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Visions & Reflections (Minireview)

Do cells think?

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Abstract. A microorganism has to adapt to changing environmental conditions in order to survive. Cells could follow one of two basic strategies to address such environmental fluctuations. On the one hand, cells could anticipate a fluctuating environment by spontaneously generating a phenotypically diverse population of cells, with each subpopulation exhibiting different capacities to flourish in the different conditions. Alternatively, cells could sense changes in the surrounding conditions – such as temperature, nutritional availability or the presence of other individuals – and modify their behavior to provide an appropriate response to that information. As we describe, examples of both strategies abound among different microorganisms. Moreover, successful application of either strategy requires a level of memory and information processing that has not been normally associated with single cells, suggesting that such organisms do in fact have the capacity to 'think'.

Keywords. Microorganisms, sporulation, MAP kinase pathway, mating pheromone, high osmolarity response, stochastic switching, logic gate, switch-like behavior.

Hedging your bets

Many microorganisms maintain a diverse population of phenotypically distinct, albeit genetically identical, individuals through stochastic switching among the different phenotypic states [1]. For instance, *Salmonella typhimurium* undergoes phase variation to maintain a subpopulation of cells that express a different flagellar antigen than the majority population [2]. Through more complex mechanisms, *Borrelia hermsii*, *Neisseria gonorrhoeae* and *Trypanosoma brucei* have developed switching systems that spontaneously generate antigenic variants in an infecting population [1, 3, 4]. *Candida albicans* switches among a variety of morphologically distinct phenotypes that affect its virulence by altering antigenicity, antifungal resistance, sensitivity to ingestion by macrophages, etc. [5]. Persistence in *Escherichia coli* involves spontaneous and reversible switching into a quasi-quiescent state that confers increased resistance to antibiotics [6].

Successful implementation of this stochastic strategy requires that the rate of phenotypic switching be tuned to the rate of environmental fluctuations. Kussell and Leiber [7] studied this problem theoretically, and concluded that stochastic switching (without any sensing of the environment) could be a possible strategy if the statistics of the environment was invariant over evolutionary timescales. They conclud-

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ed that the optimum switching rate from one phenotype to another is proportional to the probability that the relevant environmental condition will change and inversely proportional to the average duration of any one environmental condition. How does an organism then set the rate of phenotypic variation to maximize survival? Obviously, the most important contribution is evolutionary selection: those individuals that switched with the optimum rate are those that survived to produce offspring. Under certain conditions, an optimum strategy would be for cells to adjust their rate of switching on the basis of the duration and frequency of recent environmental shifts [7]. Such a strategy would obviously require that the cells 'remember' those statistics of the changes in environmental conditions. A hint that such a mechanism might be operative comes from a recent report that *Bacillus subtilis* cells exhibit a 50% higher rate of stochastic switching into a competent state upon starvation if they were starved previously [8].

Applying logic

A second strategy to address a fluctuating environment is to sense the change and then mount a response appropriate for the new condition. This requires that the cell possess a sensory mechanism for detecting the changing condition and a signal transduction apparatus for converting that perception into an appropriate response. Numerous well-studied examples of such stimulus-response systems are evident in both prokaryotic and eukaryotic microorganisms. For instance, chemotaxis in *E. coli* is a robust example of cells sensing a specific signal and responding by simply changing their rate of tumbling [9]. However, even this simple process requires that the cell be able to compare the level of the stimulating agent at two different times and to do so with equivalent sensitivity over multiple orders of magnitude of stimulant concentration [10, 11]. The ability of the cell to perform the former process means that the cell has the capability to remember a prior condition long enough to compare it to a current condition. Moreover, the dynamic range of the process rivals that of any sensory system used by metazoans.

The amount of information processing the cell has to perform increases in those cases in which mounting an appropriate response requires input from multiple sources. Induction of β-galactosidase in, *E. coli* serves as the classic paradigm demonstrating a cell's ability to perform such relatively sophisticated logic. In this case, cells initiate transcription of *lac*Z if and only if lactose is present – to inactivate the lacI repressor – and glucose is absent – to stimulate activity of the CAP activator [12]. In other words, cells serve as an [A AND NOT(B)] logic gate with regard to input signals of lactose and glucose and output response of β-galactosidase induction.

As a second example, the yeast *Saccharomyces cerevisiae* initiates a cascade of events in response to the absence of glucose and the absence of a good nitrogen source that results in meiosis. In this case, the integrating element is the Rim15 kinase, whose nuclear localization is restricted by the TOR signaling pathway in response to a rich nitrogen source and whose kinase activity is restricted by protein kinase A (PKA) in response to a rich carbon source [13]. Thus, Rim15 serves as a NOT(A OR B) (i.e., NOR) logic gate with regard to glucose and ammonia inputs and meiosis output. Other examples of naturally occurring logic gates based on similar regulatory circuits are readily obtained. Moreover, synthetic biologists have been able to create a variety of novel logic gates using regulatory elements from bacteria and yeast and stringing such logic elements together, allowing cells to perform intriguing computational feats [14,15]. However, there still remains the problem that cells could make the wrong decision!

Weighing the odds

The examples above describe the ability of microorganisms to perform simple and not so simple logic functions through natural or artificial regulatory circuits. Such events, though, may not compel one to believe that cells are capable of thinking any more than one might believe an electronic calculator is able to think. However, some recent studies provide an example of cells able not only to evaluate complex inputs but also to make individual decisions as to how to respond to those inputs so as to survive in fluctuating environments.

