Probing cellular systems with chemistry

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Chemical and genetic approaches are extensively used to probe living systems at the subcellular level and to advance therapeutic target discovery. The 2006 American Society for Cell Biology meeting highlighted the growing importance of chemical biology approaches in cell biology research.

Overlooking one of the world's finest natural harbors, the San Diego Convention Center played host to the 46th annual meeting of the American Society for Cell Biology (ASCB) on December 9–13, 2006. More than 6,000 scientists and over 3,500 presentations of scientific research spanned traditional cell biology topics and extended to include cell biology of diseases, biosensors and computational applications in cell biology. The research presented below underscores the growing role chemical biology is playing in advancing cell biology and drug discovery (**Fig. 1**) and presents some of the main challenges currently facing chemical biologists and chemical biology research.

The use of chemical genetic screening and activity-based assays was a common thread in many ASCB presentations. The goal of such techniques is to identify small molecules that will allow scientists to address a host of interesting questions in cell biology. In many cases, RNA interference (RNAi) approaches provided an important complement to these small-molecule approaches. Advances in cellular imaging methods were also featured prominently at the meeting and comprise another area in which chemical biology is having an impact on the study of cellular processes.

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Navigating cell division space

Target identification of small-molecule hits remains the primary bottleneck in chemical genetic screens. The Mitchison lab (Harvard) described RNAi as an alternative approach for investigating the mechanisms of action of small molecules that perturb cell division and uncovering their targets. They set up a genome-wide RNAi screen in HeLa H2B-GFP cells (cells stably expressing histone 2B fused to green fluorescent protein) to search for proteins that sensitize cells to a high-affinity Eg5 (a motor protein also known as kinesin-5) inhibitor that is a clinical candidate and that works by the same mechanism as monastrol[1] (**1**; **Fig. 2**). Their aim was to identify proteins involved in the cellular response to kinesin-5 inhibition and to understand the reasons behind cell sensitization. The scope of this work extends to anticancer drug discovery. New cell-division targets with improved therapeutic indices are scarce, and uncovering proteins that are required for cell division in cancer cells but not in normal cells is one route to effective new anticancer therapeutics.

The Vale lab (Howard Hughes Medical Institute, University of California, San Francisco) performed a whole-genome RNAi screen in *Drosophila melanogaster* S2 cells to search for novel genes that regulate the mitotic spindle assembly checkpoint. Using high-throughput imaging combined with automated quantitative image analysis, they identified ~220 genes that are involved in aligning chromosomes at the metaphase plate, controlling spindle length or shape, localizing γ-tubulin, and generating microtubules. Of these genes, 69 proved to be completely new—that is, they are unannotated genes with previously

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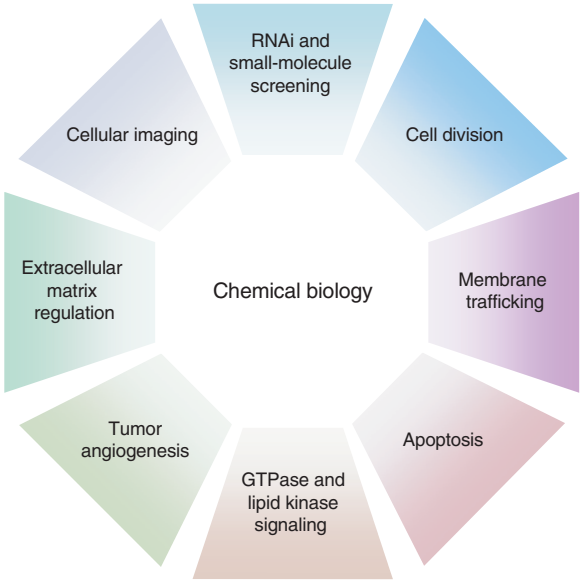

Figure 1 Chemical biology topics featured at the ASCB meeting.

unknown functions. In a secondary assay, they used GFP tagging to investigate the subcellular localization and dynamics of these genes. This study is a comprehensive survey of all the proteins that contribute to spindle assembly in *D. melanogaster*. The results underscore the power of RNAi screens in revealing new mitotic regulators and in identifying potential targets for cancer therapy. The complication in conducting these screens does not lie in the compilation of gene lists but rather in characterizing the functions of novel gene hits to ensure specificity and uniqueness—a task that was elegantly achieved by the Vale lab.

