, synthesis of both polypeptides is reduced when transcription is repressed. These findings imply that transcription of *sog* yields a single transcript which is translated from two initiation sites.

INTRODUCTION

Plasmids of the Iα incompatibility group, typified by ColI (94 kilobases, kb), are large DNA molecules that can promote their transfer between bacteria by conjugation (1). They encode a DNA primase which *in vitro* can synthesize oligoribonucleotides on single-stranded DNA of small phages to act as primers for DNA polymerase III of *Escherichia coli* (2). The enzyme is the product of a transfer gene called *sog* and it is thought to initiate plasmid DNA synthesis during conjugation (3). A remarkable feature of plasmid primase is its ability to substitute for *E.coli* primase, the product of *dnaG*, in initiating synthesis of nascent (Okazaki) DNA fragments during discontinuous replication of the bacterial chromosome (4). This property simplifies manipulation of the gene because Sog+ plasmids can be identified by their ability to suppress the temperature-sensitive *dnaG3* mutation.

Recent studies with ColI*drd-1*, which is derepressed for expression of transfer genes, and a Sog+ recombinant plasmid (pLG215) have shown that *sog* specifies two antigenically related proteins of apparent molecular weight 240,000 and 180,000 (4). Only the larger polypeptide has been shown to have primase activity. The function of the smaller protein is unknown.

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This report concerns the genesis of the two *sog* polypeptides. We have considered the possibilities of a relationship either as precursor and product of proteolytic cleavage or as products of a pair of overlapping genes which are translated in the same reading frame.

The approach has involved isolation of deletion and amber mutants of Sog^+ recombinant plasmids which contain about 8 kb of ColI*drd-1* inserted in the chloramphenicol acetyltransferase (*cat*) gene of the pBR325 vector. The effect of mutations on the size of *sog* proteins was examined using bacterial minicells. Expression of *sog* was explored by inverting the ColI-derived DNA with regard to the *cat* promoter and also by coupling this fragment to the *lac* promoter in a second vector. The results are consistent with the model that the *sog* locus contains two overlapping genes which are translated in the same reading frame from separate initiation sites on single mRNA. To our knowledge, this is the first report of such genetic complexity in a large plasmid.

MATERIALS AND METHODS

Bacterial strains and plasmids

BW84 (*dnaG3 leu thyA deoB rpsL* $ColI^r$) and plasmids ColI*drd-1*, pLG211, pLG214 and pLG215 have been described previously (3, 4). DS410 (*sup*^{o} *rpsL*) was used as the minicell-producing strain (5). pPM63 was constructed and provided by P.A. Meacock. It is a derivative of pAT153, a deletant of pBR322 (6), and was made by insertion of the 95 bp *Alu*1 fragment carrying the *lacUV*^{5} promoter between the single *Eco*RI and *Bam*HI cleavage sites in a manner analogous to that described for pPM50 (7). F'*lacI*^{q} *L8 lacZ* :: Tn*9* was obtained from G. Churchward in strain Q80C (8) and transferred to BW84 and DS410 by conjugation.

General procedures

Plasmid-containing strains were routinely grown in the presence of tetracycline (7.5 μg/ml) or ampicillin (25 μg/ml). Plasmid purification, DNA restriction, ligation, agarose gel electrophoresis and electron microscopy of heteroduplex DNA molecules were as described previously (4). Restriction endonucleases were purchased from Bethesda Research Laboratories except *Bgl*II which was a gift from J.M. Varley. The procedure of Boseley *et al.* (9) was used to end-label *Bgl*II-cleaved pLG228. Purification of minicells, SDS polyacrylamide gel electrophoresis and the solid phase immunoassay involving the method of Towbin *et al.* (10) have been described (4). Autoradiographs were scanned and the areas under peaks calculated

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using a Beckman DU-8 computing spectrophotometer.

Colony formation, DNA synthesis and DNA primase activity

Colony-forming ability, expressed as the fractional yield at the indicated temperatures, was measured using overnight, nutrient broth-grown cultures and prewarmed nutrient agar. Both media contained thymine (20 μg/ml). DNA synthesis at 41°C was measured using bacteria in a salts-glucose-Casamino acids medium (3) containing deoxyguanosine and thymine (3 μg/ml). Overnight cultures were diluted 20-fold and shaken at 31°C for 90 min. Following 5 min at 41°C, ^{3}H-thymine was added to 320 mCi/mmol. Plasmid DNA primase was assayed as before using viral strands of phage fd as template in a receptor extract containing rifampicin (2,4). 1 unit of primase incorporated 1 μmol of dTMP in the conditions used.

