Repression of phase-variable *cup* gene expression by H-NS-like proteins in *Pseudomonas aeruginosa*

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The *cupA* gene cluster of *Pseudomonas aeruginosa* encodes components of a putative fimbrial structure that enable this opportunistic human pathogen to form biofilms on abiotic surfaces. In *P. aeruginosa*, *cupA* gene expression is repressed by MvaT, a putative transcription regulator thought to belong to the H-NS family of nucleoid-associated proteins that typically function by repressing transcription. Here, we present evidence that MvaT controls phase-variable (ON/OFF) expression of the *cupA* fimbrial gene cluster. Using a directed proteomic approach, we show that MvaT associates with a related protein in *P. aeruginosa* called MvaU. Analysis with a bacterial two-hybrid system designed to facilitate the study of protein dimerization indicates that MvaT and MvaU can form both heteromeric and homomeric complexes, and that formation of these complexes is mediated through the N-terminal regions of MvaT and MvaU, both of which are predicted to adopt a coiled-coil conformation. We show further that, like MvaT, MvaU can repress phase-variable expression of the *cupA* gene cluster. Our findings suggest that fimbrial genes important for biofilm formation can be expressed in a phase-variable manner in *P. aeruginosa*, provide insight into the molecular mechanism of MvaT-dependent gene control, and lend further weight to the postulate that MvaT proteins are H-NS-like in nature.

MvaT | phase-variation

The opportunistic pathogen *Pseudomonas aeruginosa*, the leading cause of morbidity and mortality in cystic fibrosis (CF) patients (1), is a ubiquitous Gram-negative bacterium that can deploy an impressive array of virulence factors to intoxicate the human host. In the chronically infected CF lung, the organism persists as a biofilm, a surface-attached community of bacteria encased in a polymeric matrix (2). This biofilm mode of growth augments the resistance of *P. aeruginosa* to antibiotics and facilitates evasion of the host immune response (3).

Prominent among those genes that play an important role in biofilm formation in *P. aeruginosa* are the *cupA* genes, which encode components of a putative fimbrial structure that presumably facilitates surface attachment (4, 5). Expression of the *cupA* gene cluster in *P. aeruginosa* is repressed by MvaT, a putative transcription regulator found exclusively in Pseudomonads. It has been suggested, based on certain functional similarities as well as predicted structural similarities, that MvaT proteins are members of the H-NS family of nucleoid-associated proteins that typically function to repress transcription (6, 7). However, there is limited sequence similarity at the primary amino acid level between members of the MvaT protein family and those of the H-NS family (6, 8). Indeed, no obvious homolog of H-NS exists in *P. aeruginosa* or in any other Pseudomonad (6).

In *P. aeruginosa*, MvaT was originally described as a global regulator of virulence gene expression (9). Specifically, inactivation of *mvaT* was found to up-regulate expression of the *lecA* gene encoding the PA-IL lectin and cause overproduction of the toxic exoproduct pyocyanin (9). Moreover, a microarray-based transcriptome analysis has revealed that MvaT influences the expression of ≈150 genes in *P. aeruginosa*, including those identified previously together with several others that may be important for virulence (5).

Here we present evidence for a previously undescribed function of MvaT, the control of phase-variable gene expression. In particular, we show that, in the absence of MvaT, expression of the *cupA* fimbrial gene cluster phase-varies (i.e., exhibits ON/OFF expression). Using tandem affinity purification (TAP) (10) coupled with mass spectrometry, we show further that MvaT is associated with a related protein in *P. aeruginosa* called MvaU. We provide genetic evidence that MvaT associates with MvaU through a direct protein–protein contact mediated (in whole or in part) through the N-terminal regions of these two proteins. We demonstrate that both MvaT and MvaU can also form homomeric complexes and provide evidence that the ability of MvaT to oligomerize is important for its function. We show further that, like MvaT, MvaU can repress phase-variable (ON/OFF) expression of the *cupA* genes in *P. aeruginosa* and discuss the parallels between the MvaT and H-NS proteins that are highlighted by our findings.

Materials and Methods

Plasmids and Strains. Construction of the plasmids and bacterial strains used in this study is described in *Supporting Text*, which is published as supporting information on the PNAS web site.

Switching-Frequency Calculations. Switching-frequency calculations were performed essentially as described (11), except that cells were plated on LB agar plates containing X-Gal and isopropyl-β-D-thiogalactoside (IPTG) (2 mM) and grown at 37°C.

TAP. Cells were grown at 37°C with aeration in 200 ml of LB in 1-liter flasks to an OD_{600} of ≈2, then harvested by centrifugation at 4°C. TAP was then performed as described (12).

Western Blots. Purified proteins or cell lysates were separated on NuPAGE 4–12% gradient gels (Invitrogen), transferred to poly(vinylidene difluoride) membranes (Invitrogen), then probed either with antibodies against the Myc-tag (Upstate Biotechnology, Lake Placid, NY), the calmodulin-binding peptide epitope of the TAP-tag (Open Biosystems, Huntsville, AL), or the α subunit of RNA polymerase (RNAP) (Neoclone, Madison, WI). The TAP tag was detected by using soluble peroxidase–antiperoxidase complex (Sigma-Aldrich). Proteins were visualized by chemiluminescent detection by using Super-Signal West Pico chemiluminescent substrate (Pierce).

