Purification and Properties of the F Sex Factor TraD Protein, an Inner Membrane Conjugal Transfer Protein*

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Using a traD overexpression plasmid, we purified the F sex factor TraD protein in milligram quantities. The purified protein has an apparent molecular weight of 82,000 and an amino acid composition rich in acidic residues. Using specific antibodies, TraD was localized to the inner membrane of F+ cells under conditions where it is produced in physiologically normal amounts. Furthermore, the protein was soluble only in the presence of detergents, but there is evidence that the carboxyl terminus is water-soluble. The purified protein shows pH-sensitive binding to DNA cellulose columns.

F sex factor-mediated bacterial conjugation proceeds through a series of well defined stages that culminate in the formation of transconjugants (Willets and Skurray, 1980; Ippen-Ihler and Minkley, 1986). This phenomenon involves 26 or more gene products, most of which are encoded within the transfer (tra) region of the F plasmid. The in vivo functions of most tra genes are unclear (Willets and Wilkins, 1984; Ippen-Ihler and Minkley, 1986), only a few of the tra-encoded proteins having been purified and studied (Date et al., 1977; Abdel-Monem and Hoffman-Berling, 1976; Minkley and Willets, 1984; Cuozzo et al., 1984).

F traD mutants are able to carry conjugation through all of the identified cell surface interaction stages, leading to the formation of "stable mating pairs" (Manning et al., 1981); however, they are unable to transfer DNA. Using a temperature-sensitive traD mutant and a detergent, which blocks stabilization, we showed that TraD can act after the formation of stable mating pairs and that DNA transfer can be observed at this time (Panicker and Minkley, 1985). This result not only ordered these events but provided evidence that the stable mating pairs formed with traD mutants serve as true intermediates. It also strongly suggested that the traD gene product is not required at the earlier cell surface interaction stages.

It is also clear that TraD is not required either for nicking at the origin of transfer (Everett and Willets, 1980) or for initiating conjugal DNA unwinding (Kingsman and Willetts, 1978). Accordingly, one possible role for TraD is to directly mediate DNA transfer. The purpose of the present study was to purify TraD protein for in vitro characterization and to determine its precise intracellular location.

EXPERIMENTAL PROCEDURES

Materials

Bacterial Strains and Plasmids—The Escherichia coli bacterial strains used were N8300 (F' galOMP3 his ilvA relA1 str A (chld-blus) (λch157 ΔBAM ΔHl)) (Gottesman et al., 1980) and JC3272 (F lac λX74 galK his trp λ ly s str λ (ms) T6 Su) (Achtmann et al., 1971). The strains ED2336 and ED2573, which contain the las8-GSTD transducing phages ED210 (traD') and ED211 (traD83), respectively, have been described previously (Minkley and Willets, 1984).

The conjugative plasmids used were JCF10 (F lac tra'), JCF18 (F lac trd8(Am)), and JCF14 (F lac trd14(Am)) (Achtmann et al., 1971). The tra7D expression plasmid pEM1 (Minkley, 1984) was derived from the pB-containing vector pHUB2 (Bernard et al., 1979).

Media—LB broth contained 10 g of Tryptone (Difco), 5 g of yeast extract (Difco), and 10 g of NaCl/Liter.

Buffers—Buffer A is 50 mM Tris-HC1, pH 7.8, 10 mM EDTA. Buffer B is 50 mM Tris-HC1, pH 7.8, 5 mM MgCl2, 1% Triton X-100. Buffer C is 50 mM Tris-HC1, pH 7.8, 1% Triton X-100, 1 mM EDTA, 10 mM 2-mercaptoethanol, 50 μg/ml phenylmethysulfonyl fluoride. Buffer P is 10 mM sodium phosphate, pH 6.7, 1% Triton X-100, 1 mM EDTA, 1 mM EGTA, 10 mM 2-mercaptoethanol, 50 μg/ml phenylmethysulfonyl fluoride. The pH of the Tris-containing buffers was adjusted at 25°C.

Other Materials—Ultrapure urea and ultrapure ammonium sulfate were obtained from Schwarz/Mann. Triton X-100 was obtained from Research Products International Corp. Lysozyme, DNase I, protease inhibitors, and antibiotics were purchased from Sigma. Cellulose and DNA cellulose matrices were purchased from Pharmacia LBK Biotechnology, Inc. Ampholines and gel filtration matrices were obtained from Bio-Rad. Acrylamide and other gel electrophoresis reagents were purchased from Bio-Rad.

