Conjugative transfer of IncI plasmid DNA primase

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Summary. DNA primase of ColIb-P9rdl-I generates RNA primers that are thought to initiate DNA synthesis on the conjugatively transferred strand of the plasmid. To examine whether plasmid-specified primase is transferred during conjugation, we exploited the property of the enzyme to promote bacterial DNA replication in dnaG (primase-defective) mutants of Escherichia coli. It was found that dnaG3 recipient cells, treated with rifampicin to inhibit transcription, recovered ability to synthesise bacterial DNA by a process requiring an active plasmid primase gene in donor cells and a functional conjugation system. A non-transferable primase gene in the donor strain complemented a primase-negative derivative of ColIb-P9rdl-I, confirming that the enzyme responsible for recovery was supplied by donor cells. The implication is that certain proteins are transmitted from donor cells to promote conjugative metabolism of plasmid DNA in the recipients.

Introduction
Oligoribonucleotide primers that start DNA chain synthesis are generally made by the transcriptional activity of a group of enzymes termed DNA primases. The primase of Escherichia coli is dnaG protein and it is thought to act in discontinuous DNA replication in conjunction with a multiprotein complex, the primosome, which includes dnaB protein (Kornberg 1982). Conjugative plasmids of the IncI, (=I2) group, which includes ColIb-P9, specify another DNA primase that is the product of a gene designated sog (Wilkins et al. 1981). This enzyme, which is distinguishable from RNA polymerase by its resistance to rifampicin, makes short RNA primers that are elongated in E. coli by DNA polymerase III holoenzyme (Lanka et al. 1979). A physiological role of sog primase is to initiate synthesis of plasmid DNA during bacterial conjugation, but it can also substitute for E. coli primase in discontinuous replication of the bacterial chromosome, as manifest by sog-mediated suppression of temperature-sensitive dnaG mutations (Chatfield et al. 1982; Wilkins et al. 1981).

A specific strand of an IncI plasmid is transmitted from the donor to the recipient cell during conjugation and plasmid primase, which is not required for the transfer process itself, is thought to function to initiate synthesis of a replacement strand in the donor and of a complementary strand in the recipient via a dnaB-independent process. Such plasmid primase-dependent DNA synthesis on the transferred strand involves enzyme that is produced in the donor cell, since it occurs in recipient cells treated with rifampicin to inhibit expression of transferred genes and, more significantly, it can be promoted by primase specified by a non-transferable sog gene in the donor cell (Chatfield et al. 1982). Thus, RNA primers might be made in the donor on the DNA destined for transfer and be transported in a complex with the plasmid DNA, or plasmid primase itself might be transmitted to function in the recipient cell.

We report here that temperature-sensitive dnaG recipient bacteria can recover some ability to synthesise chromosomal DNA when mated with donors that are the sole source of active primases. Since an oligoribonucleotide primer is thought to function in cis on the template that serves for its synthesis, and a negligible amount of bacterial DNA is transferred from ColI-containing donors (Clowes and Moody 1966), this recovery implies that a primer-generating enzyme is transmitted from donor to recipient cells. Genetic evidence indicates that the enzyme is the product of the IncI plasmid sog gene and that it is transferred to recipients by a conjugation-dependent process. An implication of this conclusion is that proteins are selectively transmitted from the donor bacterium to promote conjugative metabolism of plasmid DNA in the recipient cell.

Materials and methods

Bacterial strains and plasmids. These are described in Table 1. BW101 was isolated as a rifampicin-resistant mutant of BW82 (Boulnois and Wilkins 1979).

Cultural conditions. Bacteria were grown in a salt-glucose-casamino acids medium termed SGC (Boulnois and Wilkins 1978). This was supplemented for thymine-requiring strains with deoxyguanosine (200 μg/ml) and thymine (2 μg/ml). Strains mutant at dna were grown at 31°C and dna+ bacteria at 37°C. To maintain selection for plasmids, strains harbouring pBR325 derivatives were grown in the presence of tetracycline (7.5 μg/ml) which was removed before mating.

DNA synthesis in recipient cells. Bacteria were diluted from overnight cultures and grown for 3 to 4 mass doublings to about 2 x 10^8 organisms per ml. Before mating, recipient cells were resuspended in phosphate buffer, UV irradiated with 400 J/m² if appropriate, and then resuspended in SGC. Donor and recipient bacteria were incubated separately at 41°C for 5 min, when rifampicin (Sigma Chemical Co.) was added to 100 μg/ml. Five minutes later, cultures were
mixed to initiate conjugation and \([^{14}C]\) thymine was added about 10 seconds before mating at this temperature.

