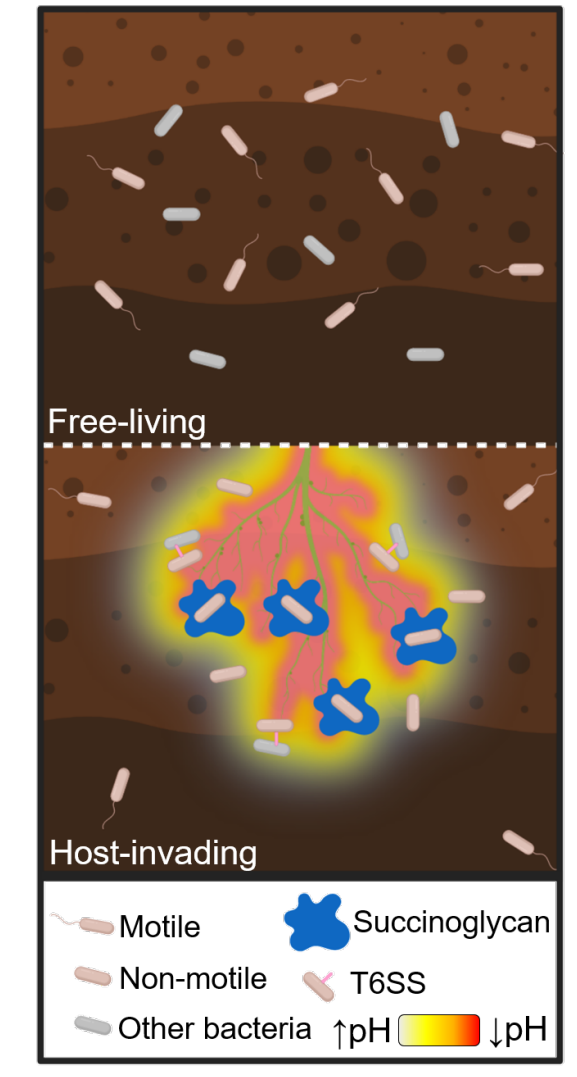


Succinoglycan Production in *Agrobacterium tumefaciens*



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What is Succinoglycan?



Succinoglycan is a negatively charged polysaccharide that is required for *Agrobacterium tumefaciens* to invade a plant host. This invasion is the cause of Crown Gall disease, where the plant grows large tumor-like growths. The mechanistic role of succinoglycan in this process remains largely unknown.

Possible succinoglycan roles:

- Acid tolerance
- Osmoprotection
- Surfactant
- Signaling molecule

Current model for plant invasion

Calcofluor white staining reveals differential regulation of succinoglycan biosynthesis.

