TatD Is a Cytoplasmic Protein with DNase Activity

NO REQUIREMENT FOR TatD FAMILY PROTEINS IN Sec-INDEPENDENT PROTEIN EXPORT

The Escherichia coli Tat system mediates Sec-independent export of protein precursors bearing twin arginine signal peptides. Genes known to be involved in this process include tatA, tatB, and tatC that form an operon with a fourth gene, tatD. The tatD gene product has two homologues in E. coli coded by the unlinked ycfH and xjv genes. An E. coli strain in in-frame chromosomal deletions in all three of tatD, ycfH, and xjv exhibits no significant defect in the cellular location of five cofactor-containing enzymes that are synthesized with twin arginine signal peptides. Neither of these mutations nor overproduction of the TatD protein cause any discernible effect on the export kinetics of an additional E. coli Tat pathway substrate. It is concluded that proteins of the TatD family have no obligate involvement in protein export by the Tat system. TatD is shown to be a cytoplasmic protein. TatD binds to immobilized Ni²⁺ or Zn²⁺ affinity columns and exhibits magnesium-dependent DNase activity. Features of the tatA operon that may control TatD expression are discussed.

The majority of bacterial periplasmic proteins are exported across the cytoplasmic membrane by the general secretory (Sec) pathway (1). However, a subset of periplasmic proteins, including many that bind redox-active cofactors, is translocated by a distinct Sec-independent mechanism. Such proteins are synthesized with N-terminal signal sequences containing a consensus (S/T)RR-X motif in which the arginine residues are invariant (2). These “twin arginine signal peptides” target the precursor protein to the recently discovered Tat (twin arginine translocation) protein export system (3) that is mechanistically and structurally related to the ΔII-dependent thylakoid import pathway of chloroplasts (4–6).

In Escherichia coli four genes, tatA, tatB, tatC, and tatE, that are required for the operation of the Tat transport system have so far been identified (7–10). Consistent with a role in transmembrane protein translocation, these tat genes are predicted to encode integral membrane proteins. Strains with mutations in tatB, tatC, or a combination of tatA and tatE are completely defective in the translocation of precursor proteins with twin arginine signal peptides.

The tatA, tatB, and tatC genes form an operon structure with a fourth promoter distal gene, tatD (7). The tatD gene is variously predicted to encode a soluble cytoplasmically located protein (8) or an integral membrane protein with a large cytoplasmic domain (7). The co-transcription of tatD with three genes known to encode essential components of the Tat export pathway strongly suggests an involvement of TatD in the Tat system (7). Here we have sought to experimentally test this prediction by examining the phenotype of a tatD mutant strain.

Two TatD homologues are coded in E. coli by the functionally unassigned and unlinked genes ycfH (29% amino acid sequence identity) and xjv (24% amino acid sequence identity). Because these additional TatD homologues may be capable of functionally substituting for TatD, we have also examined the phenotype of a strain in which all three genes coding for TatD-like proteins have been inactivated. Unexpectedly, we find that TatD family proteins are not required for protein transport by the E. coli Tat pathway.

**EXPERIMENTAL PROCEDURES**

**Genetic Constructs**—All mutants were derived from E. coli strain MC4100 (F−, ΔlacU169, araD139, rpsL150, relA1, ptsF, rbsR, fbb301 (11)). Strain TDD1 containing an in-frame deletion of tatD was constructed as follows. A 551-bp fragment covering the downstream region and the last two codons of tatD was amplified by PCR using the primers 5′-GGCCATCGATCGCTTATAGTTCTGCGAAGTCG-3′ and 5′-GGCCGATCCATGCGCTTATAGTTCTGCGAAGTCG-3′. The resulting product was digested with Clal and KpnI and cloned into the polylinker of pBluescript II KS+ (Stratagene) to give pFAST122. A 557-bp fragment covering the upstream region and the first two codons of tatD was constructed as follows. A 551-bp fragment covering the downstream region and the last two codons of tatD was amplified by PCR using the primers 5′-GGCCATCGATCGCTTATAGTTCTGCGAAGTCG-3′ and 5′-GGCCGATCCATGCGCTTATAGTTCTGCGAAGTCG-3′. The resulting product was digested with XhoI and BamHI and cloned into pFAI122 to give pFAI123. DNA from pFAI123 covering the in-frame deletion of tatD was amplified by PCR using the primers 5′-GGCCATCGATCGCTTATAGTTCTGCGAAGTCG-3′ and 5′-GGCCGATCCATGCGCTTATAGTTCTGCGAAGTCG-3′. The resulting product was digested with XhoI and BamHI and cloned into pFAI123 to give pFAI124. DNA from pFAI124 covering the in-frame deletion of tatD was excised by digestion with XhoI and KpnI and cloned into the polylinker of pMAK705 (12) to give the construct pFAI124. The mutant allele was transferred to the chromosome of MC4100 as described (12) to give strain TDD1.

