Detection of Primase Specified by IncB Plasmid R864a

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Received 7 June 1982/Accepted 29 July 1982

Plasmid R864a (IncB) contains nucleotide sequences homologous with the sog primase determinant of IncIα plasmid ColIb-P9. Extracts of Escherichia coli carrying a mutant R864a derepressed for transfer functions showed enhanced primase activity, and contained a large polypeptide identical in size (apparent Mr = 220,000) to the IncIα sog gene product.

The prototype IncIα plasmid ColIb-P9 encodes a function that can substitute for defective dnaG gene product, bacterial primase (7), in vegetative replication of the host bacterial chromosome (9). The relevant plasmid genetic determinant (sog) (1) has been cloned and shown to specify a primase that promotes complementary-strand DNA synthesis in vitro on single-stranded DNA templates (10). Plasmids of the closely related B incompatibility group, which also suppress a dnaG mutation (4), fall into two classes on the basis of colony hybridization screening, using the cloned sog determinant of ColIb-P9 as a probe. Prototype IncB plasmid R16 encodes a function detectable in the same in vitro priming assay but genetically and serologically distinct from ColIb primase. However, other IncB plasmids, for example, R864a, shows significant nucleotide sequence homology with the cloned sog gene (4). Here we report the same correlation of primase activity with regulation of transfer functions in R864a as that occurring in Ia and Iγ group plasmids (8, 9). More importantly, we demonstrate the synthesis of the same high-molecular-weight polypeptides by several Sog+ plasmids. Although not surprising in view of the extensive homology among these plasmids, this result clarifies a discrepancy in primase size estimates in the literature (5, 10).

The level of suppression of the temperaturesensitive dnaG3 allele of E. coli K-12 strain BW86 (1) by plasmids of the Iα incompatibility group is increased significantly by a plasmid mutation (drd) leading to derepression of conjugal transfer functions (9). A mutant of IncB plasmid R864a, selected specifically for thermoresistance in the dnaG3 background (8), was shown to transfer at a high efficiency comparable with that of an authentic drd mutant of R144 (IncIα), and approximately 50,000 times greater than the wild-type plasmid (Table 1). Moreover, strains harboring the mutant plasmid, accordingly designated R864adrd-1, supported plaque formation by the I pilus-specific phage PR64FS (3).

Enhanced suppression of dnaG3 by plasmid mutants also correlates with increased plasmid primase activity as detected biochemically. The method, which has been described in detail previously (1, 4, 5), involves incorporation of 1H-labeled nucleotides in the presence of circular single-stranded phage M13 DNA as template by crude extracts of plasmid-bearing bacterial strains. Cell extracts of BW86 carrying either R144adrd-3 or R864adrd-1 showed 90-fold-higher levels of primase activity compared with those of strains with wild-type plasmids (Table 1). Activity was inhibited by antiserum raised against ColIb-P9 sog gene product, as described previously (4, 10). It should be noted that this simple regulatory association between primase and transfer activity, which first suggested that the physiological role of sog primase is in conjugal DNA synthesis (9), and which was exploited

TABLE 1. Correlation of primase activity with derepression of plasmid transfer functions

<table>
<thead>
<tr>
<th>Plasmid in BW86</th>
<th>Plating efficiencya</th>
<th>Transfer efficiency (transconjugants/100 donors)b</th>
<th>Primase activity (U/mg of protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R144</td>
<td>10^-6</td>
<td>2.7 x 10^-3</td>
<td>0.17</td>
</tr>
<tr>
<td>R144adrd-3</td>
<td>NTc</td>
<td>62.5</td>
<td>16</td>
</tr>
<tr>
<td>R864a</td>
<td>2 x 10^-4</td>
<td>7.1 x 10^-4</td>
<td>0.07</td>
</tr>
<tr>
<td>R864adrd-1</td>
<td>0.2</td>
<td>34.3</td>
<td>6</td>
</tr>
<tr>
<td>pLG215</td>
<td>NTc</td>
<td><em>d</em></td>
<td>41.5</td>
</tr>
</tbody>
</table>

a Plating efficiency of BW86 derivatives is defined as the ratio of the number of colonies formed on nutrient agar at 40°C to the number at 30°C. Plating efficiency of primase-free BW86 is <10^-7.

b Donor to recipient ratio in mating mixtures was initially 1:10; incubation was at 30°C for 1 h before plating on selective medium (10 μg of tetracycline per ml for R144, or 20 μg of kanamycin per ml for R864a).

