PHENOTYPIC VARIATION AND BISTABILITY WITHIN FLAGELLAR GENE NETWORK IN SALMONELLA TYPHIMURIUM

BY

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THESIS

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In *Salmonella enterica* serovar Typhimurium, more than 50 genes divided among at least 17 operons are involved in flagellar biogenesis and chemotaxis. One of the critical features of this process is coupling of the gene expression to assembly. Like flagellar assembly, gene expression also proceeds in a sequential manner. Availability of nutrients in the cellular environment also plays a key role in switching between motile or sessile phenotypes. However, the response to nutritional cues could be very different even in closely related species. For example, *E. coli* upregulates flagellar synthesis under low-nutrient conditions through cAMP-Crp control of the class 1 operon whereas *Salmonella enterica* serovar Typhimurium downregulates flagellar synthesis under low-nutrient condition. YdiV is responsible for the repression of flagellar synthesis in *Salmonella enterica* serovar Typhimurium which acts as an anti-FlhD4C2 factor. Moreover, FliZ-dependent activation of P\text{class2} promoters is more pronounced in low nutrient condition and is achieved by repression of the *ydiV* gene by FliZ. YdiV expression is enhanced in poor media and greatly reduced in rich media. Thus, FliZ and YdiV, in poor media, form a negative regulation loop which results in a bistable motility phenotype. However, the extent of bistability is unclear. This study aims to characterize the extent of bistability within the flagellar network in *Salmonella enterica* serovar Typhimurium. Our results suggest that two distinct phenotypes, motile and sessile, coexist in an isogenic cell population under limited nutrient condition. The flagellar gene network is bistable and is mediated by FliZ-YdiV feedback loop.
ACKNOWLEDGMENTS

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CHAPTER 1

INTRODUCTION

1.1 Motivation

*Salmonella* are causative agents of a food borne disease, salmonellosis. Gastrointestinal tract of animals is their primary habitat [1]. Most of the infections results from consuming contaminated foods from animal origins or fruits and vegetables contaminated with animal faeces [2, 3]. In human, salmonellosis usually takes the form of self-limiting gastroenteritis, but occasionally could lead to systemic infection (enteric fever), bacteremia and other complications [1, 4]. It accounts for 26% of hospitalizations (15,000 per year) and 31% of deaths (400-600 per year) caused by the food borne diseases in the United States [2, 5]. Non-typhi *Salmonella* infection is also a major public health problem among children under the age of 5 in developing countries [6]. Although vaccines are readily available for typhoid fever, no vaccines are available for non-typhoidal salmonellosis [4]. Understanding *Salmonella*’s interaction and survival inside the host, through continued research in this area, is paramount for developing new vaccines and drugs.

Although only capable of causing self-limiting gastroenteritis in human under normal conditions, *Salmonella enterica* serovar Typhimurium is capable of causing systemic infection that resembles human Typhoid fever caused by *Salmonella enterica* serovar Typhi [1]. An array of classic and modern genetic tools are readily available to manipulate *Salmonella* strains. The mouse infection model and the ease of manipulation make serovar Typhimurium ideal for studying rather complex host-pathogen interactions.
1.2 Background

An ability of a cell to appropriately respond to its environmental cues ultimately decides its fate. Cells have to be able to sense chemicals in their environment and decide to move towards or away from them. Cells swim in liquid environment and drift along surfaces by assembling and rotating flagella [7, 8]. In *Escherichia coli* and *Salmonella*, flagella are long (several body lengths) thin helical filaments, usually 5-10 per cell, assembled at random sites on their body [1, 7, 8]. Each flagellar filament is driven at the base by a rotary motor capable of spinning in both clockwise (CW) and counterclockwise (CCW) directions [8]. CCW rotations of helical filaments causes a flagellar bundle formation and propels the cell along a more or less smooth trajectory called a run, whereas CW rotations disperses the bundle resulting in uncoordinated filament action with rapid somersaulting called a tumble [7].