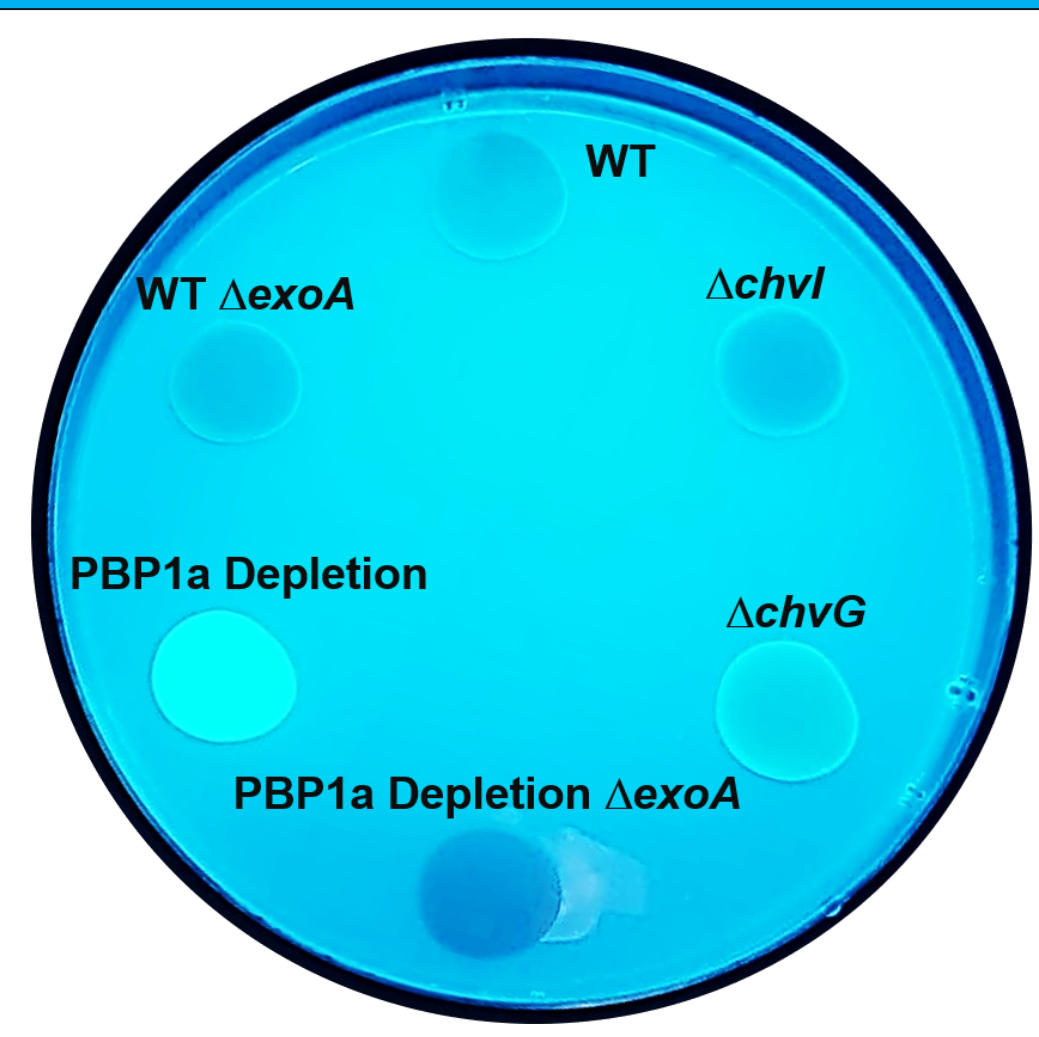


Image taken under UV light
Greater fluorescence intensity = more succinoglycan produced

Preliminary results

- Cells that depleted of the cell wall synthase PBP1a have an overproduction of succinoglycan
- $\Delta chvG$ cells showed an unexpected production of succinoglycan, suggesting the existence of additional regulatory proteins.

