traG protein of the F sex factor of Escherichia coli K-12 and its role in conjugation

(stabilization of mating aggregates/membrane protein/F pilus/traN gene/mating cycle)

PAUL A. MANNING†, GIOVANNA MORELLI, AND MARK ACHTMAN

Max-Planck-Institut für molekulare Genetik, Ihnesstrasse 63/73, D-1000 Berlin 33, Federal Republic of Germany

Communicated by A. Dale Kaiser, September 14, 1981

ABSTRACT

The traG protein of the F sex factor is an inner membrane protein with a molecular weight of 116,000. Mutants in traG (or in traN) are able to trigger conjugal DNA replication even though they cannot efficiently form stable mating aggregates with F+ cells. The traG protein (and the traN protein) probably acts in the donor at the stabilization stage of conjugation.

The tra (transfer) operon of the F sex factor of Escherichia coli K-12 is about 30 kilobases (kb) long and contains 20 known tra cistrons. A few of these tra cistrons are needed for conjugal DNA transfer (traD, tral, traM, and traN) and surface exclusion (traS and traT) (1, 2) but not for F pilus synthesis. The remaining tra cistrons, except traG, are needed for F pilus biosynthesis (3–6). The traG cistron is the interesting exception among these functional assignments. Some traG mutations prevent F pilus biosynthesis (and thus DNA transfer); other mutations allow F pilus synthesis but still prevent DNA transfer (4).

These observations could be explained if the traG protein were bifunctional (4). In agreement, a cloned DNA fragment that contains the tra genes from the beginning of the tra operon through to the promoter proximal portion of traG, but not the promoter distal portion, was sufficient to encode F pilus synthesis (7, 8) (Fig. 1). Thus the promoter distal portion of traG is not needed for F pilus synthesis. Furthermore, of the three traG mutations tested, two that do not prevent F pilus synthesis mapped in the promoter distal portion of traG (8). However, although most of the tra proteins have been identified (9, 14), the traG protein had not yet been detected and these genetic inferences were based on indirect evidence.

For other experiments, techniques were developed for counting and disrupting conjugal mating aggregates (15, 16). The results led to the proposal (1) of five sequential stages in the conjugation process: (i) F pilin on the donor cells contact the recipient cells, (ii) the cells come into shear-sensitive wall-to-wall contact, (iii) the wall-to-wall contacts are stabilized and become resistant to shear, (iv) DNA transfer occurs, and (v) the aggregated cells disaggregate. According to this scheme, F pilin are required for stage I; their relevance to the remaining stages is uncertain (16). Based on the observations with traG mutants, it seemed possible that the traG protein might play a critical role in more than one stage of conjugation.

We report here further evidence on the role of traG in conjugation as well as the identification and intracellular localization of the traG protein.

MATERIALS AND METHODS

The bacterial strains (JC3272 and JC5455) used for genetic analysis have been described (3), as have the various media and techniques used for analysis of mating aggregates and F pilis (15–17). The F factor mutants, chimeric plasmids, and mutants of the latter have been described as cited individually below. Minicells from strain M2141 (14) were purified and labeled with [35S]methionine as described (9, 16). Minicell membranes were separated by sucrose gradient centrifugation (18).

Cell Envelope Analysis. Cells were harvested by centrifugation after growth to 4 × 10⁶/ml in 100 ml of L broth at 37°C with aeration. The pellet was resuspended in 10 ml of 56/2 minimal medium, centrifuged again, and resuspended in 10 ml of 56/2 medium supplemented with 200 μl of methionine assay medium (Difco) and 100 μCi of [35S]methionine (1000 Ci/mmol; Amersham; 1 Ci = 3.7 × 10¹² becquerels). After 60 min at 37°C with gentle aeration, the cells were pelleted, resuspended in buffer (Tris-HCl, 10 mM, pH 8.0), and broken [Amino detergent pressure cell; 14,000 psi (97 MPa)]. This suspension was centrifuged at low speed to remove unbroken cells and then cell envelopes were pelleted by centrifugation (20,000 rpm; 60 min; 4°C; Sorvall SS34 rotor). The pellet was resuspended in 500 μl of water. Triton X-100 extraction was performed in 2% Triton (vol/vol) in 10 mM Tris-HCl/5 mM MgCl₂, pH 8.0, for 20 min at 20°C. Triton X-100-insoluble material was recovered as the pellet after centrifugation as above except that it was done at 20°C. The Triton X-100-soluble material was precipitated with 2 vol of ethanol (−30°C, overnight), centrifuged, and dried by desiccation. Sodium dodecyl sulfate/polyacrylamide gel electrophoresis was performed on samples boiled for 2 min in sample buffer (19) as described (15).

