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Cloning of the *trp-1* Gene from *Neurospora crassa* by Complementation of a *trpC* Mutation in *Escherichia coli*

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Studies with a hybrid plasmid containing 4.0 kilobase pairs of *Neurospora crassa* DNA cloned into plasmid pBR322 indicated that the plasmid restored to prototrophy a *trpC* mutant of *Escherichia coli* which lacked phosphoribosyl anthranilate isomerase but not a *trpC* mutant which lacked indole glycerol phosphate synthase, that the relevant transcription was initiated at a promoter within the *N. crassa* DNA, and that the phosphoribosyl anthranilate isomerase could be specified by a subcloned segment of the original DNA.

Anthranilate synthase from *Neurospora crassa* is a multifunctional protein which catalyzes, in addition to the anthranilate synthase reaction, two other reactions in the tryptophan pathway, phosphoribosyl anthranilate (PRA) isomerase and indole glycerol phosphate (InGP) synthase (1). The β-subunit of this complex is a trifunctional polypeptide which catalyzes the latter two reactions and, in combination with the α-subunit, provides the glutamine binding site for the anthranilate synthase reaction (8). As part of our studies on the role of domain structure in the trifunctional subunit, we cloned the *trp-1* gene, which encodes the β-subunit (8), into *Escherichia coli*.

Our strategy was to select for *trp-1* from *N. crassa* by using its gene product as a functional replacement for the bifunctional *E. coli* protein, encoded by the *trpC* gene, which catalyzes the PRA isomerase and InGP synthase reactions. Mutations in the *trpC* gene can lead to the selective loss of either of these two functions (19). It is possible, therefore, to select for the expression of one of these two reactions with cloned DNA. We report here the isolation of a plasmid that contains *N. crassa* DNA and that transforms to prototrophy a strain of *E. coli* which lacks PRA isomerase.

Chromosomal DNA, prepared (7) from wild-type *N. crassa* 74A and digested with *Pst*I, was inserted into the unique *Pst*I site of pBR322 (2) within the gene for β-lactamase. Hybrid plasmids thus prepared were used to transform (6) 5 $\times$ 10^9 cells of *E. coli* C9830 (*trpC1117* Tet^s Amp^s), which lacks PRA isomerase activity. Six Trp^+ Tet^r transformants grew within 6 days at 37°C after plating on Vogel-Bonner (17) minimal medium containing tetracycline (25 μg/ml). Each transformant was sensitive to ampicillin, as expected if the transformants contained derivatives of pBR322 with insertions in the *Pst*I site (16).

Each of the plasmids isolated from cleared cell lysates (3) of the six transformants was shown by restriction analysis to contain the same 4.0-kilobase-pair (kb) DNA insertion, oriented as shown in Fig. 1A within the *Pst*I site of pBR322. All of the purified plasmids transformed strain C9830 to tryptophan prototrophy. Cells of strain C9830 containing any of the plasmids grew in M9 minimal medium (12) (plus tetracycline) at about 76% of the rate at which the control cells grew (strain C9830 containing pBR322, grown in minimal medium supplemented with 25 μg of tetracycline per ml). One of these plasmids, pJK105, was characterized further.

About 5,700 cells of strain C9830 were transformed to prototrophy by 1 μg of pJK105. However, no prototrophic transformants were observed when pJK105 was used to transform strain C9941 (*trpC782* Tet^s Amp^s) (19), a strain deficient in InGP synthase. Both C9941 and C9830 took up and expressed pJK105 equally well, since both strains were transformed to tetracycline resistance with the same efficiency by the plasmid. Thus, the cloned DNA is capable of supplying enough PRA isomerase, but not InGP synthase, to *E. coli* to allow cell growth.