Introduction of in-frame xjv and ycfH deletions into TDD1 produced the triple mutant strain TDD7. Construction of the in-frame deletion of tatD was excised by digestion with XhoI and KpnI and cloned into the polylinker of pMAK705 (12) to give the construct pFAI124. The mutant allele was transferred to the chromosome of MC4100 as described (12) to give strain TDD1.

**REFERENCES**

1 The abbreviations used are: Sec, general secretory pathway; PAGE, polyacrylamide gel electrophoresis; kbp, kilobase pair; bp, base pair; MOPS, 4-morpholinepropanesulfonic acid; PCR, polymerase chain reaction; trimethylamine N-oxide.
TTG-3
9 amplification using primers 5′-GCGGAATTCATGGAGTACAGGATGTTTG-3′ and 5′-GCGGGATCCGAAGAATACCGAGTTCCGC-3′. The resultant PCR product was digested as follows. The UV5 promoter/operator on plasmid pQE60 (Qiagen) and was cloned into pBluescript II KS+ (Stratagene) to make pTATD1. pTATD1 was digested with BamHI and cloned into pKm705 to give ampicl lonic plasmid pFAT755. The strain used for tatABCD (DADE) was constructed as follows. The tatA upstream region was excised from plasmid pFA74 (8) by digestion with XhoI and BamHI and cloned into pFA712 to give plasmid pFA715. The tatA-tatD deletion region was then excised by digestion with XhoI and KplI and cloned into pMARK750 to give ampicl lonic plasmid pFA716. The deletion allele was transferred to the chromosome of J1M1 (MC4100 Δtate) (8) to give strain DADE (ΔtateBCDΔtateF). All mutant strains were verified by PCR and Southern blotting, and the chromosomal PCR products were sequenced to ensure that no mismatched bases had been introduced. Plasmid pTATD2, which contains tatD under the control of a Ptac promoter, was constructed as follows. An 837-bp PCR fragment was isolated with primers 5′-GCGGAATTCATGGAGTACAGGATGTTTG-3′ and 5′-GCGGGATCCGAAGAATACCGAGTTCCGC-3′ using pFA755 plasmid DNA as the template. The resulting fragment was digested with EcoRI and BamHI and cloned into EcoRI- and BamHI-digested pBluescript to make pTATD1. pTATD1 was digested with KpnI and XhoI and cloned into KpnI- and XhoI-digested pACT3 (15) to make pTATD2. Plasmid pFAT755 harbors the tatD gene under the control of the UV5 promoter/βlac operator on plasmid pQE60 (Qiagen) and was constructed as follows. The tatD gene and flanking sequence was amplified by PCR using pFA755 as a template and primers 5′-GCGGAATTCATGGAGTACAGGATGTTTG-3′ and 5′-GCGGGATCCGAAGAATACCGAGTTCCGC-3′. The resultant PCR product was digested with EcoRI and HindIII and cloned into pQE60 to yield pFAT755.

Culture Conditions—During all genetic manipulations, E. coli strains were grown aerobically in Luria-Bertani (LB) medium (14). Concentrations of antibiotics were as described previously (8). Biochemical characterizations were carried out in cultures grown routinely in supplemented Cohen and Rickenberg (CR) medium as described previously (8). Overproduction of TatD from plasmid pFAT755 was carried out in LB medium supplemented with 0.8% (w/v) glucose.

