The truA gene of *Pseudomonas aeruginosa* is required for the expression of type III secretory genes

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Invasive strains of *Pseudomonas aeruginosa* can cause rapid host cell apoptosis by injecting the type III effector molecule ExoS. A transposon insertional mutant bank of *P. aeruginosa* was screened to identify *P. aeruginosa* genes that contribute to the ability of the bacteria to trigger host cell apoptosis. Several isolated mutants had disruptions in the *fimV* gene. A *fimV* mutant was unable to induce the expression of exoS, exoT and exsA genes under type III inducing conditions, thus exhibiting a defect in type III protein secretion. Furthermore, this mutant was defective in twitching motility, although type IV pili were present on the bacterial surface. Complementation by a *fimV*-containing cosmid clone restored both phenotypes to the wild-type levels. However, expression of the type III genes in the *fimV* mutant was not restored by the introduction of a *fimV* gene alone, although it restored the twitching motility. A gene downstream of *fimV*, encoding a tRNA pseudouridine synthase (*truA*) homologue, was able to complement the type III gene expression defect of the *fimV* mutant. Thus *fimV* and *truA* form an operon and *fimV* mutation has a polar effect on *truA*. Indeed, a *truA* mutant is defective in type III gene expression while its twitching motility is unaffected, and a *truA* clone is able to complement the type III secretion defect. Pseudouridination of tRNAs is important for tRNA structure, thereby improving the fidelity of protein synthesis and helping to maintain the proper reading frame; thus the results imply that *truA* controls tRNAs that are critical for the translation of type III genes or their regulators.

**INTRODUCTION**

*Pseudomonas aeruginosa* is an opportunistic human pathogen that primarily infects patients with burn wounds or cystic fibrosis, or immunocompromised individuals (Asboe et al., 1998; Bodey et al., 1983; Davies, 2002; Pier, 2002). Clinical isolates of *P. aeruginosa* have been grouped into invasive and noninvasive (cytolytic) strains based on their interactions with non-phagocytic corneal epithelial cells (Fleiszig et al., 1997). The invasive and noninvasive strains encode different sets of exoenzymes that are translocated into the host cells via a type III secretion machinery (Fleiszig et al., 1997; Frithz-Lindsten et al., 1997). The type III system is a common virulence machinery of Gram-negative animal and plant pathogens, such as enterohaemorrhagic *Escherichia coli*, *Campylobacter*, *Salmonella*, *Shigella*, *Yersinia*, *Erwinia* and *Xanthomonas* species (Cornelis & Wolf-Watz, 1997; Galan & Collmer, 1999; Greenberg & Vinatzer, 2003; Grosman & Ochman, 1993; Hueck, 1998; Van Gijsen et al., 1993). The type III secretion system of *P. aeruginosa* encodes about 20 proteins, including components of a secretory apparatus which is devoted to the direct translocation of effectors into the host cell cytoplasm, and four effector molecules, ExoS, -T, -U and -Y, which alter normal host cell processes (Frank, 1997; Hauser et al., 1998; Yahr et al., 1996a, 1997). The noninvasive strains contain *exoT* and *exoU* genes, whereas the invasive strains contain *exoS* and *exoT* genes (Fleiszig et al., 1997).

ExoS and ExoT are bifunctional exotoxins with C-terminal ADP-ribosylation activities. ExoS and ExoT share 75 % amino acid identity, but ExoT possesses a lower catalytic activity, with only 0.2 % of the ADP-ribosyltransferase activity of ExoS (Yahr et al., 1996b). The ADP-ribosyltransferase activity of ExoS requires a eukaryotic cofactor termed factor activating ExoS (FAS), which is a member of the highly conserved, multifunctional 14-3-3 family of proteins, whose primary function involves the regulation of eukaryotic
enzyme activities (Aitken et al., 1995; Fu et al., 1993). The requirement of a eukaryotic cofactor for activity and the functional importance of the in vivo target proteins suggest that ExoS contributes to pathogenesis by disrupting normal cellular processes. ExoS preferentially ADP-ribosylates a number of proteins, including vimentin and several Ras family GTP-binding proteins that regulate intracellular vesicle transport, cell proliferation and differentiation (Bourne et al., 1990; Coburn & Gill, 1991). The ADP-ribosylating activity of ExoS was also shown recently to cause apoptosis in various tissue culture cells (Kauffman et al., 2000; Jia et al., 2003). Both ExoS and ExoT also cause severe cell rounding by disrupting the actin cytoskeleton with their N-terminal GTPase-activating protein domain (GAP) (Goehring et al., 1999; Krall et al., 2000; Pederson et al., 1999). Expression of these exoenzymes is coordinately regulated by a transcriptional activator, ExsA, in response to various environmental signals, including low calcium and direct contact with tissue culture cells (Vallis et al., 1999).

