[31] Systems Cell Biology Based on High-Content Screening

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Abstract

A new discipline of biology has emerged since 2004, which we call ''systems cell biology'' (SCB). Systems cell biology is the study of the living cell, the basic unit of life, an integrated and interacting network of genes, proteins, and myriad metabolic reactions that give rise to function. SCB takes advantage of high-content screening platforms, but delivers more detailed profiles of cellular systemic function, including the application of advanced reagents and informatics tools to sophisticated cellular models. Therefore, an SCB profile is a cellular systemic response as measured by a panel of reagents that quantify a specific set of biomarkers.

Background

Until recently, the focus in drug discovery and basic biomedical research has been on simplifying the complexity of the living human organism to individual genes, single metabolic pathways, single proteins, and one potential modulating molecule, such as a small chemical compound or bioproducts to regulate complex functions. This one gene, one protein, one external modulating treatment concept dominated the drug discovery process and much of basic biomedical research since the early 1990s. This paradigm grew out of the promise of the human genome project and the theory that identifying all protein-coding genes would lead to much more rapid discovery of cures for human disease (Collins *et al.*, 2003a,b; Phillips and Van Bebber, 2005).

Unfortunately, this reductionist approach to drug discovery has not delivered the promised efficiencies. Instead, the number of hypotheses validated in the clinic, as measured by novel product launches, has stagnated. In part, this failure is due to an overreliance on the continuity of the stepwise one gene, one target, one molecule reverse genetics approach described earlier. Multiple genes, including both protein coding and noncoding genes, regulate most cellular processes (Costa, 2005; Cummins *et al.*, 2006; Huttenhofer *et al.*, 2005; Volinia *et al.*, 2006), and proteins are part of complex, interacting pathways with extensive compensatory capacities. Therefore, even when a single small molecule or bioproduct has a specificity for binding to a single protein, the

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impact on cellular and, therefore, tissue and organ function is much more complex than expected (Melnick *et al.*, 2006). In addition, absolute specificity of small molecules and biologics is rarely demonstrated and ''off-target'' effects must be understood for both efficacy and potential toxicity (Hopkins and Groom, 2002; Yang *et al.*, 2004). Finally, most diseases are multifactorial where the disease phenotype arises from the dysregulation of multiple genes, pathways, and proteins (Glocker *et al.*, 2006; Jain *et al.*, 2005; Nadeau *et al.*, 2003; Tuomisto *et al.*, 2005).

Nevertheless, drug discovery has come to rely on high-throughput screening (HTS) technologies that focus on isolated targets as a means to boost productivity. Fortunately, technologies such as high-content screening (HCS) have been introduced that combine the efficiencies of HTS with superior biological context of the intact cell. HCS makes it possible to gain deeper knowledge about the effect of experimental compounds on target proteins within the context of the living cell, and thus HCS has been an important step in changing the paradigm for drug discovery. The founders and early employees of Cellomics, Inc. introduced the first HCS platform for drug discovery in 1997 and enabled the field of cellomics (see Giuliano *et al.*, 1997; Taylor, 2006; Taylor *et al.*, 2006). The discipline of cellomics can be described as the first step toward understanding how the output of the foundational ''omics'' technologies, i.e., genomics, proteomics, and metabolomics, function in a living environment (Giuliano *et al.*, 1997, 2003; Taylor, 2006). However, cellomics fall short of elucidating cellular activity as the function of a system. Taylor *et al.* (2006) summarize the application of HCS to drug discovery and systems biology.

Application of the principles of systems biology to cellomics approaches using HCS generates the discipline of systems cell biology (SCB). Systems biology is the study of a whole organism viewed as an integrated and interacting network of genes, proteins, and metabolic reactions that give rise to life (Aloy and Russell, 2005; Bugrim *et al.*, 2004; Butcher *et al.*, 2004; Hood and Perlmutter, 2004; Westerhoff and Palsson, 2004). Thus, the ''omics'' focuses on the parts, whereas systems biology deals with the functional complexity of the whole. The ''omics'' approach is an oversimplification, whereas systems biology at the organismal level is a relatively low-throughput and expensive process. SCB harnesses the higher throughput capacity of HCS technology while avoiding the expense and potential confounding species-related problems (worms, flies, fish) associated with traditional organism-based systems biology.

Systems cell biology is defined as the study of the living cell, the basic ''unit of life,'' an integrated and interacting network of genes, proteins, and a myriad of metabolic reactions that give rise to function (Giuliano *et al.*, 2005; Taylor, 2006; Taylor and Giuliano, 2005). One can think of the cell as

