PHYSIOLOGY

Putting it all on pigmentation: Heuristics of a bold and stochastic cell fate decision

Jianhua Xing* and Robin E. C. Lee*

Gradients of transmembrane potential coordinate cell-fate decisions and patterning during embryogenesis and wound-healing. Bioelectrical signaling may also be more important for adult pathologies than currently recognized. In this issue of *Science Signaling*, Lobikin *et al.* describe a role for bioelectric signals during the development of *Xenopus leavis* embryos to instruct an organism-level response reminiscent of neoplastic progression in melanoma.

Ion channels gate the flow of electrical charges across lipid bilayers to generate potential differences across cell membranes that are fundamental to life. In this issue, Lobikin and co-workers study physiological roles for endogenous bioelectric cues in *Xenopus leavis* embryos (*1*). By activating a glycine-gated chloride channel that is specific to a small and sparsely distributed subpopulation of cells (referred to as instructor cells), the resulting depolarization of the instructor cells triggers changes in gene expression and overgrowth of melanocytes, leading to a hyperpigmented phenotype in embryos. They show that melanocyte conversion is not cell autonomous, but instead mediated by serotonergic signaling and the pituitary gland. Furthermore, the phenotype is remarkably all or none: Each embryo is either hyperpigmented or not.

It is puzzling how instructor cells influence melanocytes, because the two cell populations are physically separated in developing embryos; intercellular signaling must be involved. Indeed, Lobikin *et al.* show that after depolarization of instructor cells, the pituitary gland signals, at least in part, through secretion of melanocyte-stimulating hormone; however, complete removal of the pituitary gland reduces the incidence of hyperpigmentation by only half, implying that the pituitary-mediated signals potentiate, but are not essential, to the hyperpigmentation decision. How else can instructor cells communicate with melanocytes if the pituitary gland is removed? Are these processes essential to the hyperpigmentation phenotype of intact embryos? The authors' data point toward a transcriptionally regulated epithelial-to-mesenchymal transition (EMT) program in the conversion of melanocyte behavior, shape, and proliferation, drawing parallels with human neoplasms.

EMT is a process of phenotypic transition from epithelial cells that are adherent and form the tightly adhered structures throughout the body to mesenchymal cells that are loosely connected to each other and highly motile (*2*). Studies with mice show that partial EMT induced by Snail1, a zinc-finger transcription factor of the Snail family (including Snail, Slug, and Smuc), leads to cytokine production that promotes myofibroblast differentiation and other processes related to development and progression of organ fibrosis (*3*). Indeed, depolarization of *Xenopus laevis* instructor cells induces expression of genes encoding the EMT-associated transcription factors Sox10 and Slug. Furthermore, *Sox10* transcripts are observed at foci throughout the embryo reminiscent to patterns of instructor cells, suggesting a direct link between depolarized instructor cells and the organism-level transcriptional response. The authors show that transcriptional profiles in these embryos are enriched for immune response and cell-remodeling pathways that similarly connect microenvironments of chronic inflammation with EMT-like responses in human cancers (*4*). Inflammatory signals emanating from the vicinity of instructor cells induced by Sox10 and Slug could conceivably provide diffusive factors to bridge communication between instructor cells and melanocytes. This relationship remains to be investigated, as does how closely hyperpigmentation signaling in *Xenopus laevis* mirrors that of metastasis in human cancers.

Another prominent feature of the system is that hyperpigmentation is stochastic and binary at the level of the organism. In response to depolarization of instructor cells, an individual embryo may be either hyperpigmented or normal, but does not exist in a hybrid state with a mixture of melanocyte phenotypes. As illustrated in Fig. 1A, this organism-level decision is very different from previously reported binary systems (*5*, *6*), in which the fate decision of individual cells in the same microenvironment is stochastic (Fig. 1A). Using a computational evolutionary approach, Lobikin *et al.* reverse-engineered a hypothetical physiological regulatory network with two key features to explain this all-or-none behavior. First, the network has two types of attractors corresponding to the two organism-level outcomes: hyperpigmented and wild-type pigmented. Second, intercellular signaling molecules diffuse efficiently throughout the organism. Whether the topology and the parameters of the model presented here stand the test of time will be determined as more quantitative and single-cell data are used to constrain, reevaluate, and possibly revise the model.