Hydroxylamine-induced Sog^{-} mutants

Purified pLG211 was treated *in vitro* with hydroxylamine according to Eichenlaub (11). Following transformation of BW84, Ap^{r} transformants were selected at 30°C and allowed to grow into small colonies when they were replica plated to 40°C. The temperature-sensitive strains, shown to be Ap^{r}Tc^{r}, were examined for deficiency of DNA synthesis at 41°C, using ^{3}H-thymine incorporation, and for lack of plasmid primase activity. This procedure allowed identification of Sog^{-} plasmids. Strains harbouring these plasmids were then screened for the presence of amber mutations following lysogenisation with λNM634 (λ(*srI* 1-2)$^{\nabla}$ :: *supF* :: *imm21 nin5 shn6*^{O}; 12), provided by W.J. Brammar. Purified lysogens were shown to be immune to λ*imm21cI*, resistant to λ*vir*, and able to support grown of the T4 amber mutant N82.

Construction of pLG244

The ColI-derived *Eco*RI fragment in pLG214 was purified by the procedure of Pratt *et al.*(13). *Eco*RI cleaved pPM63 was treated with calf intestinal alkaline phosphatase (Boehringer Mannheim), phenol-extracted and incubated overnight at 11°C with the purified DNA fragment (molar ratio of 1:4) and T4 DNA ligase. BW84 was transformed to ampicillin resistance with the reaction mixture. Recombinant plasmids were identified using a microscale plasmid purification procedure (14) and mapped following cleavage with *Bgl*II and *Sal*I.

RESULTS

Restriction map of pLG215

Deletion mutants were isolated from pLG215. This carries the *sog*

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gene in an *Eco*RI generated fragment of ColI*drd-1* inserted in the *cat* gene of pBR325. Figure 1 shows a restriction map of the plasmid, linearised at the unique *Sal*I site in the vector. Mapping of the *Bgl*II sites has been described (4). Construction of the *Hin*cII map involved deletant pLG214, a derivative of pLG215 which has lost about 1.5 kb of the cloned ColI DNA (4). The first approach to mapping *Hin*cII cleavage sites involved sizes of DNA fragments generated in double digests with either *Bgl*II or *Eco*RI. This allowed mapping of all of the cutting sites except that at coordinate 8.0 which is removed by the deletion of pLG214. This site was positioned using deletant pLG228, described in the next section, and the end-labeling procedure of Smith and Birnstiel (15). pLG228 was cut with *Bgl*II and 5' termini was labeled with ^{32}P using polynucleotide kinase. Following cleavage with *Eco*RI, the DNA was subjected to partial *Hin*cII digestion and the position of cutting sites was determined from the size of products following autoradiography.

Deletion analysis of *sog*

The region of pLG215 which encodes DNA primase was identified by isolating a family of deletion mutants that retained the ability to direct

