PtsI independently regulates cell length and width through multiple metabolic and signaling pathways

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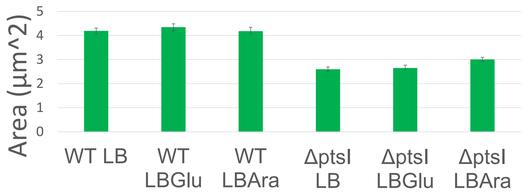
Abstract

It has been long known that Escherichia coli can adjust its cell size and growth rate to nutrient availability. Several proteins, such as OpgH (which modulates cell division based on UDP-glucose concentration), and pyruvate kinase (which affects FtsZ assembly) couple nutrition to the cell elongation machinery. We have found that Enzyme I (ptsI) of the PTS plays a critical role in dictating cell morphology through its regulation of the phosphoenolpyruvate (PEP):pyruvate ratio and its involvement in cAMP signaling. The PTS is responsible for the phosphorylation of PTS sugars (such as glucose) upon entrance into the cell; Enzyme I passes the phosphate group from PEP to the carrier protein Hpr. HPr then passes the phosphate to sugar specific Enzyme II proteins which phosphorylate specific sugars upon entry into the cell. We discovered that deletion of ptsI results in significantly shorter and thinner cells. The addition of pyruvate to media greatly corrects the cell length defects caused by ptsI deletion, while the addition of PEP further shrinks cells, suggesting that PtsI control over the PEP:pyruvate levels in the cell is important for cell length regulation. We found that interruption of cAMP production leads to similar outcomes as the absence of ptsI. These results indicate that ptsI might modulate cell size by separately influencing metabolite flux and cAMP signal transduction. In the future we hope to investigate how mutants respond to different levels of pyruvate/PEP and the specific cellular mechanisms activated by ptsI-induced cAMP.

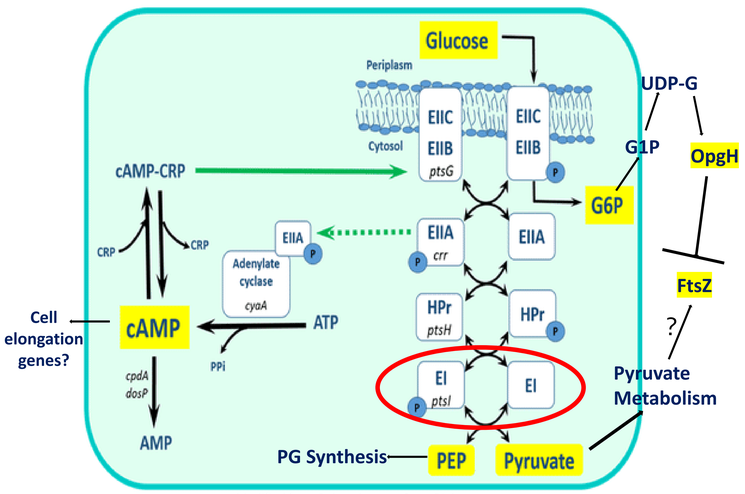
Background

PtsI regulates cell size independent of sugar uptake



WT and *ptsI* mutant cells were grown in LB medium alone or supplemented with indicated sugars.

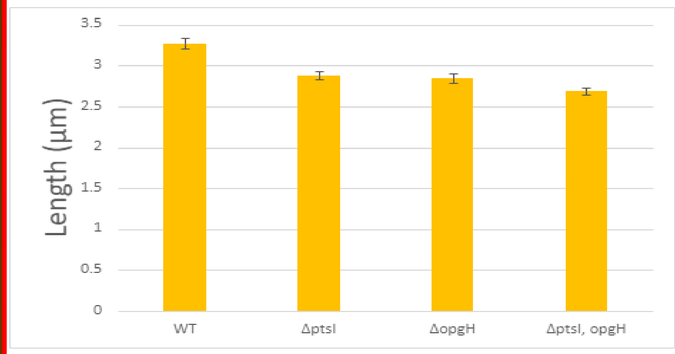
PtsI's role in the phosphotransferase system



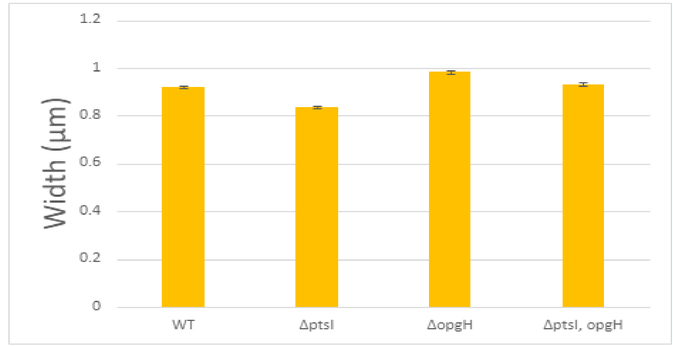
Modified from Pal et al. 2022, *Open Biology*