Bacterial Two-Hybrid Assays. Cells were grown with aeration at 37°C in LB supplemented with kanamycin (50 μg/ml), carben-

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Abbreviations: IPTG, isopropyl-β-D-thiogalactoside; TAP, tandem affinity purification; RNAP, RNA polymerase.

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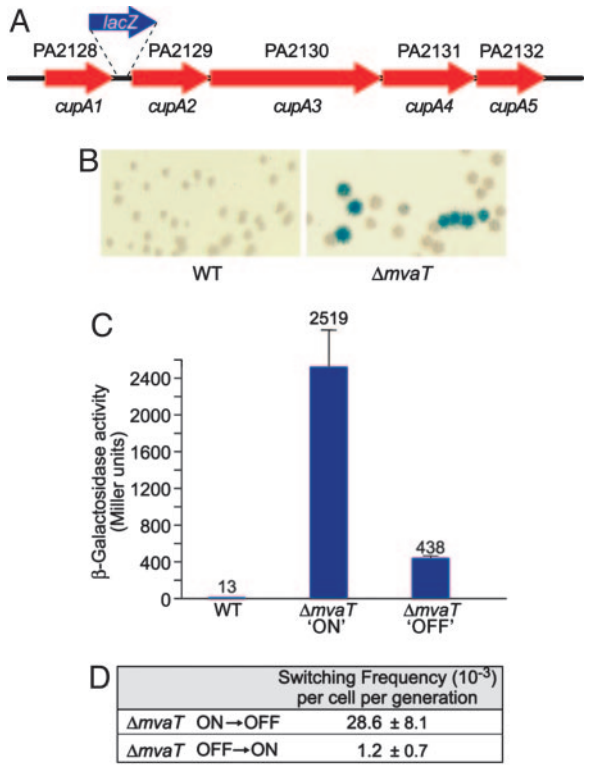

Fig. 1. The *cupA* fimbrial gene cluster exhibits phase-variable expression in the absence of MvaT. (*A*) Schematic of the *cupA* gene cluster in the *cupA lacZ* reporter strain. (*B*) Phenotypes of strains containing the *cupA lacZ* construct when plated on LB agar containing X-Gal and grown overnight at 37°C. The Δ*mvaT* mutant derivative gives rise to blue (phase-ON) and white (phase-OFF) colonies. (*C*) Quantification of *cupA lacZ* expression in cultures of the wild-type reporter strain and in cultures derived from phase-ON or phase-OFF colonies of the Δ*mvaT* mutant derivative. (*D*) Frequencies of switching from phase-ON to phase-OFF and from phase-OFF to phase-ON in Δ*mvaT* mutants.

icillin (100 μg/ml), tetracycline (10 μg/ml), and IPTG at the concentration indicated. Cells were permeabilized with SDS-$CHCl_3$ and assayed for β-galactosidase activity as described (13). Assays were performed at least three times in duplicate on separate occasions. Representative data sets are shown. Values are averages based on one experiment; duplicate measurements differed by <10%.

Results

MvaT Represses Phase-Variable Expression of the *cupA* Genes. MvaT was recently shown to repress expression of the *cupA* genes in *P. aeruginosa* (5). To study the mechanism by which MvaT represses *cupA* gene expression, we constructed a strain of PAO1 in which *lacZ* was placed downstream of the *cupA1* gene (Fig. 1*A*). When we introduced an in-frame deletion of the *mvaT* gene (Δ*mvaT*) into this reporter strain, we were surprised to find that the *cupA* genes appeared to be expressed in a phase-variable manner (Fig. 1*B*). In particular, cells of the Δ*mvaT* mutant strain gave rise to both blue and white colonies on LB agar plates containing X-Gal (Fig. 1*B*). When restreaked on LB X-Gal plates, blue colonies gave rise to both blue and white colonies, and white colonies gave rise to both blue and white colonies. This apparent ON/OFF switching of *cupA* gene expression in the Δ*mvaT* mutant strain could be complemented with the *mvaT* gene in trans (Table 1). Phase-variable expression of the *cupA* genes could not be detected in wild-type cells of the reporter strain (Fig. 1*B*). Quantification of *cupA* gene expression in Δ*mvaT* mutant cells derived from phase-ON (i.e., blue) colonies suggests there is at least an ≈190-fold difference in *cupA* gene expression between phase-ON cells and wild-type cells (Fig. 1*C*). Furthermore, determination of the frequency with which phase-ON cells switched to phase-OFF cells, and vice versa, revealed a bias in favor of the phase-ON to phase-OFF transition (Fig. 1*D*).