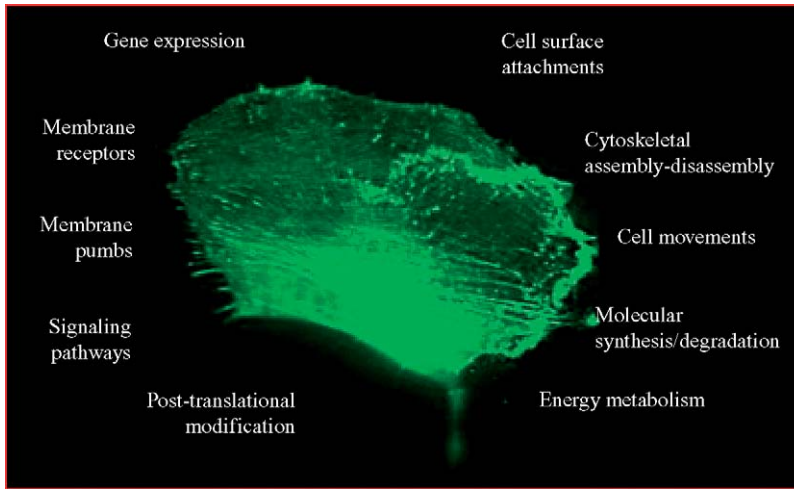

FIG. 1. Cells integrate many cellular processes, such as gene expression and energy metabolism, to yield normal functions. Diseases are due to the dysregulation of one or more of these cellular processes. Many of these processes share pathways, signals, and proteins and should be investigated as part of the cell system.

the simplest living ''system'' (Fig. 1): much less complex than a complete organism, but possessing the systemic functional complexity that facilitates a detailed understanding of the response of the integrated system to a perturbant such as a drug. Thus, the cell serves as a model in which to explore drug efficacy and potential toxicity, inexpensively and rapidly, across a broad range of doses and response times. An SCB profile is a cellular, systemic response as measured by a panel of reagents that quantify a specific set of biomarkers. The Federal Drug Administration (FDA) defines a biomarker as ''a characteristic that is objectively measured and evaluated as an indicator of normal biologic or pathogenic processes.'' Selection of the optimal cell types, whether human primary cells or cell lines, coupled with focused SCB profiling assays, reagents, and informatics analysis, is the key to making systems cell biology a valuable approach to drug discovery and development, as well as biomedical research (Fig. 2).

SCB models elucidate the impact of small molecules and bioproducts on cell functions by characterizing the cellular responses with a large number (>4) of specific cellular parameters that yield a cell system profile (Giuliano *et al*., 2005; Taylor and Giuliano, 2005). Multiplexing facilitates simultaneous capture of many parameters of cellular function, and informatics tools enable identification of the *interaction* of these parameters. These cellular responses and complex interactions can be related to a set of

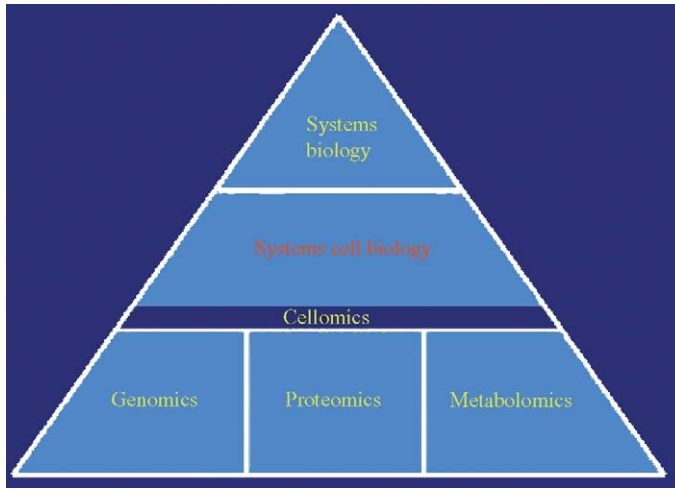

FIG. 2. Systems cell biology offers enough complexity, while allowing high-throughput and cost-effective assays. SCB and systems biology are based on the fundamental components of living systems represented by the ''omics.''

cellular function biomarkers: a unique cellular systemic profile. The cellular function biomarkers selected to represent the systemic response of particular cells are measured with a variety of fluorescence-based reagents. The response profile of a variety of human cells to perturbants is used to build a database that can be mined to relate the effect of ''new'' perturbants to those of known cellular mechanism (Fig. 3).

The Systems Cell Biology Toolbox

The toolbox for applying SCB profiling uses HCS platforms to yield data on the response of individual cells, as well as their subcellular responses. SCB leverages the power of HCS technology, which consists of readers, first-generation reagents, and basic informatics software, with proprietary panels of cellular function reagents, including more advanced reagents that can dynamically measure and manipulate cellular constituents. In addition, advanced SCB profiling informatics are used to generate new systems knowledge of disease, as well as profiles of the cellular systemic response to emerging drugs (Fig. 4).