Methods

SDS-Polyacrylamide Gel Electrophoresis and Isoelectric Focusing—SDS-polyacrylamide slab gels were run essentially as described (Panicker and Minkley, 1985) except that a 4-fold higher concentration of SDS was used in the sample buffer if Triton X-100 was present in the sample. Isoelectric focusing of purified TraD was carried out on polyacrylamide slab gels using the method of O'Farrell (1975). After focusing, the pH gradient of the gel was measured using a surface electrode. The gels were fixed with 4% sulfosalicylic acid, 12.5% trichloroacetic acid for 4-5 h and subsequently stained and destained.

Construction of Plasmids pMP1 and pMP3—The construction of the tra7D expression plasmid pEM1 from pHUB2 and ED210 has been described (Minkley, 1984). The plasmid pMP1 was constructed directly from pEM1 as a HpaI deletion derivative of pMP1 (Fig. 1) and was identified by loss of the ability to complement the F lac traD8(Am) mutant. A plasmid that was similar in size to pMP3 and contained

1 The abbreviations used are: EGTA, [ethylenebis(oxyethylene-nitrito)]tetracetic acid; SDS, sodium dodecyl sulfate.
the HpaI site but lacked the PvuII site was designated pMP2. In other work, it has been observed that the hybrid truT-truD junction at this PvuII site is prone to a 2-base pair deletion, CAGCTG to CCTG; this -2 frameshift causes translation to encounter a nonsense codon 14 bases further into truD (Perumal, 1985).

**Purification of TraD Protein from pMP1-containing Cells**—TraD was routinely purified from induced N4830/pMP1 cells. A standing overnight culture (250 ml) of strain N4830/pMP1 in LB broth + kanamycin (50 μg/ml) grown at 32°C was used to inoculate 20 liters of the same medium in a 28-liter New Brunswick Microferm fermentor. The cells were grown at 32°C to an $A_{550}$ of 0.2-0.3. The temperature of the culture was then gradually increased at a rate of 1°C every 3 min until it reached 42°C. After 4 h at 42°C, the culture was chilled and harvested. The cells were stored as a frozen paste at -60°C.

As a solid white pellet that dispersed upon thawing at 37°C, lysozyme (20 mg) was added, and the suspension was incubated for 30 min at 37°C. During this time the suspension became viscous, after which it was frozen at -70°C and thawed at 37°C. MgCl$_2$ (0.9 ml of a 1 M solution) and DNase I (0.2 ml of a 1 mg/ml solution) were added, and the suspension was incubated at 37°C for 15-30 min until it was no longer viscous. The cell lysate was centrifuged at 27,000 × g for 20 min at 4°C. All subsequent operations were carried out at 4°C.

The cell envelope pellet was resuspended in 30 ml of Buffer T and dispersed using a Teflon pestle and intermittent vortexing. This suspension was then homogenized at a time in a 30-ml Potter-Elvehjem tissue grinder. The suspension was then centrifuged at 48,000 × g for 20 min. The translucent yellow supernatant (Fraction TE, 30 ml) was separated from the pellet (Fraction P).

Fraction TE was brought to 20% of saturation with solid ammonium sulfate, which was added slowly while stirring. The solution was stirred for an additional 15 min and then centrifuged at 27,000 × g for 15 min. The supernatant was decanted from a small white granule pellet. Ammonium sulfate was then added to 30% of saturation, and the solution was stirred for 15 min and centrifuged at 27,000 × g for 15 min. This step resulted in a yellowish white scum floating on top of a clear, light yellow solution. The upper layer was separated from the lower liquid and dissolved in 8 ml of Buffer D (Fraction AS).

Protease inhibitors (N-tosyl-L-phenylalanine chloromethyl ketone, pepstatin, leupeptin, and aprotonin) were each added to a final concentration of 0.2 mM. Fraction AS was dialyzed overnight against 1 liter of Buffer D and frozen at -60°C if not used immediately.

Fraction AS (4 ml) was applied to a 5-ml blue dextran-agarose column (4.5 mg of blue dye/ml of gel) equilibrated in Buffer D. The column was washed with 2-3 column volumes of Buffer D, and then Buffer P at 4°C. The sample loads were run to the matrices.