ptsI codes for Enzyme I (EI), which is the first step of the PTS

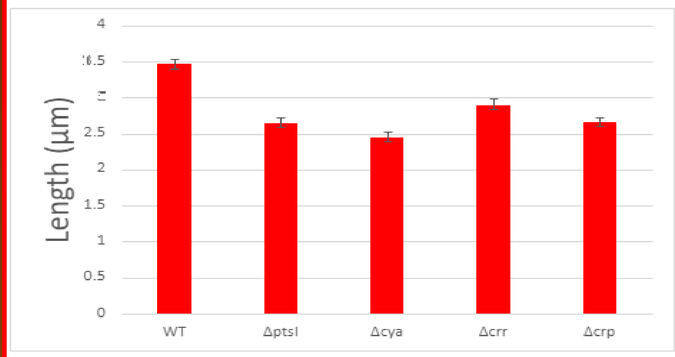
OpgH and PtsI control cell size through distinct mechanisms



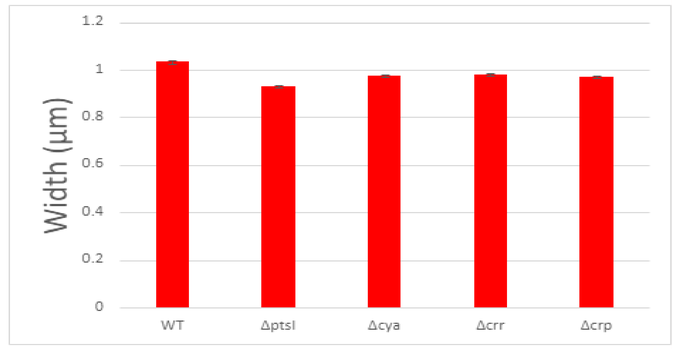
ptsI and *opgH* mutant cells were grown to exponential phase. Both mutants result in shorter cells, but they have distinct cell width phenotypes. Epistasis analysis shows an intermediate cell width phenotype and additive cell length phenotype, suggesting two different pathways.



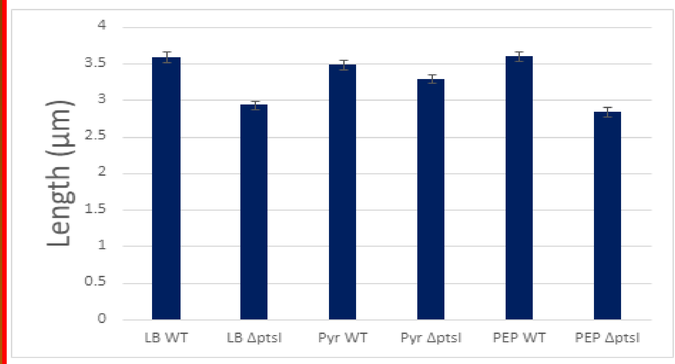
cAMP-cascade mutants display similar phenotype to PtsI mutants



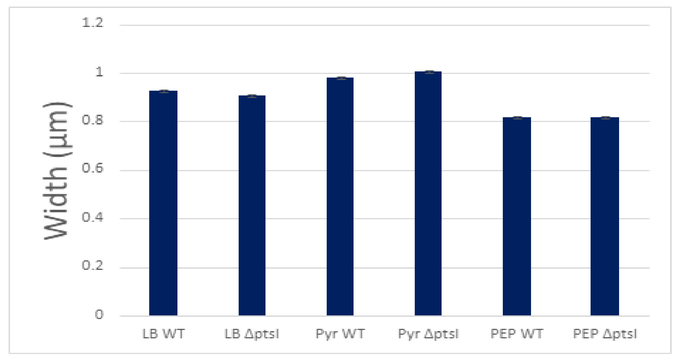
Mutants in the cAMP signaling pathway were grown in LB along with *ptsI* mutant and WT cells. *cya* mutants are shorter than Δ*ptsI* cells, while crr mutants produce cells longer cells than Δ*ptsI* cells. All three mutants produced similar width phenotypes, which are wider than the *ptsI* mutant.



Pyruvate corrects for cell size in PtsI mutants



WT cells and *ptsI* mutant cells were grown in LB and LB supplemented with 2% pyruvate or 2% PEP. Pyruvate causes an increase in both length and width in the *ptsI* mutant. The addition of PEP causes a reduction in width in both WT and Δ*ptsI* cells.



Conclusions

- PtsI and OpgH modulate cell size through distinct pathways
- Pyruvate/PEP levels regulate cell width
- cAMP signal transduction impacts cell length

Future Directions

- Test the effects of different levels of PEP and pyruvate cell size .
  - Determine if absolute or relative levels of Pyr/PEP cause cell size changes.
- Up-regulate the genes of the cAMP pathway and test double mutants to examine their morphology.
- Genetically determine the effects of PEP/Pyr by deleting genes encoding proteins that metabolize these compounds.