HCS Instrumentation and Assay Design

There are now several commercially available HCS instruments and application software packages compatible with SCB so no platform

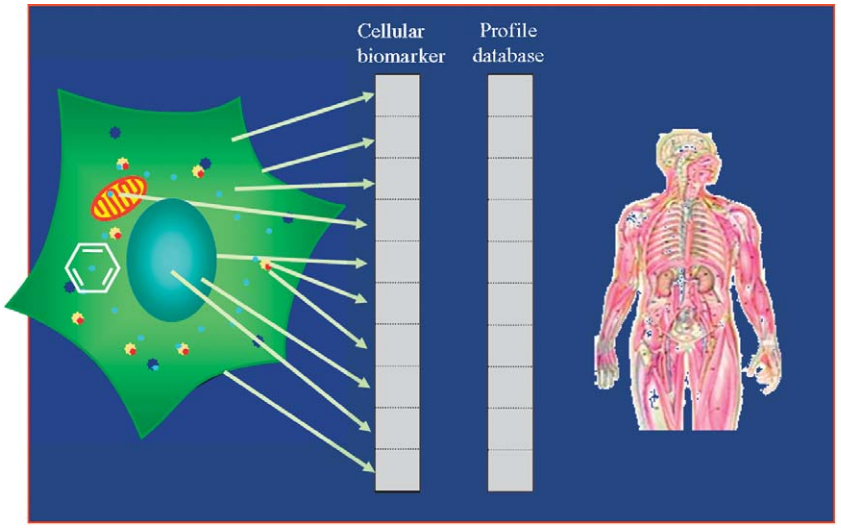

FIG. 3. Systems cell biology profiling involves the selection of the relevant cell types and cellular biomarkers of activity representing the cell as a system and not just a collection of targets and independent pathways. A database of responses can be used as a predictive tool in future profiles.

developments are required (Gough and Johnston, 2006). Although instrumentation and application software for HCS continues to evolve, there is adequate capability today for SCB.

HCS assays, the forerunner of SCB, begin with living cells that are treated with small molecules, bioproducts, and/or physical disruption. Cells are fixed at several time points and are subsequently labeled and read on an HCS reader. These assays reflect cellular activity at a moment in time and are known as ''fixed end point assays.'' Sample preparation methods are available to automate all of these steps, making the assays fast and reproducible. Thus, the fixed end point approach can be a relatively high-throughput screening method, even for SCB. However, the time domain of the biology is limited to one time point. Therefore, the investigator must either create a time series by preparing multiple plates processed over time or initially define the half-time of some cellular process of interest and set the time of fixation accordingly.

Live cell HCS is possible with the integration of an on-board fluidics environmental chamber (Abraham *et al*., 2004a,b; O'Brien and Haskins, 2006; O'Brien *et al*., 2006). This can be accomplished with an integrated platform (for a review of HCS screening platforms, see Gough and

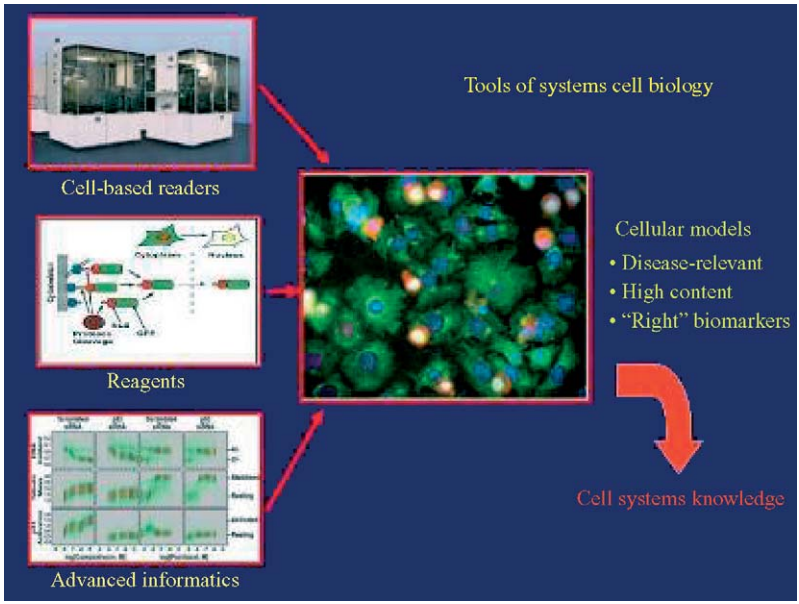

FIG. 4. The key new elements of systems cell biology profiling are the cellular models, advanced reagents, and informatics tools. High-content screening instrumentation and application software are available commercially and are useful now, although they will continue to evolve.

Johnston, 2006) or by applying add ons to a fixed end point reader. Live cell HCS assays or SCB profiles can be based on a single time point measurement using fluorescence-based reagents that are functional in living cells or the assays can be composed of multiple kinetic measurements that are made starting before the experimental treatment and continuing over time. Kinetic assays are useful in defining the half-times of specific biological processes and for critically defining the complex temporal–spatial dynamics of cells and their processes. These kinetics data are extremely valuable in the assay development phase.

Advanced Reagents to Measure and Manipulate Cellular Constituents

Tools that enable the selective and dynamic measurement and manipulation of cellular constituents and responses are important elements of the SCB approach to elucidating disease phenotypes (Table I). Multiple reagents exist for the measurement of up to six distinct parameters based on fluorescence in each well and more parameters can be analyzed by using more than one well to profile the same treatment; this is a key element of the SCB approach (Giuliano *et al*., 2006).