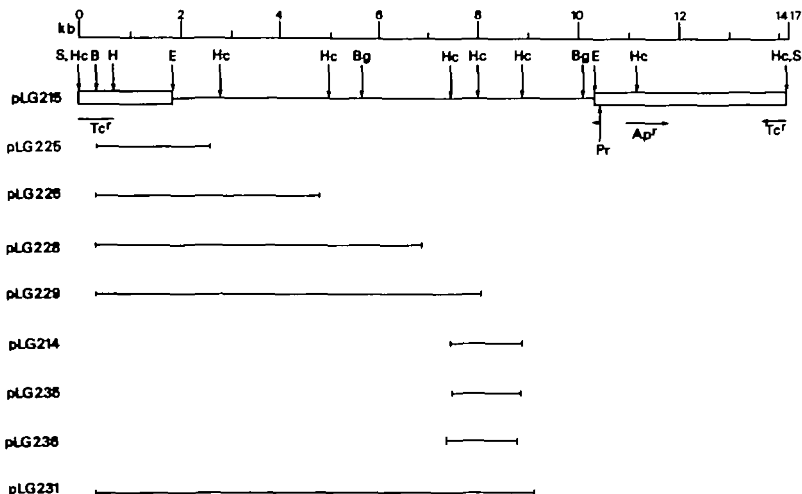

Figure 1. Restriction map of linearised plasmid pLG215 and extent of deletions in derivative mutants. The pBR325 vector component is drawn in double lines showing the position and direction of transcription of the ampicillin and tetracycline resistance genes (30). Pr indicates the promoter of the chloramphenicol acetyltransferase gene. Cleavage sites are shown for *Sal*I (S), *Hin*cII (Hc), *Bam*HI (B), *Hin*dIII (H), *Eco*RI (E), and *Bgl*II (Bg). The map shown is linearised at the unique *Sal*I cleavage site.

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synthesis of active enzyme. The first class of mutants were isolated by transforming BW84 to ampicillin resistance using pLG215 linearised at the unique *Bam*HI site located in the vector's determinant for tetracycline (Tc) resistance. This strategy allows isolation of deletants with mutations extending from the restriction enzyme site (16). Transformants that were Tet^s were classified as harboring putative deletant. Plasmids were purified and deletion end points were mapped with respect to *Hin*cII cutting sites in those plasmids that lacked the *Hin*dIII site but retained that for *Sal*I (Figure 1).

Plasmids were reintroduced into the *dnaG3* strain by transformation and the strains were tested for ability to form colonies at 40°C and for DNA primase activity. The results in Table 1 show that deletants pLG225 to 229 were Sog$^+$ by these criteria. In contrast pLG231 lacked both abilities. Thus these data define the region in pLG215 between co-ordinates 8.2 and 10.3 as essential for DNA primase activity.

This region was dissected further using a second class of mutants which had suffered spontaneous deletion of DNA from within the cloned fragment of ColI*drd-1*. The prototype plasmid is pLG214; this encodes a truncated (87 kd) DNA primase that is considerably more efficient than pLG215 in suppressing the *dnaG3* mutation (4). This type of deletant has since proved easy to isolate by transforming BW84 with pLG215 and selecting transformants directly at 40°C. pLG235 and pLG236 are two such plasmids, independently isolated. The respective deletions appeared to be remarkably similar to that in pLG214. The extent of the mutations in the three deletants was 1.4 kb, as judged from *Hin*cII fragment sizes. Their location was calculated from the length of the shorter double-stranded arm in heteroduplex DNA molecules formed between each plasmid and pLG215 following *Eco*RI cleavage. Open circular molecules of pLG215 were used as length standard. Values (n = 16-25 molecules) were pLG214/pLG215, 1.45 kb; pLG235/pLG215, 1.45 kb; and pLG236/pLG215, 1.53 kb. Thus all three mutant plasmids have deletion end points at about coordinates 7.4 and 8.8 (Figure 1). On the basis of *Hin*cII digests, a fourth deletant (pLG237) was found to contain a mutation identical to that in pLG235 and pLG236. All four plasmids were found to specify a DNA primase active *in vitro* (Table 1). Thus the analysis of the two classes of deletant plasmid maps the coding region for DNA primase activity to the region between coordinates 8.8 and 10.3. This is sufficient to encode a polypeptide of about 55 kd.

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Polypeptides specified by deletants

The *sog*$^{+}$ gene in pLG215 is known to specify two related polypeptides of 240 and 180 kd. The effect of deletions on these proteins was examined using minicells harboring appropriate plasmids. Gel electrophoresis of extracts from ^{35}S-methionine labeled minicells showed that pLG225 and pLG226 directed synthesis of a pair of polypeptides of the same size as those specified by pLG215 (Figure 2, lanes a-c). In contrast the deletion in pLG228 truncated both, giving major polypeptides of apparent molecular weight 208,000 and 132,000 (lane d). Thus the left terminus of *sog* maps between the end points of deletions in pLG226 and pLG228 (coordinates 4.8 and 7.0). Furthermore because the deletion in pLG228 reduced the size of the smaller *sog* protein, it is concluded that the determinants encoding the difference between the two polypeptides lie at the opposite end of *sog* to the deletion end points. Plasmid pLG229 has suffered a larger deletion and apparently specified only one *sog* protein of 75 kd (lane e). Because this plasmid is Sog^{+} (Table 1), this protein is presumably another truncated from of the 240 kd protein that retains DNA primase activity. If a second product is specified, it might be either masked by the low molecular weight vector proteins or degraded (17).

