P65 Truncation Impacts P30 Dynamics during *Mycoplasma pneumoniae* Gliding

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The cell-wall-less prokaryote *Mycoplasma pneumoniae* is a major cause of community-acquired bronchitis and pneumonia in humans. Colonization is mediated largely by a differentiated terminal organelle, which is also the leading end in gliding motility. Cytadherence-associated proteins P30 and P65 appear to traffic concurrently to the distal end of developing terminal organelles. Here, truncation of P65 due to transposon insertion in the corresponding gene resulted in lower gliding velocity, reduced cytadherence, and decreased steady-state levels of several terminal organelle proteins, including P30. Utilizing fluorescent protein fusions, we followed terminal organelle development over time. New P30 foci appeared at nascent terminal organelles in P65 mutants, as in the wild type. However, with forward cell motility, P30 in the P65 mutants appeared to drag toward the trailing cell pole, where it was released, yielding a fluorescent trail to which truncated P65 colocalized. In contrast, P30 was only rarely observed at the trailing end of gliding wild-type cells. Complementation with the recombinant wild-type P65 allele by transposon delivery restored P65 levels and stabilized P30 localization to the terminal organelle.

*Mycoplasma pneumoniae* is an important human respiratory tract pathogen causing community-acquired bronchitis and primary atypical or “walking” pneumonia, accounting for up to 30% of all cases of pneumonia requiring hospitalization. Chronic or permanent lung damage and extrapulmonary sequelae are not uncommon, and a growing body of evidence supports a contributing role for *M. pneumoniae* in the onset and exacerbation of asthma (1, 46). Transmission by aerosol is facilitated by a characteristically persistent cough, leading to colonization of the mucosal epithelium of the conducting airways.

*M. pneumoniae* cells have no cell wall but possess a polar terminal organelle that functions in adherence to host cells (cytadherence), gliding motility, and cell division (6, 8, 21, 39). This membrane-bound extension of the mycoplasma cell is defined by a complex, electron-dense core that is part of a Triton X-100 (TX)-insoluble mycoplasma cytoskeleton (3, 6, 17, 35) and is comprised of multiple substructures (25), including a terminal button with an arched row of discrete proteins that align with proteins on the inner and outer leaflets of the mycoplasma membrane at the distal end of the terminal organelle (Fig. 1A). These proteins are thought to correspond to cytadherence complexes that include major adhesins P1 and P30 and accessory proteins P65, B, and C, which localize to this region of the terminal organelle (4, 5, 14, 16, 26, 36, 42, 43) with sufficient proximity to allow their chemical cross-linking (31, 32).

P30 is a transmembrane protein found almost exclusively at the distal end of the terminal organelle on wild-type cells, where it has essential but distinct roles in gliding motility and cytadherence (12, 20, 40). The extracellular C-terminal domain of P30 is dominated by repeating pro-rich motifs and is required for P30 function (10, 12). Truncation of P30 at the C terminus or in-frame deletion of the pro-rich motifs renders *M. pneumoniae* cells highly branched, unable to cytadhere, and severely impaired in gliding and is accompanied by reduced stability of the cytadherence accessory protein P65 (10, 12, 20, 27, 40). Complementation of a P30 null mutant with a recombinant transposon carrying the wild-type P30 allele (MPN453) (13), or a yellow fluorescent protein (YFP) fusion thereof, restores a wild-type phenotype (20, 40).

Protein P65 lacks an obvious signal sequence and yet localizes to the mycoplasma cell surface at the distal end of the terminal organelle and partitions in the TX-insoluble cytoskeletal fraction (27, 28, 38). Like several other *M. pneumoniae* cytadherence-associated proteins, P65 has a large, low-complexity, acidic pro-rich (APR) domain (3, 11, 27, 38), which is followed by a central coiled-coil domain and a C-terminal domain of mixed secondary structures (Fig. 1B). Analysis of growing mycoplasma cultures expressing fluorescent protein fusions of P65 and P30 indicates concurrent or nearly concurrent trafficking to developing terminal organelles (21). Here we explored the P30/P65 association further, characterizing transposon insertion mutants in the MPN309 gene encoding P65 (Fig. 1C). These mutants produced truncated P65 derivatives and exhibited impaired gliding, reduced steady-state P30 levels, and downstream polar effects on terminal organelle proteins HMW2, P28, P41, and P24 but differed in their capacity to cytadhere. Despite truncation of P65, new P30-YFP foci developed at nascent terminal organelles in a manner similar to that seen in wild-type *M. pneumoniae*, but during cell gliding, a portion of the P30-YFP population appeared to drag to and subsequently detach from the trailing cell pole, creating a fluorescent trail. Complementation with the recombinant wild-type P65 allele restored normal P30 anchoring at the terminal organelle.