Table 1. Switching frequencies

Strain genotype	Plasmid	Phase transition	Frequency (per cell per generation)
WT	pMMB67EH	OFF-ON	$< 5.5 \pm 0.1 \times 10^{-6}$
Δ*mvaT*	pMMB67EH	ON-OFF	$21.4 \pm 3.6 \times 10^{-3}$
		OFF-ON	$13.5 \pm 1.3 \times 10^{-3}$
Δ*mvaT*	pM-MvaT	OFF-ON	$< 1.3 \pm 0.4 \times 10^{-5}$
Δ*mvaT*	pM-MvaU	OFF-ON	$< 2.8 \pm 2.0 \times 10^{-5}$
WT	pM-MvaT(1–61)	ON-OFF	$33.8 \pm 11.7 \times 10^{-3}$
		OFF-ON	$1.9 \pm 0.7 \times 10^{-3}$
WT	pM-MvaU(1–61)	ON-OFF	$48.3 \pm 3.2 \times 10^{-3}$
		OFF-ON	$1.1 \pm 0.7 \times 10^{-4}$

TAP of MvaT. To begin to address the question of whether MvaT performs its regulatory function in isolation or in association with other proteins in *P. aeruginosa*, we took a directed proteomic approach. In particular, to identify those proteins that associate with MvaT, we adapted the TAP strategy (10) for use in *P. aeruginosa*. We constructed a strain of *P. aeruginosa* PAO1 in which the native chromosomal copy of the *mvaT* gene had been altered such that it specified a TAP-tagged form of MvaT. To do this, we made use of an integration vector we specifically designed for this purpose (see Fig. 2*A*). The resulting strain (PAO1 MvaT-TAP) thus synthesizes MvaT with a TAP-tag fused to its C terminus at a level that likely reflects that of the wild-type protein. As a control, we constructed a strain that synthesized AceF (a subunit of the pyruvate dehdrogenase complex) with a TAP-tag fused to its C terminus (PAO1 AceF-TAP). Lysates were made from wild-type PAO1 cells, cells of the PAO1 MvaT-TAP strain, and cells of the PAO1 AceF-TAP strain. Proteins were then purified by TAP (10, 12), separated by SDS/PAGE, and stained with silver. A protein with an apparent molecular weight of ≈14 kDa was found that specifically copurified with MvaT (Fig. 2*B*). Nanoelectrospray tandem MS was used to identity the protein as MvaU (PA2667), a putative transcription regulator from *P. aeruginosa* that shares 47% identity and 65% similarity with MvaT (5).

MvaT Copurifies with MvaU-TAP. We reasoned that if MvaT and MvaU were associated with one another in *P. aeruginosa*, then MvaT would be expected to copurify with TAP-tagged MvaU. To test this prediction, we constructed two strains. One of these synthesized MvaU with a TAP-tag fused to its C terminus (PAO1 MvaU-TAP). The other strain was identical to the first, except that it harbored an in-frame deletion of the *mvaT* gene (PAO1 Δ*mvaT* MvaU-TAP) and therefore no longer synthesized MvaT. Fig. 3*A* shows the results following TAP of MvaU from these two strains. In support of the hypothesis that MvaT and MvaU associate with one another, a protein with the expected molecular weight of MvaT was found to copurify with MvaU only in the wild-type strain (Fig. 3*A*).

To test explicitly the hypothesis that MvaT copurifies with MvaU-TAP, we constructed two additional strains. Each of these synthesized MvaT with five copies of the Myc epitope fused to its C terminus (MvaT-Myc5). Whereas one strain was engineered to synthesize MvaU-TAP, the other was engineered to synthesize AceF-TAP. Immunoblot analyses of the TAP material from these two strains revealed that, although equal amounts

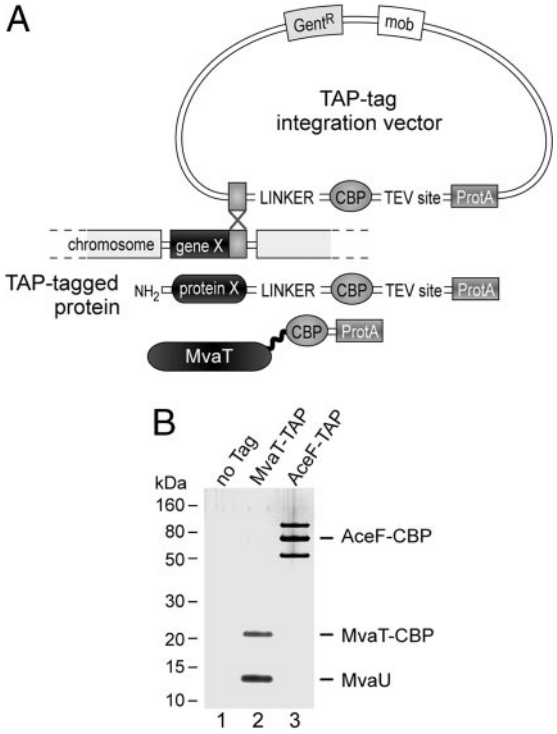

Fig. 2. TAP of MvaT from *P. aeruginosa*. (*A*) Schematic representation of TAP-tag integration vector and its use to make MvaT-TAP. The calmodulin-binding peptide (CBP), protein A moieties (ProtA), and TEV cleavage site that constitute the TAP-tag are shown (10). (*B*) SDS/PAGE analysis of proteins that copurify with MvaT-TAP and AceF-TAP from *P. aeruginosa*. Protein complexes were tandem affinity purified, electrophoresed on a 4–12% Bis-Tris NuPAGE gel and stained with silver. Lane 1, proteins purified from the nontagged PAO1 wild-type strain. Lane 2, proteins purified from strain PAO1 MvaT-TAP. Lane 3, proteins purified from strain PAO1 AceF-TAP. Molecular weights are indicated on the left.