A flagellar structure consists three individual parts: a basal body, a hook and a filament [9]. The basal body which anchors the flagellum to the cell has a complex structure embedded in the bacterial membranes. It is anchored to the inner membrane, peptidoglycan layer and the outer membrane by MS-ring, P-ring and L-ring respectively. The P-ring and the L-ring act as bushing for a hollow rod that is built onto the MS-ring and spans the periplasmic space. The cytoplasmic face of the MS-ring anchors C-ring and type III secretion apparatus which delivers majority of the protein subunits through a central channel within the growing flagellar structure [10]. The basal body also houses a motor that is driven by proton-motive force [8]. The torque produced by the motor is transmitted to the filament by a flexible joint called the hook. The filament, a long rigid helical structure approximately 5 to 15 µm in length, is capable of rotating both CW and CCW direction in sync with the motor [11]. Flagellar assembly is sequential which begins with the formation of the basal body inside out along the membranes and concludes with the formation of filament [12].

An individual flagellum is assembled by incorporating approximately 20,000 protein subunits [12]. Flagellar biogenesis, thus, is a lavish and energy intensive process that requires a high level of regulation. One of the critical features of this process is coupling of the gene expression to assembly.
Like flagellar assembly, gene expression also proceeds in a sequential manner. First, the genes required for the basal body and hook assembly are expressed, and then, only after the completion of the hook basal body (HBB), the late genes required for filament and motor assembly are produced. An unsuccessful assembly of HBB stymies the expression of late genes. This checkpoint ensures a coordinated assembly that conserves resources and energy [13, 14].

In *Salmonella enterica* serovar Typhimurium, more than 50 genes divided among at least 17 operons are involved in making chemotactic decisions. Among these, about 10 genes are involved in detecting and processing sensory cues and the rest encode for flagellar subunits and a number of regulators that synchronizes gene expression with the assembly process [7, 15]. These operons are divided into three classes forming an organized hierarchical gene cascade. Flagellar assembly is initiated by products of a single operon controlled by P<sub>class1</sub> promoter, consisting *flhDC* genes, and is therefore called the master operon [16]. The environmental signals and sensory cues manifest into the flagellar gene expression hierarchy through the master operon by the action of different global regulators on P<sub>class1</sub> promoter, which allows the cells to determine whether to be motile or not. When motility is induced, FlhD<sub>4</sub>C<sub>2</sub> hexaheteromeric complex, products of the master operon, binds to the P<sub>class2</sub> promoter region and initiates the transcription by σ<sup>70</sup>-RNA polymerase. These promoters control the expression of genes encoding HBB proteins and an array of regulatory proteins [17]. Among the regulatory proteins encoded from class 2 operons, FlgM and FliA (σ<sup>28</sup>) play major roles in enforcing HBB checkpoint. The σ<sup>28</sup> alternate sigma factor controls the transcription of class 3 operons encoding for late genes. Before HBB completion, FlgM binds to FliA and stops it from activating P<sub>class3</sub> promoters. However, after HBB completion, FlgM is secreted out of the cells allowing σ<sup>28</sup> to initiate the transcription of class3 operons [18, 19, 20]. However, mere presence or absence of functional HBBs doesn’t determine the expression of flagellar genes. Class3 gene expression is controlled by the rate of FlgM secretion mediated by the σ<sup>28</sup>-FlgM regulatory circuit [21, 22].

In addition, flagellar morphogenesis has also been shown to be regulated by two other flagellar proteins, FliT and FliZ. FliT, encoded in the *fliDST* operon, is the secretion chaperone for the filament cap protein FliD, and
negatively regulates the class 2 gene expression by binding to FlhD4C2 complex and hence inhibiting its activation of P_{class2} promoters. Class 2 gene expression has been shown to increase in a ΔfliT mutant [23]. FliZ, encoded in the fliAZY operon, is a positive regulator of class 2 gene expression [23]. It was shown to be FlhD4C2-dependent activator of P_{class2} activity and to participate in a positive-feedback loop that induces a kinetic switch in class 2 operon expression [14, 24]. Recently, a non-flagellar protein YdiV was reported to negatively regulate class 2 expression [25]. YdiV has weak homology to EAL domain proteins, which are known to be involved in regulation of cyclic-di-GMP, a second messenger molecule [26, 27].

