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COMMUNICATION

Non-genetic individuality in *Escherichia coli* motor switching

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Abstract
By analyzing 30 min, high-resolution recordings of single *Escherichia coli* flagellar motors in the physiological regime, we show that two main properties of motor switching—the mean clockwise and mean counter-clockwise interval durations—vary significantly. When we represent these quantities on a two-dimensional plot for several cells, the data do not fall on a one-dimensional curve, as expected with a single control parameter, but instead spread in two dimensions, pointing to motor individuality. The largest variations are in the mean counter-clockwise interval, and are attributable to variations in the concentration of the internal signaling molecule CheY-P. In contrast, variations in the mean clockwise interval are interpreted in terms of motor individuality. We argue that the sensitivity of the mean counter-clockwise interval to fluctuations in CheY-P is consistent with an optimal strategy of run and tumble. The concomittent variability in mean run length may allow populations of cells to better survive in rapidly changing environments by 'hedging their bets'.

S Online supplementary data available from stacks.iop.org/PhysBio/8/024001/mmedia

It has long been known that the same genotype can lead to very different phenotypes, even at the level of single cells [1]. Non-genetic individuality is often attributed to noise arising from the small number of molecules involved in gene regulation [2] or in biochemical networks. But non-genetic individuality can also arise at the level of single molecular assemblies, as strikingly illustrated by the case of prions [3]: large protein structures may fold or assemble in slightly different ways, resulting in significant phenotypic variations. Here we provide strong evidence supporting *both* kinds of non-genetic diversity in a model system suitable for detailed, quantitative study—single flagellar motors of the bacterium *Escherichia coli*. We report large cell-to-cell variations in the switching properties of single motors. We show that these variations have two independent sources: noise in concentration of a signaling molecule, and individuality of motors themselves. We find that variability primarily emerges in one of the two main properties of motor switching—the mean duration of counter-clockwise (CCW) intervals—and not the other—the mean duration of clockwise (CW) intervals. We interpret this channeling of variability in evolutionary terms in light of the asymmetric functions of the two types of intervals in *E. coli* chemotaxis.

In *E. coli*, motor switching between clockwise and counter-clockwise directions is controlled by the cytoplasmic messenger phospho-CheY (CheY-P). The concentration of CheY-P reflects changes in the cell's chemical environment, allowing cells to perform chemotaxis. It is generally believed that mean CheY-P levels fluctuate from cell to cell and over long times in a single cell [4], and that this is the source

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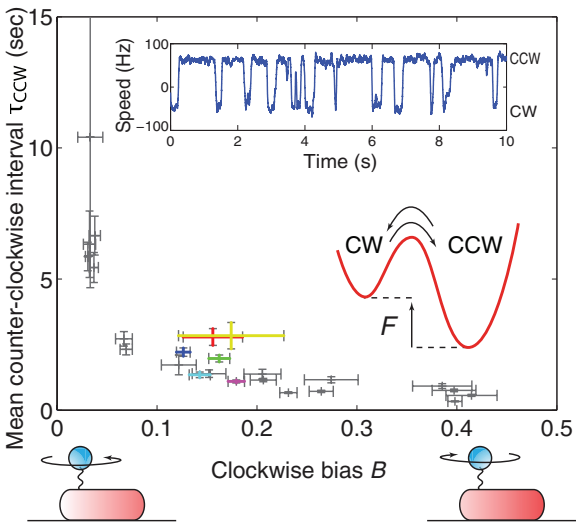

Figure 1. Cell-to-cell variability of flagellar motor dynamics. Mean CCW interval duration τ_{CCW} is plotted versus CW bias for 28 distinct cells. The CW bias is regulated by the signaling molecule CheY-P, whose concentration may vary from cell to cell due to noise in gene expression and in the chemotactic network. As the mean CCW interval τ_{CCW} depends strongly on bias, its cell-to-cell variability reflects that of the CW bias. Six representative cells with approximately the same, wild-type bias of 0.15 were colored for reference in subsequent figures. Upper inset: sample trace of bead rotation speed versus time showing CW and CCW intervals. Lower inset: schematic of the motor free-energy landscape. The motor stochastically transitions between two states, CW and CCW. Bottom: schematics of CW (right) and CCW (left) bead rotation.

