

plane positioned perpendicular to the plane of the thiazolium ring (Fig. 1).

Other ThDP-dependent enzymes that process pyruvate also generate LThDP as the predecarboxylation species. It will be interesting to see whether they also generate the S stereoisomer of this new intermediate. LThDP is presumed to be an intermediate in the reactions of pyruvate with other ThDP-dependent enzymes, including pyruvate decarboxylase, pyruvate dehydrogenase,

acetylacetyl synthase, pyruvate:ferridoxin oxidoreductase, the flavin-independent pyruvate oxidase and other pyruvate oxidoreductases. If these enzymes are all part of the same evolutionary family, one might expect S-LThDP to be a common intermediate within the family. However, the only way to determine whether this is true is by application of the biochemical and crystallographic methods used in the study of Wille and associates<sup>2</sup>.

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## Signalomic signatures enlighten drug profiling

Robert T Abraham

**When introduced into living cells, drugs frequently evoke unanticipated responses that are due either to off-target effects or to previously unknown interactions between the intended target and other biochemical pathways. The development of a panel of high-resolution sentinel assays for signal-transduction cascades in human cells promises to enhance the power of chemical genetics and increase the efficiency of drug-discovery research.**

Small-molecule drugs are developed with the singular intent of modulating the function of a molecular target implicated in the etiology of a specific human disease. However, the overall phenotypic response elicited by most drugs reflects both the ‘on-target’ interaction with the protein of interest and also a variable number of ‘off-target’ interactions involving other, usually undefined cellular components. Though ‘off-target’ effects can sometimes confer a therapeutic advantage<sup>1,2</sup>, they frequently give rise to undesirable side effects that may derail the development of the drug candidate. In most cases, researchers optimize lead candidates for their on-target activities while having only a vague understanding of the intrinsic and/or emergent off-target liabilities of the compounds under development. On page 329 of this issue, MacDonald *et al.*<sup>3</sup> describe a high-content, cell-based assay strategy that may allow chemists to exclude undesirable chemical classes early in the discovery process and optimize their lead candidates with a far more comprehensive perspective on any off-target liabilities incurred during the lead-candidate optimization process.

The protein-fragment complementation assay (PCA) strategy used in this study takes

advantage of the extensive reliance of signal-transduction pathways on constitutive or inducible interactions among component proteins. To detect changes in protein partnering, MacDonald and co-workers individually fused proteins to a complementary fragment of an intensely fluorescent variant of yellow fluorescent protein (YFP) and expressed both members of this protein pair in human embryonic kidney 293 (HEK293) cells (Fig. 1a). Fusion-protein dimerization in the transfected cells juxtaposes the complementary YFP fragments and reconstitutes the active fluorophore. The high fluorescence yield of the enhanced YFP reporter allows detection of dimerization reactions in stably transfected cells expressing biologically relevant levels of each fusion protein. Cell imaging with an automated, high-content fluorescence microscope provides both quantitative and spatiotemporal information about the protein-protein interaction itself, and it also provides an indirect readout for the status of the signaling pathway in which the protein pair resides. Drugs that interfere with the assembly of an active YFP reporter (either directly, or indirectly by blocking an upstream step in the signaling pathway) induce a decrease in total cellular fluorescence. Drugs that exert more subtle effects, such as alterations in the subcellular localization of the reporter complexes, can also be scored by high-content image analysis.

Although the PCA concept has been implemented previously<sup>4–6</sup>, MacDonald *et al.*<sup>3</sup>