Availability of nutrients in the cellular environment plays a key role in switching between motile or sessile phenotypes. However, the response to nutritional cues could be very different even in closely related species. For example, *E. coli* upregulates flagellar synthesis under low-nutrient conditions through cAMP-Crp control of the class1 operon whereas *Salmonella enterica* serovar Typhimurium downregulates flagellar synthesis under low-nutrient condition [27, 28, 29]. YdiV is responsible for the repression of flagellar synthesis in *Salmonella enterica* serovar Typhimurium which acts as an anti-FlhD4C2 factor [27]. Moreover, FliZ-dependent activation of P_{class2} promoters is more pronounced in low nutrient condition and is achieved by repression of the ydiV gene by FliZ [30]. YdiV expression is enhanced in poor media and greatly reduced in rich media [27]. Thus, FliZ and YdiV, in poor media, form a double negative regulation loop which results in a bistable motility phenotype [31]. However, the extent of bistability is unclear. This study aims to characterize the extent of bistability within the flagellar network in *Salmonella enterica* serovar Typhimurium.
CHAPTER 2

MATERIALS AND METHODS

2.1 Media, chemical reagent and growth conditions

All culture experiments were performed at 37°C unless noted otherwise. The rich and poor media used were Luria-Bertani (LB) broth and Vogel-Bonner MinE media [32] supplemented with 0.2% glucose (MG media). Various concentrations of yeast extract (YE) were supplemented to the poor media to simulate differential nutritional conditions. Agar-paltes were prepared with 12.5 g/L LB and 15 g/L agar. Antibiotics were used at the following concentrations: ampicillin at 100 µg/mL, chloramphenicol at 20 µg/mL, kanamycin at 40 µg/mL and tetracycline at 15 µg/mL. Enzymes were purchased from Fermentas or New England Biolabs and used according to the manufacturer’s recommendations. Chemicals and reagents were purchased from Sigma-Aldrich. Oligonucleotide primers were purchased from IDT Inc.

2.2 Bacterial strains and plasmid construction

Strains and plasmids used in this study are described in Tables 2.1 and 2.2, respectively. All S. enterica serovar Typhimurium strains are isogenic derivatives of strain 14028 (American Type Culture Collection) unless noted otherwise. Standard molecular DNA manipulation and cloning techniques were used [33, 34, 35, 36]. Oligonucleotide primers are listed in Table 2.3. The generalized transducing phage of S. enterica serovar Typhimurium, P22 HT105/1 int-201, was used in all transdutional crosses [36].

