Noise in gene expression as the source of non-genetic individuality in the chemotactic response of *Escherichia coli*

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Abstract A deterministic computer model of the signal transduction pathway mediating bacterial chemotaxis was used to examine the variation in both unstimulated swimming behaviour and adaptation time to stimuli in clonal populations of cells. Copy numbers of proteins in the pathway were computed from a simplified model of transcription and translation that predicts greater-than-Poissonian statistics. Simulated and experimental individuality data could be brought into good agreement on varying the noise strength of the protein copy number distributions. In the simulations, all the proteins in the pathway are involved to a significant degree in the appearance of phenotypic diversity, although there is a modest decrease in influence with increasing copy number.

Key words: Individuality; Bacterial chemotaxis; Computer model; Poisson distribution; Noise strength; Burst size

1. Introduction

Non-genetic individuality is defined as the presence of a diversity of phenotypes in a genetically identical population, and has been observed in a range of prokaryotic and eukaryotic organisms [1]. The phenomenon was first discovered to be a feature of the chemotactic response of coliform bacteria in the 1970s, with both unstimulated swimming behaviour (represented in one form in Fig. 1) and adaptation time to stimuli varying from cell to cell [2]. This variation in unstimulated swimming behaviour was confirmed experimentally and quantified in a way that allowed a first attempt to be made at modeling its origins [3].

Where might the source of such individuality lie? Spudich and Koshland [2] speculated that it might arise from Poissonian variation in molecules present at low numbers in the cell. Since the variance of a Poisson distribution is equal to its mean, the standard deviation (which is the square root of the variance) becomes relatively smaller for larger numbers of molecules: a relative standard deviation of 10% for a Poisson distribution of mean 100 falls to 1% for a distribution of mean 10 000. Ozbudak et al. [4] investigated this question experimentally by incorporating the green fluorescent protein gene (*gfp*) under the control of an inducible promoter into the chromosome of *Bacillus subtilis*. Measurement of the expression of GFP in the population using flow cytometry determined the variation in expression from cell to cell. The phenotypic 'noise strength', defined as the ratio of the variance of the distribution of phenotypes to the mean, was used in this study to quantify the spread of expression levels in the population. As Poisson distributions have variance-to-mean ratios of unity, this parameter will reveal any deviations from Poissonian behaviour. Deviations were indeed encountered experimentally and, based on an analytical model of gene expression, it was concluded that noise essentially arises at the translational, and not the transcriptional, level in the cell. The model, on which this study was based, also predicted that the spread of expression levels in a population should follow a greater-than-Poissonian distribution (one whose variance is greater than its mean) with a noise strength of $\sim b+1$, where b is the burst size or average number of protein copies synthesised per mRNA transcript [5].

1.1. Bacterial chemotaxis

Most of the proteins involved in mediating the chemotactic response of coliform bacteria are associated into two distinct membrane-bound complexes: a receptor complex, typically clustered at only one of the poles of the cell, and a flagellar motor complex, multiple copies of which are randomly distributed over the surface of the cell. The chemotaxis signal transduction pathway of *Escherichia coli* up to, but not including, the motors consists of six cytoplasmic proteins and five homologous transmembrane receptors. The genes for all the cytoplasmic components and two of the five receptor species are found in adjacent operons, *meche* (*tar tap cheRBYZ*) and *mocha* (*motAB cheAW*), on the chromosome [6].

The chemotactic receptors detect attractants and repellents in the environment and thereby indirectly control the level of the signaling molecule (phosphorylated CheY) that diffuses from the receptor complexes through the cytoplasm to the flagellar motors, where it interacts with the switch complex to increase the probability that any motor will change its direction of rotation (and hence that of its attached helical flagellum) from counterclockwise (the default) to clockwise. The consequent change in the sequence of motor reversals causes the random walk that the cell typically undergoes when unstimulated to become biased towards the sources of attractants and away from the sources of repellents. Slow adaptation of the chemotactic response is achieved by two other cytosolic proteins, CheR and CheB, which counteract the effects of binding attractants and repellents by respectively methylating and demethylating a number of specific sites on

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the cytoplasmic domains of the receptors. More detailed descriptions of various aspects of bacterial chemotaxis may be found in a number of recent reviews [7–11].

