Single-cell ultrasensitivity and stochasticity give rise to bimodal response on the population level

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***Short Abstract* — Phenotypic heterogeneity within a population of cells has been recently demonstrated to confer fitness advantage in systems ranging from bacteria treated with antibiotics to cancer cells subjected to chemotherapeutic drugs. Bimodal protein distributions are a prime indicator of such heterogeneity. We measure a bimodal distribution of phosphorylated ERK in MAPK pathway and propose a probabilistic model that gives rise to cell-to-cell variability in a cascade network system without feedbacks. Given wide implementation of this architecture in eukaryotic systems, this mechanism may play an important role in generation of phenotypic variability.**

***Keywords* — signaling, MAPK pathway, stochasticity, cellular heterogeneity, digital response**

I. Background

Genetically identical cells can respond differently to extracellular stimuli and may therefore follow different fates within cellular population. The source of this heterogeneity is typically attributed to feedback regulation in nonlinear signaling networks: a positive feedback or double negative feedback motif introduces nonlinearity, which leads to bistability [1]. Here we present a different mechanism, which also results in heterogeneous response between cells in a population but does not rely on feedback topology. Based on experiments augmented with stochastic models and computational analysis, we argue that this phenomenon results from the interplay of variability in the amount of network components and nonlinearity of the network response.

II. Results

Using flow cytometry we measure extracellular signal-regulated kinase (ERK) response to epidermal growth factor (EGF) in single human embryo kidney cells (HEK 293). Our results suggest that the level of activated ERK (doubly-phosphorylated ERK) exhibits bimodal distribution on the population level. It has been long recognized that the network involved in this response, mitogen-activated protein kinase pathway (MAPK), is capable of producing a very steep input/output relationship due to its three-tiered kinase modules, a so-called ultrasensitivity [2]. Even though MAPK cascade brings about a robust digitalization of the input on the single-cell level, not all cells in the population respond identically to the extracellular stimulus. Two factors contribute to heterogeneous response. The first, variability in gene expression of MAPK components introduces differences in steepness and threshold levels of the input/output relationship between cells. The second source of inter-cellular response variability lies is the noisy input. On the population level, EGF stimulation yields a distribution rather than a narrow peak of active Raf, the first level of MAPK cascade. Both of these contributions are capable of inducing a broad output distribution of active ERK and, under some conditions, a bimodal distribution, which leaves part of the cellular population in the low and the rest in the high active ERK state. The fraction between these subpopulations depends on the stimulation level.

III. Conclusion

Our results indicate a simple mechanism that generates heterogeneity within a population. It relies on the single-cell steep and nonlinear input-output relationship combined with stochastic effects in the signaling pathway. Population heterogeneity has been recently demonstrated to confer a fitness advantage in the face of antibiotic exposure of bacterial cells [3], chemotherapy treatment of tumors [4] or general changes in extracellular conditions [5]. Although physiological implications of bimodal distribution of active ERK is yet to be discovered, the phenomenon described here is a simple core mechanism that might underlie more complex processes that require randomization of cell phenotypes.

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References

[1] Kholodenko BN, Hancock JF, Kolch W (2010) Signaling ballet in space and time. *Nat Rev Mol Cell Biol* **11**, 414-426.

[2] Huang C-YF and Ferrel Jr. JE (1996) Ultrasensitivity in the mitogen-activated protein kinase cascade. *PNAS* **93**, 10078-10083.

[3] Blake WJ et al. (2006) Phenotypic consequences of promoter-mediated transcriptional noise. *Mol Cell* **24**, 853-865.

[4] Sharma SV et al. (2010) A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. *Cell* **141**, 69-80.

[5] Acar M, Mettetal JT, van Oudenaarden A (2008) Stochastic switching as a survival strategy in fluctuating environments. *Nat Genet* **40**, 471-475.