**MATERIALS AND METHODS**

**Mycoplasma strains and culture conditions.** Mycoplasma samples were cultured in SP-4 (45) medium and on PPLO agar plates with or without gentamicin and chloramphenicol, as appropriate (19, 20). Wild-type *M.
pneumoniae M129 (33) transformed with Tn4001 was screened for altered satellite growth (24). Transformants were filter cloned for detailed analysis, and multiple filter clones were obtained and characterized, with two representatives for each mutant shown here.

**Western immunoblotting and quantitation of hemadsorption (HA) and binding to glass.** Samples were prepared for sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting as described previously (19), with minor modifications. Briefly, membranes were blocked in 5% skim milk–Tris-buffered saline (TBS; 0.2 M Tris-HCl, 0.85% NaCl, pH 8.2), incubated with a primary antibody for 2 h at room temperature, washed 5 times for 5 min each in TTBS (TBS with 0.05% Tween 20), probed with AP-conjugated secondary antibody (Bio-Rad, Hercules, CA, or Promega, Madison, WI) at 1 h at room temperature, and again washed 5 times for 5 min each in TTBS. Monoclonal P30-specific antibody (5) was used at a dilution of 1:100, rabbit anti-P65 serum (38) at 1:3,000, and antiserum to HMW1 (44) at 1:10,000. Binding of radiolabeled mycoplasma samples to erythrocytes and to glass was assessed as described previously (20).

**Fluorescent protein fusions and construct generation.** Construction of P30-YFP and P30-CFP (P30-cyan fluorescent protein) fusions was detailed previously (20, 21). Significant transformant instability was noted with the P30-YFP construct (20) in Tn4001cat (18) transformed in M. pneumoniae, a protein band corresponding to the expected P30-YFP level (data not shown). Western immunoblot analysis with antiserum to the N-terminal region of P65 confirmed loss of the full-length protein in each mutant (Fig. 2A). No truncated P65 was detected in MPN309-261, MPN309-319, and the P65 derivative in MPN309-261 and MPN309-319 was present at nearly wild-type levels (29). PCR amplification with primers flanking the insertion sites confirmed clonal populations for each, while Southern blot hybridization with transposon-specific probes established the presence of a single copy of the transposon and no independent duplication of IS256 (data not shown).

**RESULTS**

**Identification of P65 truncation mutants.** While screening for motility-defective mutants (24), we identified three transformants exhibiting limited satellite growth and intermediate adherence to polystyrene. The transposon insertion in each mapped to MPN309, which encodes terminal organelle protein P65 and is the first of four open reading frames (ORFs) constituting the P65 transcriptional unit (29) (Fig. 1C). The insertion sites corresponded to amino acid residues 152, 261, and 319 (mutant designations MPN309-152, MPN309-261, and MPN309-319, respectively). PCR amplification with primers flanking the insertion sites confirmed clonal populations for each, while Southern blot hybridization with transposon-specific probes established the presence of a single copy of the transposon and no independent duplication of IS256 (data not shown).

**Western immunoblot analysis with antisera to the N-terminal region of P65 confirmed loss of the full-length protein in each mutant (Fig. 2A). No truncated P65 was detected in MPN309-261, MPN309-319, and the P65 derivative in MPN309-261 and MPN309-319 was present at nearly wild-type levels (29).** We also detected in each transformant, but not in wild-type M. pneumoniae, a protein band corresponding to a likely P65 multimer (Fig. 2A, arrowheads), at levels proportional to the extent of truncation.

We examined whether transposon insertion into MPN309 impacted other proteins associated with cytadherence (Fig. 2B and Table 1). Steady-state levels of downstream gene products HMW2, P28, P41, and P24 were below wild-type levels despite an outward-reading promoter in the transposon. HMW2 exhibited the greatest reduction, while P28, P41, and P24 levels were only slightly reduced, with variability observed between clonal isolates, as also previously noted with MPN310 insertion mutants (29). HMW1, HMW3, and P30 levels were below wild-type levels, but B, C, and P1 levels were unaffected (Fig. 2B and data not shown). Reduced steady-state levels of terminal organelle components HMW1, HMW3, and P30 were expected given their interdependence (29, 30, 47).