To explore the utility of 'privileged' chemical scaffolds, the Kapoor group (Rockefeller) carried out a phenotypic cell-based screen with a small collection of ~100 diaminopyrimidines (DAPs) to uncover small-molecule probes that target proteins involved in regulating mitosis[2]. They identified compounds that induce changes in spindle geometry, chromosome positioning and mitotic index. One inhibitor, named DAP-81 (**2**; **Fig. 2**), induces monopolar mitotic spindles and targets Polo-like kinases *in vitro*. These inhibitors are being pursued as tools for revealing new insights into the different roles Polo-like kinases have during the cell cycle, including their effects on microtubule dynamics with high temporal control and on regulation of assembly and maintenance of bipolar mitotic spindles.

Chemical dissection of intracellular signaling

RhoGTPases are molecular hubs that relay signaling cascades at the cytoplasmic and nuclear levels and that are involved in regulating many aspects of cancer development. The study of RhoGTPase signaling pathways would be greatly aided by the development of specific small-molecule inhibitors that target the proto-oncogenic exchange factors (RhoGEFs) that activate RhoGTPases. By using the Yeast Exchange Assay method[3], the Blangy group (National Council for Scientific Research, France) screened a 3,500-member chemical compound library and identified a potential inhibitor of Trio-GEFD1, an exchange factor of the RhoGTPase RhoG. This small molecule seems to be specific to Trio-GEFD1 and hence is being pursued as a tool for dissecting the complex mechanisms that control RhoGTPase activation[4,5]. These studies may also serve as a stepping stone into the development of drug-like molecules for this challenging class of potential anticancer targets.

Identifying small-molecule leads for drug discovery was another important theme at the ASCB meeting. With a set drug target in mind, the Wang group, in collaboration with the Harran and De Brabander groups (University of Texas Southwestern Medical Center at Dallas), designed a small molecule (**3**; **Fig. 2**) that mimics the function of Smac, a proapoptotic protein. Once Smac is released from mitochondria, it binds and masks the activity of a family of inhibitor of apoptosis (IAP) proteins in human cells, thereby promoting apoptosis. However, many cancer cells survive by blocking the release of Smac via elevated concentrations of antiapoptotic Bcl-2 and related proteins. Wang showed that the identified Smac mimetic binds IAP proteins directly and induces apoptosis in over 80% of cancer cell lines from a variety of tissue origins with no effects on normal fibroblasts and endothelial cells[6]. The use of Smac-mimetic molecules should find applications in studying IAP-dependent apoptosis signaling mechanisms and in developing new anticancer agents.

Mapping membrane trafficking and lipid signaling

Presently, there is a need for chemical tools that will allow the study of membrane trafficking events with greater flexibility than is possible with genetic techniques. Because membrane trafficking is a highly dynamic process inside cells, small molecules are very helpful in that they act on a time scale commensurate with the timing of trafficking events. The Payne group (University of California, Los Angeles) conducted an elegant chemical synthetic lethality screen in yeast to identify small molecules that mimic phenotypes of deletions of subunits of the clathrin adaptor AP-1, an important component in vesicle trafficking between the trans-Golgi network and endosomes. These piperazinyl-based small molecules block traffic specifically between the trans-Golgi network and endosomes and do not appear to perturb other membrane trafficking pathways. Because protein trafficking mechanisms are conserved between yeast and humans, these molecules can be used to study the mechanisms of clathrin-mediated protein transport at the Golgi complex.

Lipid kinases have a significant role both in normal signaling pathways such as the insulin pathway and in disease pathways such as cancer. Phosphatidylinositol-3-OH kinase (PI(3)K) is one of the key lipid signaling kinases, and it exists in three isoforms that interact with five different adaptor proteins. There is no biochemical specificity between the adaptors and the isoforms (overall there are 15 interaction possibilities), and the kinase ATP binding site is absolutely conserved among the different isoforms, which makes dissecting the biological roles of different PI(3)K-adaptor interactions very challenging. Small molecules are ideal tools for this system because they can be specific and fast-acting, thereby achieving selectivity among the various PI(3)K isoforms. Using a traditional pharmacological approach, the Shokat group (University of California, San Francisco) identified selective small-molecule inhibitors of the different PI(3)K isoforms as probes of lipid kinase signaling[7–9]. This chemical matrix of small molecules was used to demonstrate that the PI(3)Kα isoform (known as p110α) is responsible for mediating insulin-response

signaling in cultured cells; the group also found that perturbing its activity abolishes the effects of insulin treatment *in vivo*.