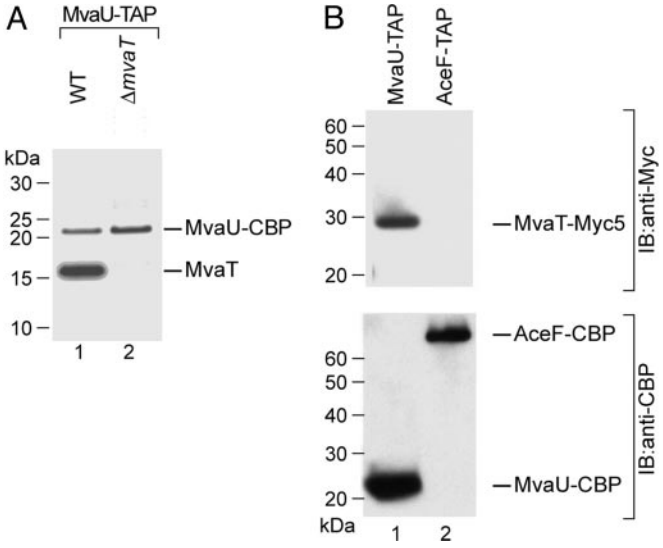

Fig. 3. MvaT copurifies with MvaU-TAP. (*A*) SDS/PAGE analysis of proteins associated with MvaU-TAP in a wild type (lane 1) or in a Δ*mvaT* mutant background (lane 2). Proteins were tandem affinity purified, electrophoresed on a 4–12% Bis-Tris NuPAGE gel, and stained with silver. Lane 1, proteins purified from strain PAO1 MvaU-TAP. Lane 2, proteins purified from strain PAO1 Δ*mvaT* MvaU-TAP. (*B*) Western blot analysis of proteins associated with tandem affinity-purified MvaU-TAP (lane 1), and AceF-TAP (lane 2). Both strains (that containing MvaU-TAP, and that containing AceF-TAP) produce Myc-tagged MvaT (MvaT-Myc5). (*Upper*) Immunoblot probed with an anti-Myc antibody demonstrates MvaT-Myc5 is only detected among the MvaU-TAP associated proteins. (*Lower*) Immunoblot probed with an anti-calmodulin-binding peptide (CBP) antibody shows comparable amounts of tandem affinity-purified material were loaded in each lane. Molecular weights are indicated on the left.

of MvaT-Myc5 were made by each strain (data not shown), MvaT-Myc5 specifically copurified with MvaU (Fig. 3*B*). From these findings, we conclude that MvaT and MvaU are associated with one another in *P. aeruginosa*.

Bacterial Two-Hybrid Analysis of Interactions Involving MvaT and MvaU. Our findings with TAP-tagged forms of MvaT and MvaU suggest these proteins may interact with one another directly to form a heteromeric complex. Because MvaT and MvaU are similar to one another at the primary amino acid level, we sought to determine whether MvaT and/or MvaU could also form homomeric complexes. To test for both heteromeric and homomeric complexes, we modified the design of a previously developed bacterial two-hybrid assay (13, 14) to permit the detection of both dimeric and higher-order complexes. This two-hybrid assay is based on the finding that any sufficiently strong interaction between two proteins can activate transcription in *Escherichia coli* provided one of the interacting proteins is tethered to the DNA by a DNA-binding protein, and the other is tethered to a subunit of *E. coli* RNAP (13, 14). In the modified version of the assay, contact between a protein (or protein domain) fused to the ω subunit of *E. coli* RNAP and another protein fused to a zinc-finger DNA-binding protein (referred to as Zif) activates transcription of a *lacZ* reporter gene (Fig. 4*A*) (13, 15). Because Zif (the Zinc-finger DNA-binding domain from the murine Zif268 protein) binds its cognate recognition site as a monomer, and because the ω subunit is monomeric in the RNAP holoenzyme complex, this modified configuration of the assay is ideally suited to detecting interactions between two protein monomers (i.e., dimer formation).

Using this newly configured two-hybrid assay (Fig. 4*A*), we first sought to detect an interaction between MvaT and MvaU from *P. aeruginosa*. Accordingly, we fused full length MvaU (residues 1–117) to the N terminus of Zif, and we fused full length MvaT (residues 1–124) to the N terminus of ω. We then determined whether the MvaU-Zif fusion protein could activate transcription from a suitable test promoter in cells containing the MvaT-ω fusion protein. Plasmids expressing MvaU-Zif and MvaT-ω chimeras were introduced into *E. coli* strain KDZif1ΔZ, which harbors the test promoter depicted in Fig. 4*A* linked to *lacZ* on an F′ episome. (KDZif1ΔZ also bears a deletion of the chromosomal *rpoZ* gene encoding ω.) In support of the idea that MvaU and MvaT interact with one another directly, the MvaU-Zif fusion protein activated transcription strongly (up to ≈18-fold) in cells containing the MvaT-ω chimera, whereas Zif did not (Fig. 4*B*). An additional control revealed that MvaU-Zif did not activate transcription from the test promoter in the presence of the unrelated Gal11P-ω chimera (Fig. 4*B*).