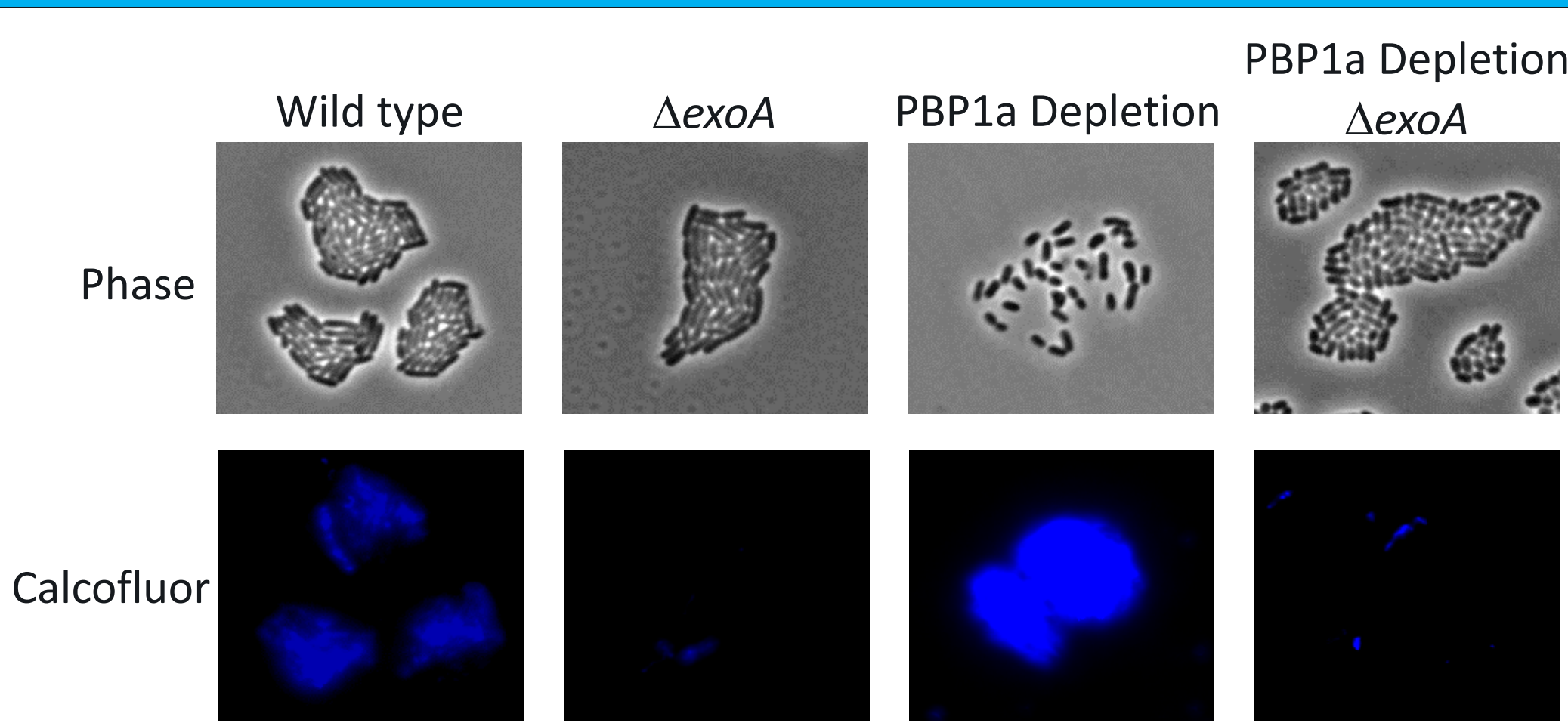
Objectives

Research Questions:

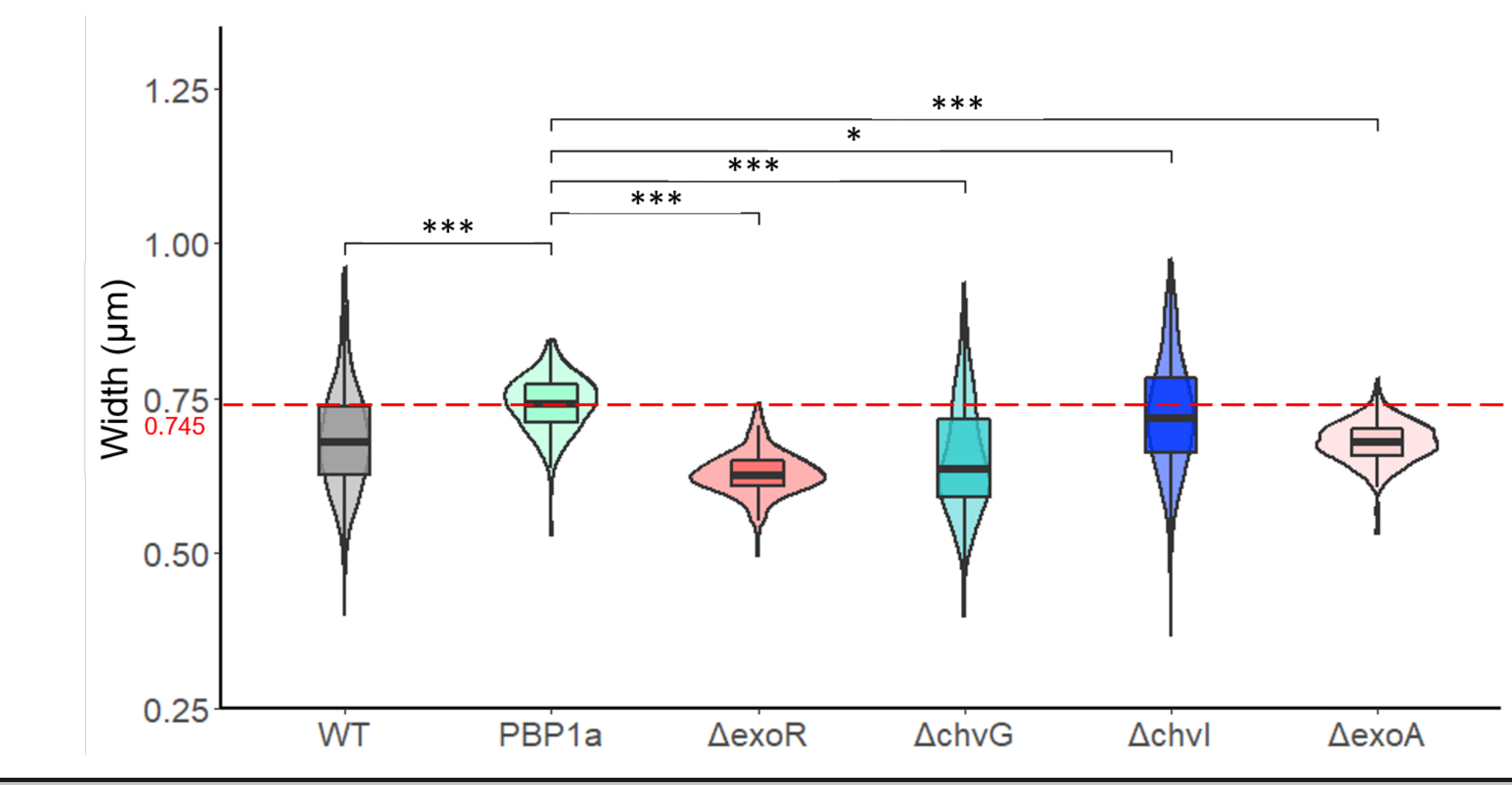
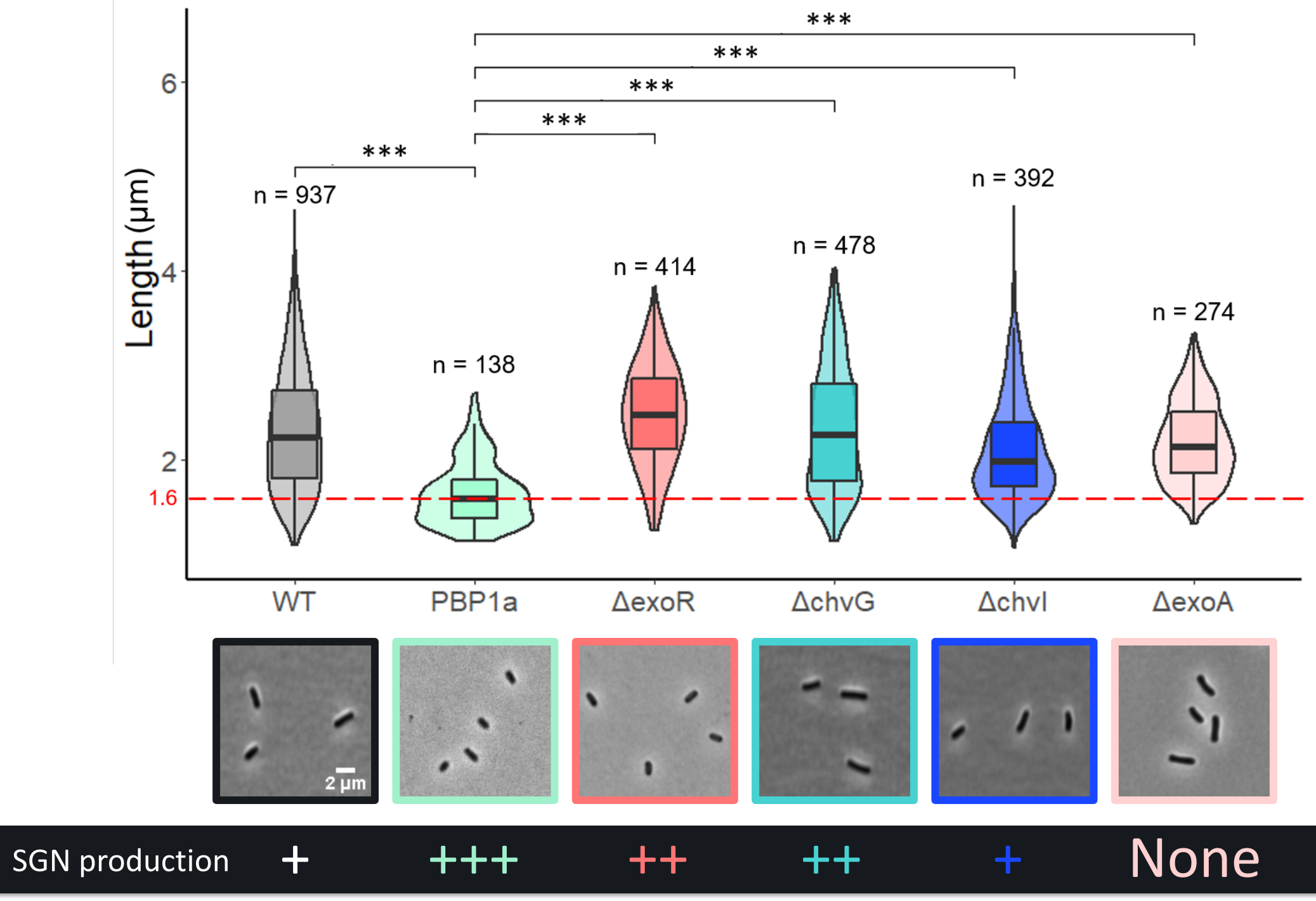
1. Does succinoglycan production regulate growth?
2. Does the impairment of growth signal for succinoglycan production?

