garding the location of the DBD relative to the initiation site and its effect on transcription activation. Significantly, the authors show that the artificial activators are only functional in the cells that contain the target protein. This level of specificity is uncommon in natural activators and constitutes a promising advance in the field [17].

The work summarized above describes continuing fundamental advances at the interface of chemistry and biology toward artificial control of gene expression. The latest addition by Mapp and colleagues provides a concrete foundation for designing a new generation of cell-type-specific and species-specific artificial activators. Certainly, much remains to be elucidated, because the ultimate goal is to generate cell-permeable transcription factors that not only turn gene transcription on or off but respond to extracellular signals as part of signal transduction cascades [3]. However, we can anticipate that chemists will continue to bring fresh perspectives and a zest for understanding biology at the molecular level to this highly fertile ground for exciting research.

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Scratch n' Screen for Inhibitors of Cell Migration

Yarrow et al. have identified a small molecule inhibitor of cell migration, 3-(4-pyridyl)indole ("Rockout"), that targets Rho-kinase via a novel screening method using a scratch wound healing assay adapted to a high-throughput format and automated microscopy.

Small molecule inhibitors are extremely useful for dissecting complex cellular events that require the coordination and regulation of many proteins. By inhibiting a specific component of a biochemical network, one can deduce its function in the process. A popular approach has been to use pure proteins and screen for inhibitors in activity assays. However, this limits the target to known elements with known activities. A more difficult and potentially fruitful approach is to design a "phenotypic screen" that assays the entire cellular process. In this issue of *Chemistry & Biology*, Yarrow and colleagues have designed an innovative, imaging-based screen for inhibitors of cell migration and have discovered the compound "Rockout," which targets the cytoskeletal regulator Rho-kinase [1].

Cell migration is attractive as a target for phenotypic small molecule screens because it relies on the integration of several processes and because it is essential in many cell types. Wound healing, tissue formation, immune responses, angiogenesis, and tumor cell metastasis all depend on cell motility [2]. The process generally begins with an extracellular stimulus that triggers a signal transduction cascade leading to polarized migration of the cell, which requires the reorganization of actin filaments and microtubules, polarization of the secretory apparatus, membrane protrusion, and dynamic adhesion to the substrate [2, 3]. Although a variety of stimuli can trigger migration, many aspects of the intracellular pathways that contribute to cell polarization and motility are conserved in multiple cell types and species [3–5]. For example, Rho family GTPases—namely Rho, Rac, and Cdc42—have conserved functions in linking extracellular signals to rearrangements of both actin microfilaments and microtubules [3, 4]. By activating downstream effectors, these GTPases regulate processes that range from microtubule/actin in-

teractions at the leading edge of a migrating fibroblast to polarizing actin structures in budding yeast [4, 6]. A major goal is to understand how these Rho GTPases function to regulate polarity in diverse cellular processes.

Small molecule inhibitors have been used to study the migration of living cells, and the most readily available compounds directly disrupt actin microfilaments or microtubules [7, 8]. Treating migrating cells with such compounds can address the requirements of microtubules or microfilaments in motile processes; however, these experiments exclude information about upstream regulation. Consequently, efforts have been made to identify small molecule inhibitors that target regulatory proteins using phenotype-based assays [9–12]. These approaches have yielded inhibitors of factors such as N-WASP, which activates the Arp2/3 complex and promotes actin polymerization [11]; Rho-kinase, which is activated by the Rho GTPase and whose targets include cytoskeletal components [10]; and myosin light chain kinase, which regulates myosin activity [9]. Because cell migration is so complex, a comprehensive understanding would be greatly facilitated by the discovery of additional, cell-permeable compounds that target other regulators, such as the Rho GTPases themselves, additional downstream actin and microtubule effectors, and factors that mediate interactions between these two cytoskeletal elements [4].

In an unbiased approach to identify novel cell migration inhibitors, Yarrow and colleagues performed a visual screen based on classical wound healing assays performed in high-throughput format [12, 13] and identified a new Rho-kinase inhibitor, Rockout [1, 14]. In the screen, BS-C-1 cell monolayers were grown in 384-well tissue culture plates. The monolayers in each well were wounded with a pin transfer device normally used for transferring small volumes of liquid to 96-well plates. Pins were placed in the wells and then moved laterally to introduce a wound of uniform size and shape to each monolayer before the addition of small molecules. A library of 16,000 small molecules was screened. Cell monolayers were allowed a 7-hr recovery period before processing for microscopy (Figure 1) [14]. An image of each well was captured using automated microscopy and compounds affecting wound healing were grouped according to phenotype. Compounds that were selected for further analysis decreased cell migration, caused aberrant morphology, increased the mitotic index, or caused a combination of these phenotypes. Compounds that caused gross disruptions of the cell monolayer were interpreted to be toxic and were not pursued.