Having demonstrated the utility of the assay to detect a direct protein–protein interaction between MvaT and MvaU, we next asked whether either MvaT or MvaU (or both) could undergo homotypic interactions. Accordingly, we made two additional fusion proteins, one in which full length MvaU (residues 1–117) was fused to the N terminus of ω, and another in which full length MvaT (residues 1–124) was fused to the N terminus of Zif. Results depicted in Fig. 4*B* show that MvaU-Zif activated transcription from the test promoter by a factor of up to ≈11 in cells containing the MvaU-ω chimera. Similarly, MvaT-Zif activated transcription by a factor of up to ≈9 in cells containing the MvaT-ω chimera (Fig. 4*B*). Control assays indicated that

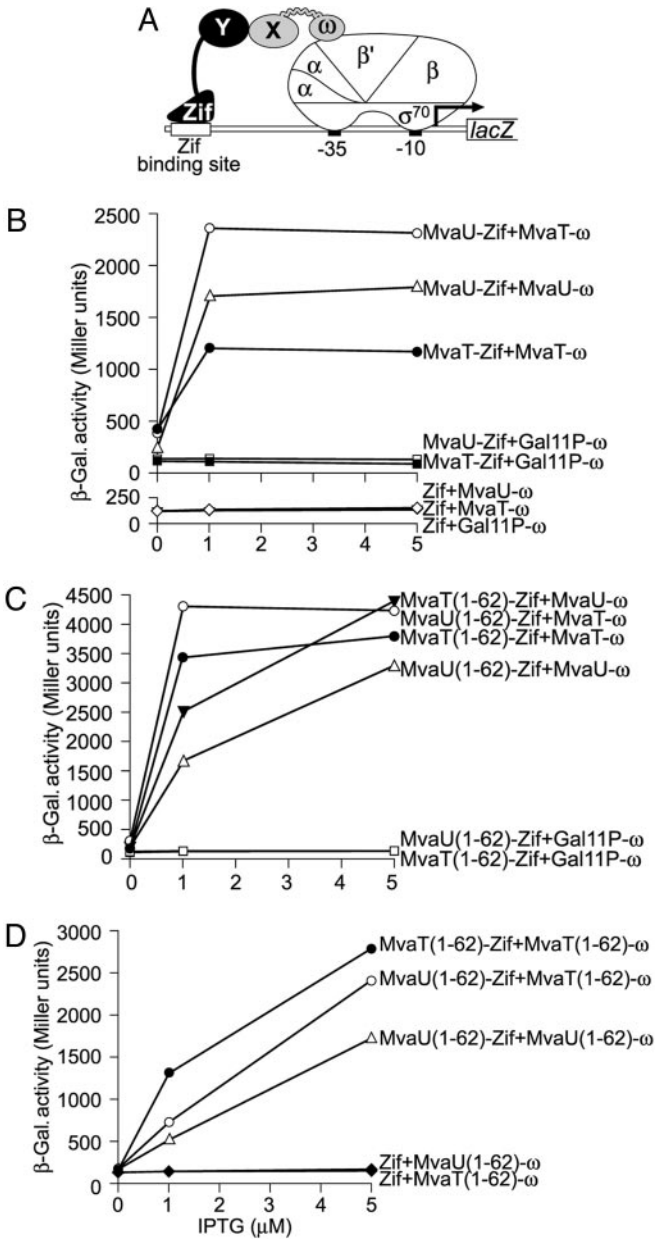

Fig. 4. Bacterial two-hybrid analysis of protein–protein interactions involving MvaT and MvaU. (*A*) Schematic representation of the two-hybrid system. Contact between protein domains X and Y fused, respectively, to the ω subunit of *E. coli* RNAP and to Zif activates transcription from the test promoter driving expression of *lacZ*. The diagram depicts test promoter p*lac*Zif1–61, which bears a Zif-binding site centered 61 bp upstream of the transcription start site of the *lac* core promoter. In *E. coli* strain KDZif1ΔZ, this test promoter is linked to *lacZ* on an F′ episome. (*B–D*) KDZif1ΔZ cells harboring compatible plasmids directing the synthesis of the indicated proteins were grown in the presence of different concentrations of IPTG and assayed for β-galactosidase activity. (*B*) Transcription activation by MvaU-Zif in the presence of the MvaT-ω or MvaU-ω chimeras and by MvaT-Zif in the presence of the MvaT-ω chimera. (*C*) Transcription activation by MvaT (1–62)-Zif or MvaU (1–62)-Zif in the presence of the MvaT-ω or MvaU-ω chimeras. (*D*) Transcription activation by MvaU (1–62)-Zif in the presence of the MvaT (1–62)-ω or MvaU (1–62)-ω chimeras and by MvaT (1–62)-Zif in the presence of the MvaT (1–62)-ω chimera.

neither Zif nor MvaT-Zif activated transcription from the test promoter in the presence of the MvaU-ω chimera, or the Gal11P-ω chimera, respectively (Fig. 4*B*). These findings suggest that both MvaT and MvaU can form homomeric complexes.

