The role of Wag31 biomolecular condensates in mycobacterial polar growth

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MYCOBACTERIAL GROWTH

Mycobacteria, including the pathogen *Mycobacterium tuberculosis* (*Mtb*), are a group of rod-shaped bacteria characterized by polar cell wall growth. DivIVA, also known as **Wag31, is an essential pole-localized cytoplasmic protein with roles in polar growth**. However, its molecular mechanisms in polar growth have not been well described.

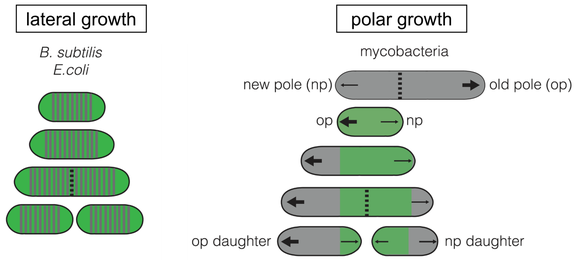


Figure 1: *B. subtilis* and *E. coli* grow by adding new cell wall (gray) along the lateral cell body. Mycobacteria grow only at the polar regions (*Baranowski et al., 2018*).

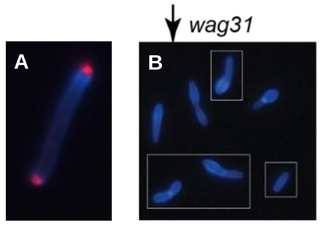


Figure 2: Micrographs of HADA stained cells. HADA is a fluorescent dye which dyes peptidoglycan metabolism.
(A) Wild-type (WT) *Mycobacterium smegmatis* (*Msmeg*) cell expressing a Wag31-mCherry construct.
(B) *Msmeg* cells during *wag31* depletion. (*Arejan et al., 2024*).

CONDENSATES

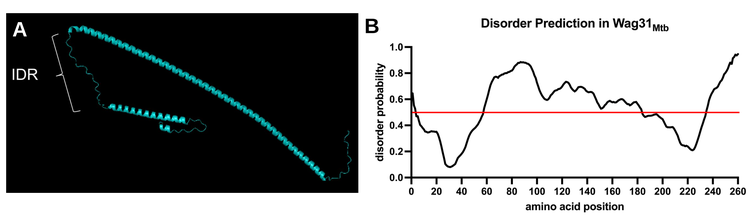


Figure 3: Structural predictions show that $Wag31_{Mtb}$ contains an intrinsically disordered region (IDR) over 100 amino acids long. IDRs are associated with the formation of biomolecular condensates. **(A)** Alpha fold predicted protein model. **(B)** Protein disorder confidence for each amino acid residue in $Wag31_{Mtb}$ predicted by PrDOS.

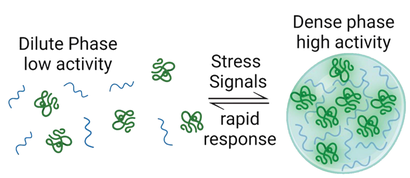


Figure 4: Biomolecular condensates are dynamic and regulatory membrane-less assemblies that organize biochemical interactions in response to various molecular signals (*Sasazawa et al., 2024*).

WAG31 CONDENSATES

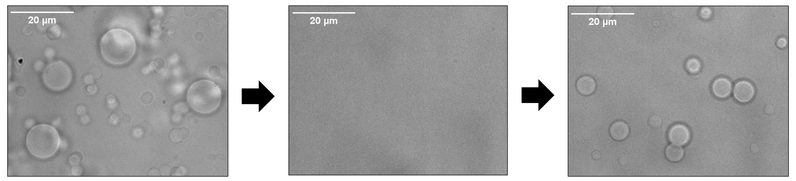
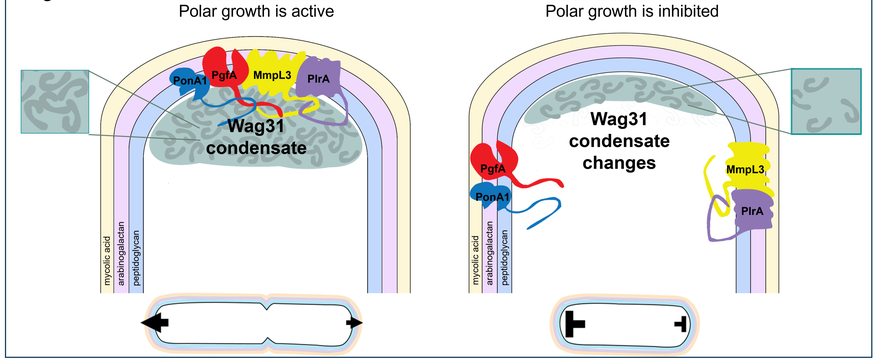


Figure 5: $Wag31_{Mtb}$ forms condensates *in vitro* in a physiological, low salt buffer. Condensates dissociate in imidazole, but reform again when returned to the same physiological buffer.