RESULTS

Mating aggregation was analyzed by electronic particle analysis [Coulter Counter technique (15)] using pilolated tra mutants as donors. Use of donor cells carrying an unmutated Flac (wild type) resulted in rapid and efficient formation of mating aggregates with F+ cells (Fig. 2). Comparable results were obtained when the F factor carried a traM102, traD60, or traR40 mutation even though DNA transfer was inefficient in these mating mixtures. Conjugal DNA synthesis is triggered under such conditions in donor cells carrying the traD mutant but not in cells carrying the traL or traM mutants (20). Because the Coulter Counter technique detects stable (but not unstable) mating aggregates (15), the results in Fig. 2 show that neither DNA transfer nor triggering of conjugal DNA synthesis is needed to form stable mating aggregates.

The results with traG and traN mutants were different. Four pilolated traG mutants and the sole traN mutant were tested. No large increase in aggregation was seen in the mating mixture.
relative to the unmated control cultures (Fig. 2). The traG100, 
traG101, and traN548 mutants synthesized as many F pilus as 
tra+ cells did and the traG42 and traG98 mutants synthesized 
almost as many (Table 1).

If these traG or traN mutants were blocked very early in the 
formation of mating aggregates, a clearly defined phenotype 
might be expected. In the absence of F pilus binding to recip-
ient cells, no cell contacts mediated by the pilus should be detected 
by electron microscopy. In the absence of wall-to-wall contact 
formation, all contacts between cells might be via F pilus. In the 
absence of stabilization, large numbers of aggregated cells with 
F pilus and wall-to-wall contacts should be detected by electron 
 microscopy but few aggregated cells should be detected with 
the Coulter Counter. Therefore, pure cultures of the traG and traN mutants as well 
as mixtures of these mutants and F− cells were examined by 
electron microscopy. In both sets of samples, about 20–40% of 
the cells were connected by F pilus contacts or wall-to-wall con-
tacts or both. The same fraction of cells was aggregated accord-
ing to the Coulter Counter measurements, and it was not signifi-
cantly higher in the mating mixture than predicted from the 
results with unmixed parental cultures alone. Thus, mixtures 
of traG or traN mutants and F− cells did not efficiently accu-
ulate stable mating aggregates but also did not accumulate 
mating aggregate precursors at a clearly defined stage in ag-
gregate formation. However, many more cells were aggregated 
than succeeded in transfer of F plasmid DNA and the aggregates seen 
resembled classical mating aggregates morphologically except for 
their lower frequency.

ompA mutants of the recipient cell are blocked in stabilization 
and hence are defective in conjugation in liquid media. How-
ever, matings conducted with ompA recipients on the surface 
of a membrane filter are efficient (15, 21). No significant in-
crease in DNA transfer was observed in membrane filter mat-
ings for the various tra mutants except for the traN mutant 
(Table 1). The traN mutant was still 1/86th as efficient on the
Table 1. F pilus synthesis by and transfer of tra mutants

<table>
<thead>
<tr>
<th>Mutation</th>
<th>F pilus no./cell*</th>
<th>Transfer efficiency, %†</th>
<th>Liquid</th>
<th>Membrane filter</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.6</td>
<td>52.0</td>
<td>67.3</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.9</td>
<td>59.2</td>
<td>172.5</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>1.4</td>
<td>48.2</td>
<td>103.1</td>
<td></td>
</tr>
<tr>
<td>traD60</td>
<td>0.6</td>
<td>6 x 10⁻⁶</td>
<td>6 x 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>traG42</td>
<td>0.5</td>
<td>8 x 10⁻⁶</td>
<td>8 x 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>traG98</td>
<td>0.8</td>
<td>3 x 10⁻⁶</td>
<td>3 x 10⁻⁶</td>
<td></td>
</tr>
<tr>
<td>traG100</td>
<td>1.4</td>
<td>4 x 10⁻⁶</td>
<td>1 x 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>traG101</td>
<td>1.8</td>
<td>6 x 10⁻⁶</td>
<td>1 x 10⁻⁴</td>
<td></td>
</tr>
<tr>
<td>traH</td>
<td>0.9</td>
<td>2 x 10⁻⁵</td>
<td>2 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>traM102</td>
<td>0.7</td>
<td>7 x 10⁻⁴</td>
<td>1 x 10⁻³</td>
<td></td>
</tr>
<tr>
<td>traN48</td>
<td>1.1</td>
<td>0.04</td>
<td>2.1</td>
<td></td>
</tr>
</tbody>
</table>

* Samples were mixed with M12 bacteriophage and analyzed by electron microscopy as described (16). The numbers represent the average number of F pilus seen when at least 100 cells were examined.
† Mixed matings performed in liquid cultures or on Nucleopore membrane filters were analyzed after 40-min incubation at 37°C as described (15). The number of Lac⁺ transconjugants detected was normalized per 100 donor cells added to the mating mixture.