TABLE I
SOME OF THE MAJOR REAGENTS USED TO MEASURE AND MANIPULATE CELLULAR CONSTITUENTS[a]

Selected reagents to measure	Selected reagents to manipulate
Antibodies	Directed siRNA to coding RNA
Fluorescent protein tagging	Random siRNA
Diffusible fluorescent probes	Gene switches
Fluorescent protein biosensors	Caged compounds
Positional biosensors	

[a] For a more extensive review, see Giuliano *et al.* (2006). These reagents are an integral part of the cellular models of disease analyzed by SCB profiling.

There are many choices of reagents to ''measure'' cellular responses (Giuliano *et al.*, 2006). For example, antibodies are readily available for a large number of targets and have been multiplexed with up to four multicolored fluorescent dyes for HCS assays. Panels of antibodies used to quantify SCB biomarkers in multiwell assays enable drug discovery applications from cellular models of disease to cytotoxicity profiling. Fluorescent proteins such as the green fluorescent protein from the jellyfish, as well as alternative versions, can be used to express fluorescently tagged target proteins in cells. Diffusible fluorescent probes are available that can label specific organelles and monitor a variety of cellular metabolites and ions. Some of the diffusible probes can be fixed for end point measurements. Fluorescent protein biosensors can be created to detect numerous protein activities through changes in the fluorescence properties (e.g., intensity, spectral shifts, anisotropy). Positional biosensors have been developed to detect some protein activities through changes in the distribution to different intracellular compartments. It is anticipated that advances in both spectral selection technologies and fluorescence-based reagents will extend the number of cellular parameters that can be selectively measured in the near future.

The number of reagents used to manipulate cell constituents is also growing rapidly (Giuliano *et al.*, 2006). For example, directed siRNAs have become important tools in ''knocking down'' the expression of specific proteins to demonstrate the role of the protein in particular pathways and disease states (Carpenter and Sabatini, 2004; Huppi *et al.*, 2005; Mattick, 2004; Sioud, 2004). The emerging importance of noncoding RNAs such as micro-RNAs in regulating cellular processes has created the opportunity to correlate any RNA, coding or noncoding, with a particular cellular process. Gene switches can be used to regulate the expression of either proteins or siRNAs (Gupta *et al.*, 2004; Karzenowski *et al.*, 2005; Kumar *et al.*, 2004; Ventura *et al.*, 2004). Multiple gene switches in the same cells permit more sophisticated manipulations of cellular processes

(Kumar *et al*., 2004). Caged compounds are molecules whose normal activity is under control of illumination at a specific wavelength of light to release the activity within cells in time and space. Several classes of this type of manipulation reagent have been reviewed (Giuliano *et al*., 2006). SCB applied to cellular models of disease integrates the ''measure and manipulate'' paradigm to better define mechanisms of disease.

Informatics for Systems Cell Biology

Informatics is an essential component of the automated work flow of HCS (Dunlay *et al*., 2006; Taylor *et al*., 2001). Commercial platforms for HCS typically include informatics software for extracting usually up to four channels of specific fluorescence measurements, but also dozens of cellular, morphometric features from each image. In addition, these basic informatics tools also include a database for managing the hundreds of images and millions of data points generated from each plate and client tools for reviewing the images and data resulting from a screen. These tools are adequate for HCS, which usually involves measuring and comparing a few features associated with a particular target (Giuliano *et al*., 1997).

However, although SCB employs the existing toolbox of HCS informatics as a starting point, the requirements for analyzing and mining data extend well beyond the present generation of HCS informatics tools. SCB makes use of panels of molecularly specific features based on fluorescence with broad coverage of cell functions and a combination of multiplexed single cell features, as well as cell population features. In addition to standard HCS informatics, the power of SCB is enhanced by multiplexing of cellular measurements; correlating measurements within single cells; analysis of subpopulations of cells; clustering and pattern matching of multifactorial data sets; and modeling cellular systems. Recent developments in automated cell analysis and systems biology are providing some additional tools to fill this gap, including some efforts to develop more comprehensive data management systems for HCS and SCB (Goldberg *et al*., 2005; Parvin and Callahan, 2002; Parvin *et al*., 2003).

SCB starts with rich, highly multiplexed biomarkers that characterize a broad range of cellular functions (Giuliano *et al*., 2005; Taylor and Giuliano, 2005). Algorithms in the portfolio of bioapplications available from HCS vendors provide some of this information but are often limited in scope to analysis of a particular cell target. There is also an unmet need for combining measurements from different algorithms in order to correlate a diversity of features in single cells. Improved methods of image analysis, such as pattern analysis, can be applied more generally to identify target localization to specific subcellular compartments, such as the nucleus,

endoplasmic reticulum, golgi, and others, while requiring only a single fluorescent channel (Boland and Murphy, 2001; Murphy, 2005). In HCS, it is often sufficient to reduce the cell population response to a single value, such as the mean or median of the measurement. However, it is well known in cell cycle analysis that additional information is available by analyzing the population and segmenting it into subpopulations, which may respond differentially (Bocker *et al*., 1996; Giuliano *et al*., 2004). It has also been shown that direct comparison of population distributions, for example, by making use of the Kolomogorov–Smirnov (KS) test, provides a much more sensitive measure of the shift in a population response than the use of standard statistical parameters, such as the mean or median of the distribution (Giuliano *et al*., 2005; Peacock, 1983).