Northern Analysis—Total cellular RNA was isolated from MC4100 grown aerobically in CR medium supplemented with glucose (0.4%) using the Qiagen RNeasy mini kit. The isolated RNA was separated by agarose gel electrophoresis in the presence of formaldehyde and capillary-blotted onto Hybond-N nylon membrane (Amersham Pharmacia Biotech). The blot was hybridized with either a 1.1-kbp DNA probe covering tatB and most of tatC, which was prepared by PCR amplification using primers 5′-GCGGAATTCATGGAGTACAGGATGTTTG-3′ and 5′-GCGGGATCCGAAGAATACCGAGTTCCGC-3′. The resultant PCR product was digested with EcoRI and HindIII and cloned into pQE60 to yield pFAT755.

RESULTS AND DISCUSSION

TatD Is a Cytoplasmic Protein—TatA, TatB, and TatC are predicted to be integral membrane proteins (4, 7, 8), while TatD is predicted to be an integral membrane protein (8, 10). Overexpression of TatD from plasmid pFAT755 was carried out in strain M15 (7, lac+, ara-, gal-, matl- (19)) that had been previously transformed with pREP4 (Kan+, lacI-, Roche Molecular Biochemicals). Overexpression of tatD was achieved following the addition of isopropyl-1-thio-β-D-galactopyranoside (final concentration 0.4 mM) to an exponentially growing 1-liter culture of M15(pREP4) + pFAT755. Cells were harvested and resuspended in 20 mM MOPS, pH 7.2, 10% glycerol, 50 mM NaCl, 2.5 mM Na2EDTA, 1 mM 2-mercaptoethanol (Buffer A) including a protease inhibitor mixture (Complete™, Roche Molecular Biochemicals). Cytoplasmic soluble proteins were prepared essentially as described (8) except exogenous DNAse I was not added to the cell suspension. The cytoplasmic extract was applied to a 5-ml HiTrap™ Q-Sepharose anion exchange column (Amersham Pharmacia Biotech) equilibrated in Buffer A. Following extensive washing with Buffer A, bound proteins were eluted with Buffer A containing 150 mM NaCl. Eluted proteins were dialyzed against Buffer A before being applied to a 1-ml Mono-Q™ anion exchange column (Amersham Pharmacia Biotech) previously equilibrated with Buffer A. Bound proteins were eluted with a linear 50-150 mM NaCl gradient, and fractions containing recombinant TatD were pooled and concentrated. The TatD pool was then subjected to molecular exclusion chromatography on a Superose-12 gel filtration column (Amersham Pharmacia Biotech) equilibrated in 10 mM MOPS, pH 7.2, 250 mM NaCl, 10% glycerol (Buffer B). Fractions containing TatD were pooled. Typically, 2.5 mg of recombinant TatD was recovered from 2 g (wet weight) of cells.

Protein Sequencing and Analysis—Protein sequencing services were provided by Alta Biosciences, Birmingham, UK.

Immobilized Metal Affinity Chromatography—A 1-ml HiTrap™ Q-Sepharose anion exchange affinity column (Amersham Pharmacia Biotech), charged with cations as required, was equilibrated in Buffer B (Protein samples were 250 µg) and were applied to a 1-ml Mono-Q™ anion exchange column (Amersham Pharmacia Biotech) previously equilibrated with Buffer A. Bound proteins were eluted with a linear 50-150 mM NaCl gradient, and fractions containing recombinant TatD were pooled and concentrated. The TatD pool was then subjected to molecular exclusion chromatography on a Superose-12 gel filtration column (Amersham Pharmacia Biotech) equilibrated in 10 mM MOPS, pH 7.2, 250 mM NaCl, 10% glycerol (Buffer B). Fractions containing TatD were pooled. Typically, 2.5 mg of recombinant TatD was recovered from 2 g (wet weight) of cells.