P. aeruginosa expresses type IV pili on the cell surface which not only function as an adhesin but also as a motility apparatus enabling the bacterium to glide on solid surfaces, a motion called twitching motility. Such motility is achieved by the retractile movement of the type IV pili (Merz et al., 2000). There are separate sets of P. aeruginosa genes devoted to pilin gene expression, processing, assembly of the pilin subunits on the bacterial surface and twitching motility (Alm & Mattick, 1997; McBride, 2001). In the course of screening P. aeruginosa genes that affect bacterial ability to inject type III effector molecules into the host cell, we have identified fimV mutants that are defective in both type III secretion and twitching motility. Complementation assay results are consistent with the notion that the fimV gene forms an operon with a downstream gene, truA, and that the fimV gene is required for twitching motility while the truA gene is required for the type III secretory gene expression. Since pseudouridination of tRNAs is known to be required for maturation of tRNAs from their precursors, aminoclylation and stabilization of the stem–loop structure through improved intramolecular base-pairing (Auffinger & Westhof, 1998; Davis, 1995; Durant & Davis, 1999; Price & Gray, 1998), our observations imply that truA controls tRNAs that are critical for the expression of type III genes or their regulators.

**METHODS**

**Bacterial strains and plasmids.** The bacterial strains and plasmids used in this study are listed in Table 1. P. aeruginosa was grown on Luria (L) agar or in L broth at 37°C. Antibiotics were used at the following final concentrations. For P. aeruginosa: carbenicillin, 150 μg ml⁻¹; spectinomycin, 200 μg ml⁻¹; streptomycin, 200 μg ml⁻¹; and tetracycline, 100 μg ml⁻¹. For E. coli: ampicillin, 100 μg ml⁻¹; spectinomycin, 50 μg ml⁻¹; streptomycin, 25 μg ml⁻¹; and tetracycline, 20 μg ml⁻¹. The pppB gene was PCR amplified using primer sets pppB-1 (5’-GCC GCA CCA GCC TGG TGG AGA AGA TGG-3’) and pppB-2 (5’-CCT GAC GCC GCT GTG GAT CCG CCA AAG ATC TA-3’). PCR products were first cloned into pCR2.1-TOPO, and then the inserts were reisolated as EcoRI—BglII fragments for cloning into C-terminal FLAG tag fusion vector pFLAG-CTC (Sigma). The pppB-FLAG clones were further isolated as HindIII—ScaI fragments and subcloned into HindIII—SmaI digests of the shuttle vector pUCP19 to generate pPpB-FLAG. Resulting plasmids were confirmed for in-frame fusions by sequencing.

**Screen of mutants defective in HeLa cell lifting.** A Tn5G transposon insertional mutant library was generated as described earlier (Wang et al., 1996). Individual mutants were grown overnight in 96-well plates with L broth containing gentamicin. The bacterial cells (0.1 ml) were used to infect freshly grown HeLa cells on 24-well plates, incubated for 6 h and washed with water to remove lifted HeLa cells. Crystal violet staining was used to visualize HeLa cells that remained adhered to the plates. Bacterial mutants that were unable to lift HeLa cells within the 6 h infection period were further characterized. First, slow-growing mutants were eliminated by testing their growth curves, then a HeLa cell adherence assay was conducted to eliminate those having mutations leading to significantly low binding (Ha & Jin, 2001). Chromosomal DNA of the mutants was digested with EcoRI and the Tn5G insertions together with the neighboring chromosomal DNA were cloned into pTZ18R. DNA sequencing analysis was further conducted to identify the transposon insertion sites.