In addition to the tools required to generate the measurement data needed for systems cell analysis, tools are required for the analysis of these rich data sets. Network modeling methods that have been applied successfully to proteomic analysis (Janes and Lauffenburger, 2006), and cell signaling networks (Araujo and Liotta, 2006) are also of use in extracting more information from SCB data. Eventually, these models will take SCB analysis beyond the value of multiparameter profiles to a tool for diagnostics and use as surrogate end points for therapies (Danna and Nolan, 2006).

One representation of biomarker data resulting from SCB is a feature vector or ''fingerprint'' of the cellular responses across many assays for a specific compound or treatment. These fingerprints can then be mined to identify correlations between response profiles, which provide a means to predict functional effects, including a detailed mode of action. For example, even using basic HCS assays to classify a library of known toxic compounds has shown that compound clusters correlate with known modes of action (O'Brien and Haskins, 2006; O'Brien *et al.*, 2006; Tencza and Sipe, 2004). In another example, about 70,000 compounds have been screened *in vitro* against 60 human cancer cell lines from different organs (Shi *et al*., 2000). Each compound is represented by a vector (e.g., ''fingerprint'') of 60 anticancer activity values, characteristic of the compound activity (Rabow *et al*., 2002). Although not an SCB application, since each assay is only a single measurement, clearly the data model and informatics approaches are similar, and the study illustrates the importance of measurements that span cell lines and cell types as well as cell functions. As a final example of systems cell analysis, compound effects on inflammation have been measured in multiple assays and shown to cluster by mode of action (Berg *et al*., 2006). Clearly some of the basic informatics tools required for SCB are already available and are being applied to related challenges, but it is equally clear that there is a need for more sophisticated tools, especially for the integration of these tools into a platform that will make SCB as accessible and useful as HCS.

Example Systems Cell Biology Profile

Cancer Model Using the Human Lung Carcinoma Cell Line (A549) Expressing Wild-Type p53

In anticancer drug discovery, the myriad of cellular events regulated by the p53 tumor suppressor protein present an invaluable set of potential HCS targets (Giuliano *et al*., 2004). That the mutation or deletion of p53 protein in many cancer cell types is often a determining factor in the chemotherapeutic outcome (Blagosklonny and Pardee, 2001; Borbe *et al*., 1999; Osaki *et al*., 2000) emphasizes the need for information on the cellular and molecular activities regulated by the p53 protein and the effect drugs have on these interrelationships. Thus, there is a need for new approaches to rapidly and precisely modulate components of the p53 signaling pathway, as well as complementary SCB methods to dissect the network of cellular and molecular activities regulated by this important tumor suppressor protein.

Therefore, the goal is to create a cell model where different components of the p53 pathway are manipulated so that the expression levels can be regulated to monitor the impact on the downstream cellular events and any off-target and/or compensatory effects. Giuliano *et al*. (2004) reported on the effects that p53 ''knockdown'' with RNAi has on the response of A549 cells to multiple anticancer agents. In this new example, p53 pathway regulation (Fig. 5) was ''manipulated'' by controlling the expression level of the p53-modulating protein HDM2 with a gene switch. A profile of the cellular response was built by ''measuring'' a panel of SCB biomarker features within the p53 pathway or other possible events either downstream (e.g., apoptosis, cell growth and division, cytoskeletal reorganization, and organelle function) or upstream (e.g., DNA damage) (Fig. 5). A single vector RheoSwitch (RheoGene; Norristown, PA) was used to regulate expression of a fluorescently labeled HDM2 protein in A549 cells using multiple concentrations of inducer molecule. Figure 6 shows that the expression level of fluorescent HDM2 was dependent on the concentration of the inducer molecule used to treat the cells as well as the total time of induction.

A fixed end point SCB assay was designed to generate a profile that elucidates the cell system response to upregulating HDM2 in the absence of any other manipulation. In addition to the quantification of HDM2 expression in each cell, Table II shows the additional cellular function biomarkers measured to build the profile. The measurements were chosen to look both at specific downstream activities and more general cellular processes. Example images of cells in which SCB reagents were used to measure and manipulate activity are shown in Fig. 7.

