

RECENT ADVANCES IN CHEMICAL APPROACHES TO THE STUDY OF BIOLOGICAL SYSTEMS

Michael A. Shogren-Knaak¹, Peter J. Alaimo¹,
and Kevan M. Shokat^{1,2}

¹*Department of Cellular and Molecular Pharmacology, University of California,
San Francisco, California 94143-0450*

²*Department of Chemistry, University of California, Berkeley, California 94720;
e-mail: alaimo@cmp.ucsf.edu, shokat@cmp.ucsf.edu*

Key Words chemical genetics, combinatorial chemistry, enzyme inhibitors,
protein-protein interactions, unnatural biopolymers

■ **Abstract** A number of novel chemical methods for studying biological systems have recently been developed that provide a means of addressing biological questions not easily studied with other techniques. In this review, examples that highlight the development and use of such chemical approaches are discussed. Specifically, strategies for modulating protein activity or protein-protein interactions using small molecules are presented. In addition, methods for generating and utilizing novel biomolecules (proteins, oligonucleotides, oligosaccharides, and second messengers) are examined.

CONTENTS

INTRODUCTION	405
PERTURBING BIOLOGICAL FUNCTION WITH SMALL MOLECULES	408
Influencing Protein Activity	408
Influencing Protein-Protein Interactions	414
SYNTHETIC APPROACHES TO NOVEL BIOMOLECULES	418
Proteins	418
Oligonucleotides	423
Oligosaccharides	424
Second Messengers	426
CONCLUSIONS AND FUTURE DIRECTIONS	427

INTRODUCTION

Chemistry has always been a central component in studies of biological systems. Yet, chemical approaches to probing biology using either small, drug-like molecules ($M_r < 700$) or synthetic macromolecules (e.g., proteins, oligonucleotides, or

oligosaccharides) have lagged behind genetic methodologies. The apparent reasons for this discrepancy are the high specificity and generalizability of genetics, that is, within an organism any single nucleotide can be changed in a background of three billion base pairs using the same technique. These advantages make both forward genetics, where random mutational events allow for the isolation of specific genes, and reverse genetics, where targeted mutations in a gene can help clarify the cellular role of a protein, exceedingly powerful tools.

In general, using small organic molecules or chemically synthesized biomolecules to recapitulate aspects of either forward or reverse genetics is challenging; it is difficult to identify small molecules that interact with a desired target with both high affinity and specificity. Generating high-affinity ligands is difficult because interactions between small molecules and proteins tend to be governed by the sum of many weak forces that are often sensitive to small perturbations. Thus, they are difficult to model and predict. Achieving specificity is challenging because of the wide range of undesirable interactions that become possible when a cell contains thousands of proteins, many with a high degree of homology. Additionally, synthesis of each small molecule or macromolecule can present a unique challenge, limiting the generality. Given this dilemma, why make chemical probes of biological systems when genetic approaches tend to be more specific and can be applied more generally?

Although genetic techniques are very powerful, there are circumstances where they are limited (Figure 1). The ideal technique for perturbing biological function is one that is highly specific, where one and only one gene product is changed; fast acting, responding on a timescale faster than the process of interest; tunable, allowing for graded phenotypes; and conditional, capable of being reversibly turned on and off at any stage of the organism's life-cycle. Gene knockouts afford exquisite specificity but are observed on the developmental timescale and are not highly tunable (except for $-/-$, $-/+$, $+/+$). Knockouts also can be problematic for genes for which compensation can occur during development and for those that are essential.

Conditional alleles can be used to address these problems but have inherent limitations of their own. Temperature-sensitive (TS) alleles are typically fast-acting conditional alleles, inducible on the minute timescale, but they are not tunable. More importantly, when TS mutants are used, the temperature shift required can induce changes to off-target proteins, such as the induction of heat-shock proteins. Moreover, the significance of the inactivation of the protein target itself may be poorly defined. Many proteins have multiple domains, where each domain contributes to different aspects of protein function. Temperature-induced inactivation can result in unfolding of some or all of these distinct functional domains, complicating analysis. Regulation of transcription, for example, using tetracycline-induced expression, is tunable and often has the high specificity of genetic knockouts. However, the tunability and timescale of induction are limited because this method acts at the transcriptional level. In contrast to genetic methods, small drug-like molecules can perturb protein activity in a manner that is