PRELIMINARY MODEL

Figure 6:



DATA AND RESULTS

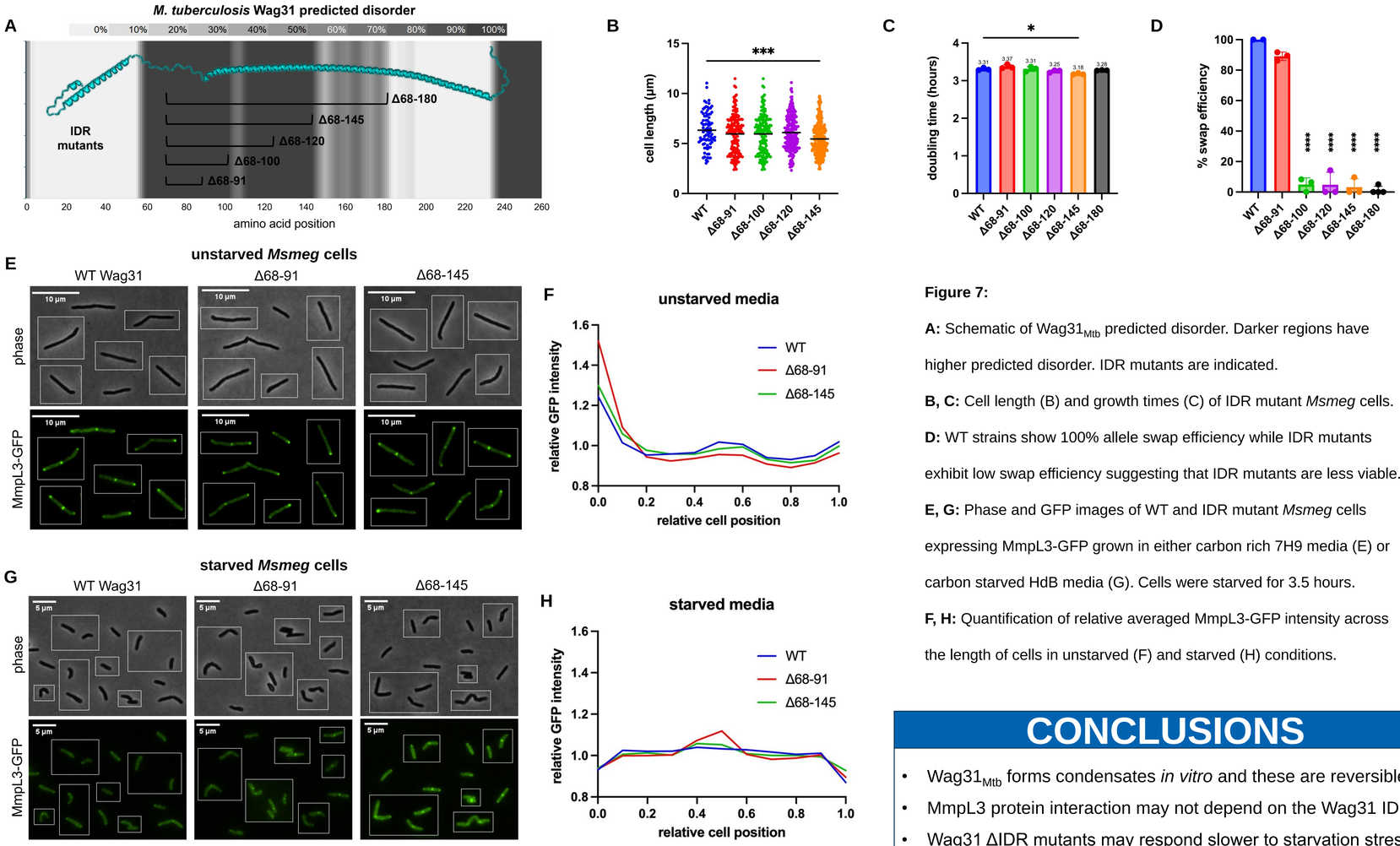


Figure 7:

A: Schematic of $Wag31_{Mtb}$ predicted disorder. Darker regions have higher predicted disorder. IDR mutants are indicated.

B, C: Cell length (B) and growth times (C) of IDR mutant *Msmeg* cells.

D: WT strains show 100% allele swap efficiency while IDR mutants exhibit low swap efficiency suggesting that IDR mutants are less viable.

E, G: Phase and GFP images of WT and IDR mutant *Msmeg* cells expressing MmpL3-GFP grown in either carbon rich 7H9 media (E) or carbon starved HdB media (G). Cells were starved for 3.5 hours.

F, H: Quantification of relative averaged MmpL3-GFP intensity across the length of cells in unstarved (F) and starved (H) conditions.

CONCLUSIONS

- $Wag31_{Mtb}$ forms condensates *in vitro* and these are reversible.
- MmpL3 protein interaction may not depend on the Wag31 IDR.
- Wag31 ΔIDR mutants may respond slower to starvation stress.