The considerable number of SCB measurements made after treatment of cells with multiple inducer molecule concentrations produced a large

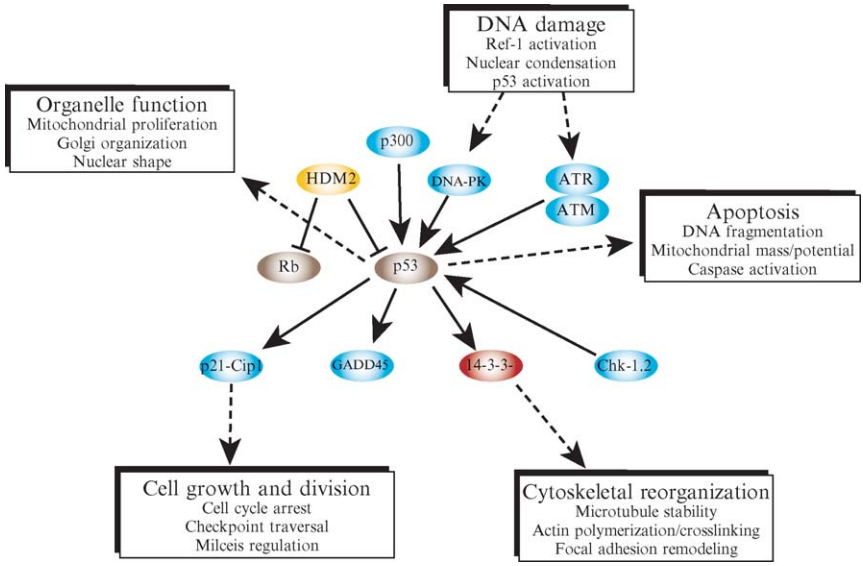

FIG. 5. Targeting a single protein activity for drug development, such as the tumor suppressor protein p53 in cancer (center), is challenging. Within the cell, this target protein and signaling pathway is part of an integrated and interacting network. Therefore, an SCB profiling approach is required to define efficacy, as well as potential off-target toxicity and compensatory activities. Thus, a cell model can be constructed where specific components of the p53 pathway can have the expression levels regulated by gene switches, short interfering RNAs, or combinations of both, and specific protein–protein interactions, molecular processes, and phenotypic responses can be measured as part of the SCB profile.

and complex SCB profile. As shown before, automated hierarchical clustering analysis based on KS population dissimilarity analysis provided an ideal first step in deciphering profiling data (Giuliano *et al.*, 2004, 2005). Figure 8 shows the clustered profiling results in the form of a heat map. Cell population responses that were not significantly different from control populations (e.g., KS <0.2) were encoded in the heat map using blue and black shades. Significant cell population responses induced by HDM2 (e.g., KS >0.2) were encoded in the heat map using yellow and red shades. In the overall response, there were three relatively distinct clusters: high responders (left), low responders (center), and intermediate responders (right).

It was expected (Fig. 8; right arrow) that the elevation of HDM2 would decrease the cellular level of p53, which did occur. However, simply raising the cellular concentration of HDM2 about five times had unexpected effects, such as stabilizing microtubules, increasing PI3 kinase activation, and increasing the level of DNA repair and redox of DNA-binding

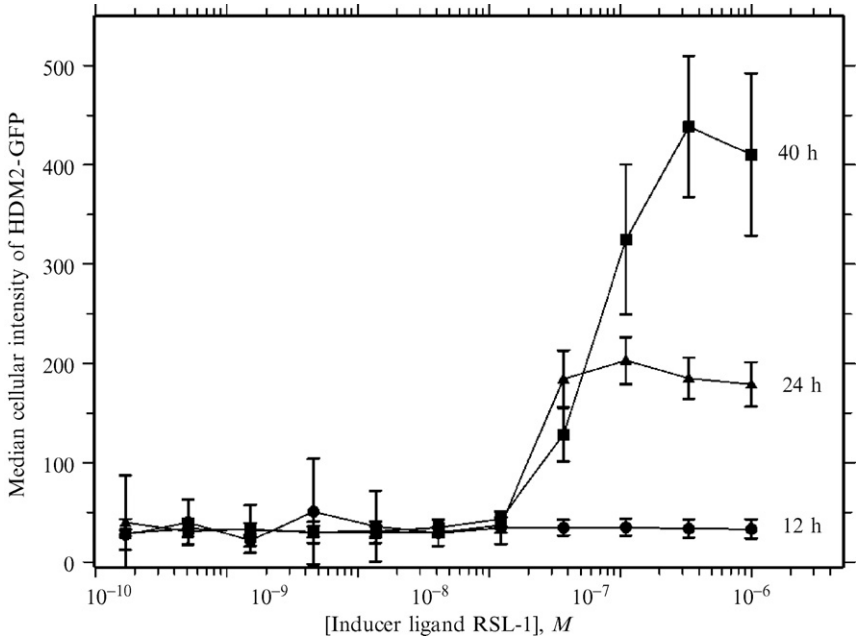

FIG. 6. Regulation of the expression level of a fluorescent-hdm2 under the control of a gene switch showing the expression levels after 12, 24, and 40 h of stimulation with the inducer.

proteins. To enable the building of connections between the cellular processes measured in the profile, we relied on the use of cell maps to visualize how two HCS feature measurements were related in the same cells under different conditions (Giuliano *et al.*, 2004, 2005). In this example, heat map data in Fig. 8 showed that the changes in phosphohistone H3 levels and the intracellular distribution of APE/Ref-1 as a function of HDM2 expression level were similar enough for them to be clustered together. Using the cell maps shown in Fig. 9, we could demonstrate the relationship between these two markers of cell cycle regulation and the DNA damage–oxidative stress response. The cell maps show that as the expression level of HDM2 increased in response to RSL-1, the phosphohistone H3 level decreased homogeneously to a lower level (vertical shift down) while the predominant nuclear localization of APE/Ref-1 increased (horizontal shift right) consistent with some common regulatory unit that coordinates the two processes. Thus, this brief example demonstrates the potential of an SCB approach to build new knowledge on the regulatory connections between cellular processes through the selective manipulation and measurement of cellular constituents.

