Salmonella flagellar regulon is controlled by a nutrient-tunable bistable switch

Santosh Koirala, Philip D. Aldridge, and Christopher V. Rao

Motility is a common strategy used by diverse organisms to adapt to changes in their environment. For instance, when proximal sources of nutrients are depleted, one simple strategy is to seek out new ones. This process has been extensively studied in the enteric bacteria *Escherichia coli* and *Salmonella enterica* serovar Typhimurium. Both employ flagella to move. Both swim towards nutrients and away from toxins by altering the rotational bias of their flagella. Both regulate the expression of their flagellar genes in response to nutrient availability, independently of chemotaxis. Interestingly, this response is quite different despite otherwise close similarities between the two motility systems. In particular, nutrients repress flagellar gene expression in *E. coli* whereas they enhance it in *Salmonella.*

Unlike *E. coli,* motility is phase variable in *Salmonella*: co-existing populations of motile and non-motile cells are observed in an isogenic cell population. This behavior is thought to arise from a feedback loop involving $FlhD_4C_2$, FliZ, and YdiV, which functions as a toggle switch. FliZ represses the transcription of *ydiV* by binding to an upstream promoter. YdiV, in turn, binds to $FlhD_4C_2$ and prevents it from activating its target promoters. YdiV also promotes the degradation of $FlhD_4C_2$ by ClpXP. Additionally *ydiV* expression is regulated by the nutrients in the media. YdiV expression is enhanced in poor media and greatly reduced in rich media. In addition to this genetic toggle switch, multiple interacting feedback loops are known to govern the expression of flagellar proteins. These feedback loops not only regulate protein expression in response to multiple environmental factors but also couple their expression to other cellular processes including the invasion and adhesion. How the global signals regulating $FlhD_4C_2$ expression and nutrients signals regulating the expression of *ydiV* give rise to heterogeneous phenotype, however, is still not well understood.

In this work, we investigated phase variation within the *Salmonella* flagellar regulon. We have shown that the response to nutrients is bistable and two distinct phenotypes, motile and sessile, coexist in an isogenic cell population. We have further shown that the response exhibits hysteresis which results from a double negative feedback loop involving two regulatory proteins, FliZ and YdiV. Additionally, we found that a positive feedback loop involving alternate sigma factor FliA is required for bistability which amplifies the expression of FliZ by enhancing its expression from class 3 component of P_{fliAZ} promoter, but two other feedback loops involving FlgM and FliT are

not. These results altogether suggest that *Salmonella* may use motility for purposes other than foraging.

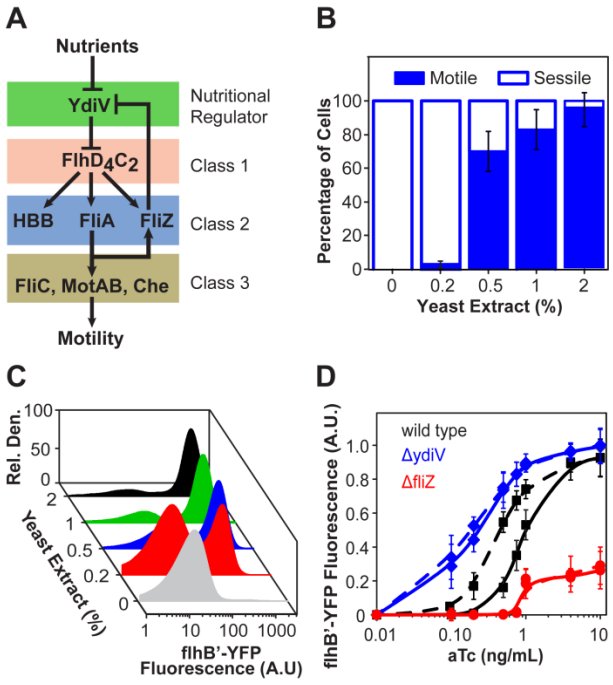


Figure. Nutrients tune the fraction of motile cells in *S. typhimurium*. A. Schematic of flagellar regulon. The flagellar genes can be arranged into three classes based on how they are transcriptionally regulated. The sole class 1 operon encodes the $FlhD_4C_2$ master regulator. $FlhD_4C_2$ activates the expression of class 2 operons, which encode the hook-basal body proteins. In addition, $FlhD_4C_2$ activates the expression of the alternate sigma factor, FliA (also known as σ^{28}) and FliZ. FliA, in turn, activates the expression of the class 3 operons, which encode the motor proteins (MotAB), flagellar filament (FliC), and chemotaxis pathway (Che). YdiV binds $FlhD_4C_2$ and prevents it from activating class 2 promoters. In addition, YdiV promotes the degradation of $FlhD_4C_2$ via ClpXP. Both nutrients and FliZ repress the expression of YdiV. **B.** Fraction of motility cells as a function of nutrient concentrations as determined by video microscopy. **C**. Flagellar gene expression as determined using single-copy transcriptional fusion to the fluorescent protein Venus for representative class 2 (P_{flhB}) promoter. **D.** Expression exhibits hysteresis. Experiments were performed in a strain where the native P_{flhDC} promoter was replaced with aTc-inducible one (P_{tetRA}). Cells were grown in presence (on, dotted line) or absence (off, solid line) of aTc and then subcultured at intermediate aTc concentrations.