DNA purification. DNA labelled in recipient cells during mating (Table 2) consisted of total DNA isolated from a 1 ml reaction mixture (Barth and Grinter 1975) for \(10^4 \text{ gg/ml} \times 10^4 \text{ cpm} \times \text{min} \). After incubation, 0.9 ml reaction mixture (Barth and Grinter 1975) for 15 min before mating at this temperature.

DNA-DNA reassociation. The method was based on that described by Barth and Grinter (1975). All DNA was sonicated to give fragments in the range of 0.3 to 0.8 kb determined by agarose gel electrophoresis. For experiments summarised in Table 2, 0.1 ml mixtures contained in 0.42 M NaCl 20 ng DNA labelled in recipient cells during mating (1050 cpm) and unlabelled BW86 DNA (20 \(\mu\)g) or plLG221 (2.5 \(\mu\)g) or salmon sperm DNA (20 \(\mu\)g; Sigma Chemical Co.). Mixtures for control experiments described in the text contained 2.5 ng labelled plLG221 (570 cpm) and 2.5 \(\mu\)g unlabelled DNA. All mixtures were heated in a bath of polyethylene glycol 400 for 10 min at 105°C to denature DNA and they were then incubated for 17 h at 75°C. The \(C_{1/2}\) value for BW86 DNA under these conditions was found to be 2.14 x 10^4 \(\mu\)g/ml x min. After incubation, 0.9 ml reaction mixture (Barth and Grinter 1975) for 15 min before mating at this temperature.

Results

Recovery of chromosomal DNA synthesis in rifampicin-treated dnaG recipient cells

To investigate whether dnaG recipient cells regain the ability to synthesise chromosomal DNA in a process dependent

ty was halted by addition of KCN (10 mM) and unlabelled thymine (100 \(\mu\)g/ml). Cells were sedimented, washed three times and resuspended in 2 ml TE (100 mM Tris-HCl, 50 mM EDTA, pH 8.0). Lysozyme (600 \(\mu\)g) was added followed, after 5 min on ice, by sodium lauryl sarcosinate to 0.2%. The lysate was treated at 37°C with ribonuclease A (150 \(\mu\)g/ml, heated at 100°C for 15 min before use) for 1 h and then with proteinase K (150 \(\mu\)g/ml) for 90 min. Lysate (2 ml) was mixed with 8 ml TE buffer and 12.9 g CsCl and centrifuged to equilibrium in a Beckman 50 Ti rotor. Fractions containing radioactivity were pooled and dialysed against four 1 l changes of 10 mM Tris-HCl, pH 8.0. Labelled plLG221 was isolated from a 7.5 ml culture of BW86 (plLG221) grown for four mass doublings with \([^{3}H]\) thymine at 6.3 Ci/mm,股价. Cells were washed, resuspended in 1 ml 10% sucrose in 100 mM Tris-HCl, pH 8.0 and lysed with lysozyme, EDTA and sodium lauryl sarcosinate, as described elsewhere (Vapnek and Rupp 1970). Following centrifugation to equilibrium in a CsCl-ethidium bromide gradient, fractions rich in closed circular DNA were pooled and then subjected to a second centrifugation in CsCl-ethidium bromide further to purify this species of DNA. The denser band of labelled DNA was recovered, treated with propan-ol saturated with an aqueous solution of CsCl, and then dialysed extensively. Unlabelled plasmid DNA was isolated by the procedure of Uemura and Mizobuchi (1982) and unlabelled bacterial DNA was prepared by a method similar to that of Jeffreys and Flavell (1977) from 11 overnight cultures of BW86 in nutrient broth.

DNA purification. DNA labelled in recipient cells during mating (Table 2) consisted of total DNA isolated from a 10 ml mating mixture of BW96 (plLG221) and unirradiated BW86 incubated for 60 min in the presence of rifampicin, deoxyguanosine and \([^{3}H]\) thymine (4.2 Ci/mm,股价). Ninety-eight percent of the radioactivity incorporated by this mixture of cells was shown to be due to conjugation-dependent DNA synthesis. After mating, incorporation of radioactivity...