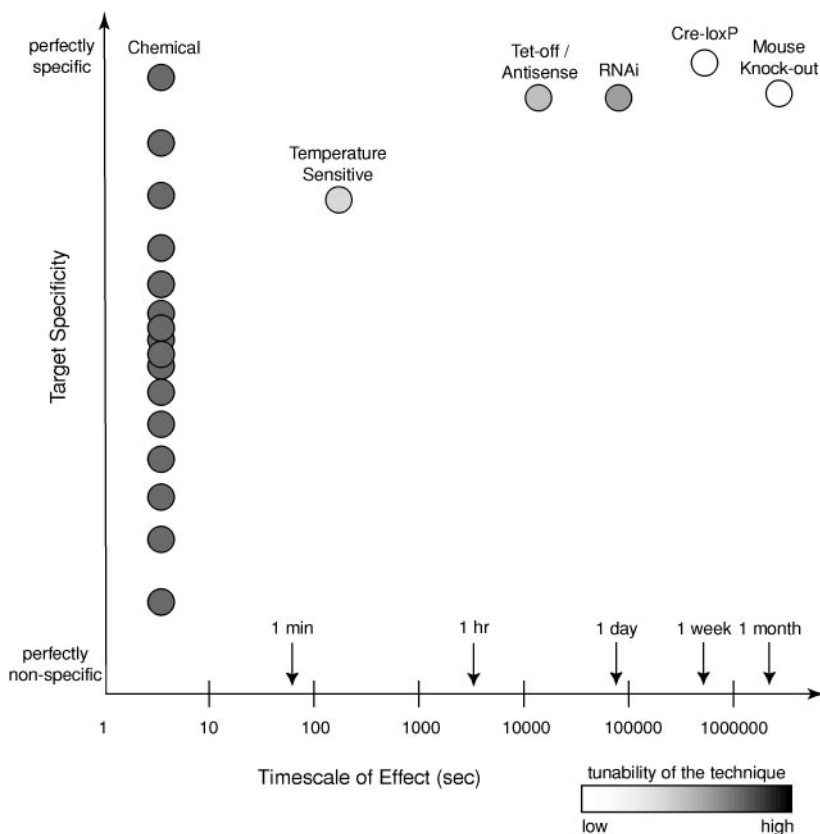


Figure 1 Qualitative comparison of various techniques for inactivating a protein of interest, where it is assumed that the technique can be applied successfully. Techniques are plotted as a function of timescale required to observe a phenotype, specificity for the protein of interest, and tunability of the method (i.e., the ability to observe graded phenotypes via partial modulation of the protein function). Highest specificity is toward the top, optimal (shortest) timescale is toward the left, and greatest tunability is shaded gray. For each genetic technique, a single point is shown to represent the average approximate timescale, specificity, and tunability. For the small-molecule approach, multiple points are depicted to highlight the potential variability in the specificity of small-molecule modulators.

highly specific, rapid (i.e., often operating at a diffusion-limited timescale), tunable (i.e., by varying concentration), and conditional (Figure 1).

An additional limitation of genetics is the range of molecules that are readily modulated. Using reverse genetics, it is possible to introduce specific DNA, RNA, and protein biomolecules into the cell. However, many biomolecules, such as posttranslationally modified proteins and cellular metabolites, require the action of multiple enzymes and may not be readily produced, for example, glycoproteins.

Additionally, unnatural derivatives of biomolecules cannot be easily generated using genetic techniques, limiting their potential for addressing structural and functional questions. Here, chemical synthesis can expand the types of biomolecules that can be generated and studied.

Given the promise of chemical biology, the use of chemical approaches for addressing biological problems, how is it possible to overcome the traditional problems of chemical approaches? In other words, can chemical methods be devised that effectively target a desired biological process with high specificity yet be sufficiently synthetically tractable to be of general use? This review focuses on recent developments in chemical biology that address this question. Current methods for finding small molecules capable of perturbing biological function are described, with an emphasis on their use to influence protein activity and protein-protein interactions. Additionally, new techniques for generating and using novel proteins, oligonucleotides, oligosaccharides, and second messengers are highlighted.

Chemical biology is a rapidly expanding field of inquiry and a review of this size cannot adequately cover its breadth. Thus we have focused on methods that perturb cell function and have not discussed chemical methods for visualizing cell processes (Zacharias et al. 2000). Additionally, we have highlighted a range of recently established and promising chemical approaches to cell biology that are potentially both highly selective and generally applicable. When possible, we describe a specific biological problem that has been addressed using a given approach as an example that illustrates the method. We hope this review will serve the reader as a starting point for further exploration of the recent developments in chemical biology.

PERTURBING BIOLOGICAL FUNCTION WITH SMALL MOLECULES

Influencing Protein Activity

Enzymes and receptors have been extensively studied because they play a central role in biological pathways and are obvious targets for developing small-molecule modulators (e.g., inhibitors, agonists, antagonists) since most have evolved to interact strongly and specifically with naturally occurring small molecules such as nucleotides, lipids, steroid hormones, and prostaglandins. Once found, small-molecule modulators are powerful tools for probing biological processes, allowing rapid, reversible, tunable, and conditional perturbation of a protein target. Currently, the most common method for identifying such molecules is to choose a protein target and a collection of compounds and then to screen the compounds for potent and selective modulators.

Historically, the screened collection of compounds has consisted of natural products, taking advantage of natural biodiversity. However, natural products are not always ideal small-molecule drugs because they can be difficult both to isolate and synthesize in large quantities. Furthermore, there are not natural products that

modulate each protein in a given pathway. To complement the use of natural product libraries, methods to synthesize combinatorial libraries of drug-like compounds have recently been developed.

Several strategies are available for the combinatorial synthesis of small-molecule libraries. One strategy pursued by many laboratories and drug companies is to start with a structural scaffold that constitutes the core of a known substrate or inhibitor (Ellman 1996, Thompson & Ellman 1996). Chemical reactions are then developed and used to elaborate this scaffold, thus producing tens to thousands of molecules that contain sterically and/or electronically diverse appendages at various positions. Because it is not always clear which scaffold will be most effective toward any particular target, a second alternative, termed diversity-oriented organic synthesis, can be used to generate small-molecule libraries that are not based on a particular scaffold, thus enabling screening of compounds of greater structural variety (Schreiber 2000). A third approach involves appropriating and modifying the endogenous biosynthetic machinery of microorganisms devoted to secondary metabolite synthesis. This method is most attractive for generating molecules *in vivo* such as polyketides (Pfeifer et al. 2001) and oligosaccharides (Yamase et al. 2000) that are especially difficult to synthesize using traditional synthetic methods. Currently, it is not clear which method is best suited for finding small-molecule inhibitors for protein targets, and it likely depends on the ultimate goal. Nonetheless, the increasing availability of chemical libraries increases the possibility of finding useful lead compounds. Once a lead compound is found, optimization usually is required to improve selectivity and affinity for the target. Optimization often involves using either structural or mechanistic information about the protein target or identifying important molecular features by correlating changes in the inhibitory activity with changes in the molecular structure. Based on these data, a new set of molecules can then be generated using either traditional synthetic methods or combinatorial chemistry. An interesting alternative approach to facilitate optimization that does not require structural or mechanistic information about the target was recently explored by Ellman and co-workers, where the target itself is used to guide the synthesis of the small-molecule library (Maly et al. 2000). In this method, weakly binding molecules are cross-linked and screened to find potent and selective bivalent inhibitors.