**Production of Rabbit Antibodies and Cellular Localization of TraD Protein**—TraD for antibody production was purified by preparative DNA-cellulose binding studies. TraD does not yet have a functional assay, we used pMP2 to produce rabbit antibodies to TraD protein resulting from the in-frame fusion of portions of the traN and traD genes (Jalajakumari and Manning, 1989) (Fig. 1). Plasmid pMP2 is similar to pMP3 but has lost the PvuII site and does not express the trihybrid protein (see "Experimental Procedures"). Since TraD does not yet have a functional assay, we used pMP2 and pMP3 as controls while developing the purification procedure.

Cell pellets from induced cultures containing pMP1, pMP2, and pMP3 were fractionated and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 2). The leftmost gel lane (lane M) contains partially purified TraD from strain ED2336 as a marker. TraD was in the cell envelope fraction, was almost totally extractable by nonionic detergent (Fig. 2, lane 12), and was separated by a 0-30% ammonium sulfate fractionation (Fig. 2, lane 15). Because of contaminating host proteins, the selective purification of TraD only becomes evident at the

**RESULTS**

**Construction and Characterization of Plasmids pMP1, pMP2, and pMP3**—Initial attempts to purify TraD were made by using induced lysogens of strains carrying ED110, a lambdaTra-transducing phage (Minkley and Willetts, 1984). However, after induction these cells lysed, and most of TraD ended up in the cell debris fraction, which was also heavily contaminated with overproduced phage proteins. We therefore decided to overexpress TraD using a plasmid-based expression vector. Cells containing pEM1 (truT* truD*) have been used to produce large quantities of TraT (Minkley, 1984) but fail to express significant amounts of TraD. Therefore, we removed truT by deleting a HpaI fragment from pEM1 to obtain pMP1 (Fig. 1). Plasmid pMP3 was constructed from pMP1 by complete PvuII deletion to provide a control traD-plasmid. Based on nucleotide sequence data, pMP3 is expected to encode a 37,000-dalton protein resulting from the

![Fig. 1. Construction of pMP1 and pMP3 from pEM1.](image-url)
A troublesome contaminant was a TraD-sized protein synthesized in all three strains upon induction (Fig. 2, lanes 1-2). This protein could be differentiated from TraD by its solubility in 30% ammonium sulfate (Fig. 2, compare lanes 10-12 with lanes 13-15).

Plasmid pMP3 showed the most dramatic effect upon induction, pronounced overproduction of a 37,000-dalton protein and some associated lower molecular weight polypeptides (Fig. 2, lanes 1 and 2). These products appear almost entirely in the soluble fraction (Fig. 2, lane 7).

**Purification of TraD Protein from pMP1-containing Cells** — A typical purification of TraD used 10 g of frozen cell paste from induced N4830/pMP1 cells. As described under “Experimental Procedures,” the purification steps included cell envelope preparation by lysozyme/EDTA (freeze/thaw) (Fraction CE), extraction of cell envelope proteins with 1% Triton X-100 (Fraction TE), and 20-30% ammonium sulfate fractionation. Upon centrifugation, the ammonium sulfate fractionated yielded a pellet, a supernatant, and a floating layer (Fig. 2, lanes 3-15). Fractionation of TraD protein on a blue dextran-agarose column. Fraction AS from 10 g of induced N4830/pMP1 cells was loaded onto a blue dextran-agarose column that was developed as described under “Experimental Procedures.” Samples of the column fractions were run on a 9.5% SDS-polyacrylamide gel and stained with Coomassie Blue. Lane M contains molecular weight markers (myosin, 205,000; α-galactosidase, 116,000; rabbit phosphorylase b, 97,400; bovine serum albumin, 66,000; and egg albumin, 45,000). Lane 1, column load (Fraction AS); lane 2, column flow through; lanes 3 and 4, two successive washes with starting buffer; lane 5, eluate with 0.5 M NaCl. The fragment of TraD that does not bind to the column is indicated.

Attempts to purify TraD further by gel filtration of Fraction AS were unsuccessful because the protein degraded, even in the presence of a mixture of protease inhibitors. However, during these experiments we found that TraD binds to the chromophore of blue dextran marker dye.