A set of seven secondary assays was then performed to test the selected compounds for their effects on the actin cytoskeleton as well as other processes. Rockout was identified as a nontoxic molecule that changed the morphology and dynamics of cells at the wound margin. In secondary assays, Rockout inhibited cell blebbing and the formation of stress fibers and focal adhesions. Importantly, Rockout did not disrupt actin filaments directly. Together these results suggest that Rockout disrupts the Rho pathway. Additional experiments showed that Rockout does not act on Rho itself, but rather inhibits the activity of Rho-kinase.

Although Rockout is less potent than the pre-existing Rho-kinase inhibitor Y-27632 [10], it provides a new scaffold that could be optimized to develop more potent and specific inhibitors of Rho-kinase. Rho-kinase

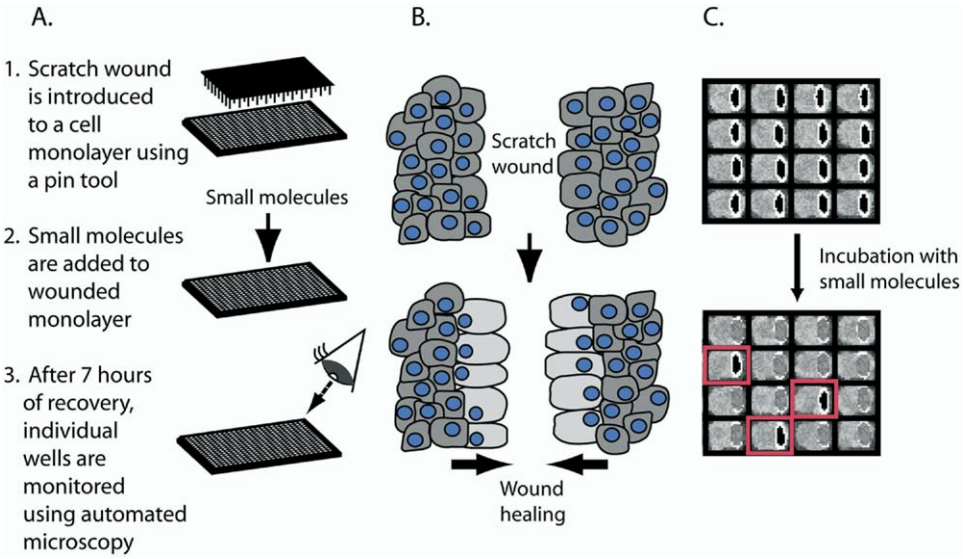

Figure 1. High-Throughput Screening Using a Scratch Wound Healing Assay

(A) Screening protocol in 384-well plate format.

(B) Schematic of wound healing at a scratch boundary, where bordering cells initiate lamellar protrusion and migration toward the center of the wound.

(C) Schematic of 16 wells before and after treatment with compounds during wound healing. Three wells, outlined in red, show defects in wound closure indicating the presence of inhibitory compounds.

is of interest as a small molecule target because it is activated by Rho and because it phosphorylates cytoskeletal proteins, including myosin light chain, which contributes directly to stress fiber formation [15]. Other substrates of Rho-kinase include factors that function in cytokinesis and membrane ruffling [16].

Like a similar screen performed previously [12], the strategy used in this study has several advantages. For one, the readout is the direct visualization of a complex phenomenon (wound healing) whose proper completion relies on several fundamental mechanisms (signal transduction, cytoskeletal reorganization, etc). This makes possible the identification of compounds that affect regulators of multiple pathways that contribute to cell migration. In addition, the screen automatically selects for small molecules that are cell-permeable. Although the current study focused on finding compounds that target actin-associated proteins, one can imagine using the same screening method to find compounds that target other processes that are important for cell migration. For example, in standard wound healing assays, the Golgi apparatus orients toward the site of polarized growth in response to the introduction of a scratch wound, and this orientation can be quantified [17]. One might discover interesting inhibitors in a screen for small molecules that disrupt Golgi orientation in this assay.

The authors of this study were able to identify a target of Rockout using a combination of careful experimentation and inference. However, target identification is an inherent challenge in phenotypic small molecule screens and it is often necessary to use other methods to identify the target or targets of a compound. For example, affinity chromatography methods can be used for target identification by coupling the compound of interest to a matrix to retrieve target proteins from cellular extracts [18]. Another approach, known as the small molecule yeast-three-hybrid system, detects compound-protein interactions in living yeast cells [19]. Here, the interaction between the compound and its target protein activates the expression of a reporter gene. Although such approaches have been used successfully, a major challenge remains to develop improved methods for identifying targets in phenotypic screens.

In summary, the study by Yarrow and colleagues demonstrates the use of a high-throughput screening approach that is based on a wound healing assay. This work has set the stage for the identification of novel inhibitors as well as potentially novel targets. Depending on how the microscopy assay is applied, it could be used to screen for compounds that affect diverse migration-related pathways associated with wound healing. We are eagerly waiting to see what crawls out next.

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