The ∆ydiV mutant strains were constructed following the protocol described by Datsenko and Wanner [33]. Wild type cells harboring helper plasmid
<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant Characteristic(s)</th>
<th>Source</th>
</tr>
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<tbody>
<tr>
<td>SK1</td>
<td>Wild-type serovar Typhimurium, 14028 strain</td>
<td>ATCC</td>
</tr>
<tr>
<td>CR201</td>
<td>ΔfliZ::FRT</td>
<td>[14]</td>
</tr>
<tr>
<td>SK61</td>
<td>ΔydiV::cm</td>
<td></td>
</tr>
<tr>
<td>CR208</td>
<td>ΔPflhDC::tetRA</td>
<td></td>
</tr>
<tr>
<td>SS1006</td>
<td>attλ::pVenus::PflhD-venus</td>
<td>[14]</td>
</tr>
<tr>
<td>CR817</td>
<td>attλ::pVenus::PflhB-venus</td>
<td>[24]</td>
</tr>
<tr>
<td>SS1009</td>
<td>attλ::pVenus::PflgM-venus</td>
<td></td>
</tr>
<tr>
<td>CR819</td>
<td>ΔfliZ::FRT attλ::pVenus::PflhB-venus</td>
<td>[24]</td>
</tr>
<tr>
<td>SK68</td>
<td>ΔydiV::cm attλ::pVenus::PflhB-venus</td>
<td></td>
</tr>
<tr>
<td>SS1008</td>
<td>ΔfliZ::FRT attλ::pVenus::PflgM-venus</td>
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<td>SK71</td>
<td>ΔydiV::cm attλ::pVenus::PflgM-venus</td>
<td></td>
</tr>
<tr>
<td>CR823</td>
<td>ΔPflhDC::tetRA attλ::pVenus::PflhB-venus</td>
<td>[24]</td>
</tr>
<tr>
<td>SS1039</td>
<td>ΔPflhDC::tetRA attλ::pVenus::PflgM-venus</td>
<td></td>
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<tr>
<td>CR825</td>
<td>ΔPflhDC::tetRA ΔfliZ::FRT attλ::pVenus::PflhB-venus</td>
<td>[24]</td>
</tr>
<tr>
<td>SK69</td>
<td>ΔPflhDC::tetRA ΔydiV::cm attλ::pVenus::PflhB-venus</td>
<td></td>
</tr>
<tr>
<td>SS1041</td>
<td>ΔPflhDC::tetRA ΔfliZ::FRT attλ::pVenus::PflgM-venus</td>
<td></td>
</tr>
<tr>
<td>SK70</td>
<td>ΔPflhDC::tetRA ΔydiV::cm attλ::pVenus::PflgM-venus</td>
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pKD46 were grown overnight at 30°C in LB supplemented with ampicillin. The overnight culture was diluted 1:100 in 50mL LB with ampicillin. The cell culture was induced with 0.2% arabinose and grown to mid-log phase. The cells were harvested by centrifuging at 4000 rpm and made electrocompetent by washing three times with ice cold 10% glycerol solution. Chloramphenicol and kanamycin cassettes with 40 basepair homology to ydiV flanking regions were PCR amplified using primers SK72F and SK72R with pKD3 and pKD4 as templates respectively. The PCR products were cleaned and transformed into the electrocompetent cells. The cells were allowed to recover at 37°C for 2 hours and then plated on kanamycin and chloramphenicol plates. Colonies obtained on the plates were checked for correct recombination events using colony PCR with primers SK73F and SK73R. All the gene deletion mutants were moved to clean wild type background using P22 transduction. Strain SK68, SK69, SK70 and SK71 were constructed by transducing ydiV::cm cassette into strains CR817, CR823, SS1039 and SS1009 respectively.
2.3 Fluorescence assays

End point and dynamic measurements of venus(yfp)-reporter systems were made using Tecan Safire II microplate reader. For fluorescence end point measurements, 200 µL of the sample was transferred to a 96-well microplate, and the fluorescence and optical density at 600 nm (OD\textsubscript{600}) were measured. Normalized fluorescence readings, given as relative fluorescence units (RFU), were calculated by dividing the bulk fluorescence with OD\textsubscript{600} and normalizing to the peak value for wild type strain.

<table>
<thead>
<tr>
<th>Plasmid</th>
<th>Relevant Characteristic(s)</th>
<th>Source or Reference</th>
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<tbody>
<tr>
<td>pKD46</td>
<td>bla P\textsubscript{BAD} gam beto exo pSC101 oriTS</td>
<td>[33]</td>
</tr>
<tr>
<td>pKD3</td>
<td>bla FRT cm FRT oriR6K</td>
<td>[33]</td>
</tr>
<tr>
<td>pKD4</td>
<td>bla FRT kan FRT oriR6K</td>
<td>[33]</td>
</tr>
<tr>
<td>pInt-ts</td>
<td>bla Int oriR6K</td>
<td>[34]</td>
</tr>
<tr>
<td>pVenus</td>
<td>kan venus att\lambda oriR6K</td>
<td>[37]</td>
</tr>
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For fluorescence-assisted cell sorting (FACS) experiments, cells were grown overnight at 37°C in MG media supplemented with 0.2% final concentration of yeast extract. Cells were subcultured to an OD of 0.05 in fresh MG media supplemented with 0.1%, 0.2%, 0.4%, 0.8%, 1.5% and 2% yeast extract. After subculture, the cells were then allowed to grow at 37°C for 4 hrs and samples were collected. The cells were pelleted by centrifuging at 4000rpm for 10 minutes and resuspended in DAPI staining buffer with 14.3 µM DAPI and 50 µg/mL chloramphenicol. The cells were then incubated at room temperature for half an hour. The cells were then analysed using BD LSR II Flow Cytometer. Fluorescence values of approximately 100,000 events were recorded using two channels, Pacific Blue channel for DAPI and FITC channel for Yfp. The cells were distinguished from other debris by gating only the population stained with DAPI. Data extraction and analysis for the FACS experiments were done using FCS Express Version 4 (De Novo Software).
Table 2.3: Oligonucleotide primers used in this study