Based on this observation, a blue dextran-agarose matrix was used as an affinity column in the purification scheme (Fig. 3). Essentially all impurities in Fraction AS flowed through the column, whereas TraD bound to the blue dextran and was eluted with 0.5 M NaCl in Buffer D. Since the samples run in all gel lanes represent the same proportion of the whole, there was apparently considerable breakdown of TraD to a 35,000-40,000-dalton fragment, (Fig. 3, compare lanes 1 and 2) which does not bind to the blue dextran-agarose (Fig. 3, lane 2). This procedure resulted in TraD that is 90-95% pure as determined by scanning densitometry of the Coomassie Blue-stained gel (Fig. 3, lane 5). The pooled peak contains approximately 1 mg of intact TraD (Fraction BD) that was obtained from 10 g (wt) of purified N4830/pMP1 cells. In the absence of a linear activity assay it was not possible to calculate an overall yield for the procedure.

**Molecular Weight, Amino Acid Composition, and Isoelectric Point of Purified TraD Protein** — The apparent molecular weight of purified TraD on a 9.5% SDS-polyacrylamide gel was estimated to be 82,000 by comparison with standard molecular weight marker proteins (Fig. 3) and is in good agreement with the molecular weight of 81,400 inferred from the trd nucleotide sequence (Jalajakumari and Manning, 1989). Because TraD is soluble only in the presence of detergents, where it presumably is incorporated into micelles, we have not yet determined a native molecular weight for the protein.

The amino acid composition of purified TraD is given in Table I, along with the composition inferred from the trd nucleotide sequence. The poor correspondence between found and predicted amino acid compositions may reflect the greater difficulty inherent in purifying a cytoplasmic membrane protein to homogeneity. When a comparison is made to the average composition of total E. coli protein (Table I), there are important consistencies in the found and predicted amino acid composition data. This includes an atypically low ratio of Lys to Arg and much higher than normal ratios of Glx to
we decided to reexamine the localization of TraD in F' cells. Predicted), TraD protein is expected to be acidic. Consistent with this, several determinations of the isoelectric point in the composition of total sequence (Dalajakumari and Manning, 1989).

Asx and Ser to Thr. (We attribute certain aberrant data, such as the Gly content, to contamination). Asx and Ser to Thr. (We attribute certain aberrant data, such as the Gly content, to contamination).

Based on amino acid composition (both found and predicted), TraD protein is expected to be acidic. Consistent with this, several determinations of the isoelectric point in the presence of urea gave values between 5.9 and 6.0.

TraD Protein Is Located in the Inner Membrane of Donor Cells—Because of possible problems in previous studies in which TraD was expressed under abnormal physiological conditions (Achtman et al., 1979; Minkley and Willetts, 1984), we decided to reexamine the localization of TraD in F' cells by using specific antibody detection. Rabbit antiserum directed against purified TraD was prepared as detailed under "Experimental Procedures." This antibody recognizes a TraD-sized protein in F' cells, but not in cells that are F- or a regulator of the tra operon) or Flac traD amber mutants (Panicker and Minkley, 1985).

We confirmed by immunoblot analysis that TraD is located largely in the cell envelope fraction of lysed F' cells (Fig. 4). There are trace amounts of TraD in the cytoplasmic fraction, but we believe that this portion of TraD is actually present in small membrane vesicles that do not pellet in 45 min at 300,000 g. Antibodies against TraT protein were used as an internal control for the fractionation, because TraT is a well characterized cell envelope outer membrane protein (Achtman et al., 1979; Minkley and Willetts, 1984). Further fractionation indicated that TraD is located only in the inner membrane of Flac traD+ cells (Fig. 5). By comparison, TraT was present exclusively in the outer membrane fraction.

TraD Protein Binds to DNA Cellulose—Using affinity chromatography on DNA cellulose, we examined purified TraD for possible DNA binding activity. In Buffer P, which contains 10 mM sodium phosphate, pH 6.7, TraD bound quantitatively to both single-stranded and double-stranded DNA cellulose (Fig. 6). In control experiments, TraD showed no binding to the equivalent cellulose matrix (Fig. 6). In an attempt to determine the relative affinity for single-stranded and double-stranded DNA, we eluted bound TraD using gradient elution.

The results indicated that TraD eluted from both DNA matrices at essentially the same salt concentration (0.17 M NaCl, data not shown). We also observed that purified TraD bound to DNA cellulose in Buffer D, which contains 50 mM Tris-HCl at pH 6.7 (prepared as pH 6.1 at 25°C), but not in Buffer D at pH 8.4 (prepared as pH 7.8 at 25°C).