In general, synthesis, screening, and optimization have been used for developing therapeutics. However, increasing effort has been devoted to applying these techniques to identify new chemical probes of biological processes. Two examples that both demonstrate and extend this basic strategy are seen in recent efforts to develop forward chemical genetics and methods that combine chemistry with genetics.

FORWARD CHEMICAL GENETICS In forward chemical genetics, the goal is to find proteins that are sensitive to small-molecule inhibition. Like forward genetics, this method can identify the proteins involved in cellular processes. Additionally, it provides new molecular tools to influence the activity of their targets. Forward chemical genetics is performed by screening libraries of molecules on cells or in

lysates, identifying those that elicit an interesting phenotypic response, and then determining the protein target. A successful example of this method comes from the laboratories of Schreiber and Mitchison (Mayer et al. 1999).

Chemicals, such as those used to arrest the yeast cell cycle, have proved invaluable for decoding the molecular details of the cell division cycle, and drugs that specifically inhibit new protein targets have the potential to further our understanding of mitosis. To date, all known compounds that directly inhibit the mitotic machinery, including Taxol and epothilone, target the same protein, tubulin. To identify cell-permeable small molecules that target other proteins involved in mitosis, Schreiber, Mitchison and co-workers screened a library of small molecules against mammalian epithelial kidney (BS-C-1) cells and looked for compounds that induced changes in mitotic spindle formation but did not affect general tubulin polymerization (Mayer et al. 1999). Using this approach they found a molecule, monastrol, that elicited the desired phenotype, inhibiting normal mitotic spindle formation (Figure 2A, C), but not normal tubulin function (Figure 2B, D). By correlating this with a known mutant phenotype and testing inhibition *in vitro*, they were able to show that the molecular motor protein Eg5 is a target of monastrol. By using a forward chemical genetic strategy, the first tubulin-independent small-molecule inhibitor of the mitotic machinery was found, providing a new clinical drug lead, a new therapeutic target, and a potential tool for probing cell biology.

Forward chemical genetics is not only useful for finding small molecules that target proteins known to be involved in a specific process, but potentially also can be used to identify proteins that were not known to be involved in a process of interest. Using the zebrafish *Danio rerio*, Schreiber and co-workers have shown that interesting developmental phenotypes can be elicited by small molecules (Peterson et al. 2000). In this study, small molecules were identified that modulate various aspects of zebrafish ontogeny, including development of the central nervous system, the cardiovascular system, the neural crest, and the ear. However, while this study indicates the potential of using forward chemical genetics to identify proteins, it also suggests a key challenge: identifying the protein targets of small-molecule inhibition.

For phenotypes that cannot be easily correlated with a unique mutation, some methods for target identification are available, including affinity purification (Taunton et al. 1996), yeast three-hybrid systems (Drees 1999, Lin et al. 2001), display cloning (Sche et al. 1999, 2001), and protein microarrays (Kodadek 2001, Zhu et al. 2000). However, these techniques are still under active development and do not yet provide a general solution for target identification. A traditional genetic solution that merits attention is the generation and isolation of mutations that abrogate drug-sensitivity. Most recently, this approach has been used to identify the target of a small-molecule plant hormone that had eluded identification for 30 years (Inoue et al. 2001). In general, forward chemical genetic approaches offer a promising means of identifying proteins and small-molecule inhibitors of these targets. Continuing efforts are certain to increase their utility.

COMBINING CHEMISTRY WITH GENETICS To determine the function of proteins *in vivo*, small molecules are attractive tools. However, finding small molecules with suitable specificity is difficult, especially for proteins within large, highly homologous families. Inhibition of kinase activity exemplifies this issue and highlights how chemistry in combination with genetics can provide the specificity of genetic knockouts while maintaining the advantages of small molecules.

Protein kinases make up a large enzyme family and have been intensely studied because they play a significant role in nearly every cellular signaling pathway (Hunter 2000). Nonetheless, knockout approaches have not been able to address the function of essential kinases (Ihle 2000), and knockouts of some kinases lead to compensation during development (Sieg et al. 1998). Studies using conditional alleles have been successfully employed in many cases. However, because of the perturbing nature of these approaches or the relatively long timescale (Figure 1), they have not been sufficient for some kinases. Reverse chemical genetics can address these problems because small-molecule inhibition of proteins can be rapid, reversible, tunable, and conditional.

Small-molecule inhibitors of particular kinases have been found using *in vitro* screening methods. Several laboratories have found small-molecule inhibitors of cyclin-dependent kinase 2 (CDK2), a key controller of cell division (Chang et al. 1999, Davis et al. 2001, Gray et al. 1998). Schultz and co-workers synthesized a library of 2,6,9-trisubstituted purines (Figure 3) and screened the molecules in a variety of human cell lines as well as in the budding yeast *Saccharomyces cerevisiae*. Several inhibitors with low nanomolar affinity and some degree of specificity (Knockaert et al. 2000) for human CDK2-cyclin A kinase complex and *S. cerevisiae* Cdc28p were identified (Chang et al. 1999, Gray et al. 1998). In this study, optimization of lead compounds was performed by iterative synthesis-screening rounds and accelerated by analysis of a crystal structure of one of the CDK2-inhibitor complexes. In another study, Ellman and co-workers have shown that by linking low-affinity kinase inhibitors together, they could identify a potent ($IC_{50} = 64$ nM) subtype-specific inhibitor of c-Src (>75-fold selectivity over closely related kinases Fyn, Lyn, and Lck) (Maly et al. 2000).