**Construction of isogenic mutants of fimV and truA.** The fimV and truA genes were PCR amplified from the P. aeruginosa PAK chromosome using the following two pairs of primers: fimV-1 (5’-GTC TCT CGC ACC GCA TGA TCG CCT TAC TGC TC-3’) with fimV-2 (5’-CAT CGT CTA TGA GGG TGC CTT CAT TTC CGG ATC AG-3’); and truA-1 (5’-TCC TCG TCG AAG AAG TCC TGG CCG AAG GTA ATG ACA GC-3’) with truA-2 (5’-TCT CGA TGG TAG CAA AAG CCC GAT TCG ACA GC-3’), respectively. The fimV and truA genes were first cloned into pCR2.1-TOPO, resulting in pHW0035 and pKS0206, respectively, and then further subcloned into the suicide vector pEX18Tc to generate pHW0199 and pKS0212, respectively. The spectinomycin/streptomycin-resistance gene cartridge (2 kb β cassette) was inserted into the BamHI site within the fimV gene of pHW0199 and also into the NdeI site of the truA gene in pKS0212, giving pHW01100 and pKS0204, respectively. For a non-polar fimV mutant, oligonucleotides fimV-sd-f (5’-GAT ATT AAA AGG GAT TAC ACT GCA GTT CGG CTT CGT ACA-3’) and fimV-sd-b (5’-CAG TGT ACG AAG CCG AAC TGC-3’) with tetracycline resistance gene (2 kb β cassette) was inserted into the pCR2.1 vector (Alm & Mattick, 1995). Briefly, the transposon insertion sites.

**Detection of ExoS and ExoT proteins in culture medium.** Bacteria were grown overnight in L broth with 5 mM EGTA to a cell density of OD₆₀₀ 4.0 and 0.5 ml culture supernatants were concentrated to 10 μl using a Centricon-30, mixed with an equal volume of 2X loading buffer, boiled and subjected to 12% SDS-PAGE (Laemmli, 1970). Protein bands were visualized either directly by Coomassie staining or following Western blotting. For Western blot analysis, proteins on the gel were transferred electrophoretically onto Hybond-C nitrocellulose (Amersham) in the Tris/glycine system as described by Towbin et al. (1979). FLAG-tagged ExoS and ExoT were detected with anti-FLAG antiserum (Sigma) followed by goat anti-mouse immunoglobulin G conjugated to horseradish peroxidase (Amersham) and an enhanced chemiluminescence (ECL) detection kit from Amersham.

**Twitching motility assay.** Twitching motility was assayed as described by Alm & Mattick (1995). Briefly, the P. aeruginosa strain to be tested was stab-inoculated through a 1% agar plate, grown...
**RESULTS**

**Identification of *P. aeruginosa* mutants defective in HeLa cell lifting**

Invasive strains of *P. aeruginosa* can cause efficient host cell apoptosis by injecting the type III effector molecule ExoS (Kaufman et al., 2000). In order to identify *P. aeruginosa* genes that affect its ability to inject ExoS into the host cell, a Tn5G transposon insertional mutant library of *P. aeruginosa* was screened. Since one of the hallmarks of HeLa cell apoptosis is cell lifting, we initially screened for mutants that are defective in causing HeLa cell lifting. At m.o.i. 20, wild-type PAK caused 100% cell lifting by 4 h of incubation whereas a type III defective mutant strain (PAK::exsA) did not cause cell lifting even after 24 h incubation. An initial screen of 5000 individual mutants identified 60 mutants that were unable to cause HeLa cell lifting by 6 h. Further analysis of those mutants revealed that 47 had a significant defect in either growth or host cell binding whereas the remaining 13 had normal growth and attachment. Sequence analysis of the transposon insertion sites revealed five different genetic loci, including genes of fimV (PA0413), hepA (PA5272) and nuoE (PA2640). cyaA encodes a protein that shares 54% similarity with adenylate cyclase of *E. coli* (Aiba et al., 1983) and was recently reported to be essential for efficient expression of the type III genes in response to the low-calcium signal.

