An Essential Endopeptidase is Required for Polar Cell Wall Synthesis in *Agrobacterium tumefaciens*

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The canonical elongation and division mechanism in *E. coli* is well-characterized; however, this mechanism isn't conserved across all bacteria, including polar-growing Alphaproteobacteria. In the Brown lab, we use *A. tumefaciens* as a model system to understand novel determinants of polar elongation. In bacteria, cell wall expansion requires both synthesis and hydrolysis. Here, we characterize Atu4178, an essential endopeptidase which may function as a space-making enzyme to allow insertion of new glycan strands by PBP1a during polar elongation. Indeed, we find that Atu4178-sfGFP localizes to the growth pole during elongation. To understand the function of Atu4178, we constructed a depletion strain in which the sole copy of the gene encoding Atu4178 is expressed when an inducer is present. Depletion of Atu4178 results in a severe cell viability defect, decreased cell length and cell rounding, consistent with a role in polar elongation. This phenotype is similar to that observed when PBP1a, an essential peptidoglycan synthase required for polar elongation, suggesting that these two enzymes may function in the same pathway. Strikingly, labeling areas of active peptidoglycan growth using fluorescent d-amino acids (FDAAs) during depletion of Atu4178 revealed that the polar cell wall biosynthesis is halted, with growth then appearing in the subpolar region of the new pole. The ld-transpeptidase Atu2336 localizes to the subpolar region and we hypothesize that its activity may contribute to the detection of subpolar growth. We plan to treat cells depleted of Atu4178 with antibiotics which block ld-transpeptidase activity to investigate the mechanism of subpolar growth. Furthermore, analysis of changes in the peptidoglycan composition during depletion of Atu4178 will help determine if ld-transpeptidase activity is increased. Collectively, these results indicate that the essential Atu4178 endopeptidase participates in polar growth of *A. tumefaciens*

and suggest that ld-transpeptidases may play an important role in a previously undescribed mode of subpolar growth.