Venturing outside the cell

Remodeling of the extracellular matrix (ECM) has an important role in numerous human diseases. Investigating the molecular factors that regulate the mechanisms of ECM remodeling is critical to our understanding of tumorigenesis. Using a small-molecule approach, the Whitman group (Harvard) set forth to identify the target and mode of action of halofuginone (**4**; **Fig. 2**), a potent small-molecule regulator of ECM gene expression. They synthesized halofuginone analogs in order to gain insight into their structure-activity relationships, and then they immobilized halofuginone on a polymer for affinity purification. They identified a complex of nuclear chromatin remodeling factors as the interacting target of halofuginone and probed the domains involved in the interaction. These studies are an important step toward developing selective therapeutic agents against ECM disorders and will aid in exploring ECM biology.

One of the cutting-edge technologies that is driving cell biology research to a new frontier is the ability to regiospecifically control the physical and chemical microenvironments of cells as a means to address key biological questions. Though many lithographic methods have been developed to control the spatial relationships between adherent cells, most of them cannot be used to redefine microenvironments in real time to confine developing or motile cells. To address this issue, the Shear lab (University of Texas, Austin) used two-photon photochemistry to microfabricate in real time high-resolution matrices (less than 1 μm in feature dimensions) that are suitable for monitoring cell growth and development[10,11]. By manipulating the topographic and chemical microenvironment of cultured cells, they were able to confine actively motile cells such as *Escherichia coli*[12], and they also showed directed neurite outgrowth *in situ*. These results illustrate the potential to trap and manipulate cells of interest using three-dimensional microfabrication.

In an effort to understand how human tumor cell invasion and angiogenesis occur, the Klemke lab (University of California, San Diego) developed a tumor xenograft model in transgenic zebrafish. By taking advantage of the optical transparency of zebrafish tissue, the group used quantitative high-resolution multicolor three-dimensional imaging to directly visualize tumor growth, angiogenesis and invasion in real time at the subcellular level. Using this cancer model, the group studied the effects of two kinase inhibitors, SU5416 (**5**; an inhibitor of the proangiogenic vascular endothelial growth factor receptor) and PP1 (**6**; an inhibitor of the oncogenic Src tyrosine kinase), on tumor progression (**Fig. 2**). Their data reveal that the former compound causes shrinkage of tumor cells and regression of tumor-induced vasculature whereas the latter mainly perturbs tumor cell survival. The development of a zebrafish cancer model is an important step in illuminating tumor cell invasion and angiogenesis processes, because existing cancer models do not allow for such direct high-resolution visualization *in vivo*. This model also has great potential to provide a cost-effective means for pharmacological testing of the effects of small molecules on tumor cell growth and angiogenesis.