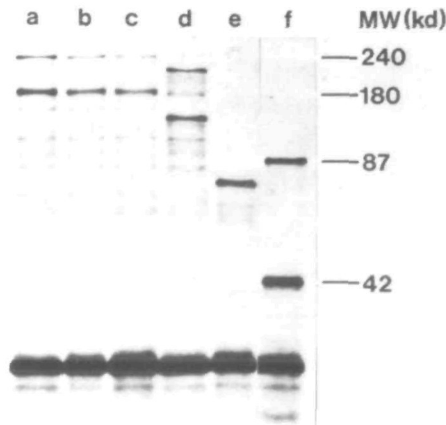

Figure 2. SDS-polyacrylamide gel electrophoresis of polypeptides synthesized in minicells harboring pLG215 and deletant plasmids. Minicells were labeled with ^{35}S-methionine for 60 min and extracts were subjected to electrophoresis and autoradiography. The plasmids in DS410 were pLG215 (a), pLG225 (b), pLG226 (c), pLG228 (d), pLG229 (e) and pLG214 (f).

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Table 1. The Sog phenotype of deletant plasmids in a *dnaG3* host

Plasmid in BW84	Colony formation 40°C/30°C	Plasmid primase activity[a]
None	2.0×10^{-8}	N D
pLG215	1.1×10^{-4}	315
pLG225	1.9×10^{-4}	583
pLG226	2.0×10^{-4}	735
pLG228	1.0×10^{-5}	406
pLG229	2.0×10^{-3}	390
pLG231	$< 3.9 \times 10^{-8}$	N D
pLG214	1.0	333
pLG235	1.0	544
pLG236	1.0	394
pLG237	1.0	371

[a]mU per mg crude cell extract. N D: not detectable

The *sog* proteins specified by the second class of mutants, which contain a deletion within the cloned fragment, were examined by a solid phase immunoassay using antiserum raised against the 87 kd DNA primase of pLG214 (4). Figure 3 gives results showing that pLG235-7 directed synthesis of a pair of antigenically related proteins apparently identical to those of pLG214 (87 and 42 kd).

Because the two classes of deletant plasmids show that the left end of *sog* can be deleted without eliminating either polypeptide synthesis or primase activity, it is inferred that *sog* is transcribed from right to left in the map in Figure 1.

Analysis of *sog* with amber mutants

Amber mutants have provided the most conclusive evidence on the genesis of the two *sog* proteins. Mutants were isolated from pLG211. This Sog^+ plasmid is identical to pLG215 with regard to conferred phenotype but, following isolation of mutants, it was found to contain an additional small *Eco*RI fragment of ColI*drd-1* of about 270 bp. Plasmid DNA was mutagenised *in vitro* with hydroxylamine and then used to transform BW84. 16 out of 2460 transformants were identified as temperature sensitive for DNA synthesis and deficient in plasmid primase. The Sog^- plasmids were then

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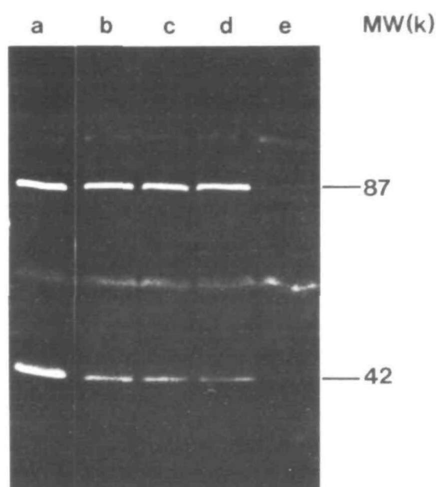

Figure 3. Solid phase immunoassay of polypeptides in extracts of plasmid-containing bacteria. Cell extracts were subjected to SDS-polyacrylamide gel electrophoresis. Polypeptides were transferred electrophoretically to a sheet of nitrocellulose which was incubated with antiserum raised in a rabbit against the 87 kd DNA primase specified by pLG214. Bound antiserum was detected using fluorescein-conjugated goat anti-rabbit IgG. The lanes give results obtained with BW84 containing pLG214 (a), pLG235 (b), pLG236 (c), pLG237 (d) and pLG240 (e).

screened for the presence of amber mutations by lysogensing transformants with a lambdoid recombinant phage carrying the *supF* translational suppressor gene, and testing the lysogens for regained ability to synthesise both DNA at 41°C and active plasmid primase. Two amber mutants (pLG218 and pLG219) were identified (Table 2). Plasmid pLG217 is representative of the other 14 mutants and it presumably carries an amino acid-substituting mutation.