There does exist a caveat. Even in computational models that are well constrained by carefully measured biophysical parameters, additional quantitative data can lead to sets of parameter or topology constraints that favor other regulatory architectures (*7*). For instance, a hypothetical feedback mechanism (Fig. 1B) includes a third feature by which each melanocyte (normal or converted) influences nearby melanocytes to assume the same cell state (or alternatively to suppress opposite states, which produces the same output). The molecular mechanism for the cell-cell interactions can be either through secretion of intercellular signaling molecules—for example, melanocyte-secreted inflammatory cytokines—or through local physical contact. In this example, if the intercellular interactions are sufficiently strong, cells will collectively adopt the normal or converted state. Depolarized instructor cells play the role of biasing melanocytes, which are collectively in the normal state in the early embryo, to adopt the converted state, either directly or in combination with pituitary signals. Similar mechanisms are widely used to explain collective phenomena that are ubiquitous in nature. Some examples include liquid-gas phase transition (*8*), helix-coil transition of

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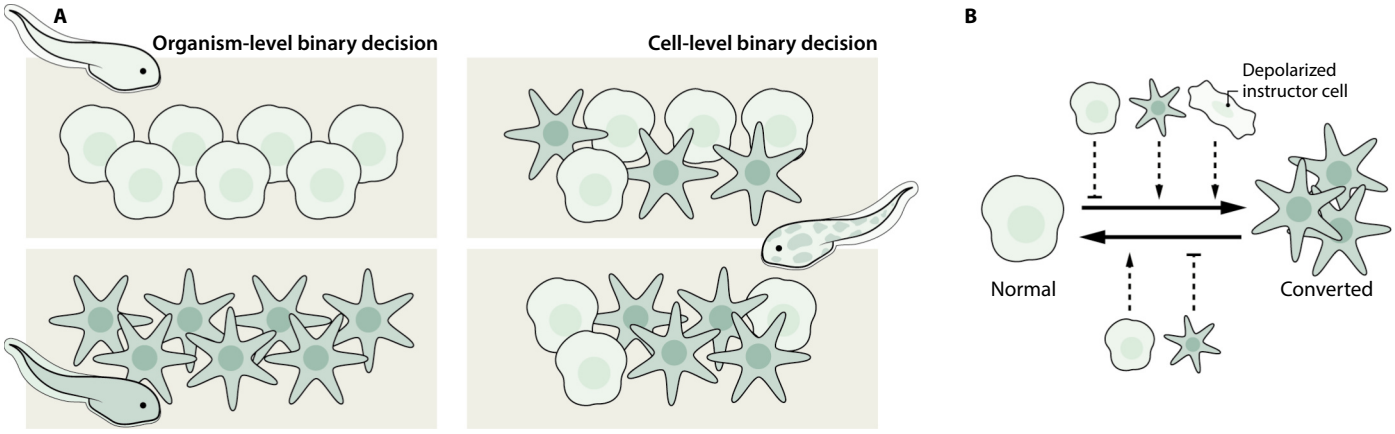

Fig. 1. Schematics of binary cell fate decision. (**A**) Examples of organism-level (left) and (hypothetical) cellular-level (right) binary decision. (**B**) Possible mechanism of the all-or-none normal versus converted hyperproliferative melanocyte transition induced by the depolarized instructor cells and influenced by other melanocytes in the normal or converted state. Arrowed lines indicate activation, and blunt-ended lines indicate inhibition.

peptides (*9*), and even maintenance of epigenetic histone covalent modifications (*10*). Such feedback is also apparent on human scales, particularly in the often observed rhythmic synchronization of applause from an audience of clapping individuals. Our experience tells us that it is rare to have people in the incoherent clapping mode surrounded by a majority of people in the synchronized mode, and vice versa, just like the absence of *Xenopus laevis* tadpoles with mixed normal and converted melanocytes.

The work of Lobikin *et al*. demonstrates the functional roles of intercellular signals, such as serotonin, on cell fate decisions in *Xenopus laevis*. Similar mechanisms may play roles in other developmental and disease-associated processes, and through heuristics, we gain a glimpse at the possible underlying biological design principles. Further quantitative studies, that combine experimental and computational approaches, will refine the details of these regulatory principles, thus paving the way to a deeper mechanistic understanding of cell fate decisions in development and disease.

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10.1126/scisignal.aad2816

Citation: J. Xing, R. E. C. Lee, Putting it all on pigmentation: Heuristics of a bold and stochastic cell fate decision. *Sci. Signal.* **8**, fs17 (2015).

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Science Signaling **8** (397), fs17. [doi: 10.1126/scisignal.aad2816]

Science Signaling (ISSN 1937-9145) is published weekly, except the last December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.