Correction Subcellular Localization of Tat-dependent Proteins

TatD Is a DNase—TatD, TatB, and TatC are predicted to be integral membrane proteins (4, 7, 8), while TatD has been proposed to have either a cytoplasmic (8) or a membranous location (7). To explore the cellular location of the Tat proteins, expression of plasmid-encoded tatABCDE was induced in the presence of [35S]methionine, and the cells were fractionated by ultracentrifugation. Membranes were washed once in 50 mM Tris-HCl, pH 8.0, 500 mM NaCl. Samples were analyzed by autoradiography following SDS-PAGE.

PreSuff was expressed under the control of the phase 7 T7 promoter from pLA2001 (18), pNR14 and pG1-P2 were expressed from CM1400 or TDD7, and synthesis of plasmid-encoded gene products was induced by a temperature shift from 30° to 42°C, followed by labeling with [35S]methionine for 5 min, according to the procedure described by Tabor and Richardson (17). Cells were chased with unlabeled methionine (750 µg/ml final concentration) and further incubated at 30°C for 0–60 min. The effect of TatD overproduction on the export of SufI was assessed in strain BL21(DE3) carrying pNR14 and pTATD2. Pulse-chase experiments were carried out as above with the exception that the cells were kept at 37°C for all steps except the heat-shock (which was retained to allow efficient uptake of rifampicin) and that 0.4 mM isopropyl-1-thio-β-D-galactopyranoside was included in the growth medium from 2 h prior to the heat-shock step to induce both TatA and TatD expression. Overproduction and Purification of the tatD Gene Product—Plasmid pFAT755 was transferred into strain M15 (7, lac+, ara-, gal-, matl- (19)) that had been previously transformed with pREP4 (Kan+, lacI-, Roche Molecular Biochemicals). Overexpression of tatD was achieved following the addition of isopropyl-1-thio-β-D-galactopyranoside (final concentration 0.4 mM) to an exponentially growing 1-liter culture of M15(pREP4) + pFAT755. Cells were harvested and resuspended in 20 mM MOPS, pH 7.2, 10% glycerol, 50 mM NaCl, 2.5 mM Na2EDTA, 1 mM 2-mercaptoethanol (Buffer A) including a protease inhibitor mixture (Complete™, Roche Molecular Biochemicals). Cytoplasmic soluble proteins were prepared essentially as described (8) except exogenous DNAse I was not added to the cell suspension. The cytoplasmic extract was applied to a 5-ml HiTrap™ Q-Sepharose anion exchange column (Amersham Pharmacia Biotech) equilibrated in Buffer A. Following extensive washing with Buffer A, bound proteins were eluted with Buffer A containing 150 mM NaCl. Eluted proteins were dialyzed against Buffer A before being applied to a 1-ml Mono-Q™ anion exchange column (Amersham Pharmacia Biotech) previously equilibrated with Buffer A. Bound proteins were eluted with a linear 50-150 mM NaCl gradient, and fractions containing recombinant TatD were pooled and concentrated. The TatD pool was then subjected to molecular exclusion chromatography on a Superose-12 gel filtration column (Amersham Pharmacia Biotech) equilibrated in 10 mM MOPS, pH 7.2, 250 mM NaCl, 10% glycerol (Buffer B). Fractions containing TatD were pooled. Typically, 2.5 mg of recombinant TatD was recovered from 2 g (wet weight) of cells.
TMAO reductase is a soluble periplasmic enzyme containing a molybdopterin guanine dinucleotide cofactor. Me₂SO reductase and nitrate-inducible formate dehydrogenase are membrane-bound molybdopterin guanine dinucleotide-containing enzymes. The precursors of all three reductases bear twin arginine signal peptides. Correct subcellular localization of these enzymes has previously been shown to be blocked in \( \text{tatE} \), \( \text{tatA} \), and \( \text{tatC} \) strains (8–10). Both the \( \text{tatD} \) and \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) mutants were capable of anoxic growth on the non-fermentable carbon source glycerol with TMAO or Me₂SO as sole electron acceptor, and the triple mutant showed identical patterns of TMAO and Me₂SO reductase localization to the parental strain (Table I). The absence of TatD-like proteins does not, therefore, affect targeting and maturation of TMAO and Me₂SO reductases. Enzymatic assays indicate that assembly of the TatD-dependent nitrate-inducible formate dehydrogenase is likewise unaffected in the \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) mutant (Table I).