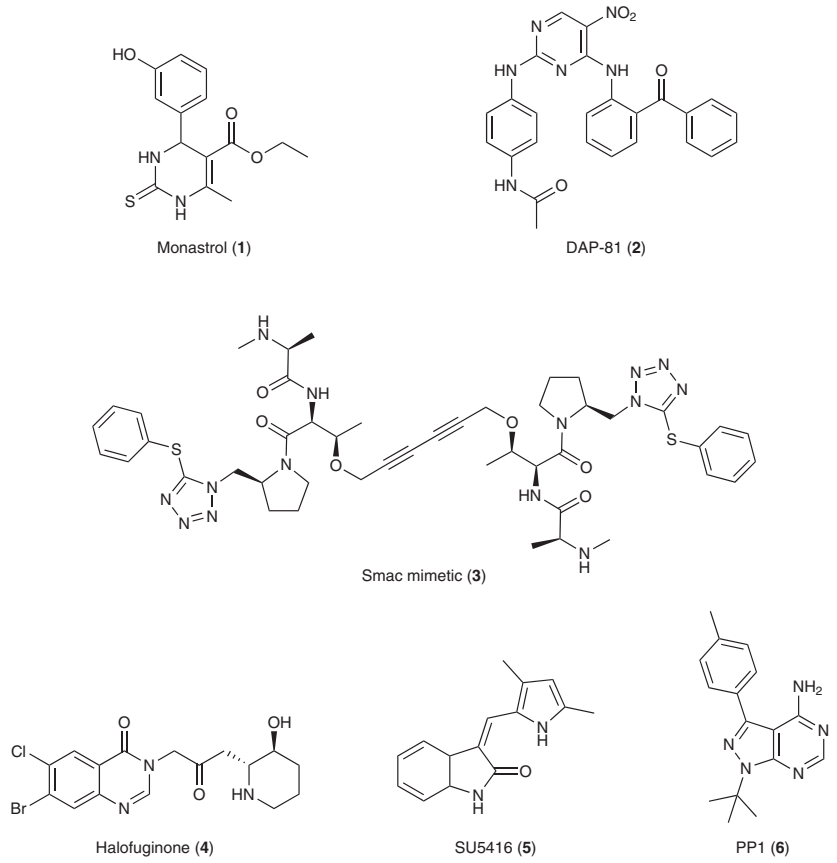

Figure 2 Small molecules used for exploring biology and for therapeutic target discovery. Monastrol (**1**) inhibits the mitotic kinesin Eg5 (also known as kinesin-5 or KSP); DAP-81 (**2**) modulates Polo-like kinase activity *in vitro*; Smac mimetic (**3**) binds IAP and induces apoptosis in human cancer cells via caspase activation; halofuginone (**4**) regulates extracellular matrix gene expression; SU5416 (**5**) inhibits vascular endothelial growth factor receptor tyrosine kinase; and PP1 (**6**) inhibits Src tyrosine kinases.

Biosensor applications and new imaging technologies

As interest grows in studying protein-protein interactions in living cells, there is need for new imaging tools and optical reporters that can examine interactions at multiple levels. The Tsien group (Howard Hughes Medical Institute, University of California, San Diego) developed a new method as a longer-range alternative to fluorescence resonance energy transfer (FRET) for imaging larger protein complexes inside cells. The method uses singlet oxygen diffusion to a GFP-based reporter and allows detection of tens of nanometers of spatial proximities (an improvement over the FRET method, which detects up to 100 Å in donor-acceptor spatial separation). The Tsien group has also developed a new zinc-based polyhistidine dye pair (HisZiFit) that is orthogonal (that is, chemically complementary) to previously reported tetracysteine biarsenicals[13]. They also reported on a method named TimeSTAMP (time-specific tagging and age measurement of proteins), which relies on sudden pharmacological inhibition of a genetically fused *cis*-acting protease to allow high-resolution retrospective visualization of new synaptic protein synthesis under various stimulation conditions.

Altogether, these developments should further enable the use of chemical tools to enlighten complex cell biology.

The Danuser (Scripps Institute) and Hahn (University of North Carolina) labs joined efforts to combine computational and multiplex imaging approaches to uncover the spatiotemporal characteristics of RhoGTPase signaling. Using this approach to reconstruct the timing of activation of the small GTPases Rac1, RhoA and Cdc42 during cell protrusion, they found that RhoA activation occurs simultaneously with protrusion initiation, whereas Cdc42 activation is delayed and is followed by Rac1 activation. The Danuser lab also reported on a software package to be used in combination with phase-contrast imaging that allows for time-resolved quantitative analysis of the spatial dynamics of cell movement. This is especially useful for studying *in vitro* cancer metastasis models that involve loss of cell-cell junctions upon hepatocyte growth factor stimulation and subsequent scattering of cells in space. This live-cell microscopy-based approach also enables screening of small molecules against tumor metastasis and facilitates the evaluation of their effects on migration regulation and cell-cell adhesion.

Perspectives

An overall unifying theme of the 2006 ASCB meeting was to promote interdisciplinary science and to encourage creativity and innovation among scientists. The continual merging of distinct yet overlapping disciplines in chemistry and biology is driving progress in scientific discoveries. The ASCB meeting highlighted chemical biology approaches that are being pursued in cutting-edge cell biology and drug discovery. The research presented at this meeting engendered in its attendees a powerful sense of optimism for the future of chemical biology.

COMPETING INTERESTS STATEMENT
The author declares that he has no competing financial interests.

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