Membrane filter than the wild-type sex factor.

Identification of traG Protein. Radioactively labeled cytoplasmic and cell envelope fractions were analyzed by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. No effects of traG mutations were seen in the cytoplasmic extracts. However, a high molecular weight protein was absent from the cell envelopes of most of the Flac traG mutants examined (Fig. 3A). This protein disappeared with amber (traG81 and traG24) or frameshift (traG100 and traG101) mutations but was present with a missense (traG98) mutation. This protein comigrated with β-galactosidase, corresponding to a molecular weight of 116,000. The evidence presented below demonstrates that it is not β-galactosidase and we shall refer to it as TraGp. The gels indicated that 500–5000 copies of TraGp were present per cell (proteins I and II* used as standards) (22, 23).

The tra operon is transcriptionally regulated under the control of the traf gene. Most traf mutations (90, 120, 150, 256, and 259) (24) resulted in the total absence of the traG protein (Fig. 3B); the traJ52 mutation reduced its concentration by two-thirds. Bacteriophage Mu-1 insertions in traG resulted in the loss of TraGp. Polar, promoter-proximal Mu-1 insertions in traf and traH led to a 50% reduction in TraGp whereas promoter-distal insertions in trad had no effect. For comparison, Mu-1 insertions in traF, traH, and traG resulted in a 25–33% reduction in TraTp whereas traD::Mu-1 had no effect. Two deletions removing the end of the tra operon of F8 (an F'gal), P17 and P43 (25), have also been examined by using cells with a deletion of the lacZ gene on the chromosome. P43 is tra−traD− and P17 is tra−traD+ traG−. A protein corresponding to TraGp was absent only from P17.

Much of traG is an EcoRI fragment fl but part is on the neighboring DNA fragment fl (8). We examined cell envelopes from lacΔ cells harboring the chimeric plasmid pRS27, pRS29, pRS31, or pRS26 (see Fig. 1). Only pRS26 contains an intact traG cistron, and only pRS26-containing cells produced a protein comigrating with TraGp. However, the plasmid pRS29 which contains fragment fl did produce a protein slightly smaller than TraGp, which we have designated TraGp*. The same results were obtained when proteins encoded by these chimeric plasmids were specifically labeled in minicells. Thus, TraGp is encoded by the traG cistron (as opposed to being a modified chromosomally encoded protein). TraGp* is probably derived from a gene fusion between the NH₂-terminal portion of traG contained on fragment fl and the COOH-terminal portion of a pSCI101-encoded gene.

Recently, Willetts and Maule (26) identified a protein with the apparent subunit molecular weight of 100,000 as the traG gene product (26). The difference in molecular weight from that reported here may reflect differing conditions of sodium dodecyl sulfate/polyacrylamide gel electrophoresis but has not yet been resolved.

![Fig. 3](image1.png)

**Fig. 3.** Analysis of the cell envelopes of tra mutants of F·lac by sodium dodecyl sulfate/polyacrylamide gel electrophoresis. (A) Envelopes of cells labeled with [35S]methionine harboring either a wild type (wt) or a traG mutant of F·lac; visualization by autoradiography. (B) Envelopes of cells containing either a wild type (wt) or a traJ90 mutant of F·lac; staining with Coomassie blue G250.

![Fig. 4](image2.png)