**Impact of P65 truncation on HA and gliding motility.** Mutants MPN309-152 and MPN309-261 but not MPN309-319 exhibited modest reductions in HA (Table 2). In contrast, gliding velocity and frequency were dramatically reduced in all three mutants, with MPN309-152 and MPN309-261 exhibiting the greatest decline (Table 2). Despite differences in their HA and gliding phenotypes, the P1 major adhesin protein localized to the terminal organelle in a manner similar to that seen with the wild type in all three P65 mutants (data not shown). These mutants were identified originally by their poor binding to polystyrene, but their glid-
Protein profiles of MPN309 insertion mutants and mutant MPN309-261 complemented with rP65

<table>
<thead>
<tr>
<th>Strain</th>
<th>P65</th>
<th>P65 multimer</th>
<th>HMW2</th>
<th>P28</th>
<th>P41</th>
<th>P24</th>
<th>P1/B/C</th>
<th>HMW1</th>
<th>HMW3</th>
<th>P30</th>
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<tr>
<td>Wild type</td>
<td>++</td>
<td>-</td>
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<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
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</tr>
<tr>
<td>MPN309-152</td>
<td>+/−</td>
<td>+</td>
<td>++</td>
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<td>MPN309-261</td>
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<td>MPN309-319</td>
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<td>+</td>
<td>++</td>
<td>++</td>
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<td>++</td>
<td>++</td>
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<td>++</td>
</tr>
<tr>
<td>MPN309-261 + rP65</td>
<td>++</td>
<td>+</td>
<td>+/−</td>
<td>++</td>
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<td>++</td>
<td>++</td>
<td>++</td>
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</table>

*Assessment of protein levels relative to wild-type levels based on band intensities from the analysis of multiple immunoblots. ++ , protein present at wild-type levels; + , protein present at decreasing levels; +/− , protein barely detectable; − , protein absent.

Protein level

A

B

C

FIG 2 Western immunoblotting analysis of wild-type *M. pneumoniae* and MPN309 insertion mutants. (A) Immunoblot analysis of P65. WT, wild-type *M. pneumoniae* (left) and wild-type *M. pneumoniae* with Tn4001.2065 in intergenic location (right); open arrow, P65; solid arrows, truncated P65 in mutants; arrowheads, multimeric truncated P65. Each lane represents a different clonal isolate of the indicated mutant. Size standards are given to the left. (B) Immunoblot analysis of indicated terminal organelle-associated proteins. Lanes are the same as described for panel A, with each panel presenting a different immunoblot. Some variability between clonal isolates was observed for certain proteins. (C) Immunoblot analysis of MPN309-261 producing recombinant WT P65. Open arrow, P65; arrowhead, multimeric truncated P65.

TABLE 2 HA, gliding motility, and glass binding by MPN309 insertion mutants and mutant MPN309-261 complemented with rP65

<table>
<thead>
<tr>
<th>Strain</th>
<th>HA (% WT ± SD)</th>
<th>Gliding motility</th>
<th>Binding to glass (% WT ± SD)</th>
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<tbody>
<tr>
<td></td>
<td>Velocity (WT)</td>
<td>% time resting</td>
<td></td>
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<tr>
<td>WT</td>
<td>100 ± 1.5</td>
<td>100</td>
<td>100 ± 0.1</td>
</tr>
<tr>
<td>MPN309-152</td>
<td>77.4 ± 3.2</td>
<td>11.3</td>
<td>140.0 ± 22.2</td>
</tr>
<tr>
<td>MPN309-261</td>
<td>56.4 ± 6.1</td>
<td>6.4</td>
<td>104.4 ± 15.5</td>
</tr>
<tr>
<td>MPN309-319</td>
<td>102.2 ± 4.2</td>
<td>21.6</td>
<td>110.9 ± 12.0</td>
</tr>
<tr>
<td>MPN309-261 + rP65</td>
<td>86.0 ± 5.7</td>
<td>38.9</td>
<td>170.4 ± 28.2</td>
</tr>
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</table>

* WT, wild type.

Minimum of 100 total/30 gliding cells examined.

P < 0.10.

P < 0.05.

Assessment of protein levels relative to wild-type levels based on band intensities from the analysis of multiple immunoblots. ++ , protein present at wild-type levels; + , protein present at decreasing levels; +/− , protein barely detectable; − , protein absent.