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**Table 1. Bacterial strains and plasmids used in this study**

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<tr>
<th>Strain or plasmid</th>
<th>Description*</th>
<th>Source</th>
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<td><strong>P. aeruginosa</strong></td>
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<td>D. Bradley†</td>
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<td>Promoterless lacZ fusion vector; Sp′ Sm′ Tc′</td>
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<td>Cosmid clone containing fimV and truA genes in pLA2917; Tc′</td>
<td>Croft et al. (2000)</td>
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*Ap′, ampicillin resistance marker; Km′, kanamycin resistance marker; Sm′, streptomycin resistance marker; Sp′, spectinomycin resistance marker; Tc′, tetracycline resistance marker.
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A *fimV* mutant is defective in type III gene expression

In order to confirm that mutation in *fimV* is responsible for the observed defect in HeLa cell lifting, a new defined *fimV* mutant was generated in the PAK background by inserting an Ω fragment, encoding resistance to streptomycin and spectinomycin, into the *fimV* locus by allelic exchange utilizing the sacB counterselection marker (Schweizer, 1992). The *fimV* mutant grew as well as wild-type PAK in L broth with (type III inducing condition) or without 5 mM EGTA. As reported by Semmler *et al.* (2000), the *fimV* mutant was completely defective in twitching motility (Fig. 1), although type IV pili were observed on the surface by scanning electron microscopy. The new *fimV* mutant also showed a defect in HeLa cell lifting, just like the original transposon insertion mutants (data not shown).

Next, we tested whether the secretion of the type III effector molecules is defective in the *fimV* mutant. Plasmids with *exoS*-FLAG or *exoT*-FLAG fusions (pHW0029 or pHW0027) were transformed into wild-type strain PAK, PAK* fimV::Ω* and PAK*exsA::Ω*, respectively. These strains were grown under type III inducing conditions (L-broth plus 5 mM EGTA) and Western blot analysis was conducted on both culture supernatants for secreted form and bacterial cell extracts for intracellular level using antibody against the FLAG-tag. As a control, FLAG-tag fusion to the C-terminal end of a known cytoplasmic protein, PppB, was used (Ha & Jin, 2001) to monitor cell-lysis mediated protein release. As shown in Fig. 2, in both supernatant and cellular fractions, the ExoS and ExoT protein levels were much lower in the *fimV* mutant compared to that of wild-type PAK, suggesting that type III expression might be defective in the *fimV* mutant. Absence of PppB-FLAG in the supernatant eliminated the possibility of cell-lysis mediated protein release.

To further test if the expression of the type III genes is defective in the *fimV* mutant, plasmid constructs containing transcriptional fusions between promoters of the *exoS*, *exoT* and *exsA* genes and a promoterless *lacZ* reporter gene, pHW0005, pHW0006 and pHW0032, respectively, were

![Fig. 1. Twitching motility assays. Bacteria were inoculated on L agar, incubated overnight at 37 °C and placed at room temperature for a further 2 days. Strains: wt, wild-type PAK; *fimV*<sup>−</sup>, *fimV* mutant; *truA*<sup>−</sup>, *truA* mutant. Plasmids: */fimV* and */truA* represent complementation by *fimV* clone pHW01116 and *truA* clone pKS0207, respectively.](image1)

![Fig. 2. Secretion of type III proteins is defective in the *fimV* mutant. Bacteria were grown overnight in L broth with 5 mM EGTA and both culture supernatants and cell extracts were separated by SDS-PAGE. The proteins were transferred onto nitrocellulose membrane and blotted with anti-FLAG antibody. Strains: wt, wild-type strain PAK; *fimV*<sup>−</sup>, *fimV* mutant; *exsA*<sup>−</sup>, *exsA* mutant. Plasmids: S, T and pppB are *exoS*, *exoT* and pppB with FLAG-tag, respectively.](image2)
used. The three fusion constructs were transformed into PAK, PAKfimV<sup>-</sup>, and a type III mutant strain PAKexsA<sup>-</sup>, and β-galactosidase activities were monitored following induction in L broth with 5 mM EGTA. As shown in Fig. 3, the induction of exoS, exoT and exsA was abolished in the fimV mutant background, similar to that in the exsA mutant background, confirming that the fimV mutant is indeed defective in the expression of the whole type III secretion system. Furthermore, since these reporter constructs were transcriptional fusions, the results indicate that type III gene expression is affected at the transcriptional level.