Hypothesis: Succinoglycan production is increased as a result of decreased growth.

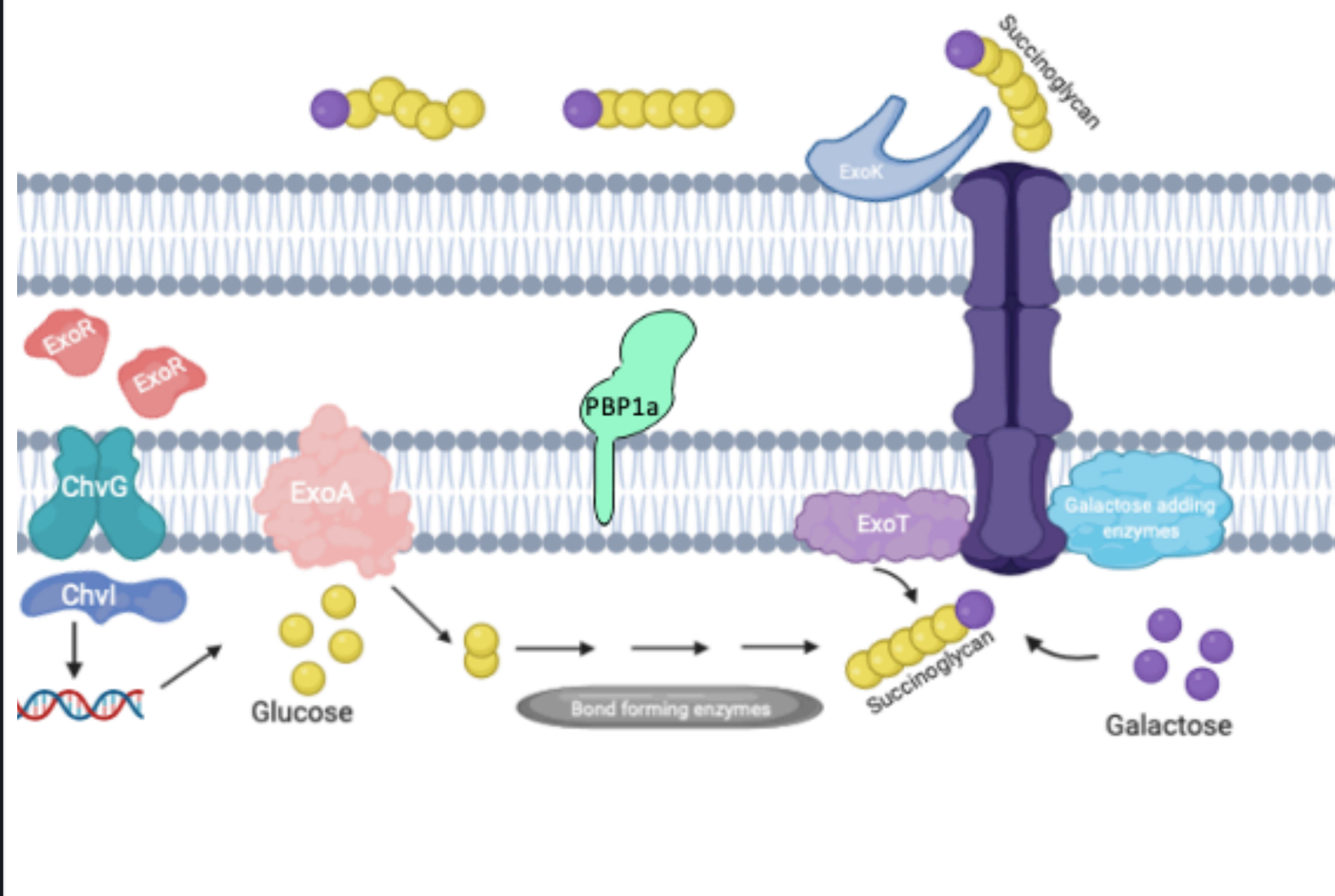
Does succinoglycan production regulate growth?