Polypeptides specified by these plasmids were examined using sup^o minicells. Gel electrophoresis showed that pLG217 encodes the 240 and 180 kd proteins (Figure 4, lane a) as does the parental plasmid pLG211 (lane d). In contrast pLG218 and pLG219 failed to specify the larger *sog* protein whilst still causing synthesis of the smaller (lanes b and c). The novel polypeptide (36 kd) in lane b is presumably an amber fragment of the 240 kd protein. Assuming that the two proteins differ at the N-terminus these findings preclude the possibility that the smaller *sog* protein is derived from the larger by proteolytic cleavage.

Expression of *sog*

The two *sog* proteins could be derived either from a single message

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Table 2. DNA synthesis at 41°C and plasmid primase activity in *dnaG3* strains containing Sog⁻ mutants of pLG211

Plasmid in BW84	DNA synthesis[a]		Plasmid primase activity[b]	
	sup°	*supF*	*sup*°	*supF*
None	9	10	N D	N D
pLG211	308	341	206	187
pLG217	11	14	N D	N D
pLG218	11	201	N D	70
pLG219	13	94	N D	34

[a] 10^{-2} cpm ^{3}H-thymine incorporated per ml of culture in 90 min at 41°C.

[b] mU per mg crude cell extract. N D: not detectable

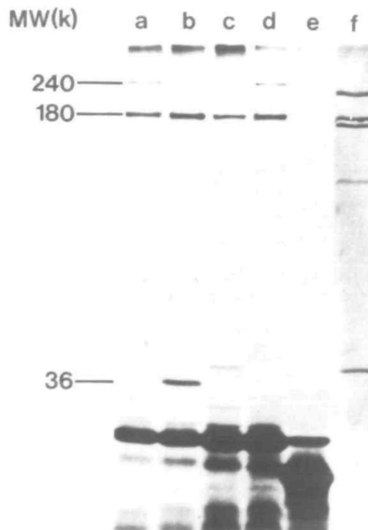

Figure 4. SDS-polyacrylamide gel electrophoresis of polypeptides synthesized in minicells containing pLG211 and Sog⁻ derivative plasmids. Minicells were labeled with ^{35}S-methionine for 90 min and extracts were analysed by electrophoresis and autoradiography. Extracts were prepared from DS410 containing pLG217 (a), pLG218 (b), pLG219 (c), pLG211 (d) and pBR325 (e). Lane F shows Coomassie blue-strained standards of myosin (212 kd), RNA polymerase subunits β' (165 kd), β (155 kd), σ (90 kd) and α (39 kd).

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translated from two different initiation sites or from separate messages initiated at different promoters. To distinguish between these possibilities, the gene was cloned in vector plasmid pPM63 (Figure 5) to place expression under control of the *lac* promoter. pPM63 is a multicopy plasmid that contains a 95 bp *Alu*I-generated fragment of *lac* carrying the UV^5 promoter, operator and all but two base pairs of the sequence specifying the leader of the *lacZ* message (18). Construction of this vector was arranged so that the terminus of the *Alu*I fragment which is downstream of the promoter forms part of a reconstituted *Eco*RI cleavage site.

Plasmid pLG244 is the appropriate recombinant consisting of the 6.5 kb *Eco*RI fragment of pLG214 inserted at the *Eco*RI site in pPM63 (Figure 5). The relative orientation of the two components was determined from sizes of *Bgl*II - *Sal*I fragments. pLG244, like pLG214, promoted complete suppression of the *dnaG3* mutation at 40°C, it specified a large amount of DNA primase and, in minicells, it caused synthesis of the 87 and 42 kd polypeptides (Table 3, Figure 6). These phenotypes were largely unaffected by addition of IPTG, presumably because host-specified *lac* repressor was already titrated by the multiple copies of the plasmid-borne operator (19).