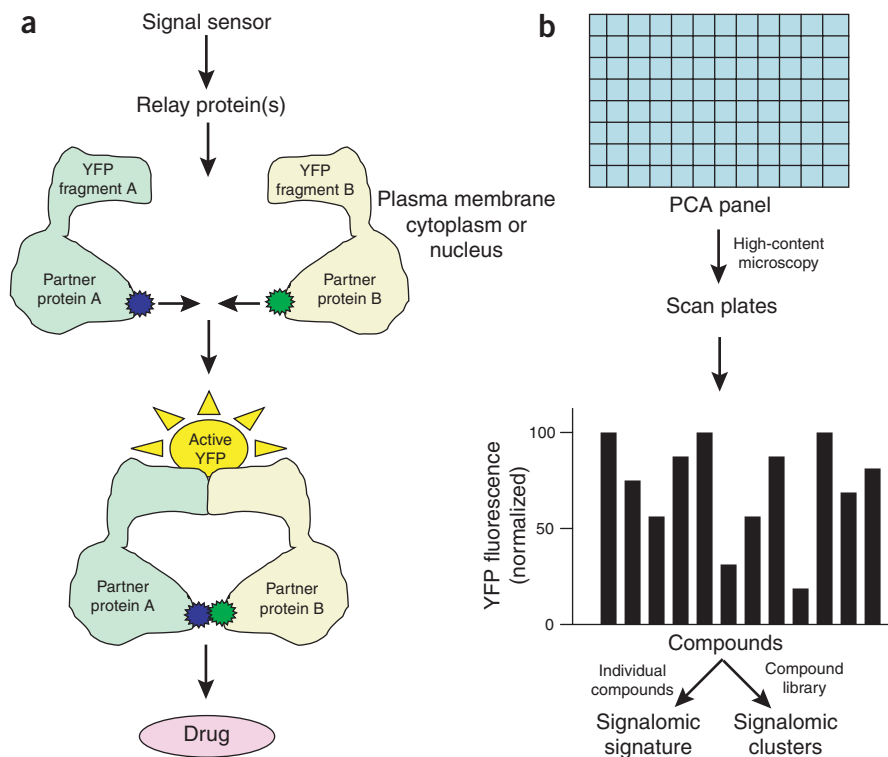
have pushed this technology to a new level by constructing a diverse set of parallel PCAs in HEK293 cells (one PCA per transfected cell line). The 49 PCA assays in this panel cover a broad spectrum of cytoplasmic and intranuclear signaling pathways. Using these assays as pathway sentinels, the authors examined a panel of 107 compounds associated with several distinct therapeutic areas. The summed PCA results yield a ‘signalomic signature’ for each drug, and hierarchical analysis of the results allows clustering of compounds based on their overall activity profiles (Fig. 1b). As predicted, the PCAs faithfully grouped drugs with similar chemical structures and/or identical molecular targets, for example  $\beta$ -adrenergic receptor agonists. The predictive power of these sentinel PCAs was established with the demonstration that the statins, which inhibit cholesterol biosynthesis by blocking 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, clustered together in spite of the fact that their actual molecular target was absent from the PCA panel. Furthermore, the PCA assays revealed that chemically similar agonists for the nuclear hormone receptor PPAR- $\alpha$  manifested distinct profiles of off-target activities. This finding could lead to discoveries of previously undetected or ‘hidden’ phenotypes<sup>3</sup> that are provoked by well-established therapeutic agents.

Cell proliferation is a highly complex phenotypic response that is orchestrated by a

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multiplicity of intracellular signaling cascades. MacDonald *et al.*<sup>3</sup> have identified a supercluster of compounds with distinct on-target activities but also with overlapping effects on PCAs linked to cell-cycle progression or DNA damage responses. The members of this supercluster include known antiproliferative agents (such as geldanamycin) as well as drugs that have not previously been characterized as cell growth inhibitors. One striking example is the selective serotonin reuptake inhibitor sertraline, which is used clinically as an antidepressant. The signature from the PCA panel predicted that sertraline has off-target cell-cycle-inhibitory activities, and subsequent proliferation assays confirmed that this drug suppressed cell growth at concentrations similar to those required for the on-target activity against the serotonin reuptake machinery. The authors later identified a subset of 25 specific PCAs that provide a reliable signature for on- or off-target-related antiproliferative activities—a remarkable feat, given that the PCA panel was not constructed with a particular focus on cell-cycle pathways. One can now envision the assembly of a battery of subsignalomic panels geared toward the discovery of new agents that meet specific therapeutic objectives (for instance, agents that inhibit cholesterol biosynthesis) or enable the early exclusion of compounds that elicit undesirable responses (such as cytotoxicity or metabolic abnormalities).

Pharmaceutical companies are intensely interested in decreasing the high attrition rates of their drug candidates, which frequently fail during preclinical testing in animal models or in phase 2 clinical trials in humans<sup>7</sup>. The extent to which off-target effects contribute to drug failures is unknown, because of the fact that such effects go largely undetected in the current paradigm. The ability to ‘kill’ at an earlier stage of development compounds destined to induce show-stopping adverse effects *in vivo* would save both time and money and would allow resources to be diverted quickly to more promising compound classes. In addition, cell-based assays are becoming increasingly popular tools for drug discovery efforts in both the academic and industrial settings. Oftentimes, the most daunting challenge is to identify the molecular targets of



**Figure 1** PCA strategy. (a) A generic signal-transduction pathway is depicted in this panel. Activation of the endogenous pathway triggers domain interactions (blue and green spiked circles) that lead to the dimerization of partner proteins A and B in living cells. Ectopic expression of chimeric proteins containing the dimerization domains of proteins A and B, each of which is fused to a complementary fragment of an enhanced version of YFP, creates a cell line in which changes in pathway activity are monitored in time and space. Fusion-protein dimerization generates an active YFP enzyme whose fluorescent output is detected by automated high-content microscopy. (b) Generation of a panel of sentinel cell lines allows broad PCA coverage of intracellular signaling pathways. Treatment of the cell lines with drugs or other bioactive compounds yields a signalomic signature for each compound. Comparison of the signatures derived for a set of test compounds allows clustering based on either shared on-target effects or the induction of often hidden off-target phenotypes.

active compounds that emerge from these screens. Gene microarray analysis has proven its utility in both the off-target and on-target scenarios described above; however, it does not provide adequate spatiotemporal resolution for changes in mRNA expression, and it scores events that may be far downstream of or only tangentially related to the intended target of the compound. The PCA methodology described by MacDonald *et al.*<sup>3</sup> represents a promising starting point for the development of signalomic arrays that could

substantially increase both the efficiency and the success rates of current drug discovery efforts.

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