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A Role for RKIP in Cell Motility

The target of locostatin, a small-molecule inhibitor of cell movement, has been identified as RKIP, a Raf-1 kinase modulator [1]. In addition to advancing our understanding of cell locomotion, this work represents a major landmark in the development of chemical genetics.

Cell locomotion is a fundamental feature of many normal and pathological processes including morphogenesis, wound healing, and metastasis. Consequently, a detailed understanding of cell movement is a major goal for modern biology and medicine. Crawling cells move forward due to tightly choreographed interplay between actin filament assembly and disassembly, molecular motor-based movement, and differential cell adhesion. To date, we know a considerable amount about the actomyosin-based machinery and cell adhesion molecules essential for cell locomotion, and more than a little about some of the immediate regulators of this machinery. We do not, however, know much about regulators farther upstream, except in some systems that undergo chemotaxis [2]. However, in this issue of *Chemistry & Biology*, Zhu et al. report that locostatin, a small-molecule cell motility inhibitor [3], inhibits cell migration by covalent modification of RKIP (Raf kinase inhibitor protein; Figure 1). Because RKIP has no known direct cytoskeleton or adhesion protein targets, and because it is a metastasis suppressor [4], it likely represents a general upstream regulator of cell motility.

The case for RKIP as the locostatin target is very strong [1]: RKIP is one of only four proteins specifically bound by locostatin and the only one of the four whose binding profile for locostatin analogs correlates with the effects of the analogs on cell locomotion. Further, RKIP knockdown suppresses cell motility, thus mimicking the locostatin phenotype, while RKIP overexpression promotes cell migration and reduces the effects of locostatin. Indeed, RKIP overexpression changes the basic features of epithelial cell growth, causing them to lose cell-cell adhesions and generally resemble other, more motile cell types.

How does RKIP function during cell migration? As its name implies, RKIP is best known as an inhibitor of Raf-1 [5, 6], a serine/threonine kinase, and Zhu et al. [1] show that locostatin suppresses RKIP-Raf-1 binding. Because raf kinases are essential players in cell division promoted by the small GTPase, ras, RKIP might be supposed to exert its effects through inhibition of cell division. However, this is not the case, since cell division is not required for movement in the assay used by Zhu et al. and gene knockout experiments indicate that Raf-1 is not the raf isoform required for ras-dependent cell division [7]. A more direct link between Raf-1 and cell locomotion comes from the demonstration that Raf-1 can control cell migration by controlling the localization of another kinase, Rock-α [8]. However, the Raf-1-Rock-α interaction is kinase independent, so it's not clear how inhibition of RKIP, which is expected to result in activation of Raf-1 kinase activity, suppresses cell locomotion.

An alternative mechanism is suggested by the demonstration that, when phosphorylated by protein kinase C, RKIP ceases to bind Raf-1, and instead binds to and inhibits G protein-coupled receptor kinase 2 (GRK2 [9]) (Figure 1). GRK2 is a serine/threonine kinase known to downregulate a variety of G protein-coupled receptors [10]. Significantly, such receptors play a key role in cell locomotion in other cell types [2]. Further, in leukocytes, increased GRK2 activity is associated with suppression of cell locomotion [11], while GRK2 loss is correlated with increased cell locomotion [12]. In this case, the locostatin-RKIP interaction would have to disrupt RKIP-GRK2 binding, a point which needs to be tested.

In addition to providing important insight into cell locomotion, this work also fulfills the promise of chemical genetics as an approach with many of the strengths of classic genetics, but which is not limited to a few model systems. To be sure, the chemical genetic approach can suffer from a relative lack of specificity and reduced potency of many small-molecule effectors and, in many cases, difficulty in cellular target identification. Nevertheless, this report [1], when considered with the authors' previous work [3], satisfies several key criteria that define a successful genetic study.

First, classic forward genetics can work with any process, no matter how complex, as long as an appropriate screen is employed. While much recent effort in chemical genetics has focused on the use of automated, high-throughput screens, with a particular bias toward biochemical readouts [13, 14], here a screen based on visual inspection of epithelial cell migration was performed. Manual screens are inherently slower and less objective than automated screens; however, they are also simpler and higher content [15], and their potential for success is confirmed by the identification of locostatin. Given the relative accessibility of manual screens to the average investigator, and the fact that small-molecule libraries can now be obtained commercially, or even for free, it follows that chemical genetics is an avenue available to anyone who cares to try it.

Second, genetic studies have the potential to identify any player that controls a particular process, regardless of that player's mode of action. However, many previously identified small-molecule inhibitors target enzymes. Further, of those that target nonenzymes by disrupting protein-protein interactions, most, if not all, of them were identified in screens directed at finding agents to disrupt binding of two particular proteins (e.g., Myc and Max; see [13]). Locostatin not only targets a nonenzyme (RKIP), disrupting the interaction of RKIP with Raf-1, but was also identified in a target-blind approach. This indicates that small molecules may prove generally useful as regulators of nonenzymes by disrupting protein-protein interactions and, by extension, that this approach can potentially identify any relevant player in a pathway.

Third, the ideal genetic study is definitive, with a par-

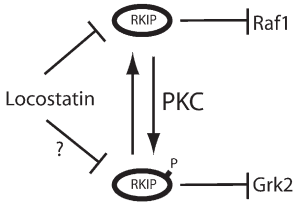

Figure 1. Schematic Diagram Showing Relationship of RKIP to Its Targets and Locostatin

Active RKIP inhibits Raf-1 kinase (Raf1). When RKIP is phosphorylated by protein kinase C (PKC), it no longer inhibits Raf-1 but instead inhibits Grk2. The question mark indicates that whether or not locostatin inhibits phosphorylated RKIP is currently unknown.

ticular gene and gene product linked to a phenotype by mapping (or a related approach) and by rescue. Here, rather than first narrowing the field to "likely suspects" and potentially missing unexpected targets, Zhu et al. [1] took advantage of the fact that locostatin covalently modifies its targets, and then subjected the entire proteome to labeling with tritiated locostation. Only four proteins were thus identified, and of these, only RKIP subsequently satisfied further tests (see above).

Fourth, some of the most useful genetic screens are for conditional (e.g., temperature-sensitive) mutants, which permit disruption of a particular protein at the investigator's convenience. But conditional mutants can be hard to come by. Chemical genetics, however, provides the pharmacological equivalent of a conditional mutant, in that a drug can be applied at any time. This is a particular advantage for proteins likely to play multiple roles during the lifetime of a cell, tissue, or organism. RKIP, for example, has previously been implicated not only in cell division, but also in differentiation and programmed cell death [4, 16]. Thus, simply disrupting RKIP function by gene knockout in a developing organism might fail to reveal its role in cell locomotion. Since most proteins play multiple roles, such pharmacological conditionality is a great advantage.

Just how far can functional analogies between chemical genetics and classic genetics be pushed? After the success of these studies, one can't help but wonder if other genetic strategies will work for chemical genetics. For example, it might be possible to identify other players that work in the RKIP pathway by screening for small-molecule suppressors or enhancers of locostatin-dependent inhibition of cell movement. Not only might such approaches reveal exactly how RKIP regulates cell motility, the former also has the potential to identify novel therapeutic agents, as RKIP levels are sharply reduced in a number of tumors, and it has recently been suggested that small molecules that modulate RKIP targets offer a promising approach for cancer treatment [4, 16].

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