PBP1a-depleted cells are short and round, suggesting they are growth deficient. They also overproduce succinoglycan, suggesting a link between succinoglycan production and growth.

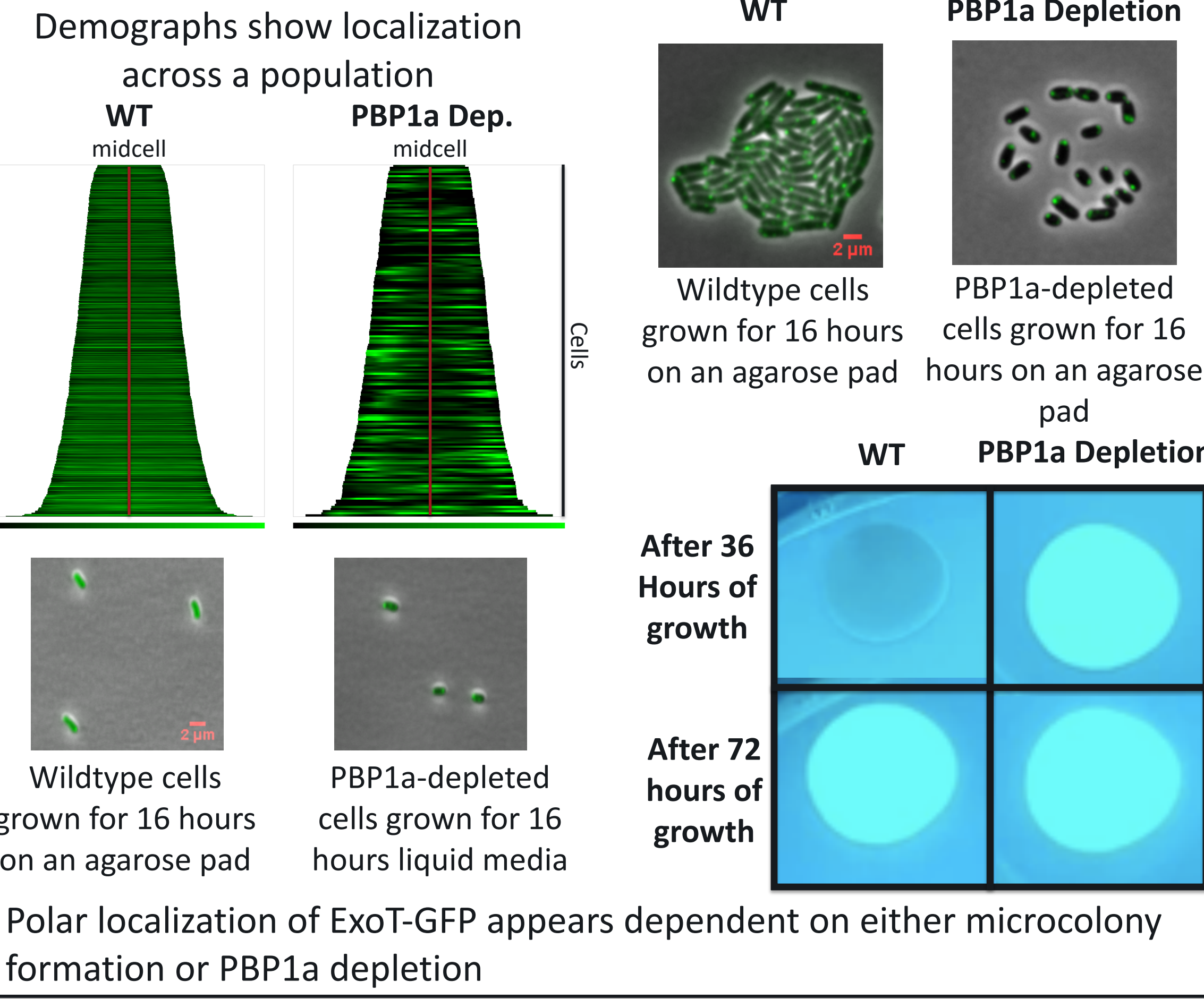


Succinoglycan Biosynthesis Pathway



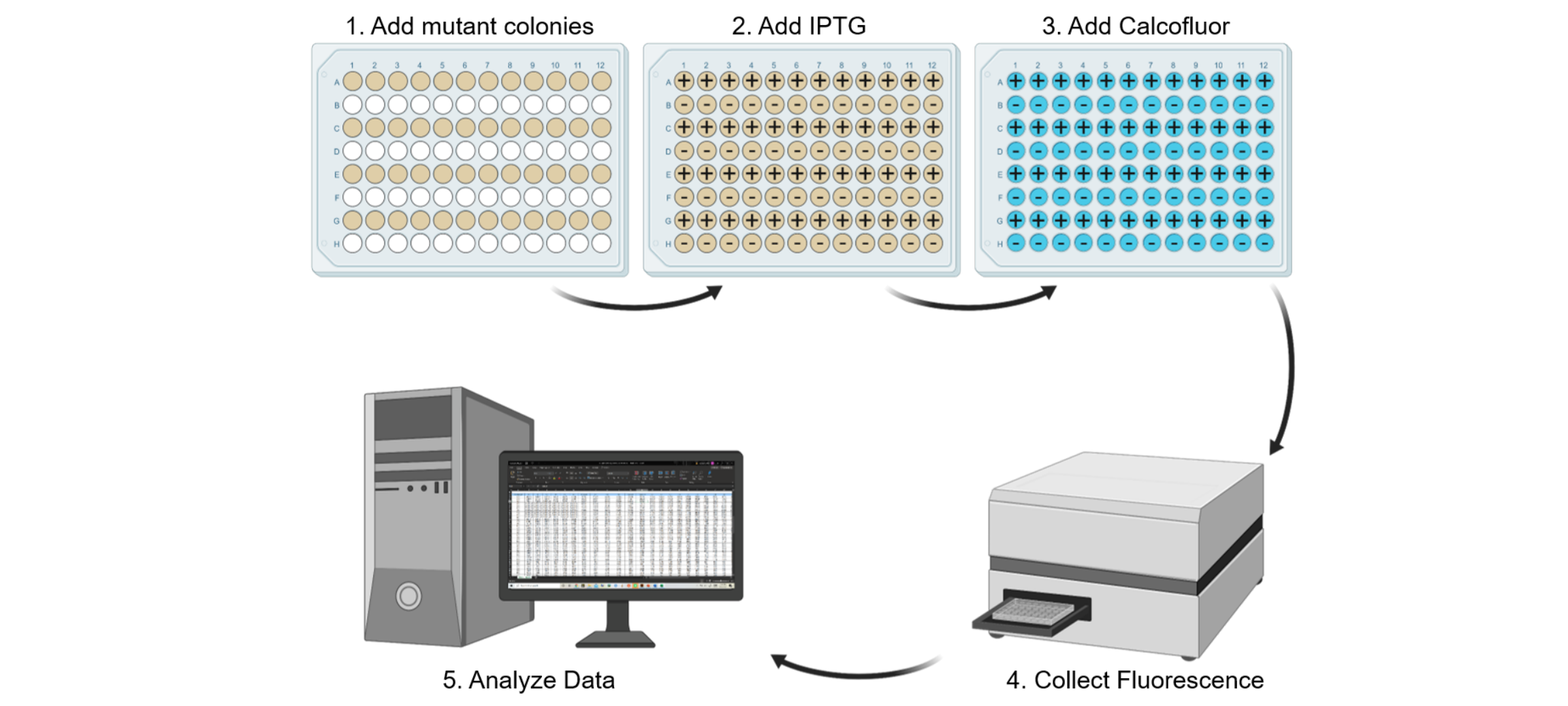
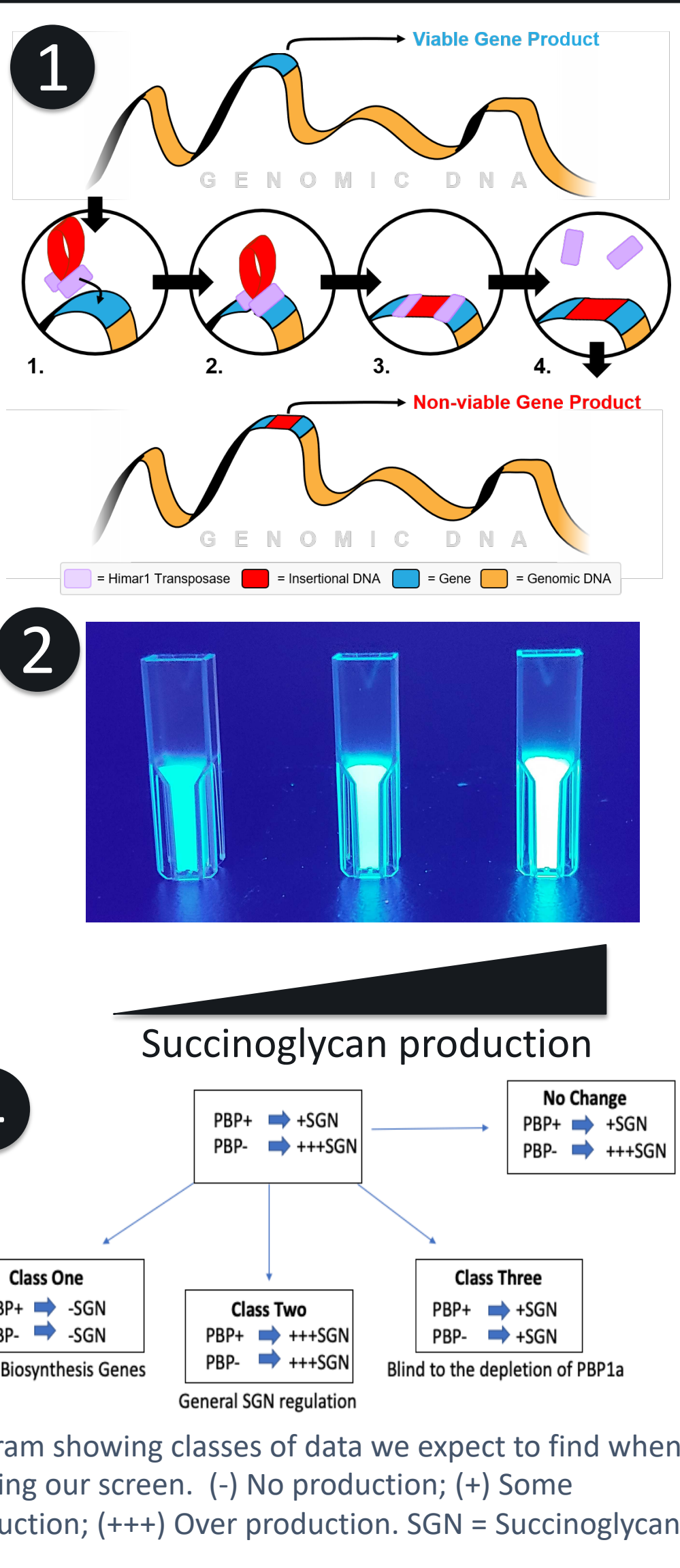
How is succinoglycan secretion machinery coordinated in the cell?

ExoT-GFP fusion localizes the succinoglycan secretion complex.



Development of a screen to detect succinoglycan regulatory mutants

1. A library of transposon insertion mutants will be generated using a method for random transposon mutagenesis.
2. Using the fluorescence intensity readout of calcofluor bound to succinoglycan, the library will be screened for mutants that no longer overproduce succinoglycan when depleted of PBP1a.
3. PBP1a expression will be controlled using a chemical inducer known as IPTG.
4. Mutants will be classified and then sequenced for further characterization.



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