When microarrays Met epidermal-cell migration

Kevin A Janes1,2

1 Department of Cell Biology, Harvard Medical School, Boston, MA, USA and 2 Department of Biomedical Engineering, University of Virginia, Charlottesville, VA, USA

Molecular Systems Biology 1 July 2008; doi:10.1038/msb.2008.41

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Cell movements are important for many physiological processes. Whether it is the development of an embryo, the lethal progression of a cancer, or the healing of a paper cut, a common biological theme is the ability of cells to migrate in complex environments. Cell migration occurs through a series of protein modification and macromolecular assembly–disassembly cycles that are mechanochimically coupled (Lauffenburger and Horwitz, 1996; Ridley et al., 2003). Now, a recent publication in Molecular Systems Biology adds transcription to the mix of regulatory pathways that impinge upon a cell’s decision to migrate or remain stationary (Busch et al., 2008).

Cell migration is a field that has a rich history of advancing through computational and quantitative studies (Carlsson and Sept., 2008). Most of this work, however, has involved cells that are already actively migrating. Usually, cells require a delay of several hours after exposure to a motogenic stimulus to interpret it and gear up their migratory machinery. The study of Busch et al focuses on the molecular events that take place during this ‘processing time’ in a co-culture model of epidermal wound healing.

The authors use hepatocyte growth factor (HGF) to induce migration of a primary human keratinocyte monolayer that has been ‘wounded’ by a pipette scratch. HGF rapidly binds to its receptor (Met), but Met signaling does not noticeably promote migration of the monolayer until nearly 1 day after stimulation. To hone in on the relevant signaling events during this period, Busch et al invoke a ‘slaving principle’ from physics. The slaving principle states that when complex dynamical systems undergo an abrupt transition, they actually simplify by becoming highly dependent on their slowest variables. For many cell-state transitions, the slowest variable is regulated gene expression, and indeed the authors show that new transcription is absolutely required for HGF-induced wound healing.

To identify the relevant gene expression network that initiates and sustains keratinocyte migration, the authors use transcriptional profiling together with a stringent selection criterion. Similar to other motogens such as epidermal growth factor (EGF) (Wolf-Yadlin et al., 2006), HGF activates both migration and proliferation, thus confounding the identity of migration-specific genes. Busch et al circumvent this problem by filtering the HGF-induced expression patterns against the genes induced by growth factors that promote keratinocyte proliferation but have no effect on migration. Doing so reduces tens of thousands of measurements to a set of 20 candidate regulators that may coordinate cell motility as a network.

The authors then construct a dynamic neural-network model from measurements of the leading gene candidates to analyze possible interrelationships among migration-specific genes. Neural-network models have a less-than-favorable reputation among experimentalists for being heavily parameterized, but they can be useful in certain contexts (Krogh, 2008). Busch et al arrive at their model by combining a genetic search algorithm with a variety of diagnostic tests to assess the qualitative stability of their parameter estimates. The model identifies a core network of three genes with strong interconnections—the immediate-early gene egr3, the protein kinase A (PKA) scaffold akap12, and the cyclooxygenase ptgs2. The authors confirm by experiment that the protein product of ptgs2, Cox-2, is a positive regulator of early HGF-induced migration. Conversely, the catalytic activity of the Akap12 binding partner, PKA, is shown to inhibit migration. Busch et al did not manipulate egr3 directly; however, egr3 has recently been reported to be important for growth factor-induced migration of endothelial cells (Liu et al., 2008). Together, these results suggest that the authors’ model has captured some of the inducible mechanisms for HGF-driven motility.

Besides the core egr3–akap12–ptgs2 network, another six transcripts had somewhat weaker links within the model and thus possibly served as modulators of migration. Of particular interest were the EGF family ligand hbegf and the EGF-induced feedback inhibitor erffi1 (Hackel et al., 2001). The co-occurrence of hbegf and erffi1 among HGF-induced migration genes suggested that autocrine signaling could be taking place through the EGF receptor (EGFR). The authors did not measure HB-EGF release or EGFR activation directly, but inhibition of EGFR family kinase activity completely stopped HGF-induced migration, even when added nearly a day after HGF.

By updating their neural-network model, Busch et al show that a sustained motogenic input (presumably through members of the EGFR family) is required for maintaining elevated expression of the genes most closely linked to migration. As a final test for their updated model, the authors predict a context-specific role for PKA signaling in migration, which they then verify by experiment. In the model, akap12 (the authors’ surrogate for PKA activity) antagonizes ptgs2 but positively interacts with egr3. Both ptgs2 and egr3 promote migration (see above), meaning that the apparent role of PKA depends on the transcriptional context.
provided by \textit{ptgs2} and \textit{egr3}. Instantaneous blockade of PKA slightly enhances cell migration because of the network ‘state’ specified by HGF (high \textit{ptgs2}, low \textit{egr3}). By contrast, late inhibition of PKA abrogates migration because of the state specified by long-term autocrine signaling (low \textit{ptgs2}, high \textit{egr3}). Thus, small subnetworks of inducible genes have the ability to ‘tune’ the role of pleiotropic signaling cascades such as PKA depending on the microenvironment.

The work of Busch \textit{et al} adds further evidence for the important role that autocrine factors play in mediating ‘slow’ cellular decisions (Janes \textit{et al}, 2006). The authors’ migration model is primitive in the way that the growth factor inputs and migration output are encoded. Nevertheless, it is a good step toward combining signaling and transcription in a common data-driven framework. HGF was the focus of the authors’ study, but it appears only to act as a trigger for a cascade of events leading to sustained migration of keratinocytes. Indeed, when systems approaches are applied to ‘well-defined’ problems \textit{in vitro}, often the underlying biology is more than Met the eye.

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