These molecules have the potential to be useful tools for probing kinase function *in vivo*; however, whether most small molecules are sufficiently specific to be used in a cellular context is still an open question. An *in vitro* study of the specificity of 28 commercially available protein kinase inhibitors, some with supposedly high specificity, revealed that all but 2 drugs had more than 1 protein target (Davies et al. 2000). Admittedly, kinases have a high degree of homology in their active sites (Sicheri et al. 1997), and other protein targets could be more forgiving, but drug specificity remains a major challenge to chemical biology.

Our laboratory has addressed this specificity problem by borrowing from genetics. We reasoned that by combining the best features of genetics, target specificity and generalizability, with the best features of chemistry, rapid inhibition and tunability, we could create a new way to probe complex biological systems. Our approach relies on changing the active site of one kinase to make it distinct from

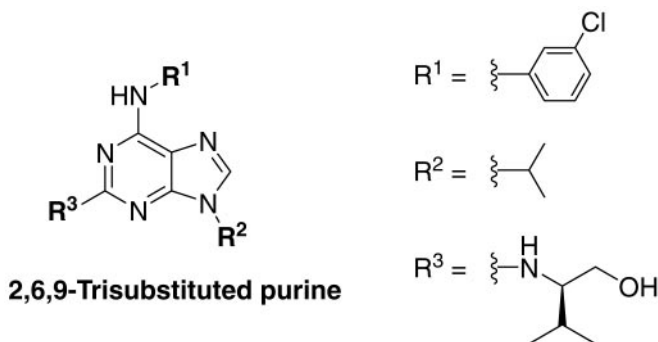


Figure 3 Chemical structure of the 2,6,9-trisubstituted purine scaffold. The tightest binding inhibitor is shown for which the IC_{50} against cdc2-cyclin B is 4 nM.

all other kinases in a cell. This mutant kinase provides a much simpler molecular recognition problem that we can more easily solve. Additionally, this approach is amenable to a wide variety of kinases (Bishop et al. 1998, 1999; Bishop & Shokat 1999).

Practically, this involves using site-directed mutagenesis to engineer a functionally silent yet structurally significant mutation into the active site of a kinase of interest. Typically, this mutation is the replacement of a homologous, conserved, large, hydrophobic residue in the ATP-binding site with glycine or alanine, thus creating a new pocket in the active site. Importantly, these mutations usually do not significantly affect kinase activity (Figure 4C). In parallel, a nonspecific kinase inhibitor of the wild-type enzyme (Hanke et al. 1996) (Figure 4A, E) is chemically modified with substituents that specifically complement the mutation introduced into the active site (Figure 4D, F). Importantly, the new inhibitor analogs are unable to inhibit any wild-type kinases because of steric clashes with the aforementioned bulky active site residue (Figure 4B). In a cellular context, it is difficult to prove perfect specificity for any drug; however, experiments have shown that the developed inhibitors do not inhibit wild-type kinases *in vitro*. Furthermore, addition of our inhibitors to wild-type *S. cerevisiae* shows few transcriptional changes *in vivo*, suggesting that the molecule has few, if any, off-target effects (Bishop et al. 2000). Using this combined chemical genetic approach, several analogs of a pyrazolo[3,5-*d*]pyrimidine inhibitor have been found that inhibit engineered kinases with nanomolar IC_{50} s but do not significantly inhibit wild-type kinases (Bishop et al. 1999).

This combined chemical genetic strategy can be used to study many kinases, but several requirements must be met. First, it must be possible to introduce the desired mutant allele into the organism of interest. Second, the stability and activity of the engineered enzyme must be unaltered. Third, the inhibitor must have acceptable

bioavailability. Work in both our laboratory and others has demonstrated that these requirements often can be met. This technique has been used to probe the functional roles of CaMKII α in learning and memory in the mouse, *Mus musculus* (J.Z. Tsien, unpublished data); v-Src in transformation of NIH 3T3 mouse fibroblasts (Bishop et al. 1999); and Cla4 and Cdc28 in cell cycle regulation in *S. cerevisiae* (Weiss et al. 2000, Bishop et al. 2000).

An example that highlights the ability of chemical genetics to reveal roles not observed using traditional genetics is the use of our technique to clarify the role of Cdc28 in the cell cycle of *S. cerevisiae*. Cdc28 is the primary cyclin-dependent kinase (CDK) involved in cell cycle control. Use of temperature-sensitive (TS) mutants of Cdc28 generated by traditional genetics suggests that the most critical role for Cdc28 is to control the transition from G1 to S phase. Surprisingly, experiments done in collaboration with the Morgan laboratory showed that inhibitor-sensitive Cdc28 mutants arrested at the G2/M transition when treated with the mutant specific inhibitor, 1-naphthylmethyl PP1 (1-NMPP1). This discrepancy was not due to off-target effects of the drug because this compound induced no toxicity or cell cycle arrest in wild-type yeast lacking the analog-sensitive Cdc28 allele. Thus, the difference in cellular phenotype was the result of a fundamental difference in how Cdc28 function was altered by the two approaches.

The discrepancy between the chemical- and temperature-induced inhibition of Cdc28 may be explained from a structural perspective. Cdc28 functions in two ways, as a catalyst and as a scaffold for other components of the cell cycle machinery. Typically, TS mutants unfold at elevated temperature, which results in a loss of both of these functions. ATP competitive inhibitors, such as 1-NMPP1, block only kinase catalytic activity. Thus, in this case, the chemical method may be a more specific probe of protein function.

Moreover, the observed discrepancy is consistent with known Cdc28 catalytic activity and cell cycle progression. The kinase activity of Cdc28 is maximal at the G2/M transition and is therefore expected to be most sensitive to inhibition at this stage, consistent with observed G2/M arrest at low doses of 1-NMPP1. This model predicts that higher inhibitor concentration should result in earlier cell cycle arrest, which was also demonstrated. Thus the ability of this chemical genetic approach to more specifically probe protein function highlights the advantages of using chemical genetics as a complement to traditional genetics.