Saccharomyces cerevisiae possesses a number of signal transduction pathways designed to assess different environmental conditions, including nutritional availability, high or low osmolarity or the presence of mating pheromones. Many of these signal transduction pathways consist of kinase cascades homologous to mammalian mutagen-activated kinase (MAP) kinase modules [16, 17]. One in particular senses pheromones produced by cells of the opposite mating type, while another senses increased external osmolarity, such as that resulting from addition of sorbitol to the media, for example. Stimulation of the pheromone pathway elicits multiple responses, including reorientation of cell growth toward the source of pheromone and transcriptional activation of genes required for mating. Stimulation of the high osmolar-

ity (HOG) pathway elicits transcriptional activation of a number of genes whose products enhance glycerol accumulation to increase the internal osmolarity of the cell.

A remarkable feature of these two MAP kinase pathways is that they share a single intermediate kinase (Ste11) and have homologous terminal kinases (Fus3 and Hog1). Given the shared component, why doesn't activation of the HOG pathway elicit a pheromone response and vice versa? This question has puzzled investigators for many years [18]. In addressing this question, we have recently shown that specificity is maintained through a mechanism of lateral inhibition [19]. That is, stimulation of the pheromone pathway not only elicits its downstream effectors but also inhibits signal transmission through the HOG pathway, and vice versa. A remarkable consequence of the mechanism of specificity is that in single cells the pathways behave like a switch, such that the cell responds to only one stimulus even when exposed to both. Accordingly, we found that in a population of genetically identical cells presented simultaneously with both stimuli, some cells responded solely to the pheromone signal while others responded solely to the high osmolarity signal. No cell responded to both signals. Moreover, the percentage of cells responding to one signal versus the other varied in proportion to the relative concentrations and the time of addition of the two signals, although the overall response was always bimodal. This cell-to-cell variability in the response allows for heterogeneity of the decision in genetically identical populations.

Given that *Saccharomyces* is perfectly capable of constructing MAP kinase signaling cascades without having shared components, why would it retain signaling pathways that have to rely on mutual inhibition to maintain specificity? One possibility could be that cells stimulated by pheromone and sorbitol can productively respond to one stimulus or the other but would not be physiologically capable of responding simultaneously to both. This would not be surprising, since mating involves partial removal of the cell wall, rendering the cell vulnerable to hyper- or hypotonic lysis. In fact, previous work has shown that increased internal glycerol blocks zygote formation, suggesting that the mating process is quite sensitive to increased internal osmotic pressure [20]. Accordingly, this observation suggests that a cell presented with both pheromone and high osmolarity has to choose whether to activate the mating pathway and initiate a mating response or activate the osmolarity pathway to increase internal osmolarity. Apparently for physiological reasons, it cannot do both.

While cells confronted with both stimuli could be hard wired so that all responded by mating or all responded by increasing internal osmolarity, our results suggest that the response is significantly more sophisticated. The signaling pathways appear configured so that some cells in the population respond by initiating the mating response while others initiate an osmotic response. Thus, the population can achieve what individual cells cannot, an osmolarity response on one hand and a mating response on the other. The mechanism that allows for this variability may facilitate enhanced survival by allowing identical cells to pursue different courses in response to a changing environment, thus exploring a much wider behavioral space. This suggests that evolution has not selected for cells that 'know' the right response to every complex or competing set of stimuli. Rather, evolutionarily successful cells are those that are capable of exploring multiple, alternative responses to complex situations. While only a subset of responses might prove successful in a specific situation, the genetic endowment of all the cells would be propagated through those siblings that happened on the right solution in that particular situation. Moreover, since the percentage of cells choosing one response over the other is in proportion to the relative strengths of the signals, the cells are essentially 'weighing the odds' that one response or the other is the more favorable solution to the complex situation.

Yeast cells with identical genetic endowment and presented with the same complex set of stimuli exhibit distinct and stable behaviors, in that some cells respond to pheromone while others to sorbitol. We don't yet know why some cells decide to pursue one course and other cells pursue a different course. However, the ability of individual cells to exhibit distinct responses to the same stimuli suggests the existence of another, as yet unidentified, layer in the decision making circuitry. This higher-order level of information processing begins to approach the complexity of a true 'thought' process.

Finally, is this a unique situation or can we identify other cases of individual cell autonomy in decision making? As mentioned above, starvation of *B. subtilis* elicits two distinct responses in genetically identical cells: the vast majority of cells sporulate, while a small percentage (4–6%) do not sporulate but acquire competence for DNA uptake [8]. In addition, other MAP kinase pathways operate in *Saccharomyces*, some of which also use the Ste11 component shared between the HOG and pheromone pathways [16]. Thus, these pathways may also exhibit lateral inhibition that would render them subject to a switch-like behavior in individual cells. In fact, in preliminary observations, we find that glucose starvation – a signal for the pseudohyphal MAP kinase pathway – inhibits signaling through the pheromone pathway [19]. This

may reflect the fact that these two responses – mating versus pseudohyphal development – are alternative, mutually exclusive behaviors for individual cells presented with both stimuli. Finally, our computational and experimental analyses of pheromone and osmolarity response highlight the importance of confronting cells simultaneously with multiple stimuli as a means of revealing dualistic behavior. Thus, further experiments using multivariate stimuli may well reveal additional cases of cells having deep thoughts.

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