To demonstrate that the inserted DNA came from *N. crassa*, we hybridized ^{32}P-labeled pJK105, prepared by nick translation (11), to restriction enzyme-digested chromosomal DNAs from *N. crassa* and from two strains of *E. coli* which had been immobilized on diazobenzyloxymethyl paper (18). Radioactively labeled pJK105 hybridized, as expected, with restriction fragments of unlabeled pJK105 (Fig. 2, lanes 1 and 5). The labeled plasmid also hybridized with

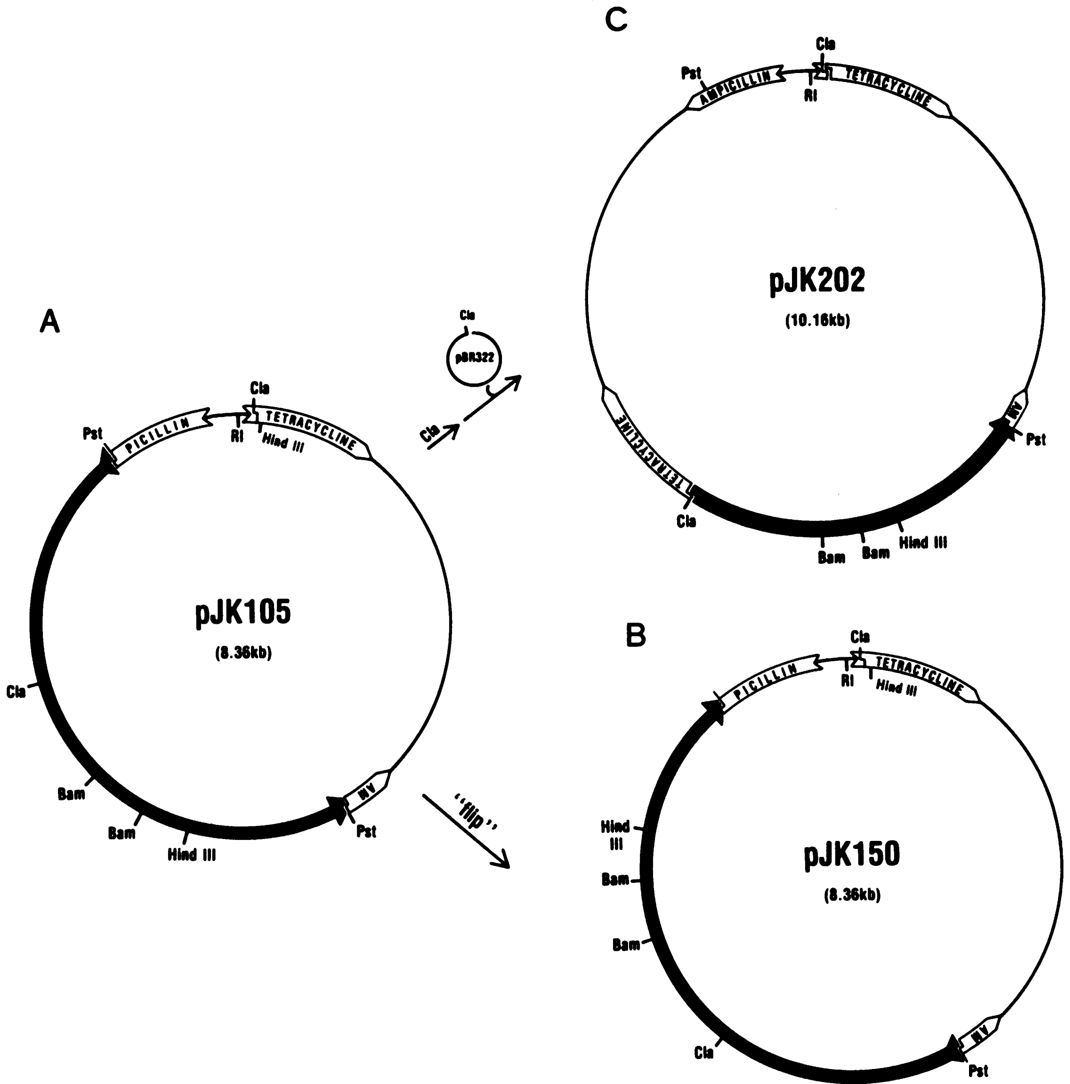

FIG. 1. Structural relationships of plasmids which rescue strain C9830. Heavy black lines indicate *N. crassa* DNA. Arrowheads on heavy lines indicate the relative orientation of *N. crassa* DNA within plasmids. Genes specifying drug resistance are shown as labeled arrows which point in the direction of gene transcription. RI, *Eco*RI.

a 4.0-kb piece of DNA from *N. crassa* (lane 3). No sequences complementary to pJK105 were detected in *E. coli* strains (lanes 2 and 4).