TABLE I

| Residue | Found* | Predicted* | Typical* |
|---------|--------|------------|----------|
| Asx     | 8.9    | 10.5       | 9.0      |
| Glx     | 11.9   | 13.0       | 9.8      |
| Thr     | 4.6    | 4.2        | 4.7      |
| Ser     | 8.5    | 6.2        | 4.0      |
| Pro     | 4.8    | 5.2        | 4.1      |
| Gly     | 11.1   | 6.3        | 11.5     |
| Ala     | 9.7    | 7.3        | 9.6      |
| Cys     | 1.6    | 1.3        | 1.7      |
| Val     | 6.6    | 7.0        | 7.9      |
| Met     | 2.1    | 2.9        | 2.9      |
| Ile     | 5.2    | 6.3        | 5.4      |
| Leu     | 8.6    | 7.5        | 8.4      |
| Tyr     | 2.2    | 3.6        | 2.6      |
| Phe     | 3.1    | 3.8        | 3.5      |
| His     | 2.0    | 1.7        | 1.8      |
| Lys     | 4.6    | 5.2        | 6.4      |
| Arg     | 4.5    | 6.4        | 5.5      |
| Trp     | ND     | 1.8        | 1.0      |

* Amino acid composition was determined as described under "Experimental Procedures." Tryptophan was not determined (ND).

The predicted amino acid composition of TraD protein is calculated from the amino acid sequence inferred from the truD nucleotide sequence (Jalajakumari and Manning, 1989).

The amino acid composition of a "typical" protein is taken from the composition of total E. coli protein (Neidhardt, 1987).

Based on amino acid composition (both found and predicted), TraD protein is expected to be acidic. Consistent with this, several determinations of the isoelectric point in the presence of urea gave values between 5.9 and 6.0.

TraD Protein Is Located in the Inner Membrane of Donor Cells—Because of possible problems in previous studies in which TraD was expressed under abnormal physiological conditions (Achtman et al., 1979; Minkley and Willetts, 1984), we decided to reexamine the localization of TraD in F' cells by using specific antibody detection. Rabbit antiserum directed against purified TraD was prepared as detailed under "Experimental Procedures." This antibody recognizes a TraD-sized protein in F' cells, but not in cells that are F- or a regulator of the tra operon) or Flac traD amber mutants (Panicker and Minkley, 1985).

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The results indicated that TraD eluted from both DNA matrices at essentially the same salt concentration (0.17 M NaCl, data not shown). We also observed that purified TraD bound to DNA cellulose in Buffer D, which contains 50 mM Tris-HCl at pH 6.7 (prepared as pH 6.1 at 25°C), but not in Buffer D at pH 8.4 (prepared as pH 7.8 at 25°C).
TraD DNA Transfer Protein

Fig. 6. DNA binding studies of TraD protein using affinity column chromatography. Purified TraD (Fraction BD) was loaded onto three cellulose columns, which were developed as described under “Experimental Procedures.” Samples from these columns were run on a 9.5% SDS-polyacrylamide gel and stained with Coomassie Blue. The numbered lanes contain fractions from either the control cellulose column (no superscripts), the single-stranded DNA cellulose column ('), or the double-stranded DNA cellulose column ("'). Lane 1, sample load applied to each column; lane 1', column dead volume; lane 2, flow through; lanes 3 and 4, two successive washes with 1-column volume of starting buffer; lane 5, eluate with 0.5 M NaCl; lane MW, molecular weight marker proteins as in Fig. 3.

DISCUSSION

We have developed a method for purification of TraD protein in quantities sufficient for biochemical analyses. Our most important findings were that purified TraD is an integral inner membrane protein and that it is capable of binding to DNA as determined by affinity chromatography on DNA cellulose. Since TraD lacked a biochemical assay, we used SDS-gel electrophoresis to guide the purification. Deletion plasmids that overexpressed truncated versions of TraD served as controls. In particular, plasmid pMP3, which expresses only the carboxyl terminus of the protein that we band, and inner membrane fractions of the cell envelope in column chromatography.

Our observation that purified TraD binds to DNA affinity columns is particularly interesting in light of the phenotype of traD mutants. Although binding to DNA cellulose is not a catalytic activity, it is our best evidence to date that TraD has been purified in a native conformation. Because TraD is an acidic protein and binds to DNA cellulose above its determined isoelectric point, we suspect that the observed binding is specific and not simply a charge-related phenomenon. Our finding that TraD binds equally well to single- and double-stranded DNA cellulose may not be significant, given the ill defined nature of double-stranded DNA on a cellulose matrix.