c NT, Not tested. Values of 10^-2 to 3 x 10^-3 have been reported previously for R144adrd-3 in another dnaG3 strain (9). The plating efficiency of BW86 carrying pLG215 has been reported as 1.8 x 10^-4 (10).

d Plasmid pLG215 is nonconjugative.
FIG. 1. SDS-polyacrylamide gel electrophoresis of crude cell extracts of plasmid-bearing BW86 derivatives. Samples containing up to 500 μg of protein were analyzed on 7% polyacrylamide gel (monomer to dimer ratio, 44:0.8) in 0.75 M Tris (pH 8.8) containing 0.2% SDS, with a 3% stacking gel in 0.25 M Tris (pH 6.8, also with 0.2% SDS.) The electrophoresis buffer was 25 mM Tris (pH 8.3)–20 mM glycine–0.2% SDS. Samples: (a) myosin (Mₐ = 212,000); (b) BW86 (R64); (c) BW86 (R64drd-11); (d) BW86 (R144); (e) BW86 (R144drd-3); (f) BW86 (R864a); (g) BW86 (R864adr-1); (h) BW86, plasmid-free; (i) BW86 (pLG215). β indicates subunit E. coli RNA polymerase (Mₐ = 165,000). β indicates subunit E. coli RNA polymerase (Mₐ = 155,000). After electrophoresis, gels were stained with Coomassie blue, destained, and photographed. Since all tracks were overloaded with protein, only the upper portion of the gel is shown.

to generate IncP plasmid mutants capable of more efficient mobilization of the host bacterial chromosome (6), does not hold for another IncB plasmid, R16, or for other plasmids that specify the same or similar primase activity (B. P. Dalrymple and P. H. Williams, manuscript in preparation).

Plasmid pLG215 is a 7.0-kilobase (kb) EcoRI fragment of ColIb-P9drd-1 carrying the sog determinant cloned in vector plasmid pBR325 (10). Comparison of restriction enzyme digests of several IncIa plasmids indicates about 15 common EcoRI-generated fragments (2), including one of 7.9 kb carrying the sog gene (2, 4) and two HindIII fragments (13.5 and 4.5 kb) which show significant homology with pLG215 (4). Although the IncB plasmid R864a lacks several of the “core” fragments characteristic of IncIa plasmids (B. P. Dalrymple, unpublished data), it does possess the 7.9-kb EcoRI fragment and two HindIII fragments to which pLB215 hybridizes (4). We have compared R864a sog gene expression with that of particular IncIa plasmids because of reports indicating that plasmids with obviously extensive nucleotide sequence similarity specify apparently dissimilar products. Thus, plasmid R64 encodes primase activity with a sedimentation coefficient (in glycerol density gradients) of less than 4S, and reportedly with two polypeptides having apparent molecular weights of 180,000 and 140,000 (5). However, plasmid pLG215 specifies a 4.2S primase and the synthesis of two large, serologically related polypeptides said to be 240,000 and 180,000 daltons, of which only the larger exhibits activity (10).

This larger species is detectable by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis of crude cell extracts of a strain carrying pLG215 (10). With the same assay conditions as those used to derive the values shown in Table 1, a crude extract of BW86 carrying pLG215 contained 41.5 U of primase per mg of cell protein. So by overloading gel tracks with extracts of BW86 carrying the drd mutant of R864a (Fig. 1), it was possible to demonstrate the presence of a polypeptide with about the same mobility as the larger molecule encoded by pLG215. We estimate the apparent Mₐ of this polypeptide to be 220,000. Polypeptides of the same electrophoretic mobility were also observed in extracts of strains carrying drd mutants of R64 and R144 (Fig. 1). In view of this result, which was expected from the genetic and serological evidence (4), it is not clear why discrepant size estimates exist in the literature.

We are grateful to Brian Wilkins for Incl plasmids R64, R64drd-1, R144, and R144drd-3, and to Esther Lederberg (Plasmid Reference Center) for IncB plasmid R864a. We thank J. Coetzee for providing phage PR64FS and Erich Lanka for the generous gift of antiserum against ColIb primase. We are indebted to Elisha Orr for invaluable assistance with primase assays.

B.P.D. was supported by Science Research Council CASE studentship no. B2236 awarded in collaboration with F.D. Walker (Wellcome Research Laboratories).

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