**Fig. 4.** Localization of TraGp minicell membrane separation on sucrose gradients. cyt, Cytoplasm; WM, whole envelopes; OM, outer membrane; L2, L1, subfractions of the inner membrane. The proteins labeled range in molecular weight from 170,000 (traI) to 18,000 (traS).
Location of TraGp Within the Cell Envelope. The nonionic detergent Triton X-100 has been shown to solubilize cytoplasmic membrane proteins (27). When cell envelopes of Flac containing cells were solubilized with Triton X-100, TraGp was found in the soluble fraction, suggesting that it is an inner membrane protein. When envelopes of cells containing Flac were fractionated on sucrose gradients according to Osborn et al. (28), large amounts of β-galactosidase (which comigrates with TraGp) were trapped in the inner plasma vesicles. No conclusion was possible other than that TraGp was not detected in the outer membrane. Therefore, we performed membrane separations on radioactively labeled minicells harboring either pRS26 (traG*) or pRS29 (synthesizes TraGp*). The technique used yields enriched outer membrane and two enriched inner membrane fractions referred to as L2 and L1 (18). TraGp was enriched in the inner membrane fraction L2 (Fig. 4). Interestingly, TraGp* was also located within the inner membrane even though it has a different COOH terminus. The locations of previously analyzed tra proteins within the minicell membranes were as reported (18). The proteins encoded by traC, traF, and traH had not yet been analyzed. TraFp was located in the outer membrane and TraCp, in the inner membrane. Three inner membrane bands disappeared in traH mutants, and it is possible that, in fact, TraHp is a tra operon protein produced in a precursor form which is subsequently processed.

The results presented here demonstrate that (i) traG and traN are needed for the efficient formation of mating aggregates with F- cells, (ii) traG encodes a protein of 116,000 daltons, and (iii) the traG protein is located in the cell envelope inner membrane.

The inefficient formation of mating aggregates by traG or traN mutants contrasts with the observation (20) that conjugal DNA replication in donor cells carrying these mutants is triggered with normal efficiency upon contact with F+ cells. Furthermore, traI and traM mutants which efficiently formed stable mating aggregates (Fig. 2) did not trigger conjugal DNA replication (20). Thus, conjugal DNA replication and formation of stable mating aggregates appear to be independent events because each can occur in the absence of the other. This provides the basis for depicting parallel and independent stages in the mating cycle (1) as shown in Fig. 5. The choice of showing triggering and stabilization in parallel is tentative: triggering might occur concurrently with previous steps of aggregation. Currently it is impossible to assign the traG and traN proteins definitively to a single stage of the mating cycle because their phenotype does not match exactly any of those predicted. Because F pilus and wall-to-wall contacts were seen (although in reduced numbers) and because normal numbers of homosexual mating aggregates with wall-to-wall contacts were seen, we ten-

**DISCUSSION**

The mating cycle. Proteins needed for conjugation by the donor are indicated inside the circle. The OmpA protein and an intact LPS are required by the recipient. Hatched arrows indicate blocking. Cells carrying the F factor are shown with small circles to represent the plasmid DNA; F pili are represented by lines projecting from the cell surface. The original donors in a mating mixture are represented as ovals, and the original recipients are drawn as circles. During the mating cycle the recipients come into contact with the donors via several stages leading to stable mating aggregates. Within these stable mating aggregates, the recipients acquire an F factor and express tra cistrons, thus becoming converted to potential donors. Disaggregation occurs and the new donors are ready to reinitiate the mating cycle with new recipient cells. The individual stages and proteins have been reviewed elsewhere (2).

![Diagram](image-url)
tatively assign the primary defect of *traG* and *traN* mutants to the stabilization stage. However, *ompA* recipients which define stabilization resulted in accumulation of shear-sensitive (unstable) mating aggregates unlike *traG* donors.

The results could be explained if continuous aggregation and disaggregation occurred with these *traG* or *traN* cells. Possibly, the primary role of these two proteins in the donor cells would be to render the formation of the stable mating aggregates irreversible. We note here that the *traG* or *traN* phenotype is also observed when the "recipient" cell expresses the *traT* surface exclusion cistron (ref. 29; unpublished data) and tentatively have also assigned this protein to the stabilization stage (Fig. 5). Triggering of DNA replication in the donor cells was not stimulated when surface exclusion was expressed (30). We therefore postulate that the *traS* surface exclusion protein, which has only minimal effects on mating aggregation, prevents triggering in the donor cell. The resulting working hypothesis for conjugation is summarized in Fig. 5. The predictions of this hypothesis are that *traS* recipients or *ompA* recipients will trigger DNA replication in donor cells but *traT* recipients will not.

*traG* is also required for F pilus synthesis. The location of the *traG* protein in the inner membrane suggests that possibly it is part of an F pilus (basal?) organelle which spans inner and outer membranes and is involved both in F pilus synthesis and in converting initial F pilus contacts into stable wall-to-wall contacts. It is conceivable that F pilus retraction normally leads directly to wall-to-wall contacts and then to stabilization and that the *traG* protein is directly involved in these functions.

The excellent technical assistance of Barica Kusecek is greatly appreciated. We thank Neil Willetts for supplying the *traN* mutant of Flac and for sending unpublished manuscripts. During this project P.A.M. was supported by Grant Ac 36/2 from the Deutsche Forschungsgemeinschaft.