When an $F'lacI^q$, which causes increased synthesis of repressor, was introduced into the strains, expression of *sog* was reduced by about 96% as judged by *dnaG* suppression and DNA primase activity (Table 3). There was also diminished synthesis of both *sog* proteins in minicells (Figure 6,

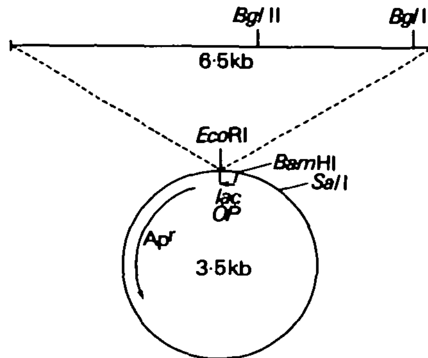

Figure 5. Restriction map of plasmid pLG244. Vector pPM63 is drawn as a circle and the direction of transcription from the *lac* promoter is shown. The 6.5 kb ColI-drived *Eco*RI fragment from pLG214 is inserted at the unique *Eco*RI cleavage site in pPM63. The two components are drawn to different scales.

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Table 3. Expression of *sog* from the *lac* promoter in pLG244

Plasmid in BW84	Colony formation 40°C/30°C		Plasmid primase activity[a]	
	-IPTG	+IPTG[b]	-IPTG	+IPTG[c]
pPM63	$< 10^{-7}$	$< 10^{-7}$	N D	N D
pLG244	1.07	1.1	112	286
pLG244 + F'*lacI*^{q} [d]	0.06	1.0	7.5	95

[a] mU/mg crude cell extract. N D: not detectable

[b] IPTG (0.5 mM) was added to the overnight cultures and nutrient agar.

[c] Crude cell extract was prepared from bacteria grown in the presence of IPTG (0.5 mM).

[d] F'*lacI*^{q} *L8 lacZ* :: Tn*9*

Figure 6. SDS-polyacrylamide gel electrophoresis of polypeptides synthesized in minicells containing pLG244 and parental plasmids. Minicells were labeled for 60 min with ^{35}S-methionine and extracts were analysed by electrophoresis and autoradiography. DS410 contained pPM63 (a), pLG244 (b) and pLG214 (c). Lanes d and e show results from a separate experiment involving DS410 harbouring pLG244 and F'*lacI*^{q} *L8 lacZ* :: Tn*9*. Minicells for lane e were incubated with IPTG (0.5 mM) for the 90 min preincubation and throughout the subsequent 60 min labeling period.

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lane d). The effect of the high level of *lac* repressor in reducing *sog* expression was reversed by addition of IPTG (lane e). Densitometer scans indicated that the amount of the 87 and 42 kd polypeptides was reduced by 95% and 71% respectively when IPTG was absent. Presumably the 37 kd protein detected in this gel was *lac* repressor, implying segregation of the F' into minicells.

Similar results were obtained when extracts of BW84(pLG244, F*lacI*^{q}) were screened using the solid phase immunoassay (data not shown). The 87 kd polypeptide was hardly detectable in extracts of bacteria grown in the absence of IPTG but the 42 kd polypeptide was slightly more intense. In the presence of inducer, both polypeptides were as pronounced as in extracts of cells containing either pLG244 or pLG214.

Thus, in pLG244, efficient synthesis of both *sog* proteins requires transcription from the *lac* promoter in the vector. The residual synthesis of these proteins in the presence of high levels of *lac* repressor could reflect either incomplete repression and, or, activity of a low level promoter in the 6.5 kb DNA fragment which contains *sog*. The former is suggested by the intermediate colour of BW84(pLG244, F*lacI*^{q}) colonies on Xgal indicator plates (20), implying incomplete repression of β-galactosidase synthesis. However it is clear that predominant synthesis of the small *sog* protein does not require an independent promoter within the *sog* gene.

This conclusion is supported by the properties of pLG214 derivatives that contain the ColI-derived insert inverted with regard to the pBR325 vector. Four plasmids (pLG240-3) were isolated following ligation of an *Eco*RI digest of pLG214. They conferred the phenotype of Ap^r Tc^r Cm^s and digestion with both *Hin*dIII and *Bgl*II gave fragments consistent with an insertion of orientation opposite to that in Figure 1. The plasmids slightly enhanced the colony-forming ability of BW84 at 40°C (to 3.9×10^{-6}) and cell extracts contained 1% of the primase activity specified by pLG214. Neither 87 nor 42 kd protein was detected using the solid phase immunoassay (Figure 3, lane e), probably because the method is insufficiently sensitive. Thus the major promoter for expression of *sog* in pLG214 lies in the vector, the nearest being that of *cat* which maps at about coordinate 10.5.