N-Terminal Regions of MvaT and MvaU Mediate Protein–Protein Interactions. H-NS-like proteins typically contain coiled-coil motifs in their N-terminal regions that can mediate the formation of both homodimeric and heterodimeric complexes (16, 17). An MvaT homolog from *Pseudomonas* strain Y1000, which has been described as an H-NS-like protein, is predicted to contain a coiled-coil in its N-terminal region (6). Moreover, analysis of *P. aeruginosa* MvaT and MvaU using COILS (18) predicts that residues 1–39 of MvaT and residues 1–35 of MvaU are likely to adopt coiled-coil conformations (not shown). We therefore asked whether the N-terminal regions of MvaT and MvaU harboring these putative coiled-coil motifs could mediate both the heteromeric and homomeric interactions of these proteins. To do this, we fused the N-terminal regions of MvaT (residues 1–62) and MvaU (residues 1–62) to Zif and then assayed the ability of the resulting fusion proteins to interact with full length MvaT and MvaU. In reporter strain KDZif1ΔZ, the MvaU (1–62)-Zif fusion protein activated transcription strongly in cells containing either the MvaT-ω chimera (up to ≈32-fold) or the MvaU-ω chimera (up to ≈25-fold) (Fig. 4*C*). Similarly, the MvaT (1–62)-Zif fusion protein strongly stimulated reporter gene expression in cells containing either the MvaT-ω or the MvaU-ω chimera by factors of up to ≈29 and ≈33, respectively (Fig. 4*C*). Control assays revealed that both MvaU (1–62)-Zif and MvaT (1–62)-Zif failed to activate transcription in the presence of the Gal11P-ω chimera (Fig. 4*C*).

We then wished to determine whether the N-terminal regions of both MvaT and MvaU could suffice to mediate both the heteromeric and homomeric interactions of MvaT and MvaU. Accordingly, we made two additional chimeras in which either MvaT (residues 1–62) or MvaU (residues 1–62) were fused to ω. We then assayed the ability of each of these chimeras to interact with the MvaU (1–62)-Zif and MvaT (1–62)-Zif fusion proteins in reporter strain KDZif1ΔZ. Fig. 4*D* shows that MvaT (1–62)-Zif strongly activated transcription from the test promoter in cells containing the MvaT (1–62)-ω chimera (by a factor of up to ≈18). Moreover, whereas MvaU (1–62)-Zif activated transcription by factors of up to ≈16 and ≈10 in cells containing the MvaT (1–62)-ω and MvaU (1–62)-ω chimeras, respectively, Zif did not (Fig. 4*D*). We can therefore conclude that the N-terminal regions of both MvaT and MvaU are sufficient to mediate the formation of the respective heteromeric and homomeric complexes.

Dominant-Negative Effect of Overproducing N-Terminal Regions of MvaT and MvaU. We have shown, using a bacterial two-hybrid assay, that the N-terminal regions of both MvaT and MvaU can interact with both full length MvaT and full length MvaU (Fig. 4*C*). In *E. coli*, overproduction of the N-terminal portion of H-NS results in dominant-negative effects on gene expression because this portion of H-NS sequesters the full length protein in an inactive heterodimer that is incapable of binding DNA (19, 20). We therefore wished to determine whether overproduction of the N-terminal regions of MvaT and MvaU would result in a dominant-negative effect on *cupA* gene expression in *P. aeruginosa*. To do this, we constructed two vectors, pM-MvaT (1–61) and pM-MvaU (1–61), carrying genes encoding MvaT (1–61) and MvaU (1–61), respectively, under the control of the IPTG-inducible *tac* promoter. Each vector, as well as the empty parent vector (pMMB67EH), was then introduced into the *cupA lacZ* reporter strain. Overproduction of either MvaT (1–61) or MvaU (1–61) resulted in phase-variable expression of the *cupA* genes (Table 1). Furthermore, overproduction of MvaT (1–61) in cells of a Δ*mvaU* mutant resulted in phase-variable expression of the *cupA* genes (data not shown), demonstrating that MvaU itself is not absolutely required for phase-variable expression of the *cupA* genes. Therefore, akin to the situation with truncated versions of H-NS in *E. coli* (19, 20), overproduction of the N-terminal portions of either MvaT or MvaU can result in dominant-

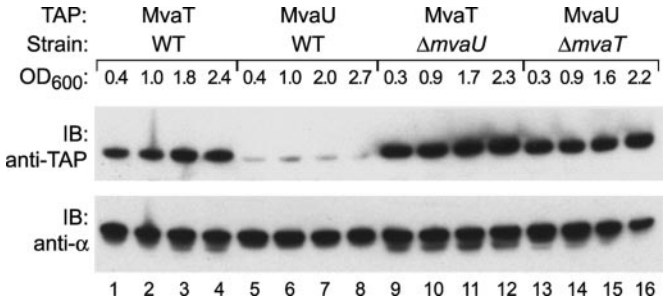

Fig. 5. Western blot analysis of the effects of deleting *mvaU* or *mvaT*, respectively, on the intracellular concentrations of MvaT or MvaU. The amount of MvaT-TAP protein was monitored at different points in the growth curve of a wild-type strain (lanes 1–4) and a Δ*mvaU* mutant derivative (lanes 9–12). The amount of MvaU-TAP protein was monitored at different points in the growth curve of a wild-type strain (lanes 5–8) and a Δ*mvaT* mutant derivative (lanes 13–16). Samples were taken at mid-log (lanes 1, 5, 9, and 13), late-log (lanes 2, 6, 10, and 14), early stationary (lanes 3, 7, 11, and 15), and stationary (lanes 4, 8, 12, and 16) phases of growth. The OD_{600} at which each sample was taken is indicated, and the corresponding growth curves are shown in Fig. 6, which is published as supporting information on the PNAS web site. Equivalent amounts of cell lysates were loaded for each sample. (*Upper*) Immunoblot probed with anti-TAP. (*Lower*) Immunoblot probed with antibody against the α subunit of RNAP serves as a control for sample loading.

negative effects on gene expression in *P. aeruginosa*. These findings are consistent with a model in which MvaT exerts its negative effect on *cupA* gene expression in oligomeric form.