In addition to using combined chemical genetics to inhibit kinases, it is also possible to probe kinase activity using modified agonists and substrates. By synthesizing γ [³²P]-labeled analogs of ATP that contain a sterically bulky functional group and using the engineered kinases previously described, we can selectively label the direct phosphorylation substrates of a kinase of interest (Habelhah et al. 2001, Shah et al. 1997). This allows for the identification of direct kinase substrates.

This combined chemical-genetic approach also has been powerful for specifically modulating the function of proteins outside the kinase family. Conklin and co-workers have applied a similar chemical genetic method to the study of seven-transmembrane G protein-coupled receptors (GPCRs). A detailed understanding of

the processes that are mediated by these receptors is desirable because GPCRs affect a broad range of physiological events including heart rate, proliferation, chemotaxis, neurotransmission, and hormone secretion. However, studying the effects of GPCR signaling *in vivo* is complicated by the large number of GPCRs that are expressed on the cell surface and the presence of endogenous ligands. By introducing changes in the agonist-binding domain of a particular GPCR, receptors were generated that are activated by a synthetic agonist, but not by the endogenous peptide hormone ligand. By providing exclusive control over the activation of these receptors, influence over a number of physiological processes, including heart rate and cell proliferation, has been demonstrated (Coward et al. 1998, Redfern et al. 1999).

Influencing Protein-Protein Interactions

Although efforts to perturb biological function using small molecules have focused predominantly on developing enzyme inhibitors and receptor agonists/antagonists, similar strategies (e.g., library screening against protein targets *in vitro*, screening for full cell phenotype changes *in vivo*, and techniques that combine chemistry and genetics) have been utilized to find small molecules to control protein-protein interactions. Protein-protein interactions, from transient to long-lived, are important in controlling numerous cellular functions. In fact, recent studies in yeast using two-hybrid methods and other techniques suggest that at least 2700 interactions exist between the 6000 yeast genes (Schwikowski et al. 2000, Uetz et al. 2000). With the prevalence of such interactions, these protein-protein interfaces become obvious targets for perturbing cell function. Moreover, because many interacting proteins consist of multiple domains, where catalysis and regulation are distinct and potentially can be controlled separately, small molecules that specifically target these domains would be useful. With small molecules that can specifically and conditionally initiate or disrupt a desired protein-protein interaction, many opportunities exist to influence and illuminate the intricate details of a wide range of cellular processes.

DISRUPTING PROTEIN-PROTEIN INTERACTIONS Despite the appeal of such targets, general techniques for finding tight-binding small molecules that can disrupt protein-protein interactions have been elusive, and specific examples of such molecules, especially those that act within cells, are relatively limited. Contributing to this may be the fact that many protein-protein interactions are mediated by a large number of protein contacts and are therefore difficult to disrupt through the binding and competition of a small molecule (Cochran 2000). Successful strategies for using relatively small molecules have focused on disrupting contacts that access few amino acids such as RGD peptide sequences and Src homology 2 (SH2) binding domains (Liuzzi et al. 1994), or utilizing peptides and protein domains that access larger surfaces (Shultz et al. 2000, Zutshi et al. 1998). In both cases, peptides have typically served as lead compounds, with extensive effort often required to find nonpeptidal analogs.

As an alternative to the redesign of peptide leads, screening of small-molecule libraries for molecules that directly disrupt protein-protein interactions has also been pursued. In a recent example from the laboratories of Yuan and Mitchison, small molecules with low micromolar affinity for the BH3 peptide-domain binding pocket of Bcl-x_L were found by screening *in vitro* (Degterev et al. 2001). When used *in vivo*, this molecule induces apoptosis, functioning in a manner similar to peptides derived from the BH3 domain. An interesting and potentially more general screen for disrupting protein-protein interactions *in vivo* was described by Franco and co-workers (Young et al. 1998). By screening a small-molecule library for members that could disrupt interacting protein partners in a yeast two-hybrid system, they found a micromolar inhibitor of the association between the $\beta 3$ and $\alpha 1B$ subunits of a human N-type calcium channel.

An intriguing complement to finding small molecules that disrupt interactions between proteins at their interface is to screen for small molecules that stabilize proteins in a non-associating conformation. Kelly and co-workers found that in screening a relatively small library of commercially available drugs, they were able to find molecules with nanomolar dissociation constants that stabilized the native conformation of the thyroxine carrier transthyretin, preventing it from adopting a conformation that promotes undesirable protein-protein interactions to form amyloid plaques (Peterson et al. 1998). Similarly, screening of a library of small molecules for nitric oxide synthase (NOS) inhibitors revealed a nanomolar binder that inhibited induced NOS activity in cells, but not *in vitro* (McMillan et al. 2000). Analysis revealed that this inhibitor acted by binding to the heme cofactor of the enzyme, inducing a conformational change in the protein and preventing formation of the catalytically active homodimer.

Because of the crucial role protein-protein interactions play in mediating a wide range of cell processes, focus on the development of general methods for finding small molecules to disrupt protein-protein interactions is becoming more intense. The discovery of additional disrupters of protein-protein interaction is certain to provide new tools for probing questions in cell biology.

INITIATING PROTEIN-PROTEIN INTERACTIONS Library screening has provided some small molecules capable of inducing protein-protein interactions, such as the homodimerization of receptors (Qureshi et al. 1999, Tian et al. 1998). However, techniques that combine chemistry and genetics have provided more general methods for initiating and stabilizing protein-protein interactions. Studies directed at elucidating the mechanism and functional role of oligomerization of the Fas receptor complex highlight the utility of such methods. Fas, a type I membrane protein, is a cell surface receptor capable of mediating apoptosis (Itoh et al. 1991). Binding of the Fas ligand, FasL, results in the initiation of this pathway (Tanaka et al. 1995) and is believed, although not directly established, to be mediated by receptor trimerization. In terms of its functional role, Fas is involved in thymocyte differentiation, including differentiation of peripheral thymocytes (Klas et al. 1993). However, its full role in these processes is unclear.