To test the effect of orientation on the functional expression of the DNA insertion, we cut pJK105 with *Pst*I, religated the mixture at a high concentration (50 μg/ml) of DNA, and used this mixture to transform strain C9830. Plasmids prepared (10) from 22 of the Tet^r Amp^s Trp$^+$ colonies arising from the transformation were screened by restriction analysis to determine the orientation of the 4.0-kb insertion in each. Of the plasmids screened, four had multiple insertions and were not analyzed further. A total of 7 plasmids carried the insertion in the same orientation found in pJK105 (Fig. 1A), whereas 11 had restriction patterns (Fig. 3) consistent with the insertion of the 4.0-kb DNA piece in the reverse orientation. One plasmid in the latter group (pJK150, Fig. 1B) transformed strain C9830 to tryptophan prototrophy equally as well as did pJK105. These data suggest that transcription of the *N. crassa* DNA originates within the insertion itself rather than from a specific pBR322 promoter.

A detailed restriction map of the DNA inser-

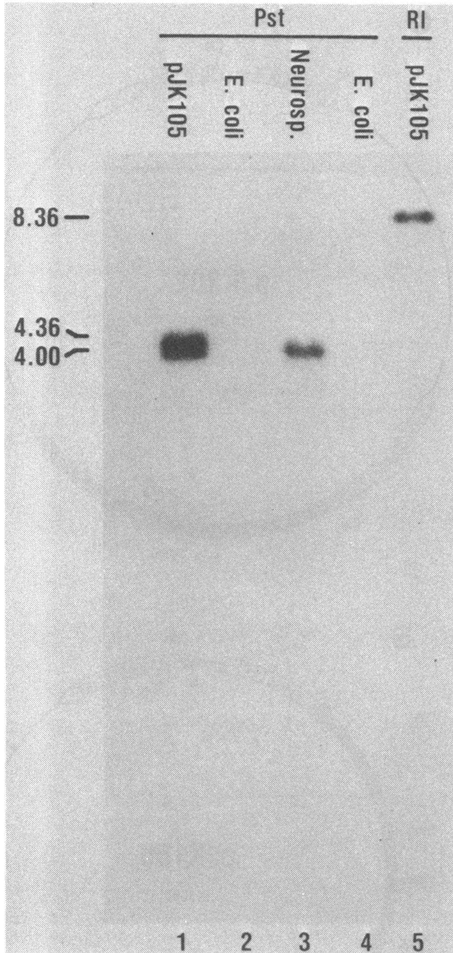

FIG. 2. Hybridization of plasmid pJK105 to *N. crassa* chromosomal DNA fragment. ^{32}P-labeled molecules of pJK105 were detected by autoradiography of a sheet of diazobenzyloxymethyl paper containing covalently bound unlabeled DNA after the sheet was incubated with the radioactively labeled plasmid. Labels at the top of the figure indicate the origin of the DNA on each lane of the paper and the restriction enzyme with which the DNA was cut. The sizes (in kb) of the radioactive bands are indicated at the left side of the figure. Neurosp., *N. crassa*. RI, *Eco*RI.

tion, prepared by the method of Smith and Birnsteil (14), is shown in Fig. 4. On the basis of this map, a subcloning experiment was carried out. The larger of two *Cla*I fragments of pJK105, when inserted into the unique *Cla*I site of pBR322, produced a new plasmid (pJK202, Fig. 1C) which provided PRA isomerase to strain C9830. The 2.25 kb of *N. crassa* DNA contained within this fragment of pJK105 had, therefore, sufficient information to restore strain C9830 to prototrophy.

Surprisingly, no PRA isomerase activity could be detected in cells carrying pJK105 by either spectrophotometric (4) or periodate (15) assay techniques. Also, no unique proteins encoded by *N. crassa* DNA were detected when cells containing pJK105, pJK150, or pJK202 were analyzed by the maxicell (13) technique, even though proteins produced from pBR322 transcripts could be seen (data not shown).

It is possible that pJK105 encodes, rather than a protein, a tRNA which suppresses the *trpC* (point) mutation in strain C9830 and allows production of active *E. coli* PRA isomerase. Yet, if suppression is the effect seen here, it does not produce significant levels of *E. coli* PRA isomerase, a relatively stable enzyme (5).