MvaU Can Substitute Functionally for MvaT in the Control of *cupA* Gene Expression. Because MvaU is similar to MvaT, we next wished to determine whether MvaU could functionally substitute for MvaT with respect to its role in *cupA* gene expression. For this purpose, we constructed a vector, pM-MvaU, in which expression of the *mvaU* gene is under the control of the IPTG-inducible *tac* promoter. We found that when overexpressed, *mvaU* could complement the Δ*mvaT* mutant cells and repress phase-variable expression of the *cupA* genes (Table 1).

Deletion of *mvaU* Increases the Amount of MvaT in the Cell. Although MvaU associates with MvaT in *P. aeruginosa* (Figs. 2 and 3), and although MvaU can repress phase-variable expression of the *cupA* genes (Table 1), deletion of *mvaU* evidently has no effect on *cupA* gene expression (ref. 5 and data not shown). We wondered why this was the case. Because deletion of *mvaT* results in an increase in expression of the *mvaU* gene (5), we wished to determine whether the converse situation might be true; if deletion of *mvaU* were to result in an increase in the amount of MvaT in the cell, this compensatory change might be sufficient to mask any role MvaU might normally play in repression of the *cupA* genes.

To determine whether deletion of *mvaU* influences the amount of MvaT in the cell, we introduced an in-frame deletion of *mvaU* into our strain synthesizing TAP-tagged MvaT (PAO1 MvaT-TAP) to create strain PAO1 Δ*mvaU* MvaT-TAP. Immunoblot analysis of the amount of MvaT-TAP in these two strains, at different points of the growth curve, revealed that deletion of *mvaU* results in a modest increase in the intracellular concentration of MvaT-TAP (Fig. 5). In addition, using our PAO1 MvaU-TAP strain together with a Δ*mvaT* mutant derivative, we also found that deletion of *mvaT* results in an increase in the amount of MvaU-TAP in the cell (Fig. 5). This finding is consistent with the earlier observation that deletion of *mvaT* results in increased expression of the *mvaU* gene (5). Our findings with the Δ*mvaU* mutant suggest that one unanticipated consequence of deleting *mvaU* is that the intracellular concentration of MvaT increases. This might confound the interpretation of phenotypes (or lack thereof) of Δ*mvaU* mutants.

Discussion

MvaT influences the expression of a large number of genes in *P. aeruginosa*, including several that are important for virulence (5, 9). We present evidence that a cluster of genes involved in biofilm formation exhibits phase-variable expression in the absence of MvaT. Using TAP (10) in combination with tandem MS, we have found that in *P. aeruginosa* MvaT interacts with a related protein called MvaU. This finding potentially implicates MvaU in the control of target gene expression by MvaT. Furthermore, using a bacterial two-hybrid system specifically designed to study protein dimerization, we have shown that not only can MvaT and MvaU interact with one another, but they can also interact with themselves, raising the possibility that different homo- or heterooligomeric species have distinct functions in the cell.

Phase-Variable Expression of the *cupA* Fimbrial Genes. Of the 150 or so genes in *P. aeruginosa* whose expression is altered as a result of deleting *mvaT*, the *cupA* genes are among those whose expression is most strongly affected (5). The *cupA* genes constitute a putative fimbrial operon whose products are involved in the early stages of biofilm formation (4). In a previous study (5), it was found that MvaT represses expression of the *cupA* genes, either directly or indirectly. We present evidence that, in the absence of MvaT, the *cupA* genes are expressed in a phase-variable manner. Thus, within a population of *mvaT* mutant cells, some cells will express the *cupA* fimbrial genes, whereas others will not. Although phenotypic variation is known to occur in *P. aeruginosa* (21–23), to our knowledge this is the first explicit demonstration of phase-variable gene expression in *P. aeruginosa*. In addition, although MvaU has not previously been implicated in the control of *cupA* gene expression (5), we have shown that, when overexpressed, *mvaU* can complement an *mvaT* deletion and repress phase-variable expression of the *cupA* genes (Table 1). Because deletion of *mvaU* results in an increase in the amount of MvaT in the cell, this compensatory change might be sufficient to mask any role MvaU might normally play in repression of the *cupA* genes. Thus, we have yet to establish whether MvaU contributes to the repression of *cupA* gene expression. We note that phase-variable expression of the *cupA* genes in the absence of MvaT was not detected in an earlier study (5); possibly the difference between our observations and the previous observations lies in the fact that the *cupA1* promoter region was present on a multicopy plasmid in the prior study.