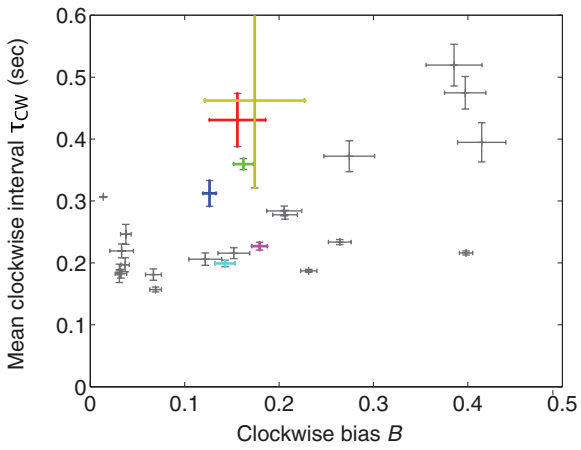

Figure 2. Motor individuality. Mean CW interval duration τ_{CW} versus CW bias for the same 28 cells with the same colors as figure 1. In contrast to τ_{CCW} (figure 1), τ_{CW} is approximately independent of CW bias. The large variability in τ_{CW} (even for nearly the same bias) reflects motor individuality.

of variation in motor activity. However, in addition motors themselves can differ. Each flagellar motor is a large molecular assembly made of 28 distinct proteins, all present in multiple copies [5]. This precise assembly can vary from motor to motor, in particular in the number of copies of the circularly arrayed proteins that form the rotor, as revealed by electron microscopy [6, 7]. Moreover, this assembly is not necessarily static. Key proteins such as MotB and FliM are constantly being replaced, with the rate of turnover of FliM depending on the CheY-P concentration [8, 9].

To explore the variability of motor switching dynamics, we attached a latex bead (1.0μm diameter) to the flagellar stub of single motors in a non-chemotactic environment (figure 1, schematics at bottom), and imaged the rotation of 28 single motors each for 30 min using a high-resolution detection system. The rotation speed as a function of time was extracted (a sample trace is shown in the upper inset of figure 1), and interpreted as a sequence of intervals of CCW and CW rotation (see the supplementary material available at stacks.iop.org/PhysBio/8/024001/mmedia for details).

At fixed bias, the mean CW and CCW interval durations τ_{CW} and τ_{CCW} are distributed exponentially [10], or rather as a sum of exponentials [4, 11, 12] (see the supplementary material available at stacks.iop.org/PhysBio/8/024001/mmedia). This observation is consistent with equilibrium switching between CW and CCW states, as schematized in the lower inset of figure 1. A previous study [10] has shown that [CheY-P] controls the CW bias through τ_{CW} as well as through τ_{CCW}, in a way that is symmetrical around the CW bias $B = \tau_{CW}/(\tau_{CW} + \tau_{CCW}) = 1/2$. Our results in figure 1 reveal large cell-to-cell variations in both the CW bias and the mean CCW interval, which are strongly anticorrelated, consistent with the hypothesis that [CheY-P] controls both quantities, and varies from cell to cell due to expression and chemical noise [13]. Similarly, the mean CW interval τ_{CW} is also found to vary from cell to cell (figure 2).

However, if [CheY-P] was the only source of cell-to-cell variation, the scatter-plot of τ_{CCW} versus B (figure 1) and of τ_{CW} versus B (figure 2) would each necessarily fall onto a single curve. Instead, in both cases we find significant spread of the data in two dimensions. Moreover, we find that the cell-to-cell variation of τ_{CW} in figure 2 is essentially independent of bias. This additional, bias-independent variation points to motor individuality.

Could extrinsic sources explain variations of τ_{CW}? (i) Switching rates have been reported to depend on motor speed [14, 15]. However, we found little variation in motor speed in our 28 recordings, and no significant correlation between motor speed and switching rates (figure 3(*A*)). (ii) Another possible source of variation is rotation heterogeneity. Bead rotation is usually not perfectly uniform. Instead, the rotation speed may depend on the angular position of the bead on the ellipse of the bead's trajectory. We define an angle heterogeneity index as the standard deviation of the angle distribution normalized by the mean distribution (see the supplementary material available at stacks.iop.org/PhysBio/8/024001/mmedia). Again, we found negligible correlation with switching rates (figure 3(*B*)). Quantitatively, we estimated the dependence of mean CW interval with respect to bias, speed and heterogeneity index by linear regression, and found that these three dependences only explained 9% of the observed variance in mean CW interval, while experimental noise accounted for another 9.7% (see the SI text available at stacks.iop.org/PhysBio/8/024001/mmedia). (iii) The proton-motive force (the strength of the energy source powering the motor) could also affect switching rates. However, the proton-motive force is also proportional to