To address the mechanistic details of Fas-mediated signaling, as well as its functional role in thymocyte selection, Schreiber and co-workers utilized a combined chemical-genetic approach that allowed initiation of Fas-mediated signaling using a designed small molecule (Spencer et al. 1996). It has been shown that the natural product FK506 (Figure 5A) induces heterodimer formation between the 12-kDa FK506-binding protein (FKBP) and a portion of the 289-kDa FKBP12-*rapamycin*-associated protein (FRAP) in an FK506-dependent fashion (Stockwell & Schreiber 1998). By using a chemically synthesized FK506 dimer (FK1012) (Figure 5B), homodimerization between FKBP12 proteins can be induced (Diver & Schreiber 1997, Spencer et al. 1993). By fusing the FKBP protein to a protein of interest, it becomes possible to conditionally induce oligomerization of the desired protein in a drug-dependent fashion *in vitro* and *in vivo*.

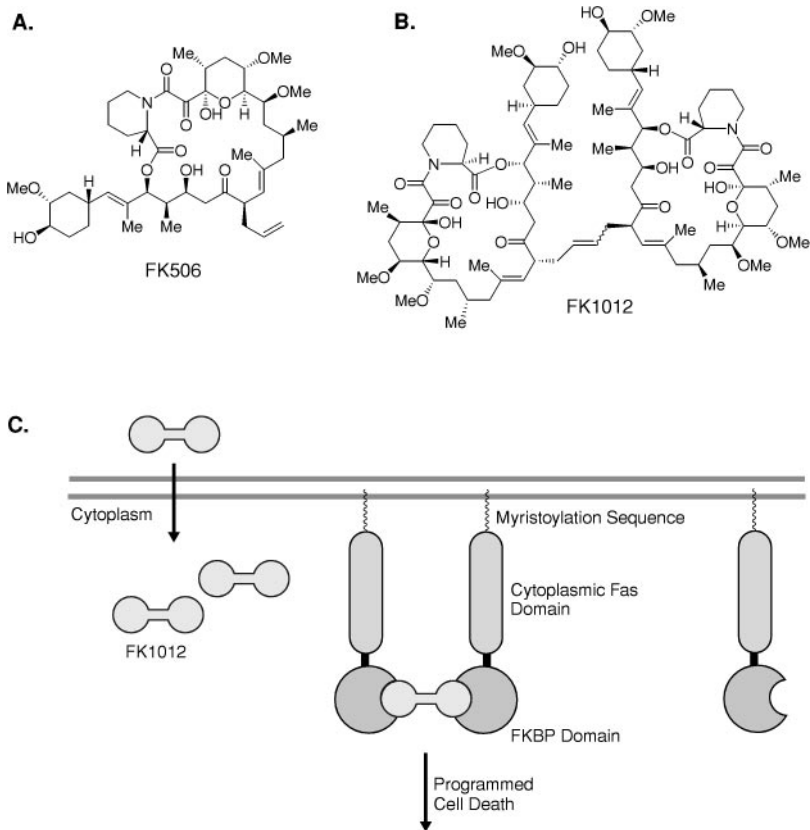


Figure 5 (A) Chemical structure of FK506. (B) Chemical structure of FK506 dimer, FK1012. (C) Schematic of FK506 mediated dimerization of a chimeric Fas-FKBP receptor.

Using this chemical-genetic approach, Schreiber and co-workers probed the mechanism of Fas-mediated apoptosis (Spencer et al. 1996). By grafting one or more FKBP domains to the intracellular portion of the Fas receptor and anchoring this fusion to the cell membrane (Figure 5C), they showed that addition of FK1012 resulted in apoptotic cell death, either in transiently transfected Jurkat Tag T cells or in transgenic mice expressing the fusion protein under the control of a T cell-specific promoter. Moreover, they were able to demonstrate that dimerization, not trimerization, was sufficient, although less efficient, in eliciting an apoptotic response. Additionally, they demonstrated that membrane localization of the intracellular Fas domain was required for this response.

The Fas-FKBP also was used to address a potential functional role of Fas in peripheral thymocyte viability. Although chimeric Fas-FKBP was expressed throughout thymocyte ontogeny in transgenic mice, only double-positive thymocytes were sensitive to Fas-mediated killing upon exposure to FK1012 *in vitro* and *in vivo*. While this potential role in T cell development had previously been observed using anti-Fas monoclonal antibodies, the additional induction of a profound fulminant hepatitis-like syndrome complicated the analysis of these results (Ogasawara et al. 1995).

Taken together, this inducible dimerization strategy provides a general method for specifically and conditionally inducing protein-protein interactions and offers a tool for studying their mechanism and functional role. The specific nature of the small-molecule-binding protein interaction, coupled with the ability to express the binding protein in a tissue-specific manner, allows an interaction of interest to be studied in isolation from other factors. Moreover, because many different small-molecule-binding protein pairs are now available, it is possible to engineer and study complexes composed of both homomeric and heteromeric protein interactions (Stockwell & Schreiber 1998). In addition to their use with other receptor complexes (Spencer et al. 1993), the approach has allowed the study of other proteins including Src (Spencer et al. 1995), Sos (Holsinger et al. 1995), Raf (Farrar et al. 1996), and transcriptional activator interactions (Ho et al. 1996, Holsinger et al. 1995), as well as interactions that lead to subcellular localization (Farrar et al. 1996, Ho et al. 1996, Holsinger et al. 1995). Interestingly, recent results from Clackson and co-workers show that these small-molecule-binding domains are not only useful for inducing dimerization, but can be utilized to disrupt protein-protein interactions *in vitro* and *in vivo* (Rivera et al. 2000). By fusing engineered FKBP12 to secreted proteins, aggregation of the protein could be maintained until release was initiated by a ligand to FKBP.