TABLE II
FEATURES MEASURED IN THE SCB PROFILE AND THEIR ROLES IN THE REGULATION OF CELLULAR SYSTEMS

Measurement	Abbreviation for heat map	Cellular system regulation
Total nuclear intensity and variation of Hoechst 33342 label	dna, nu4	DNA content: cell cycle arrest DNA degradation
Projected nuclear area and shape defined by Hoechst 33342 label	nu1, nu2, nu3	Nuclear condensation or fragmentation
Nuclear intensity of phosphohistone H3 label	ph3	G_2/M cell cycle transition
Cytoplasmic intensity of α-tubulin label after detergent extraction	tub	Microtubule stability
Cytolasmic vinculin label at substrate	vin	Focal adhesion remodeling
Nuclear/cytoplasm ratio of p53 label	p53	Tumor suppressor involved in DNA damage response. HDM2 inhibits p53 upon complex formation
Nuclear/cytoplasm ratio of p21 label	p21	Tumor suppressor inhibitor of cell cycle progression. Active p53 upregulates p21 expression
Cytoplasmic intensity of phospho-Akt label	akt	Activated Akt promotes cell survival by causing the inhibition of apoptosis targets
Nuclear intensity of APE/Ref-1 label	ape	APE/Ref-1 is activated upon DNA damage or oxidative stress
Cytoplasmic level and organization of 14-3-3 protein	x43	14-3-3 proteins regulate cell survival, apoptosis, proliferation, and checkpoint control through phosphorylation-dependent protein–protein interactions
Organization and intensity of golgi and mitochondrial organelles	gol, mit	Organelles such as mitochondria play specific roles in many cellular processes, including apoptosis

Summary and Conclusions

The development of HCS was a major step in improving the drug discovery and development process, as well as creating a complementary high-throughput method for research imaging microscopy. The integrated use of HCS, as well as semiautomated microscopy and high-performance research imaging techniques, will continue to be a powerful set of tools used to define cellular functions of cell constituents and whole cells. SCB takes advantage of basic HCS platforms, while creating a cell ''systems'' response instead of focusing on one target or a few related cell factors

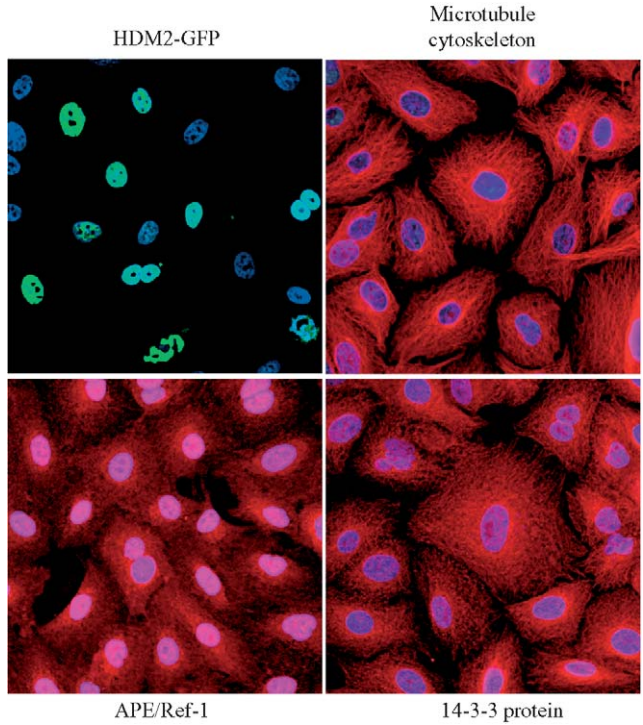

FIG. 7. Example images of cellular feature labeling for SCB analysis. HDM2-GFP is shown with expressing cells (green) overlaid with Hoechst 33342-labeled nuclei (blue). An antibody to α-tubulin was used to measure changes in the stability of the microtubule cytoskeleton. Antibodies against APE/Ref-1 labeled the dual function enzyme that takes part in DNA repair as well as regulating the redox state of DNA-binding proteins. Changes in the nuclear distribution of APE/Ref-1 were used to measure the extent of DNA-repair pathway activation. The 14-3-3 protein, through its association with the cytoskeleton, regulates the localization and activity of other proteins (e.g., Cdc25). The phosphorylation level of the cytoplasmically localized 14-3-3 protein was used as a measure of its protein–protein interaction activity.

in simple assays. More advanced classes of reagents that measure and manipulate cellular constituents, as well as more integrated and powerful informatics tools, will be the driving forces of SCB.