E. coli hydrogenases-1 and -2 are membrane-bound enzymes with cofactor-binding subunits located at the periplasmic face of the plasma membrane. In each enzyme a large catalytic subunit binding a Ni-Fe cofactor is partnered by a small subunit containing iron-sulfur clusters. Export of both subunits is stable the plasma membrane. In each enzyme a large catalytic subunit binding a Ni-Fe cofactor is partnered by a small subunit containing iron-sulfur clusters. Export of both subunits is end with the convergently transcribed region is capable of forming a stable stem-loop structure with a predicted \( \Delta G \) of \(-16 \) kJ/mol. It can be inferred from the characteristic run of uridine residues to the stem-loop structure that active hydrogenase-1 and -2 are found exclusively in the membrane fraction of both parental and \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) mutant strains (Fig. 2, a and b, lanes 3 and 4) and thus that assembly of these enzymes does not require proteins of the TatD family.

**Export Kinetics of a Tat-dependent Precursor Protein in a \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) Mutant**—The experiments described above demonstrate that TatD-like proteins have no obligate role in the targeting of proteins with twin arginine signal peptides. Nevertheless, it remains possible that proteins of the TatD family play a non-essential role in export. Such a function might manifest itself as an effect on export kinetics in the \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) mutant. In order to investigate this possibility, pulse-chase experiments were undertaken using the water-soluble, peripheral Tat substrate preSufI (10, 18). No significant differences in the export kinetics of SufI in the \( \text{tatD}_{\Delta \text{yjjV}} \text{yycfH} \) background relative to the parental strain could be detected (Fig. 3). This experiment suggests that proteins of the TatD family are not required for a rate-limiting step in the export of preSufI and provides further evidence that TatD-like proteins are not essential for Tat pathway export.

**TatD May be Expressed at Substantially Lower Levels Than the Other Products of the tatA Operon**—On the basis of reverse transcriptase PCR experiments, Weiner and co-workers (7) were previously able to establish that tatABCDO can be transcribed as a single messenger RNA and therefore constitute an operon (Fig. 4a). Northern analysis of whole cell mRNA was undertaken using a tatBC probe to investigate the possible existence of alternative tatA operon transcripts. In addition to the expected 2.6-kbp tatABCDO species, a much more abundant 1.6-kbp transcript was also identified (Fig. 4b, lane 1). Only the 1.6-kbp transcript could be detected in the \( \text{tatD} \) strain TDD1 (Fig. 4b, lane 3) suggesting that smaller mRNA corresponds to a message covering tatABC alone. This interpretation was confirmed by Northern analysis with a tatD-specific probe. The tatD probe recognized only the larger 2.6-kbp transcript and only in a tatD+ background (Fig. 4b, lane 4). Thus, under the growth condition tested, tatD is transcribed at a lower level than the other genes of the tatA operon.

The tatC and tatD genes are separated by 41 bp (the tatD start codon is definitively identified below), and tatD shares a 2-bp overlap at its 3′ end with the convergently transcribed \( \text{rfaH} \) gene (Fig. 4a). Fig. 4c shows that the \( \text{tatC-tatD} \) intergenic region is capable of forming a stable stem-loop structure with a predicted \( \Delta G \) of \(-16 \) kJ/mol. It can be inferred from the characteristic run of uridine residues to the stem-loop structure on an \( \text{rfaH} \) (but not a tatABC) transcript (Fig. 4c) that the stem-loop structure functions primarily as a Rho-independent transcription terminator for \( \text{rfaH} \). However, the stem-loop structure is probably also responsible for the lowered levels of tatD-containing transcripts detected by Northern analysis (Fig. 4b) either by attenuating transcriptional readthrough from tatC or, since mRNA is degraded from the 3′ end (20), by stabilizing the tatABC portion of the message. Moreover, \( \text{rfaH} \) transcripts terminating at the stem-loop structure would reduce the levels of tatD mRNA available for translation by an antisense mechanism (and vice versa). Together these various transcript features suggest that TatD is produced at considerably lower levels than the other products of the tatA operon.