A potentially more subtle way of controlling dimerization is to create a small-molecule switch at the intrinsic receptor/ligand interface of the protein of interest. Schultz and co-workers showed that by introducing a cavity into both the human growth hormone and its associated receptor, they were able to screen a chemical library to find a small molecule that suppresses the introduced mutations, allowing much of the original binding affinity to be restored (Guo et al. 2000).

In summary, genetic techniques are very useful, but no single method provides a means to perturb protein activity or protein-protein interactions in a highly specific, rapid, reversible, tunable, and conditional manner (Figure 1). In contrast, the use of small molecules can offer all of these features. However, the primary challenge is to find molecules with sufficient affinity and specificity. With the development of methods for generating molecular diversity and high-throughput screening, it may be possible that small molecules with sufficient affinity and specificity for any desired protein target will be found. Methods that combine chemistry and genetics have shown that such specificity can be achieved and can complement existing methods. Further developments should provide even more chemical tools that should aid in deconvoluting complex biological processes.

SYNTHETIC APPROACHES TO NOVEL BIOMOLECULES

Sometimes the advantages of small molecule approaches (e.g., conditional inhibition/activation, reversibility, dose-dependent perturbation, delivery *in vivo*) are not necessary to probe biological activity. In these cases, the direct alteration of biomolecules can be attractive. Because these changes are covalent, a high degree of intrinsic target specificity is possible. Additionally, because many of the biomolecules of interest are polymers composed of regular units (e.g., amino acids, nucleic acids, sugars), techniques to alter their composition can be general to large-molecular-weight species.

Although genetic methods offer the potential to generate many biomolecules, chemical synthesis offers additional opportunities, including access to natural biomolecules not easily obtained and to biomolecules that are unnatural in origin. Recently, significant progress has been made in generating and altering biomolecules to address structural and functional questions. In this section, we focus on a sampling of methods available for generating novel proteins, oligonucleotides, oligosaccharides, and small-molecule second messengers.

Proteins

The ability to study many biological processes depends on the expression and/or purification of a protein of interest. However, access to some proteins can be limited. Many proteins are post-translationally modified to present single or multiple copies of groups that are not genetically encoded: these modifications include phosphorylation, glycosylation, acetylation, methylation, lipidation, and sulfation. The pattern of these modifications can also vary for a given protein, further complicating analysis. Additionally, access to proteins containing residues that aid in functional or structural characterization is often limited to amino acids that can be genetically encoded or modified with chemical labeling groups (often by cysteine or lysine modification). Recent chemical advances allow some of these problems to be addressed.

Techniques such as expressed protein ligation provide access to a wide range of natural and unnatural proteins and highlight the progress that has been made in gaining access to post-translationally modified proteins (Cotton & Muir 1999). For example, signal transduction mediated by the type I transforming growth factor β receptor (TGF β R-I) requires phosphorylation of four to five serine and threonine residues in the amino-terminal regulatory domain (Wieser et al. 1995). This hyperphosphorylation activates the kinase activity of the TGF β R-I receptor and allows it to mediate further signal transduction. However, the mechanistic role of this phosphorylation change in regulating TGF β R-I kinase activity is unclear. Structural studies of the unphosphorylated intracellular portion of TGF β R-I suggest that the unphosphorylated amino-terminal domain is inhibitory, where hyperphosphorylation "de-represses" TGF β R-I kinase activity (Huse et al. 1999). However, phosphorylation of this region may also play an activating role. Attempts to prepare a uniformly hyperphosphorylated cytoplasmic domain of the receptor using either coexpression of the kinase of TGF β R-I or phosphorylation *in vitro* have been unsuccessful (Huse et al. 2000). In contrast, expressed native ligation provided access to this molecule.

Expressed protein ligation combines the strengths of solid phase peptide synthesis (SPPS), recombinant protein expression, and native ligation chemistry. Historically, SPPS has been successful at generating peptides and small proteins containing both natural and unnatural amino acids. However, synthesis of larger proteins often is not possible without chemical or enzymatic methods of peptide condensation (Braisted et al. 1997, Wallace 1995). One of the more successful examples of such strategies is native chemical ligation (Dawson et al. 1994). In this approach, SPPS is used to generate an amino-terminal fragment containing a carboxy-terminal thioester and a carboxy-terminal fragment containing an amino-terminal cysteine (Figure 6A). To ligate these residues, the unprotected peptides are simply mixed in the presence of thiols. In the first step of the reaction, selective transesterification occurs between the carboxy-terminal thioester and cysteine residues (Figure 6B). This step occurs reversibly in the presence of the thiols. However, if the thioester is formed with the amino-terminal cysteine residue, attack of the amino-terminal amine is possible and results in irreversible main-chain rearrangement to generate the desired ligation product (Figure 6C). Although this approach is amenable to large proteins, multiple ligation steps can be difficult.