Assessment of protein levels relative to wild-type levels based on band intensities from the analysis of multiple immunoblots. ++ , protein present at wild-type levels; + , protein present at decreasing levels; +/− , protein barely detectable; − , protein absent.

Mutants MPN309-261 and MPN309-319 attached to glass at wild-type levels under the conditions used to assess gliding, and MPN309-152 bound to glass at levels well above the wild-type level (P < 0.10) (Table 2).

**P65 localization and P30 trafficking.** P65 is typically found at the distal end of the terminal organelle of wild-type *M. pneumoniae* cells (27, 28) but exhibited a punctate pattern in MPN309-152 and MPN309-261, with the latter very faint and often with few foci detectable (Fig. 3). Polar P65 foci were apparent in both mutants, but P65 distribution overall appeared largely random. In contrast, MPN309-319 cells had a distinct P65 focus at the terminal organelle similar to that seen with wild-type cells but also often a faint focus at the opposite cell pole. P65 foci not specifically associated with cells were common for all three mutants but rare for wild-type *M. pneumoniae*. As addressed below, chains of cells were common in the P65 mutant cultures but not in wild-type cultures.

P30 and P65 colocalize in wild-type *M. pneumoniae* (21, 27, 28, 42), and we examined P30 distribution relative to P65 distribution in MPN309-261 and MPN309-319 mutants by means of P30-YFP or P30-CFP fusions (Fig. 4). Both mutants typically exhibited an intense P30 focus at the terminal organelle but often also a weaker focus at the opposite cell pole. P65 foci not specifically associated with cells were common for all three mutants but rare for wild-type *M. pneumoniae*. As addressed below, chains of cells were common in the P65 mutant cultures but not in wild-type cultures.

Assessment of protein levels relative to wild-type levels based on band intensities from the analysis of multiple immunoblots. ++ , protein present at wild-type levels; + , protein present at decreasing levels; +/− , protein barely detectable; − , protein absent.
We monitored P30-YFP trafficking in all three MPN309 mutants over time during growth in culture, where new P30-YFP foci formed at developing terminal organelles in a manner similar to that seen with wild-type cells. However, during gliding, some fluorescence was seen moving rearward from the terminal organelle and accumulating at the trailing pole, often subsequently detaching to yield a fluorescent trail behind the cells (Fig. 5 and data not shown). We assessed quantitatively the frequency of P30 localization to the trailing end on cells that glided during the observation period, as gliding appeared to contribute to displacement of P30-YFP to the trailing end of the cell. Significantly, cells with two or more terminal organelles typically do not exhibit whole-cell gliding (21). Only 1% of gliding wild-type cells but over 50% of gliding MPN309-261 mutant cells had a P30-YFP focus at the trailing end (Table 3). Immunofluorescence analysis with P30-specific antibodies confirmed that the P30 localization patterns were not merely a function of the YFP fusion (Fig. 6).

**Complementation with recombinant wild-type P65.** The polar effects of MPN309 disruption complicated the assignment of cause and effect, given the limited genetic tools available for manipulating *M. pneumoniae*. We introduced a recombinant wild-type P65 allele (rP65) by transposon delivery into MPN309-261 and characterized multiple transformants, with representative results shown. rP65 levels were comparable to that of P65 in wild-type *M. pneumoniae* (Fig. 2C) and partially restored P30 but, as expected, not HMW1, HMW2, or HMW3 levels (summarized in Table 1). rP65 restored both HA and gliding frequency to nearly wild-type levels, whereas gliding velocity increased 6-fold but still remained well below wild-type levels and binding to glass was

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**FIG 3** Immunofluorescence analysis of P65 in representative wild-type and MPN309 mutant *M. pneumoniae* isolates. Left panels, phase-contrast and P65-specific immunofluorescence images merged; right panels, P65-specific immunofluorescence only; arrowheads, examples of P65 foci not cell associated. Bar, 2 μm.

**FIG 4** Immunofluorescence analysis of P65 localization relative to P30 and P41 fluorescent protein fusions. Top row, merged phase-contrast and P65-specific immunofluorescence images; middle row, merged phase-contrast and P30-YFP (columns 1 and 3) or P30-CFP (column 2) images; bottom row, merged phase-contrast and P41-YFP fluorescence images. Red circles, cell-free P65 foci colocalizing with P30 foci (blue circles); white circles, unpaired cell-free P65 (top) or P30 (middle) foci; red arrows, cell-associated P65 foci not colocalized with P30 foci; yellow arrow, cell-associated P30 focus not colocalized with P65 focus; blue arrow, cell-associated P30 focus not colocalized with P65 focus. Bars, 2 μm.
unusually high (Table 2). MPN309-261 transformants with rP65 remained morphologically similar to the parent mutant strain, with chains of cells still common, but exhibited little evidence of P30 foci at the trailing end or detached from cells (Fig. 6). Given the issues with recombinant transposon stability noted above, we did not attempt to introduce rP65 and P30-YFP simultaneously into MPN309 mutants to quantify gliding cells with bipolar P30-YFP foci, as measured for wild-type and mutant cells in Table 3.