DISCUSSION

The *sog* gene, present in ColI*drd-1* or the recombinant plasmids, has previously been shown to encode two immunologically related polypeptides of

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240 and 180 kd with the larger protein containing primase activity (4). The implication that the smaller polypeptide shares primary structure with part of the larger is supported by our unpublished findings of common peptides following digestion of the proteins with *Staphylococcus aureus* V8 protease using the method of Cleveland *et al.* (21).

Because amber Sog⁻ mutations prevented synthesis of the larger protein without affecting the smaller, it is concluded that the two *sog* polypeptides are separate translation products. The finding that both polypeptides were truncated by deletions affecting the promoter-distal end of the locus implies that the small protein corresponds to the C-terminal portion of the larger molecule. Our previous enzyme purifications, coupled with the effect of the amber Sog⁻ mutations on the larger product, imply that DNA primase activity requires an amino acid sequence unique to the N-terminal part of the 240 kd polypeptide. Results with the Sog⁻ amber mutant pLG218 and the Sog⁺ deletant pLG229 suggest the size of the domain to be between 36 and 75 kd.

Efficient transcription of *sog*, when present in the recombinant plasmids, requires an active promoter in the vector. Because synthesis of both *sog* proteins was diminished in the absence of such promoter activity, it is inferred that the two polypeptides are generated from a single transcript with translation occurring from separate initiation sites. Translation from these sites would be in phase to produce the sequence-related polypeptides. Processing of a primary transcript to give different mRNA species would be an elaboration of this model.

Overlapping genes that are translated in different phases to yield sequence-unrelated products have been detected in small genomes such as that of ϕX174 (22, 23). It has been argued that such genetic organisation increases the coding capacity of small DNA molecules. More relevant to *sog*, a variety of genomes have been shown to contain overlapping genes that are translated in phase. Included are genes A and A* of ϕX174 (24), *C* and *Nu3* of phage λ (25) and the *cheA* locus of *E.coli* (26). A comparable situation holds for the expression of DNA encoding VP2 and VP3 of SV40 and polyoma virus (27, 28). As suggested by Shaw and Murialdo (25) and Smith and Parkinson (26), it seems unlikely that this type of genetic arrangement has arisen in bacteria and large replicons in response to selection for maximized use of coding capacity. Thus this type of overlapping genes may offer other advantages: for instance, the portion common to both polypeptides might participate in one reaction, possibly involving mutual

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interaction, whilst the unique sequence could have a separate function. This may be the case for the two *sog* polypeptides.

The function of the larger *sog* protein is partially clear. It was suggested to act in the transfer of the plasmid during bacterial conjugation: DNA primase or primers, made in the donor cell, would act in the recipient bacterium to initiate synthesis of DNA complementary to the transferred strand of the plasmid (2, 3). This hypothesis has been confirmed using defined Sog$^-$ mutants of ColI*drd-1* in conjunction with Sog$^+$ recombinant plasmids (L.K. Chatfield, G.J. Boulnois, E. Orr and B.M. Wilkins, to be published). The 240 kd polypeptide is probably multifunctional since only about one third is required for DNA primase activity. The function of the remainder of this polypeptide and the related 180 kd product is unknown. This sequence is apparently required in higher concentration than the primase domain implying that it could play a structural role in conjugal DNA transfer.

Recent evidence suggests that a range of conjugative plasmids encode DNA primases which are not consistently homologous (29, B.P. Dalrymple, G.J. Boulnois, B.M. Wilkins and P.H. Williams, to be published). The respective plasmids belong to the Iα, Iγ, Iδ, Iζ, B, J, K, M and P incompatibility groups. It is significant that the IncP plasmid, RP4, specifies a primase of 118 kd and a second related polypeptide of about 80 kd which may also have priming activity (29). Possibly the organisation of the RP4 primase gene is analogous to that of the *sog* gene of ColI.

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