Prospectus

Systems cell biology will be applied to the whole continuum of the drug discovery and development process. Cellular models of disease are being created to better understand the selected targets, along with the systems

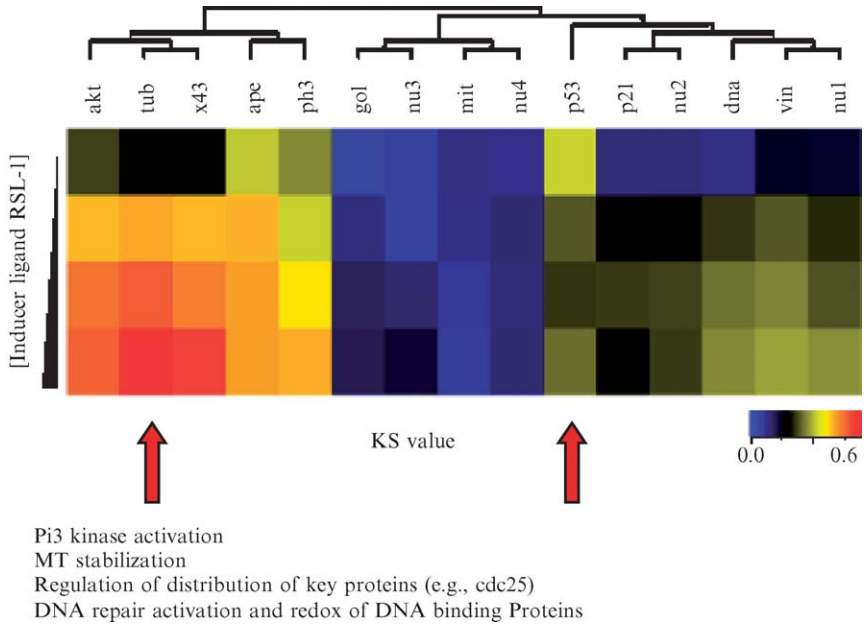

FIG. 8. An SCB profile of a cellular model of cancer where a human lung cancer cell line was transduced with a fluorescently tagged protein, HDM2, a modulator of p53 that was regulated with the RheoSwitch gene switch. The level of expression of this tagged HDM2 was regulated by increasing the concentration of the inducer ligand (increased concentration of expression on the *y* axis). This allowed the ''manipulation'' of HDM2 levels in cells. The cellular systems response was monitored by measuring the KS statistics of 15 distinct cellular parameters (see abbreviations of parameters on the top with clustering) in a fixed end-point assay using high-content screening. The KS statistics were color coded to show those cellular parameters that did not change (blue), changed moderately (black), and changed extensively (yellow-red).

response of the cell models to optimize selection of lead compounds. In addition, cytotoxicity profiling, using the SCB approach, will provide a critical ''filter'' for prioritizing lead compounds and could evolve into a predictive tool. Patient sample profiling at the cell and tissue levels will become an important approach in defining patient subpopulations for more focused clinical trials and could also become a valuable cell-based diagnostic method. SCB will also have a major impact on basic biomedical research where complex processes such as the differentiation of stem cells are clearly a systems challenge. A major effort will be focused on creating powerful reagents that measure and manipulate specific biochemical/molecular events in a reversible manner, such as protein–protein

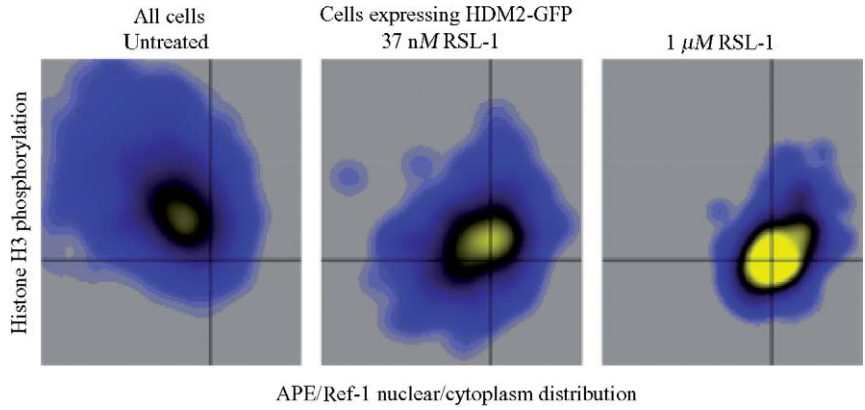

FIG. 9. Cell maps demonstrate the relationships between cellular processes within the same cells. Cell maps were prepared from SCB profiling data as described (Giuliano *et al.*, 2004, 2005). HCS measurements of histone H3 phosphorylation and APE/Ref-1 intracellular distribution made in the same cells were plotted against each other at various HDM2 expression levels that were induced with RSL-1. The fiduciary lines, which are constant in each map, show that cells exhibiting decreased phosphohistone H3 levels were, in large part, the same cells that exhibited predominantly nuclear-localized APE/Ref-1.

interactions in relation to other dynamic cellular processes. In addition, a new generation of informatics tools will allow understanding of the complex systems responses of cells to various manipulations.

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