DISCUSSION

The P65 transcriptional unit encodes terminal organelle proteins P65, HMW2, P28, P41, and P24, but transposon mutagenesis in this locus yields strikingly different phenotypes, depending on the gene disrupted. Insertions in MPN310 result in loss of HMW2 (30) and failure to form a terminal organelle core (7, 42). In contrast, MPN311 disruption impairs terminal organelle anchoring to the cell body, leading to its detachment from gliding cells (22), while loss of P24 results in reduced gliding frequency and failure to form new terminal organelles at a wild-type rate (23). Here, the truncation of P65 impacted cytadherence, gliding motility, and surface dynamics of the adhesin P30, but a cascade of polar effects from transposon insertion affecting the stability of other terminal organelle proteins (Table 1) complicated efforts to link cause and effect. Reduced levels of HMW2 and P28 were likely a direct result of transposon insertion in MPN309 (15, 29), as neither was restored by complementation with rP65 (Table 1). Transposon insertions in MPN310 have been previously shown to impact P41 and P24 levels downstream (29), and the same was true here for insertions in MPN309 just upstream. As with MPN310 insertions (29), the presence of an outward-reading promoter in the transposon likely contributed to variable expression of downstream genes. Decreased levels of HMW2 in turn likely impacted HMW1.

**TABLE 3** P30-YFP localization at the trailing end of motile wild-type *M. pneumoniae* and mutant MPN309-261

<table>
<thead>
<tr>
<th>Strain</th>
<th>Total no. of cells</th>
<th>No. of motile cells</th>
<th>% of motile cells with head and tail foci</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT + rP30YFP</td>
<td>902</td>
<td>177</td>
<td>1</td>
</tr>
<tr>
<td>MPN309-261 + P30YFP</td>
<td>751</td>
<td>189</td>
<td>51</td>
</tr>
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</table>

*WT, wild type.*
and HMW3 levels (2, 15, 37, 47), which returned to wild-type levels with the introduction of rHMW2 (data not shown). Loss of HMW2 also affects P30 levels (29, 30), although probably by a different mechanism than that observed here, as mutants lacking HMW2 are nonmotile. Neither rP65 (Table 1) nor rHMW2 (data not shown) alone fully restored P30, suggesting that both are required to stabilize P30 at fully wild-type levels, although rP65 alone was sufficient to reduce P30 dragging to and detachment from the trailing end of gliding cells (Fig. 6).

Wild-type P65 localizes to the distal end of the terminal organelle (27, 28), but localization of truncated P65 derivatives differed with the extent of truncation. Thus, severely truncated P65 largely appeared to localize randomly. In contrast, the modestly truncated P65 in mutant MPN309-319 localized at the terminal organelle in a manner similar to that seen with wild-type cells but also typically exhibited a faint focus at the trailing end of gliding cells. Cell-free P65 foci were common with all three mutants but rare in wild-type M. pneumoniae. Based on the sites of truncation (Fig. 1C), these data suggest that the P65 coiled-coil and C-terminal mixed domains are required for its localization and anchoring, respectively, to the terminal organelle. All three truncated P65 derivatives partitioned primarily in the TX-insoluble fraction in a manner similar to that seen with wild-type P65 (data not shown); thus, P65 association with this fraction, which includes mycoplasma cytoskeletal elements, is likely distinct from its localization to the terminal organelle and may involve the APR domain. For reasons that are not clear, truncated P65 was prone to forming apparent multimers, and we were unable to determine if multimeric P65 and monomeric P65 localized distinctly.