The truA gene is required for the induction of type III secretion genes

A cosmid clone containing a 27 kb insert covering the fimV region of the P. aeruginosa chromosome (pMO010631) was able to complement the fimV mutant for both twitching motility and type III expression. However, a PCR clone of the intact fimV gene, pHW01116, was only able to complement the twitching motility defect (Fig. 1) but not the type III secretion defect (Fig. 4), suggesting the requirement of additional genes for rescuing type III expression. We searched for neighbouring genes that might be affected by a polar effect upon insertional disruption of the fimV. An open reading frame (truA) with high homology to the hisT gene of E. coli, encoding tRNA pseudouridine synthase, was identified downstream of the fimV gene on the P. aeruginosa genome sequence (http://www.pseudomonas.com). Since there is no obvious promoter sequence 5’ to truA, it is likely that the truA gene forms an operon with the upstream fimV gene. To test if the truA gene is required for complementation of the type III defect in the fimV mutant, plasmids harbouring truA, alone or together with fimV, pKS0207 and pKS0209, were constructed. As shown in Fig. 4, in contrast to the fimV-only clone, secretion of the ExoS and ExoT proteins in the fimV mutant was restored by the introduction of truA-containing clones (pKS0207 and pKS0209). However, the truA-only clone (pKS0207) was unable to complement the twitching motility defect of the fimV mutant (Fig. 1). These results demonstrate that fimV is required for the twitching motility while truA is required for the type III gene expression. The two genes are likely to form an operon and disruption of fimV interferes with truA expression through a polar effect.

A non-polar fimV mutant strain of PAK was further generated by double crossing into the chromosome a fimV mutant whose translational start codon ATG was changed into CTG. The resulting non-polar fimV mutant strain was completely defective in twitching motility while its type III gene expression was unaffected (data not shown).

To further confirm the role of truA in type III gene expression, a truA null mutant was generated in the PAK background by the insertion of the Ω fragment. This mutant did not show a significant difference in growth from that of the wild-type. As expected, the truA mutant did not show a
twitching motility defect (Fig. 1), but the expression of type III secretory genes was almost non-responsive to type III inducing conditions, similar to the phenotype of the fimV mutant, as shown by β-galactosidase activities (Fig. 5). Consistent with this, we failed to detect the ExoS and ExoT from culture supernatants of PAKtruA− under type III inducing conditions (data not shown). Furthermore, the truA mutant showed the same defect as the fimV mutant in the HeLa cell lifting assay. These type III defect phenotypes were corrected to that of wild-type by introducing a truA-containing clone (pKS0207) (Fig. 6), but not by the fimV-only clone (pHW01116). Taken together, these results clearly demonstrate that the truA gene is indeed a novel modulator regulating the expression of type III secretion genes in P. aeruginosa.

DISCUSSION

Semmler et al. (2000) previously demonstrated that the fimV gene is required for twitching motility in P. aeruginosa. The FimV protein has a peptidoglycan-binding domain, predicted transmembrane domains, a highly acidic C terminus and unusual primary or secondary structure. Overexpression of fimV resulted in an unusual phenotype where the cells were massively elongated and migrated in large convoys at the periphery of the colony. It was suggested that FimV may be involved in remodelling of the peptidoglycan layer to enable assembly of the type IV fimbrial structure and machinery (Semmler et al., 2000). In the current study, a fimV mutant was found to be also defective in type III protein secretion, a defect which could not be complemented by the fimV clone, although the clone complemented the twitching motility defect. This prompted us to test the downstream gene truA, which turned out to be the gene required for the type III gene expression under inducing conditions, both under low calcium conditions and upon contact with host cells. The complementation test results as well as the non-polar mutant phenotype clearly demonstrated that fimV is required for twitching motility while truA is required for type III gene expression. As summarized in Fig. 7, fimV and truA form an operon; therefore disruption of fimV has a polar effect on the truA gene – the fimV mutant is defective in both twitching motility and type III gene expression while the truA mutant has a normal twitching motility but is defective in type III gene expression.

In P. aeruginosa, the fimV gene is positioned between two genes, usg-I and truA (hisT). Interestingly, in E. coli, usg-I is located directly upstream of truA and the two genes form an operon (Stover et al., 2000). It is likely that the fimV gene in P. aeruginosa was acquired through horizontal gene transfer, and its insertion site as well as direction ensured the co-regulation of the twitching motility and type III secretion apparatus; therefore, these two functions might be closely related. In support of this, all of the mutants that have been identified from the Tn5G insertion bank in the current study, fimV, pilL, cytA, hepA and muoE, seem to have defects in twitching motility. Despite the lack of twitching motility, none of these mutants are defective in the HeLa cell binding; in fact the fimV mutant seems to have a higher binding activity than the wild-type. Efforts are under way to elucidate the mechanism underlying this observation.