In the light of this apparent regulatory complexity, it was conceivable that control of the cellular TatD to TatABC stoichiometry is important for Tat-dependent export. This possibility was assessed by investigating whether raised levels of TatD interfere with the export process. Plasmid pTATD2 in which tatD expression had been placed under the control of a strong promoter was used to overproduce TatD in the Tat+ strain BL21(DE3). This increase in TatD synthesis had no effect on the kinetics of preSufI export (data not shown). It is therefore unlikely that either highly regulated or deliberately low level expression of TatD is important for Tat-dependent export.
Characterization of the tatD Gene Product—In order to gain further insight into its biochemical properties, TatD was purified from the high speed supernatant of cells overexpressing the tatD gene. The N terminus of the purified protein was determined to be XFDIGV (where X is a non-definable residue) by direct sequencing. This sequence corresponds to that of TatD starting at the second of two putative tatD initiation codons (Fig. 4c). Additional positive identification of the purified protein as TatD was obtained by peptide mass fingerprinting following trypsinolysis (data not shown). The purified TatD protein was sized at 30 kDa by gel permeation chromatography under native conditions suggesting that the 28,955-Da TatD polypeptide is a monomer in solution.

TatD family proteins show significant sequence similarity to proteins assigned to the polymerase and histidinol phosphatase superfamily of metal-dependent phosphoesterases (21). For example, E. coli TatD exhibits 33% amino acid identity to the YabD protein of Chlamydomphila pneumoniae. Purified TatD bound to affinity matrices bearing immobilized Ni\(^{2+}\) or Zn\(^{2+}\) ions (Fig. 5a, lanes 5 and 7) indicating that TatD has at least one high affinity metal ion-binding site. Interestingly, the TatD protein exhibited DNase activity in the presence of Mg\(^{2+}\) (Fig. 5b, lane 4) but not Ca\(^{2+}\) or Zn\(^{2+}\) (Fig. 5b, lanes 3 and 5). Thus TatD can function as a magnesium-dependent DNase.

The ability of TatD to degrade RNA was not tested. Purified TatD, with or without divalent metal cation supplementation, showed no nonspecific protein hydrolyzing activity with casein as substrate.

Concluding Remarks—Previous research has shown that tatB, tatC, and tatA plus tatE are each required for export by the Tat pathway of E. coli (7–10). In contrast, the results presented here demonstrate that tatD and two further genes encoding TatD homologues are dispensable for Tat-dependent protein transport. In addition, neither loss of these gene functions nor overproduction of the TatD protein had any discernible effect on the export kinetics of a Tat-dependent precursor. A non-essential and indeed Tat-nonspecific role for TatD-like proteins is consistent with phylogenetic analysis of the occurrence of tat genes. Although homologues of the essential TatA/B/E and TatC proteins are encoded in all complete genomes of prokaryotes possessing proteins with twin arginine signal peptides, a TatD-like protein is missing from the genome of one such organism, the Archaeon Archaeoglobus fulgidus (22). Additionally, TatD-like proteins are coded by the genomes of the yeasts Saccharomyces cerevisiae (Swiss-Prot accession number P01603; 35% amino acid identity) and Schizosaccharomyces pombe (Swiss-Prot accession number 2257524; 36% amino acid identity), neither of which possesses proteins with twin arginine signal peptides or homologues of the essential Tat proteins. Thus no exact correlation exists between the ability of an organism to synthesize a TatD-like protein and whether that organism has a Tat protein transport pathway. Furthermore, although the TatD-coding gene forms an operon with tatABC in E. coli, suggesting a functional inter-relationship between
TatD and the essential Tat proteins, this genetic linkage is not maintained in bacteria other than the closely related *Salmonella typhi* and *Yersinia pestis*. An additional circumstantial argument in support of a non-essential role for TatD in Tat-dependent export is that thylakoid import by the structurally and mechanistically related ΔpH-dependent transport pathway of plant chloroplasts does not require, and is not enhanced by, any water-soluble stromal factors (23, 24), yet it is shown here that TatD is a water-soluble cytoplasmic protein. Finally, we have also shown that purified TatD exhibits a magnesium-dependent DNase activity, a biochemical function that is difficult to reconcile with a role in protein transport. A possible function of TatD-like proteins in DNA metabolism is supported by linkage analysis as one *E. coli* gene coding for a TatD homologue, *ycfH*, is apparently co-transcribed with a gene encoding the δ′ subunit of DNA polymerase III (*holB*, Fig. 4a), and a similar linkage is seen for the single *tatD*-like gene of *Rhodobacter capsulatus* (open reading frame RRC02703).