2. Materials and methods

For the individuality simulations, a modified version of the deterministic, bacterial chemotaxis simulation program BCT (available from http://www.zoo.cam.ac.uk/comp-cell) was used. The output of the program follows the standard convention for characterising swimming behaviour in terms of the rotational bias of the flagellar motor, i.e. the fraction of time the motor spends rotating counterclockwise (Fig. 1). For adaptation times, however, the definition used by Alon et al. [12] (given in terms of tumbling frequency) was modified to be the time taken following stimulation for the bias to return halfway back to its pre-stimulus level. The program uses a robust adaptation mechanism [13] to ensure that the bias always returns to its pre-stimulus steady state (exact adaptation) over a wide range of initial protein concentrations. Each in silico experiment comprised 10 000 individual simulations, in each of which the copy numbers of all the proteins were chosen randomly from a set of independent, normal distributions of equal noise strength, as defined in Section 1.

3. Results

3.1. Individuality in unstimulated swimming behaviour

The computer program was used to simulate the distribution of unstimulated swimming behaviour displayed by a population of wild-type *E. coli* cells, for which experimental data exist [3]. The choice of noise strength was based on the genetic organisation of the *meche* and *mocha* operons described in Section 1. In the absence of quantitative data, and for reasons of simplicity, the two operons were assumed to be transcribed at the same rate, giving a burst size for each protein of its copy number divided by the (constant) number of transcripts. The results of a series of individuality simulations based on

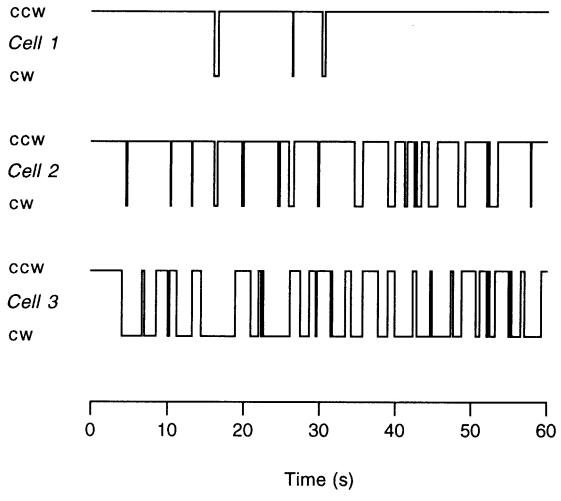

Fig. 1. Individuality of flagellar motor switching. Cells from a population of the wild-type strain were shorn of their flagella and attached to glass coverslips by anti-flagellin antibodies. Each cell was videotaped through a microscope for 1 min and the times at which the direction of rotation of the cell changed from clockwise (cw) to counterclockwise (ccw) and vice versa were recorded. Time courses for three cells are shown, with cell 1 displaying a high motor bias (0.98), cell 2 a bias close to the population mean (0.85), and cell 3 a low bias (0.43), during the observation period. Note that motor switching occurs stochastically, with cw and ccw intervals distributed exponentially [19].

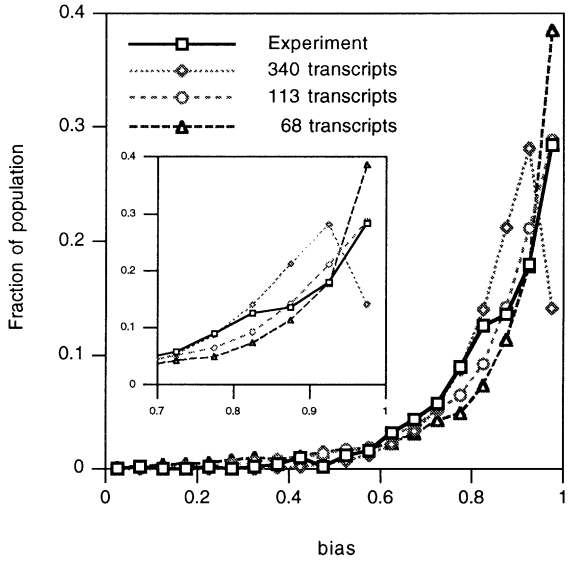

Fig. 2. Simulations of individuality in unstimulated flagellar motor bias. The in vivo data set is replotted from fig. 5A of Levin et al. [3]. For the in silico data, each individual simulation was run for 200 s of simulated time, sufficient to bring the bias to steady state. The experimental and simulated distributions, plotted as histograms, all have mean biases of ~0.85. For clarity, only three in silico individuality histograms are shown. The inset redisplays the data for biases between 0.7 and 1.0.