<table>
<thead>
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<th>Primer</th>
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<tr>
<td>SK72F</td>
<td>ydiV knockout forward</td>
<td>actggatggcgaatagcgccctaaccatggactggcgtaGTGTAGGCTGGAGCTGCTTC</td>
</tr>
<tr>
<td>SK72R</td>
<td>ydiV knockout reverse</td>
<td>agacggttaatcaccggttaaacaccggcaaacagagaaggCATATGAATATCCTCCTCTTAG</td>
</tr>
<tr>
<td>SK73F</td>
<td>ΔydiV check forward</td>
<td>gaatattggtttataatcag</td>
</tr>
<tr>
<td>SK73R</td>
<td>ΔydiV check reverse</td>
<td>gggtaaaagcgcgggtatacg</td>
</tr>
</tbody>
</table>

2.4 Microscopy

Zeiss Standard RA Microscope outfitted for use in phase contrast microscopy was used to determine the fraction of motile and sessile cells. The microscope was outfitted with a CCD Hyper HAD B&W Video Camera. Samples were collected in a similar manner to FACS experiments. Glass slides and coverslips were soaked in 1M KOH for 15 minutes and washed with deionized water prior to use. 5 µL of appropriately diluted sample, such that there would be roughly 50 cells in a frame when looked under the microscope, was put on glass slide and covered with coverslip. The edges of the coverslips were sealed with epoxy. The slide was placed under the microscope to capture a movie (1000 frames) of motile and non motile cells. The movie was then analysed using custom matlab routine. The matlab routine ignores all the cells that are stuck on the glass slides and only analyses the swimming cells (motile) and the cells drifting cells in the liquid media (sessile).
3.1 Two distinct phenotypes coexist under nutrient limited condition.

Under nutrient limited condition, *Salmonella enterica* serovar Typhimurium downregulates flagellar synthesis, thus motility is reduced [27]. To investigate the effects of nutrients on motility phenotype, overnight cultures were grown with wild type SK1 and *ydiV* mutant SK61 strains, shaking, in MG media supplemented with 0.2% YE at 37°C from a single colony on LB-Agar plates. The overnight cultures were subcultured to 1:100 dilution in MG media supplemented with various concentration of YE and grown for 5 hours, shaking at 37°C. The percentage of motile and sessile cells were determined using microscopy (see Materials and Methods).

It was observed that wild type cells are completely sessile when grown with no YE supplement and completely motile when grown with 2% YE (Figure 4.1). Also, the percentage of motile cells increase as the amount of YE increases in the growth media. These results show that sub-populations of cells exhibiting motile and sessile phenotypes coexist in an isogenic culture under nutrient limited conditions. Additionally, percentage of population committed to motility are determined by the availability of the nutrients in cellular environment.

YdiV expression is enhanced in poor media. It acts as an anti-FlhD4C2 factor and is responsible for the repression of flagellar genes in poor media [27]. To investigate the effect of YdiV, the percentage of motile and sessile cells in *ydiV* mutant strain was determined. As expected, all the cells were motile even during nutrient limited condition. These data suggest that the
heterogeneous phenotype is facilitated by YdiV.

3.2 The flagellar class 2 and class 3 gene expression profiles are bimodal.

According to Monod and Jacob, the phenotype of cells responding to different cues and stimuli could, in theory, be explained by studying the underlying gene regulatory network [38]. After successful demonstration of the existence of two distinct phenotypes modulated by the availability of nutrients, gene expression were analysed at single cell resolution. Chromosomally integrated transcriptional fusions to the yellow fluorescent protein (Venus) were used as measures of promoter activities. The gene expression dynamics were analysed using representative promoter fusions \( P_{flhD} \) (SS1006), \( P_{flhB} \) (CR817) and \( P_{flgM} \) (SS1009) of class 1, 2 and 3 genes respectively.