The human urogenital tract pathogen Mycoplasma genitalium and avian pathogen Mycoplasma gallisepticum both have P65 orthologs. Loss of this ortholog (MG217) in M. genitalium has no effect on cytadherence or gliding velocity but results in a high incidence of erratic gliding, suggesting that MG217 contributes to the characteristic curved gliding path in this species (9). However, MG217 appeared not to have a surface-accessible location (9), in contrast to P65 in M. pneumoniae and its ortholog (PlpA) in M. gallisepticum, which also binds fibronectin (34). It is not known whether MG217 colocalizes with the P30 ortholog in M. genitalium or if these proteins exhibit the interdependency seen with P65 and P30 in M. pneumoniae. Regardless, these findings suggest that evolutionary pressure has resulted in distinct roles for the P65 family of proteins in mycoplasma samples.

P30 and P65 are predicted to incorporate late in terminal organelle assembly (30), and indeed, fluorescent protein fusions of each localize to nascent terminal organelles in growing cells nearly concurrently and after P41 (21). Electron cryotomography analysis of wild-type cells (25) reveals a high density of protein complexes lining the inner and outer faces of the cell membrane at the distal end of the terminal organelle, where P30 and P65 colocalize (20, 27, 28). As P30 and P65 exhibit reciprocal requirements for stabilization and as P30 often colocalized in MPN309 mutants with truncated P65 at the trailing end of cells and in cell-free foci (references 10 and 40 and this study), we conclude that P30 and P65 may form a complex on the mycoplasma surface. The presence of unpaired P30 and P65 foci with the MPN309 mutants was therefore unexpected but might be a function of stoichiometry if multimer formation by truncated P65 affects its ability to partner with P30.

Our data are consistent with a scenario where P30 bound to an inert surface fails to anchor properly to the terminal organelle in the absence of full-length P65 and consequently during gliding is dragged to and detaches from the trailing end of the mycoplasma cell. Significantly, omission of gelatin from the culture medium resulted in parallel reductions in both the gliding frequency and the percentage of cells having a P30 focus at the trailing end (data not shown). We cannot rule out the possibility that the presence of P30 at the trailing pole is the result of dysfunctional terminal organelle assembly with P65 truncation, but time-lapse images suggest otherwise. In addition, the lack of detectable cell-free P41 or P1 (Fig. 4 and data not shown) indicates that cell-free P30/P65 foci are not detached terminal organelles such as occur in the absence of P41 (22). The P30-YFP at the trailing end of gliding cells constituted only a small portion of the total P30-YFP and may specifically reflect the P30 population bound to the glass substrate. Nevertheless, this P30 population attached to the substrate sufficiently to separate from the trailing end of gliding cells, to impact steady-state levels of cell-associated P30, and likely to create drag, contributing to the reduced gliding velocity and the chains of cells observed with MPN309 mutants.

The M. pneumoniae terminal organelle constitutes the gliding apparatus (22), but the mechanism of gliding is unknown. According to one model (25), extension and contraction of the terminal organelle core is leveraged to generate a motive force that is transmitted via membrane protein complexes to the mycoplasma surface. Each cycle of extension and contraction might require

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**FIG 6** Immunofluorescence analysis of P30 localization in wild-type M. pneumoniae (A), the MPN309-261 mutant (B), and the same mutant complemented with rP65 (C). Circles, P30 foci not cell associated. Bar, 5 μm.
that membrane adhesins move downward across the front of the cell and engage the gliding surface, replacing previous points of contact as they move toward the rear. Seto et al. [41] suggest that the P1 adhesin specifically binds to and releases from the substrate surface repeatedly with gliding. By this treadmill scenario, adhesins disengaging the gliding surface normally return to the tip of the terminal organelle to repeat the process. P30 is required for both adherence and gliding, and our observations here suggest that P30 treadmills in a manner requiring P65. The continued movement of P30 to the trailing end of gliding mutant cells may reflect a defect in P30 release from the substrate and/or its return to the tip of the terminal organelle in the absence of full-length P65. This scenario might also explain the elevated binding to glass by mutants MPN309-152 and MPN309-261 + P65. It is noteworthy, however, that P1 did not colocalize with trailing or detached P30 foc. P30 is required for P1 function, and full-length P65 may provide stable linkage of P1 and P30. P30 may remain functionally linked to P1 only transiently, accounting for intermediate HA capacity in the P65 mutants, or unlinked P30 may be sufficient to confer some measure of functionality to P1.

In conclusion, P65 truncation impacted cytadherence, gliding motility, and surface dynamics of the adhesin P30. The results revealing phenotypic changes associated with P65 truncation provide important new insights into terminal organelle architecture and function, in particular, into the mechanism by which this structure confers gliding motility. The testable models emerging from these findings are expected to yield additional details regarding the gliding motor of this novel prokaryote.

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