340, 113 and 68 transcripts of the above operons are shown in Fig. 2, with the value of 113 (corresponding to a range of burst sizes from 1.5 for CheR to 150 for CheY) producing the best match with the experimentally determined distribution. To investigate the relative contributions of the individual proteins to the overall phenotypic variation in the population, further simulations were performed with the copy number of each of the proteins in turn held constant, which resulted in a set of distributions lying between the 340- and the 113-transcript distributions of Fig. 2 (data not shown). Thus, in silico, all of the proteins make a significant contribution to the generation of individuality in unstimulated swimming behaviour, with no single protein, or small group of proteins, predominating.

3.2. Individuality in adaptation time

Experimental data are not available in sufficient quantity (or the appropriate format) to be used meaningfully in a modeling study of this type of individuality. A limited amount of data is, however, available on the closely related topic of how adaptation times are affected by changes in the concentration of CheR (when expressed from an inducible plasmid in a strain deleted for *cheR*) [12]. With incremental steps through a range of CheR concentrations, the adaptation times of the simulations could be brought into good agreement with these experimental data (Fig. 3). A full individuality simulation, as described in Section 3.1, but with the same parameters as those used to generate the data in Fig. 3, is shown in Fig. 4. Although there is a spread of adaptation times for any given CheR concentration, the shape of the overall distribution follows the deterministic curve in Fig. 3, with the magnitude of the spread indicating the contribution of the other proteins to the variation in adaptation time. Note that the in silico adaptation times are normally distributed (Fig. 5),

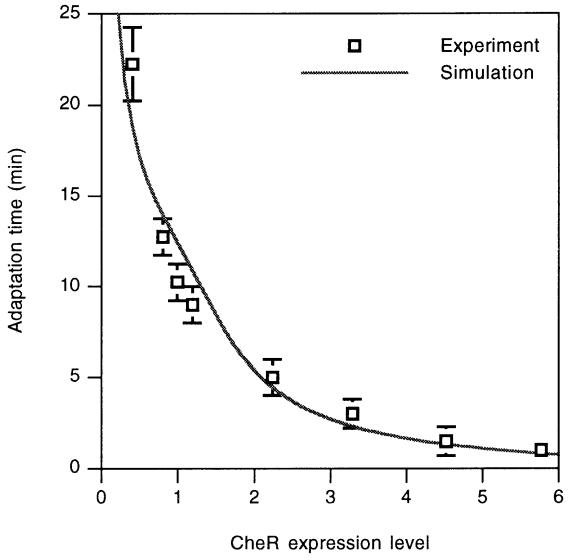

Fig. 3. Effect of expression of CheR on adaptation time. Each simulation was run to steady state, whereupon the concentration of the attractant aspartate was increased from zero to 1 M to ensure saturation of the receptors and then held at that level for the remainder of the simulation (see Section 2). The rates of the adaptation reactions (mediated by CheR and CheB) had to be reduced four-fold from those used in the currently available version of the program to bring the in silico adaptation times in line with the experimental data (not shown). Note that the discovery of an inverse relationship between expression of CheR and adaptation time corroborated a prediction made in an earlier theoretical study of robust adaptation [13].

in agreement with one of the findings of the original investigation into this phenomenon [2].

