Signal Manipulation Coordinates Cellular Decision Making at the Population Level

Eric L. Haseltine and Frances H. Arnold[1]

***Short Abstract* — Autocrine signaling, a form of cell-cell communication, is commonly thought to coordinate population behavior. To understand how the structure of the signaling regulatory network affects this coordination, we rewired one such network from the quorum-sensing bacterium *Vibrio fischeri*. In the presence of cell to cell heterogeneity, positive feedback on the signaling molecule coordinates a stable population-level response, whereas positive feedback on the transcriptional activator amplifies this heterogeneity and is unstable. A mathematical model suggests that population heterogeneity as opposed to mutation sufficiently explains the experimental results. These findings highlight the importance of signal manipulation in coordinating cellular decision making.**

***Keywords* — cellular decision making, autocrine signaling, quorum sensing, transcriptional regulation**

Autocrine signaling, the process by which cells of the same type communicate with one another, is commonly thought to coordinate population decision making [1, Ch. 15, p. 835]. One recurring motif in autocrine signaling is positive feedback on the signaling molecule. This motif is present in *Drosophila* development (e.g., Spitz) [**Error! Reference source not found.**2] and interferon signaling [**Error! Reference source not found.**4]. For both of these cases, coordinating cellular decision making is critical for ensuring proper development and effectively fighting infections, respectively. Many quorum-sensing bacteria, the most primitive cell-cell communicators, exhibit this motif as well [**Error! Reference source not found.**3].

We decided to investigate how the structure of the communication regulatory network affects population coordination by rewiring the *lux* quorum-sensing operon of the bacterium *Vibrio fischeri*. This operon consists of genes encoding LuxI, the signaling molecule synthase, and LuxR, the transcriptional regulator activated by the signal. Previously, we experimentally demonstrated that such rewiring could yield graded, threshold, and bi-stable gene expression [3]. Here, we consider two subtly different architectures employing positive feedback:

1. constitutive expression of *luxR* and *lux*-mediated expression of *luxI* (hereafter denoted as *+luxI*), and
2. constitutive expression of *luxI* and *lux*-mediated expression of *luxR* (hereafter denoted as *+luxR*).

Simply extending the modeling framework we previously developed [**Error! Reference source not found.**3] suggests that both of these architectures should yield the same threshold-type response.

We used constant growth-rate experiments to explore how these different network architectures affected the population coordination. Surprisingly, we discovered that none of the *+luxR* circuits equilibrated over the course of the experiment. In contrast, only one of the tested *+luxI* circuits failed to equilibrate. Flow cytometry revealed the presence of bi-modality in the responses of each *+luxR* circuit, whereas only one of the *+luxI* circuits exhibited significant bi-modality.

To further explore this phenomenon, we examined simpler circuits in which we removed the *luxI* gene and exogenously dosed the signaling molecule. Population-level measurements of these simpler circuits indicated that the growth rate decreases upon circuit induction. Additionally, both circuits (either constitutive expression or positive feedback of *luxR*) exhibited the same level of growth-rate inhibition as a function of circuit induction. Studying these circuits using time-lapse microscopy confirmed the presence of heterogeneous circuit induction and growth-rate inhibition at the single-cell level.

We also constructed a mathematical model accounting for growth-rate inhibition due to circuit induction. The model considered two populations, one population being more sensitive to the signaling molecule than the other, and employed precisely the same parameters for both the *+luxI* and *+luxR* architectures. The model reproduced the observed experimental results of observable bi-modality in the population-level response. Additionally, the model suggested that the *+luxI* architecture can coordinate coexistence of the two populations, whereas the *+luxR* architecture selects for the less sensitive population at some cellular densities. Notably, mutation is not necessary to explain the experimental results.

Our findings indicate that signal manipulation can coordinate population behavior in the presence of cell to cell heterogeneity. Positive feedback on the signaling molecule facilitates coordination of stable population-level responses in spite of this heterogeneity, whereas positive feedback on the transcriptional activator amplifies the population heterogeneity, leading to less effective coordination. Hence signal manipulation appears to be a fundamental principle for coordinating cellular decision making.

References

[1] B. Alberts, A. Johnson, J. Lewis, et al. Molecular Biology of the Cell. Garland Science, New York, NY, fourth edition, 2002.

[2] M. Freeman. Feedback control of intercellular signalling in development. Nature, 408(6810):313–319, 2000.

[3] E. L. Haseltine and F. H. Arnold. Implications of rewiring bacterial quorum sensing. Appl. Environ. Microbiol., 74(2):437–445, 2008.

[4] T. Taniguchi and A. Takaoka. A weak signal for strong responses: interferon-alpha/beta revisited. *Nat. Rev. Mol. Cell. Biol.*, 2(5):378–386, 2001.

[1]Division of Chemistry & Chemical Engineering M/C 210-41, California Institute of Technology Pasadena, CA 91125. E-mail: frances@cheme.caltech.edu