In preliminary experiments we found that our preparation of purified TraD contains a DNA-dependent ATPase activity. At present we cannot exclude the possibility that this ATPase activity is associated with a low level contaminant. Interestingly, it has been noted that the TraD amino acid sequence inferred from the traD nucleotide sequence of the related plasmid R100 contains an ATP-binding site consensus sequence (Yoshioka et al., 1990). Future experiments need to address the questions of whether and how DNA binding and DNA-dependent ATPase activities of TraD are related to its function in conjugation.

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REFERENCES

Abdel-Monem, M., and Hoffmann-Berling, H. (1976) Eur. J. Biochem. 65, 431-440
Achtman, M., Willetts, N., and Clark, A. J. (1971) J. Bacteriol. 106, 529-538
Achtman, M., Manning, P. A., Edelbluth, C., and Herrlich, P. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 4837-4841
Bernard, H.-U., Renaud, E., Hershfield, M. V., Das, H. K., Helsinki, D. R., Yanofsky, C., and Franklin, N. (1979) Gene (Amst.) 5, 59-76
Capaldi, R. A., and Vanderkooi, G. (1972) Proc. Natl. Acad. Sci. U. S. A. 69, 930-932
Cuozzo, M., and Silverman, P. M. (1986) J. Biol. Chem. 261, 5175-5179
Cuozzo, M., Silverman, P. M., and Minkley, E. G., Jr. (1984) J. Biol. Chem. 259, 6659-6666
Date, T., Inuzuka, M., and Tomoeda, M. (1977) Biochemistry 16, 5579-5585
Everett, R., and Willetts, N. (1980) J. Mol. Biol. 136, 129-150
Franklin, N. C., and Bennett, G. N. (1979) Gene (Amst.) 8, 107-119
Garvey, J. S., Cremer, N. E., and Suslow, D. H. (1977) Methods in Immunology: A Laboratory Text for Instruction and Research, 3rd

2 M. M. Panicker, B. A. Traxler, and E. G. Minkley, Jr., unpublished observation.
TraD DNA Transfer Protein

Ed., pp. 218–229, W. A. Benjamin, Inc., Reading, MA
Gottesman, M. E., Adhya, S., and Das, A. (1980) J. Mol. Biol. 140, 57–75
Ippen-Ihler, K. A., and Minkley, E. G., Jr. (1986) Annu. Rev. Genet. 20, 593–624
Jalajakumari, M. B., and Manning, P. A. (1989) Gene (Amst.) 81, 195–202
Jalajakumari, M. B., Guidolin, A., Buhk, H. J., Manning, P. A., Ham, L. M., Hodgson, A. L. M., Cheah, K. C., and Skurray, R. A. (1987) J. Mol. Biol. 198, 1–11
Kennedy, N., Beutin, L., Achtman, M., Skurray, R., Rahmsdorf, U., and Herrlich, P. (1977) Nature 270, 580–585
Kingsman, A., and Willetts, N. (1978) J. Mol. Biol. 122, 287–300
Manning, P. A., Morelli, G., and Achtman, M. (1981) Proc. Natl. Acad. Sci. U. S. A. 78, 7497–7499
Manning, P. A., Kusecek, B., Morelli, G., Fisseau, C., and Achtman, M. (1982) J. Bacteriol. 150, 76–88
Minkley, E. G., Jr. (1984) J. Bacteriol. 158, 464–473
Minkley, E. G., Jr., and Willetta, N. (1984) Mol. & Gen. Genet. 196, 225–235
Neidhardt, F. C. (1987) in Escherichia coli and Salmonella typhimurium: Cellular and Molecular Biology (Neidhardt, F. C., Ingraham, J. L., Low, K. B., Magasanik, B., Schaechter, M., and Umbarger, H. E., eds) pp. 3–6, American Society for Microbiology, Washington, D. C.
O'Farrell, P. H. (1975) J. Biol. Chem. 250, 4007–4021
Panicker, M. M., and Minkley, E. G., Jr. (1985) J. Bacteriol. 162, 584–590
Perumal, N. B. (1985) Biochemical Characterization of the F Sex Factor traT Gene Product. Ph.D. thesis, Carnegie Mellon University
Willetts, N., and Skurray, R. (1980) Annu. Rev. Genet. 14, 41–76
Willetts, N., and Wilkins, B. (1984) Microbiol. Rev. 48, 24–41
Yoshioka, Y., Fujita, Y., and Ohtsubo, E. (1990) J. Mol. Biol. 214, 39–53