Furthermore, a cloned fragment of *N. crassa* DNA which includes much of the DNA within the 4.0-kb insertion of pJK105 has been sequenced by M. Schechtman and C. Yanofsky (personal communication). The sequence con-

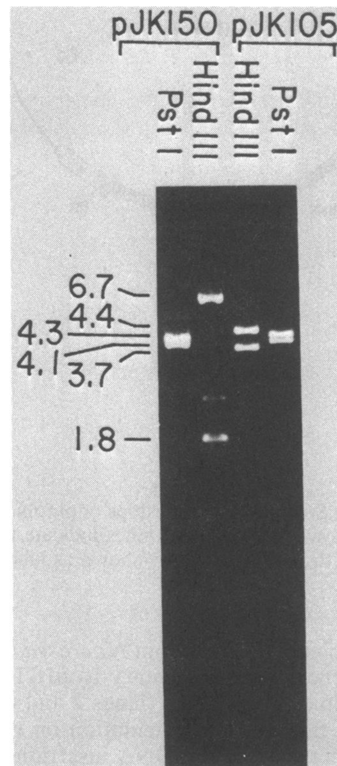

FIG. 3. Electrophoretic analysis of restriction fragments from plasmids pJK150 and pJK105. Plasmids which contained the same *N. crassa* DNA insertion in two different orientations were cut with restriction enzymes as indicated at the top of the figure, run on an 0.8% agarose gel, and stained with ethidium bromide. The sizes (in kb) of the bands on the gel are indicated at the left side of the figure.

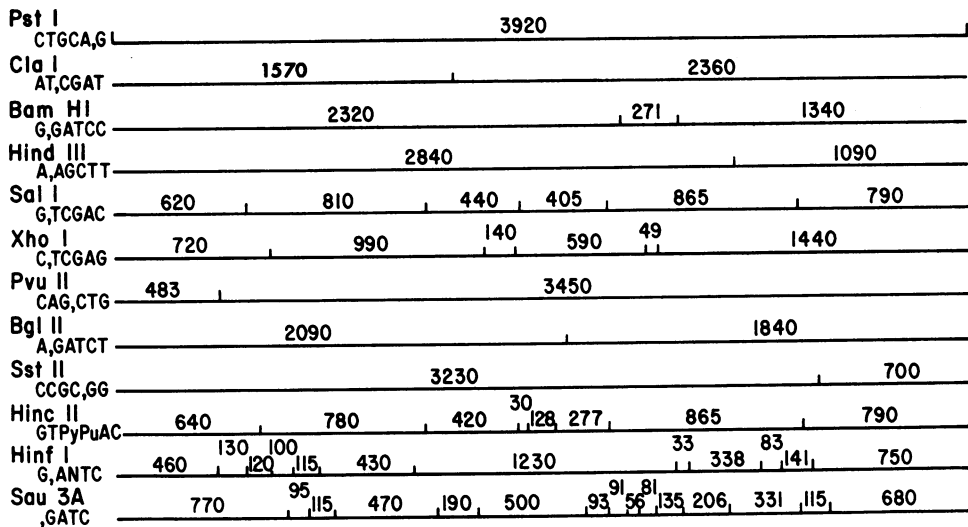

FIG. 4. Restriction map of *N. crassa* DNA carried on plasmid pJK105. Numbers indicate the estimated size of each restriction fragment in base pairs. The base sequence recognized and the cleavage site are indicated below each restriction enzyme listed. Enzymes which do not cleave the *Pst*I fragment are *Eco*RI, *Sma*I, *Xor*II, and *Xba*I.

tains a reading frame capable of encoding a protein of 85,000 daltons which is highly homologous with the *trpC* gene, suggesting that the DNA fragment contains the *N. crassa trp-1* gene.

In the *N. crassa* protein, PRA isomerase is probably the C-terminal domain, just as it is in the *E. coli* protein (9). It seems most likely that pJK105 transcripts encode a fragment of the β-subunit of *N. crassa* anthranilate synthase, which contains only the PRA isomerase domain. Since the β-subunit has distinct enzymatic domains which can function independently (1), such an explanation is plausible.

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