Many examples exist in bacteria of surface structures such as fimbriae being produced in a phase-variable manner (reviewed in ref. 24), and some of these are involved in biofilm formation (see, for example, ref. 25). We speculate that phase-variable (ON/OFF) expression of the *cupA* genes might contribute to the fitness of the cell population as a whole. For example, in the host, expression of the *cupA* genes (in phase-ON cells) might facilitate initial biofilm formation, whereas any subsequent switch to the phase-OFF expression state might better enable cells to persist, because they no longer produce a potentially immunogenic surface structure. Although we have been unable to detect phase-variable expression of the *cupA* genes in the presence of MvaT (i.e., in wild-type cells), it is tempting to speculate that there may be specific environmental cues that promote phase-variable expression of the *cupA* genes. Our demonstration that overproduction of the N-terminal region of MvaT or MvaU had a dominant negative effect on *cupA* gene expression establishes MvaT sequestration as a potential mechanism that could be used to promote phase-variable expression of the *cupA* genes in wild-type cells.

Parallels Between MvaT and H-NS. Our findings with MvaT and MvaU strengthen the claim that, despite the lack of sequence similarity, members of the MvaT protein family functionally resemble H-NS (6). Like H-NS, we found that MvaT and MvaU contain protein–protein interaction motifs in their N-terminal regions that can mediate the formation of both heteromeric and homomeric complexes. Moreover, we found that overproduction of the N-terminal portions of either MvaT or MvaU can result in dominant negative effects on gene expression in *P. aeruginosa*; similar observations have been made with truncated versions of H-NS in *E. coli* (19, 20). Our findings are consistent with the idea that MvaT exerts its negative effect on *cupA* gene expression in oligomeric form.

In *E. coli*, H-NS can interact with a related nucleoid-associated protein called StpA (reviewed in ref. 17). StpA shares 58% identity with H-NS at the primary amino acid level and can evidently form both homodimers and heterodimers (with H-NS) through a predicted coiled-coil motif present in its N-terminal domain (reviewed in ref. 16). We suggest that the interaction of MvaT with MvaU in *P. aeruginosa* is formally analogous to the interaction of H-NS with StpA in *E. coli* (see ref. 17). It is also striking that the reciprocal regulatory interaction we observe between MvaT and MvaU in *P. aeruginosa* (Fig. 5) resembles that seen between H-NS and StpA in *E. coli*; H-NS and StpA can each repress the gene encoding the other protein (17, 26, 27). Although MvaT is known to repress expression of *mvaU* (5), it remains to be determined whether the effect of MvaU on MvaT protein levels (Fig. 5) is mediated at the level of *mvaT* gene expression.

Phase-variable expression of the *cupA* fimbrial genes in the absence of MvaT provides yet another parallel between MvaT and H-NS. In *E. coli*, H-NS represses both the phase-variable expression of genes encoding type 1 fimbriae (28, 29), and the phase-variable expression of genes encoding the Pap pili (30). However, we do not yet know the mechanism governing the apparent phase-variable expression of the *cupA* fimbrial genes that occurs in the absence of MvaT, nor do we know whether MvaT mediates its effects on *cupA* gene expression directly or indirectly.

Association Between MvaT and MvaU. Our findings with MvaT and MvaU in *P. aeruginosa* may be relevant to several observations made regarding MvaT homologs from other Pseudomonads. Specifically, MvaT in *Pseudomonas mevalonii* was originally described as a heteromeric transcription regulator composed of two subunits, P16 and P15, with molecular weights of 16 and 15 kDa, respectively (31). The P16 subunit of MvaT from *P. mevalonii* is 82% similar to MvaT from *P. aeruginosa* (5). Although the identity of the P15 subunit of *P. mevalonii* MvaT was not determined (31), based on our findings, we speculate that P15 is the equivalent of *P. aeruginosa* MvaU. More recently, in *Pseudomonas putida*, a homolog of MvaT called TurA was identified that repressed transcription from the TOL plasmid *Pu* promoter (7). TurA was found to copurify with a smaller related protein called TurB (7). Although an alternative explanation was proposed (7), we suggest that TurA and TurB in *P. putida*, like MvaT and MvaU in *P. aeruginosa*, may copurify, because they physically interact with one another.

Our findings that MvaU associates with MvaT in *P. aeruginosa*, and that MvaU can complement one of the phenotypes of an *mvaT* mutant, raise the possibility that MvaU may influence the expression of a subset of the genes that are controlled by MvaT in *P. aeruginosa*. Indeed, expression of the *lecA* gene, which encodes the PA-IL lectin, appears to be repressed by both MvaT and MvaU (5). It will be interesting to determine the extent to which those genes that belong to the MvaT regulon overlap with those of the putative MvaU regulon. Because MvaT appears to be more abundant in the cell than MvaU (Fig. 5), we speculate that the majority of MvaU will be complexed with MvaT. Therefore, although MvaU homomers may form, we expect that MvaT homomers and MvaT-MvaU heteromers will predominate in the cell. In *E. coli*, heterodimers of the nucleoid-associated protein HU (composed of HUα and HUβ subunits) have been shown to have functions distinct from those of the corresponding homodimeric species (reviewed in ref. 17). It will be important to determine whether MvaT-MvaU heteromers have properties distinct from those of either MvaT or MvaU homomers.

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