tRNA pseudouridine synthase (Ψ synthase I) catalyses the conversion of uridine in tRNA to its C-glycoside isomer, pseudouridine (Ψ) (Ramamurthy et al., 1999). Pseudouridine (5-ribosyluracil) is a ubiquitous yet enigmatic constituent of structural RNAs (transfer, ribosomal, small
nuclear and small nucleolar). Although pseudouridine was the first modified nucleotide to be discovered in RNA, and is the most abundant, its biosynthesis and biological roles have remained poorly understood since its identification as a ‘fifth nucleoside’ in RNA (Charette & Gray, 2000). Ψ is found in almost all tRNAs, notably as the nearly universal Ψ 55, after which the TΨC stem–loop is named. Ψ also occurs at the D stem, the anti-codon stem and loop in all three domains of life (archaea, eubacteria and eukaryotes) as well as in organelles (mitochondria and chloroplasts) (Auffinger & Westhof, 1998). Pseudouridinylation plays an important biological role in fine-tuning the structure of those tRNAs in which it occurs, thereby influencing their decoding activity, improving the fidelity of protein biosynthesis, and helping to maintain the proper reading frame (Harrington et al., 1993). In the context of translation, the stability of a tRNA population is conferred in part by the broad range of known tRNA nucleoside modifications, which affect tRNA structure and function in a number of subtle ways.

Recently, a strong and specific involvement of tRNA modifications in the adaptation of virulence gene expression to the nutritional quality of the growth medium has been reported (Durand & Bjork, 2003; Urbonavicius et al., 2002). The presence of modified nucleosides in tRNA was also shown to be important for virulence of *Shigella* (Durant & Davis, 1997), *Agrobacterium tumefaciens* (Gray et al., 1992), *Pseudomonas aeruginosa* (Sage et al., 1997; Urbonavicius et al., 2002) and *Pseudomonas syringae* (Kinscherf & Willis, 2002). Isolation of mutants with defective tRNA modification activity has frequently been associated with altered pathogenicity or metabolic activity phenotypes. The genes *miaA* and *tgg*, encoding tRNA isopentyladenosine synthase and tRNA-guanine transglycosylase, respectively, were identified as pathogenicity modulators in *Shigella* (Durand et al., 1994, 1997). The *tgg* mutant of *Shigella flexneri* lacks epoxy-Q nucleoside in its subset of tRNA, showing an altered virulence gene expression pattern. The lack of Q in tRNA does not influence the synthesis of *virF* mRNA but reduces its translation capacity by an unknown mechanism, resulting in a reduced expression of the downstream genes in the cascade required for virulence (Durand et al., 1994). In the *orf* mutant of *P. aeruginosa* (a homologue of *E. coli truB* mutant), which exhibits temperature-sensitive growth on solid media and reduced salt tolerance, experimental data suggested that tRNA modification may play a role in potentiating the translation of specific mRNA molecules in osmotically stressed cells (Sage et al., 1997; Urbonavicius et al., 2002). Clearly, a fully modified tRNA seems to be required for the pathogenicity of at least some organisms by affecting the translation of specific mRNAs, the products of which are key elements in the expression of virulence.

It has been proposed that the common function of the tRNA pseudouridinylation is to improve reading frame maintenance. The improvement occurs in two principal ways: by promoting the recruitment of the ternary complex to the A-site codon and thereby shortening the pause in the A-site; or by preventing slippage of the peptidyl-tRNA (Urbonavicius et al., 2001). Recently, the pseudouridination of tRNA molecules was implicated in the function and stability of certain tRNA molecules in plant mitochondria, where it is required for processing into mature tRNA, aminoclaylation and stabilization of the secondary structure through improved pairing in the stem–loop structures (Auffinger & Westhof, 1998; Durant & Davis, 1999; Fey et al., 2001). Based on the proposed functions of tRNA pseudouridination in plant mitochondria and the fact that TruA affects type III genes at the transcriptional level, it is likely that the TruA of *P. aeruginosa* is essential for the stability and/or function of certain tRNA molecules that are critical for the translation of a transcriptional regulator which is required for the expression of type III genes. Identification of this unknown transcriptional regulator as well as the target tRNA of the TruA will help us to better understand the mechanism by which the *truA* gene affects the type III gene expression.

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