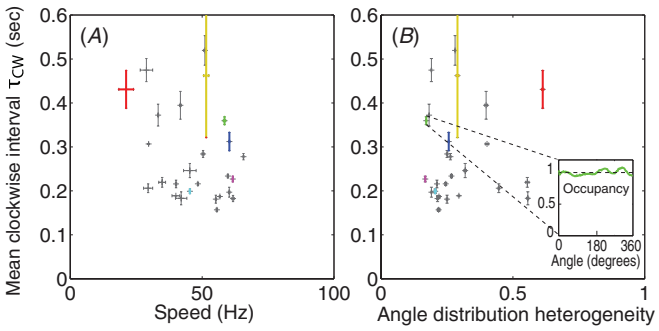

Figure 3. Variability of CW-interval duration τ_{CW} is not due to motor speed or angle heterogeneity. (*A*) τ_{CW} versus motor speed, for the same 28 cells with the same colors as in figures 1 and 2. (*B*) τ_{CW} versus angle heterogeneity index, defined as the normalized standard deviation of the angle occupancy during motor rotation (see the text). There is little or no correlation between motor speed or angle heterogeneity and interval duration. Inset: typical angle distribution in a single recording.

the motor speed [16], which we just showed has negligible effect on τ_{CW} variation. (iv) It has recently been shown that the second messenger cyclic di-GMP could influence motor switching via YcgR [17], but, like the proton-motive force, it would also affect motor speed, which we do not observe. Taken together, these results support the hypothesis that motors made of genetically identical proteins can be behaviorally different.

To summarize, our data show that the chemotactic signal [CheY-P] controls τ_{CCW}, but not τ_{CW} in the physiological regime of low CW bias, in agreement with previous reports [10, 18, 19]. Nevertheless τ_{CW} still varies from cell to cell due to motor individuality. Why should changes in [CheY-P] affect only τ_{CCW} while leaving τ_{CW} fixed? We argue that to maximize the cell's sensitivity to chemical gradients, [CheY-P] should act primarily on τ_{CCW}, related to the run length, while keeping τ_{CW}, related to the tumble time, constant. Indeed, in an optimal run-and-tumble process, the run length should fully register changes in the cell's chemical environment, while tumbles should only serve to randomly reorient the cell. Thus, the mean CW interval should be just long enough to let the cell reorient. This constraint sets functional bounds on the mean CW interval. Previous works report tumble times of $\sim$0.14 s, and a mean reorientation angle of 60° [20, 21]. A smaller CW interval and correspondingly shorter mean tumble time would lead to lower average reorientation angles, thereby harming the cell's chemotactic ability. Yet, we do see variations in τ_{CW}. These could arise from variations in the flipping rates of the individual proteins FliM/N and FliG which control rotation direction, or from previously reported motor-to-motor variations in the number of copies of these proteins [7]. Remarkably, τ_{CW} is always larger than 0.15 s. When more than one flagellum is present, the mean tumble time depends on the number of flagella, and can be smaller than τ_{CW}, as sometimes more than one motor is required to rotate CW in order to initiate a tumble. The twofold factor in the variations of τ_{CW} is consistent with variations in the number of flagella (typically from 2 to 5) [21].

For maximum chemotactic drift, gradient-induced changes in [CheY-P] should be entirely channeled into changes in τ_{CCW}. But that still leaves the question—Why are cell-to-cell variations in adapted [CheY-P], and thus in τ_{CCW}, so large? Variations are expected from noise in gene expression and in the chemotactic network. Since the cell is not growing, the molecules involved in the chemotactic network (CheR, CheB, CheA, etc) should have more or less constant concentrations during the 30 min of the recording. The steady-state concentration of CheY-P is regulated by two proteins, the kinase CheA and the phosphatase CheZ, whose concentrations vary widely from cell to cell, and show only moderate correlation in their expression levels despite being both regulated by FlgM [13]. Since the CW bias is very sensitive to [CheY-P] (with a Hill coefficient of $\sim$ 10 [22]), variations in [CheY-P] get greatly amplified, resulting in large variations in CW bias [13, 23].

What is the biological function of this variability? Fine tuning of parameters such as the mean CW and CCW intervals may be hard to achieve reproducibly, notably because of the high Hill coefficients involved in the chemotactic pathway, and cell-to-cell variations might stress the limits of the control mechanisms implemented by the cell to achieve robustness. Alternatively, phenotypic diversity is often proposed as a bet-hedging mechanism, whereby a clonal population of cells maximizes its survival rate under rapidly changing conditions by exploring diverse phenotypic solutions. From that perspective, betting on diverse mean CCW intervals might prove more useful: it allows for a variety of mean run lengths, each of which could be optimal for different environmental conditions [24, 25]. By contrast, no real advantage would be conferred to very short or very long tumble times. Although our study cannot decide whether the magnitude of variations in interval durations is incidental or advantageous from an evolutionary perspective, it emphasizes that [CheY-P] variation has been channeled into τ_{CCW}, whereas motor variation affects τ_{CW}, observations consistent with optimized run and tumble behavior.

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