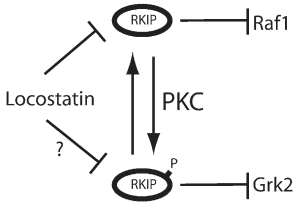

Figure 1. Schematic Diagram Showing Relationship of RKIP to Its Targets and Locostatin

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].

William M. Bement
Department of Zoology and
Program in Cellular and Molecular Biology
University of Wisconsin-Madison
1117 West Johnson Street
Madison, Wisconsin 53706

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Live Chemical Reports from the Cell Surface

Understanding the dynamic roles that proteins play in cell biology requires tools to selectively label these biomolecules in living systems. Yin and colleagues [1] showcase the power of a novel chemical labeling technology for tracking receptor-ligand interactions in native cellular environments.

Historically, the tagging of proteins for optical analysis in cells and organisms has been accomplished through genetically encoded fusion to fluorescent proteins (e.g., green fluorescent protein [GFP]) [2]. GFP fusion proteins have profoundly impacted cell biological research,

where these reagents are now routinely employed to monitor dynamics in protein localization, trafficking, stability and interactions. Despite their versatility and power, GFP fusion proteins do exhibit some shortcomings. First, GFP and related fluorescent proteins are large (>200 amino acids) and therefore may disrupt the function of their fusion partners. Second, only a limited number of fluorescent protein variants are available for routine applications, thus restricting the multidimensionality of molecular imaging experiments. Finally, these probes provide exclusively an optical readout of protein behavior; additional tags, such as photocrosslinkers or biotin, are desired that can be used to profile a wider range of protein functions (e.g., protein-protein interactions, activity, and state of modification). To address these challenges, chemical biologists have introduced several exciting strategies for site-specific chemical modification of proteins in native environments [3–5]. In this issue of *Chemistry and Biology,* Yin and colleagues showcase the power of one of these chemical labeling strategies for visualizing interactions between the transferrin receptor 1 (TfR1) and its natural ligand transferrin in living cells [1].

To accomplish the site-specific labeling of TfR1 in living cells, the authors first fused an 80 residue peptide carrier protein (PCP) excised from a nonribosomal peptide synthetase to the extracellular C terminus of the receptor. Cell surface-expressed TfR1-PCP was then chemically modified by the enzyme phosphopantetheinyl transferase Sfp, which attaches a phosphopantetheine group derived from coenzyme A (CoA) to a specific serine residue on PCP via a phosphodiester bond [6]. Importantly, Sfp has been found to accept a wide range of CoA derivatives conjugated to different molecular probes, including biotin and several fluorophores [7, 8]. Incubation of TfR1-PCP-transfected cells with Sfp and biotin-CoA resulted in rapid (< 20 min) and robust biotinylation of TfR1-PCP as judged by streptavidin blotting. Endogenous streptavidin binding proteins were also observed in this experiment, suggesting that a two-step labeling profile, where fluorescent streptavidin is used to image biotinylated TfR1-PCP, may produce high background signals. Accordingly, the authors elected to label TfR1-PCP directly with a fluorophore by treatment of transfected cells with Sfp and an Alexa Fluor 488-CoA conjugate.

To monitor TfR1-transferrin interactions over time in living cells, the authors conducted fluorescence resonance energy transfer (FRET) studies with Alexa Fluor 488-labeled TfR1-PCP (donor) and Alexa Fluor 568-labeled transferrin (TF-Alexa 568; acceptor). Cell surface FRET signals were observed almost immediately (<1 min) upon addition of TF-Alexa 568. Interestingly, within 15 min, large intracellular aggregates of FRET signals were detected, likely representing TfR1-transferrin complexes accumulating in recycling endosomes. Thus, these studies have provided the first direct visual confirmation that the TfR1-transferrin interaction is maintained during the endocytosis/exocytosis cycle.

Overall, these studies offer a tantalizing glimpse of the potential impact that chemistry can have on the study of cell biological systems. The labeling of cell surface proteins on specific extracellular sites with fluorophores (or other reporter tags) has historically proven to be a challenging technical endeavor. The Sfp-PCP system, along with related approaches like the phosphopantetheinyl transferase-ACP [9] and biotin ligase-keto-biotin [10] technologies, promise to reduce such experiments to a routine practice, providing researchers with an unprecedented level of spatial and temporal control to probe the function of proteins in living systems. Although the requirement of exogenously added enzymes (phosphopantetheinyl transferases and biotin ligase) currently limits these strategies to the analysis of extracellular/plasma membrane proteins, other complementary chemical labeling systems have been described that function inside cells. For example, Johnsson and colleagues have shown that cytosolic proteins fused to the O^6-alkylguanine transferase (hAGT) enzyme can be covalently modified with a variety of reporter tagged-O^6-benzylguanine suicide substrates [11]. Additionally, noncovalent ligand-receptor pairs, such as trimethoprim-dihydrofolate reductase, can be also used for chemical tagging of intracellular fusion proteins [12]. For a more detailed discussion of various strategies for site-specific chemical labeling of proteins in living cells, the reader is referred to one of several excellent reviews that have recently appeared in the literature [3–5].

When contemplating the future direction of this exciting field, one provocative challenge immediately becomes apparent—the extension of site-specific chemical labeling from the culture dish to living animals. While many biological processes can be characterized in cell culture, there are also surely a plethora of remarkable changes in protein localization, transport, and complexation that occur selectively in the intricate three-dimensional environment of intact tissues. Advanced imaging methods like two-photon microscopy [13] offer a wonderful opportunity to explore such dynamic molecular events in vivo. However, site-specific chemical labeling methods will first need to overcome some technical hurdles before they can be used to report on the functional state of proteins in vivo, including the stable endogenous production of fusion proteins, as well as the efficient delivery of modification enzymes and reporter-tagged substrates. The former issue should be addressable using targeted transgenic technologies like gene "knockins," where the gene encoding a protein of interest is replaced by homologous recombination with a gene encoding a "chemically taggable" variant of this protein. This approach would also have the desirable effect of placing the fusion protein under control of the endogenous promoter of the parent gene, thus resulting in native levels of fusion protein expression and avoiding potential biological artifacts that can accompany protein overexpression. The in vivo production of labeling enzymes like Sfp and biotin ligase could also conceivably be accomplished by transgenic methods—in this case, simple overexpression would presumably suffice (and perhaps be preferred). Success with delivering (and eventually washing out) reporter-tagged labeling reagents will likely depend on systemic or local pharmacological administration, and thus be subject to the notoriously unpredictable whims of compound distribution, metabolism, and excretion kinetics. Nonetheless, this author is optimistic that, through rigorous experimental inquiry, an empirical solution will be

found for each of these problems to enable the extension of site-specific chemical labeling in vivo. Can you then imag(e)ine the possibilities?

Benjamin F. Cravatt
The Skaggs Institute for Chemical Biology
Departments of Cell Biology and Chemistry
The Scripps Research Institute
10500 North Torrey Pines Road
La Jolla, California 92037

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