P-2.29

Investigating the Ph-gating Mechanism of Bacterial Urea Channels: Implications for Pathogen Survival in Acidic Environments

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Bacterial urea channels, especially HpUreI in Helicobacter pylori, are crucial for survival in acidic environments by transporting urea, which is then hydrolyzed into ammonia to neutralize stomach acid. With rising resistance to standard H. pylori treatments, HpUreI is a promising therapeutic target. Despite structural studies of its open and closed states, the precise pH-gating mechanism remains elusive. In our study, we used yeast complementation assays to examine the role of protonatable residues on both the periplasmic and cytoplasmic sides of HpUreI, alongside homologous channels from Helicobacter hepaticus and Streptococcus salivarius. Expressing these constructs in a urea/ammonia uptake-deficient Saccharomyces cerevisiae strain allowed us to compare channel functionality and pH gating within a physiological range (pH 4.0–7.0). Yeast growth in varying pH conditions with urea or ammonia as the sole nitrogen source directly linked channel permeability to cell survival. Our results reveal a complex network of residues governing pH-gating, challenging the idea that periplasmic histidines alone serve as pH sensors. This yeast system provides a robust platform for testing protein functionality under diverse solute and pH conditions, offering insights that may inform future drug development targeting HpUreI channels.