Flagellar assembly is initiated by products of a single operon controlled by class 1 \( P_{flhD} \) promoter, transcribing \( flhDC \) genes. Flow cytometry revealed unimodal distribution of class 1 promoter activity (Figure 3.2 A). Moreover,
the promoter is active regardless of the availability of nutrients in the culture medium. However, class 2 and class 3 gene expression profiles are bimodal (Figure 3.2 B,C). The bimodality suggest the existence of two stable states, one in which cells exhibit sessile phenotype and the other in which cells exhibit motile phenotype. This is consistent with the observation of two distinct phenotypes in an isogenic cell population.

Figure 3.2: The flagellar class 2 and class 3 gene expression profiles are bimodal. The signal feeding into the flagellar network encompassed through the class 1 operon, \( flhDC \), however, is unimodal. Class 1 \( P_{flhD} \) (A), class 2 \( P_{flhB} \) (B) and class 3 \( P_{flgM} \) (C) promoter activity in a wild type strain as determined using flow cytometry.

When looked under the microscope, all the \( ydiV \) mutant cells were found to be motile even during nutrient limited condition. Next, we investigated the effect of YdiV in the gene expression profile of class 2 genes at single cell resolution (Figure 3.2). Flow cytometry data indicated that the gene expres-
pression profile is unimodal in ydiV mutant strain (SK68). This is consistent with the observation of only one phenotype in ydiV mutant strain.

![Graph showing flow cytometry data](image)

**Figure 3.3**: Breaking the YdiV-FliZ feedback loop makes the system monostable. Class 2 P_{flhB} promoter activity in a ydiV mutant as determined using flow cytometry.

The bistability is mediated by a regulation loop involving YdiV. Specifically, FliZ and YdiV form an overall positive feedback loop in poor media which results in bistability. Breaking the YdiV-FliZ feedback loop makes the system monostable. From these observations, it can be concluded that flagellar network is bistable with coexisting populations of motile and sessile cells.

### 3.3 YdiV provides threshold for class 2 genes activation.

For a system to exhibit bistability, it is required to display nonlinear kinetics and at least one positive feedback loop or even numbers of negative feedback loops. The nonlinearity could be result of multimerization or cooperative binding of transcriptional regulators to target sequence. It is evident by a sharp increase in the output response beyond some threshold level of input signal [39].

In flagellar gene circuit, gene cascade starts on the top from class 1 gene products FlhD and FlhC. Four copies of FlhD and two copies of FlhC form a heterohexomer, FlhD$_4$C$_2$ which initiates the transcription of class 2 genes.
To investigate the kinetics of flagellar gene expression system, $P_{\text{flhDC}}::tetRA$ strain was used where the native $P_{\text{flhDC}}$ promoter is replaced with $tetRA$ element from transposon Tn 10. This allows for a controlled expression of class 1 gene product FlhD$_4$C$_2$ by inducing $P_{tetA}$ promoter with anhydrotetracycline (aTc) [14]. Concentration of aTc gives an indirect measure of FlhD$_4$C$_2$ concentration inside cells. Chromosomally integrated $P_{\text{flhB}}$-Venus transcriptional fusion was used as indirect measure of $P_{\text{class2}}$ promoter activity. Cells were grown overnight at 37°C in LB media without aTc from a single colony on LB-Agar plates. The overnight cultures were subcultured to 1:100 dilution in LB media supplemented with various concentration of aTc and grown for 4 hrs. The endpoint fluorescent measurement was made as described in Materials and Methods. The experiments were repeated on three separate days and the average values are reported with standard deviation shown as error bars.

Figure 3.4: Flagellar gene circuit displays non linear kinetics. YdiV provides the threshold to prevent the feedback loops from activating all the cells. Class 2 $P_{\text{flhB}}$ promoter activity as a function of anhydrotetracycline concentration (measure of FlhD$_4$C$_2$ concentration inside the cells) in a $P_{\text{flhDC}}$::tetRA and $P_{\text{flhDC}}$::tetRA ΔydiV mutant determined using a bulk fluorescence assay.