4. Discussion

The same model for generating variation in chemotaxis protein copy numbers was used to simulate the individuality

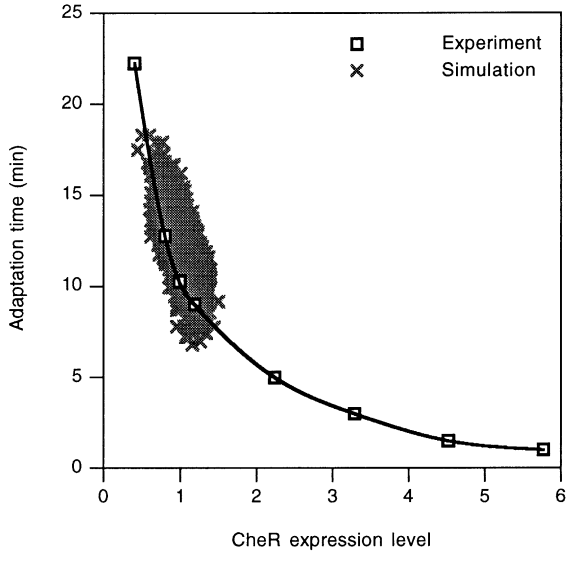

Fig. 4. Simulation of individuality in adaptation time with variation in all chemotaxis proteins (see Fig. 3 for details). The solid curve is an interpolation through the experimental data points.

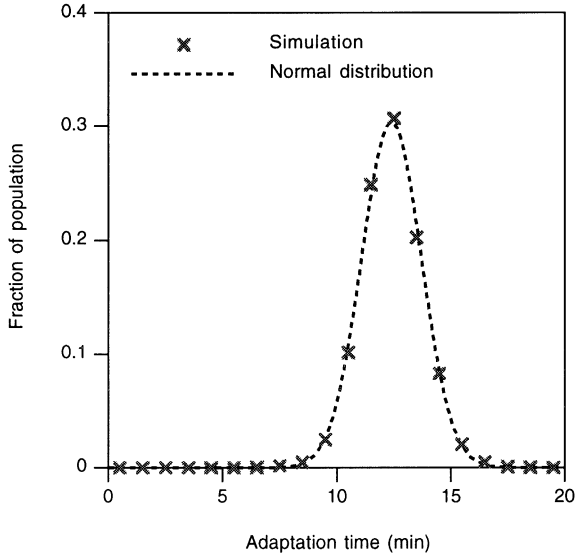

Fig. 5. Distribution of in silico adaptation times. The mean and standard deviation of the normal distribution are the same as those calculated from the in silico data in Fig. 4 (744 ± 79 s).

in steady-state bias and adaptation time exhibited by clonal populations of *E. coli* cells. The model consists of a set of seven independent, normal distributions (one for each type of chemotaxis protein), with variance-to-mean ratios ranging from 2.5 for CheR to 151 for CheY. According to the theory of stochastic gene expression described in Section 1 [5], such noise strengths would arise from burst sizes, or average number of protein molecules per transcript, of approximately 1.5 and 150, respectively (for comparison, the burst sizes for *lacA* and *lacZ* are 5 and 40, respectively [14] and for *trp*, 20 [15]). The results of the individuality simulations described in Section 3.1 show that all the proteins have a part to play in the appearance of individuality in the chemotactic response. How can this be when their noise strengths differ by a factor of 60? The reason lies in the fact that the shapes of the distributions depend on their standard deviations, and a comparison of the spread of the distributions (by normalising the standard deviation with the mean) produces a much more modest range of values from 0.12 for CheR to 0.09 for CheY. It should be noted, however, that this argument is based on the assumption that the *meche* and *mocha* operons produce equal numbers of transcripts and would not hold true if the number of transcripts of the two operons differed greatly from one another.

In more general terms, what are the possible costs and benefits of individuality? Gene expression is an inherently noisy process, due to the high degree of stochasticity associated with transcriptional and translational events. Various strategies, such as negative feedback, have been proposed (and experimentally tested) for attenuating this source of noise (for recent reviews, see [1,16]), although they all impose some cost on the cell in terms of the energy requirement for protein synthesis. From an evolutionary perspective, however, individuality seems to provide a mechanism that would allow a small fraction of a population that might survive exposure to severe stress to repopulate the environment after the stress has eased, or to migrate to and colonise new environments [17]. An example where this might occur is provided by the

bacterium *Myxococcus xanthus* [18]. Because this species secretes amino acids into the environment that are chemoattractants for *E. coli*, individual cells are innocently lured towards the myxobacteria up the resultant attractant gradient and are subsequently devoured when they make contact. Under these circumstances, cells that respond weakly or not at all to this signal have a clear survival advantage. In short, there may be evolutionary benefits for populations arising from individuality, but also costs for the cell in counteracting it if it causes no deleterious effects.

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