Given that TatD has a function unrelated to protein transport, why, as confirmed in the current work, is *tatD* co-transcribed with *tatABC*? It is in fact not uncommon in *E. coli* for genes encoding components of the protein secretion apparatus to form operons with genes of unrelated function. For example, the gene for the Sec translocase SecE subunit is part of an operon that includes a gene, *nusG*, encoding a transcriptional anti-terminator protein (25), while the SecY subunit of the

**FIG. 4.** The tatA operon produces two transcripts. *A*, organization of the *E. coli* genomic loci encoding TatD family proteins. *aarF* codes for a protein required for ubiquinone biosynthesis, *rfaH* codes for a transcriptional anti-terminator involved in the regulation of among other functions hemolysin synthesis; *holB* codes for the δ′ subunit of DNA polymerase III; *tmk* codes for deoxyxynucleosidase lyase; *ptsG* codes for glucose permease component IIBC; *osmY* codes for osmotically inducible protein Y. *b*, Northern blot of total cellular RNA isolated from the following: lanes 1 and 4, the parental strain MC4100; lanes 2 and 5, the ΔtatABCD* tatE* strain DADE; and lanes 3 and 6, the ΔtatD strain TDD1 and hybridized to a probe covering either *tatBC* (lanes 1–3) or *tatD* (lanes 4–6). *c*, predicted mRNA secondary structure in the *tatCD* intergenic region for both *tatA* (upper strand) and *rfaH* (lower strand) transcripts. The *tatC* stop codon, the putative *tatD* ribosome-binding site, and the polyuridine regions that may form part of a Rho-independent terminator for the *rfaH* transcript are indicated.
same translocase is encoded by an operon otherwise dedicated to ribosomal proteins (26). Another example is that the gene coding for the signal recognition particle receptor FtsY is part of an operon encoding two other genes, ftsE and ftsX, that are involved in cell division rather than protein transport (27). In these cases, and perhaps also that of tatD, the cell may be grouping functionally unrelated genes with similar regulatory requirements.

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FIG. 5. Purified TatD binds to immobilized divalent metal cations and exhibits DNase activity in the presence of magnesium ions. a, testing the ability of purified TatD to bind to chelating affinity media. Purified TatD was passed through a chelating affinity column without bound metal (lanes 2 and 3) or charged with Ni^{2+} (lanes 4 and 5) or Zn^{2+} (lanes 6 and 7). SDS-PAGE was used to analyze purified TatD (lanes 1 and 8), material that did not bind to the columns (wash through (WT), lanes 2, 4, and 6), and material that was eluted from the columns by 500 mM imidazole (IM, lanes 3, 5, and 7). b, linearized pBluescript plasmid was incubated in the absence (lanes 1 and 7) or presence (lanes 2–6) of TatD and a variety of metal ions for 60 min at 37 °C. DNA was visualized by agarose gel electrophoresis in the presence of ethidium bromide. Lane 1, linearized DNA only; lane 2, as lane 1 in the presence of 5 μg of TatD; lanes 3–6, as lane 2 but with 5 mM of the following added: lane 3, CaCl_{2}; lane 4, MgCl_{2}; lane 5, ZnSO_{4}; lane 6, EDTA. Lane 7, DNA incubated in the absence of TatD but with an equivalent volume (1 μl) of Buffer B in the presence of 5 mM MgCl_{2}.