In wild type cells (CR823), $P_{\text{class2}}$ promoters are not activated until a certain threshold level of FlhD$_4$C$_2$ is reached inside cells (Figure 3.4). This threshold mechanism provides the nonlinear kinetics required for the system to be bistable. Next, to determine the role of YdiV in flagellar gene expression kinetics, $P_{\text{class2}}$ promoter activity was measured in ydiV mutant strain (Figure 3.4). In absence of YdiV, class 2 genes are activated immediately by FlhD$_4$C$_2$. These data suggest that YdiV provides the threshold to prevent
the feedback loops from activating all the cells. Initially formed FlhD$_4$C$_2$ heterohexamers are sequestered by YdiV proteins. Only after the copies of FlhD$_4$C$_2$ inside cells exceed a threshold amount are they able to activate $P_{\text{class2}}$ promoters and initiate transcription of class 2 genes.
Various microbial systems exhibiting phenotypic variation have been studied in great details including lysis-lysogeny switch in phage lambda, cellular differentiation and competence for genetic transformation in *B. subtilis* and lactose utilization system in *E. coli* [39]. This study focused on the chemotactic decision making process of *Salmonella enterica* serovar Typhimurium, specifically aimed to investigate the existence of different motility phenotypes and the underlying genetic circuitry controlling this phenotypic variation.

It was demonstrated that two distinct phenotypes coexist in an isogenic cell population under nutrient limited condition. During poor nutrient condition all of the cell population are non motile. The percentage of motile cells increase with the availability of nutrients. Finally when the nutrient is abundant all of the cell population is committed to motility. The gene expression data reinforces the view first proposed by Monod and Jacob that the phenotype of cells responding to different cues and stimuli, in theory, is explained by the architecture of underlying gene regulatory network [38]. Class 1 promoter activity has unimodal distribution and the promoter is active regardless of the availability of nutrients in the culture medium. However, class 2 and class 3 gene expression profiles have bimodal distribution. From these results one can deduce that nutritional control of flagellar operon is at the level of class 2 gene transcription and propagated to class 3 gene expression whose expression is controlled by the class 2 gene product σ^{28}. The bimodality suggest the existence of two stable states, one in which cells exhibit sessile phenotype and the other in which cells exhibit motile phenotype. The flagellar gene circuit also exhibits non linear kinetics with threshold mechanism, an important requirement for bistable systems. FliZ and YdiV form an overall positive feedback loop in poor media which results in bistability. Breaking the YdiV-FliZ feedback loop makes the system monostable. YdiV provides
the threshold to prevent the feedback loops from activating all the cells.

Class 1 P_{flhD} promoter is constitutively expressed and manifests the action of various global regulators. Class 1 gene products FlhD_{4}C_{2} can not activate the class 2 gene transcription immediately as initially formed FlhD_{4}C_{2} heterohexamers are sequestered by YdiV proteins. This effect is more pronounced in poor nutrient condition because YdiV expression is very high. As the amount of nutrients increases, the YdiV expression decreases and some of FlhD_{4}C_{2} complexes can’t be bound by YdiV which are then free to kick off the transcription of class 2 genes. Finally in rich media, YdiV expression is so diminished that most copies of FlhD_{4}C_{2} are able to initiate the transcription from P_{class2} promoters. FliZ acts as an activator of class 2 genes. By binding to YdiV, FliZ decreases FlhD_{4}C_{2} sequestration and enhances its own expression along with other class 2 genes. Thus there is a double negative feedback loop formed by YdiV and FliZ. This feedback loop is strengthened in nutrient limited condition and hence the system becomes bistable.

These results reflect that microbial strategy is to conserve resources and energy during challenging condition by committing only a portion of popu-
lation to motility. These observations reinforce the view that bacteria vary
the phenotypes as a form of bet-hedging strategy [39]. The architecture of
underlying genetic circuit enables them to achieve this goal.
REFERENCES