By combining this technique with recombinant protein expression, it is possible to take advantage of the flexibility SPPS provides, while gaining easier access to large proteins. It has been shown that some proteins undergo spontaneous backbone splicing using a mechanism similar to the native ligation chemistry described above (Paulus 1998). In this rearrangement, an intein domain initiates a backbone transfer to a cysteine residue to generate a thioester intermediate (Figure 7A). This activated intermediate then undergoes chemical reactions similar to those described earlier to excise the intein domain (Figure 7B). Muir and co-workers have found that this intein domain can be fused to a protein of

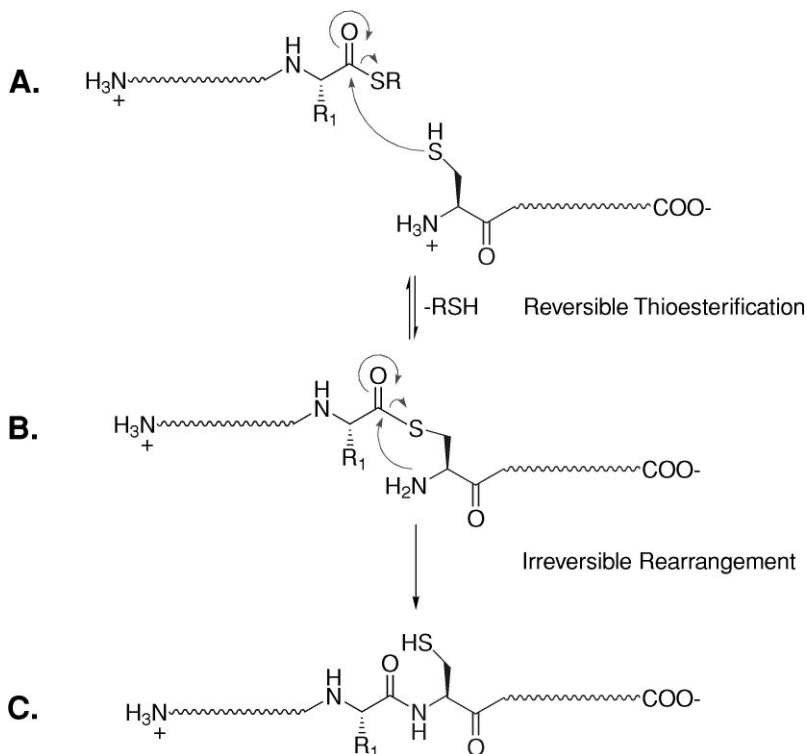


Figure 6 Chemical scheme of thioester mediated native ligation. (A) Amino- and carboxy-terminal peptide fragments and their reversible thioesterification. (B) Thioester peptide intermediate and the subsequent irreversible thio- to amino-acyl transfer. (C) Final full-length peptide product.

interest, and that the activated thioester intermediate can be chemically intercepted with thiols to generate an expressed protein fragment containing a carboxy-terminal thioester group (Figure 7C) (Cotton & Muir 1999). With this fragment, condensation between chemically synthesized peptides and expressed proteins is possible.

Ligating a chemically synthesized hyperphosphorylated peptide fragment and a recombinantly expressed protein fragment, Muir and co-workers generated the desired hyperphosphorylated TGF β R-I intracellular domain (Huse et al. 2000). With this construct they were able to demonstrate that phosphorylation of the amino-terminal domain had more than a simple inhibitory effect because activity of the hyperphosphorylated protein was significantly greater than protein lacking the phosphorylation domain. Although the exact mechanism of this enhancement is still not clear, the synthesis of this hyperphosphorylated form allows further functional and structural studies to be pursued.

In general, expressed protein ligation offers an effective tool for generating proteins containing amino acids not typically encoded and has been used to generate other proteins with novel post-synthetic modification (Tolbert & Wong 2000). Moreover, the changes possible are not limited to those found in post-translational modifications. Unnatural amino acids can be included to aid in functional and structural characterization and have been utilized as an aid in the heteronuclear NMR spectroscopic analysis of segmentally labeled proteins (Xu et al. 1999), and for monitoring the phosphorylation state of the adapter protein Crk-II (Cotton & Muir 2000). Continued use and refinement of this technique should facilitate our understanding of protein structure and function.

While expressed protein ligation is especially well-suited for the study of proteins *in vitro*, other techniques for generating proteins containing unnatural amino acids provide a useful complement. In nonsense suppression mutagenesis (Noren et al. 1989, Dougherty 2000), a desired point mutation is generated by replacing the codon of interest with an amber stop codon (Figure 8). Separately, a tRNA is prepared that can recognize the amber codon. This tRNA is then charged with the desired amino acid derivative using both chemical and enzymatic coupling steps (Figure 8). Together these components are translated either *in vitro* or *in vivo*, and the unnatural amino acid is incorporated at the desired site.

Since this technique utilizes the native translational machinery of the cell, it is well suited to probe a number of processes including those involved in and concurrent with translation. Brunner, Rapoport, and co-workers have utilized nonsense suppression mutagenesis to probe when and how transmembrane proteins leave the Sec61p channel during translation and become integrated into the endoplasmic reticulum (Heinrich et al. 2000). Initial studies of the *in vitro* translation of a model transmembrane protein suggest that partitioning into the membrane from the Sec61p channel occurs relatively soon after translocation begins (Heinrich et al. 2000). To substantiate this observation, a photo-cross-linking phenylalanine analog was incorporated into the translating protein using nonsense suppression mutagenesis. Using this photosensitive probe, they were able to demonstrate a corroborating rise and then fall of photo-cross-linking between translated protein and Sec61p as more of the transmembrane protein is translated. Moreover, they observed photo-cross-linking directly with lipid, something difficult to accomplish using other techniques. This protein-lipid interaction lagged slightly behind the initial interaction observed between the translated proteins and Sec61p, and persisted after decreased Sec61p interaction was observed. Brunner, Rapoport, and co-workers were then able to utilize this system to probe for the factors necessary to achieve and coordinate integration of transmembrane proteins into the endoplasmic reticulum.

Nonsense suppression mutagenesis also has been employed to probe biological processes *in vivo*. Expression and functional characterization of transmembrane proteins, such as the nicotinic acetylcholine ion channel in *Xenopus laevis* oocytes, provided insights into the role a range of amino acids play in gating and ion transport (Dougherty 2000, Zhong et al. 1998). To expand the utility of

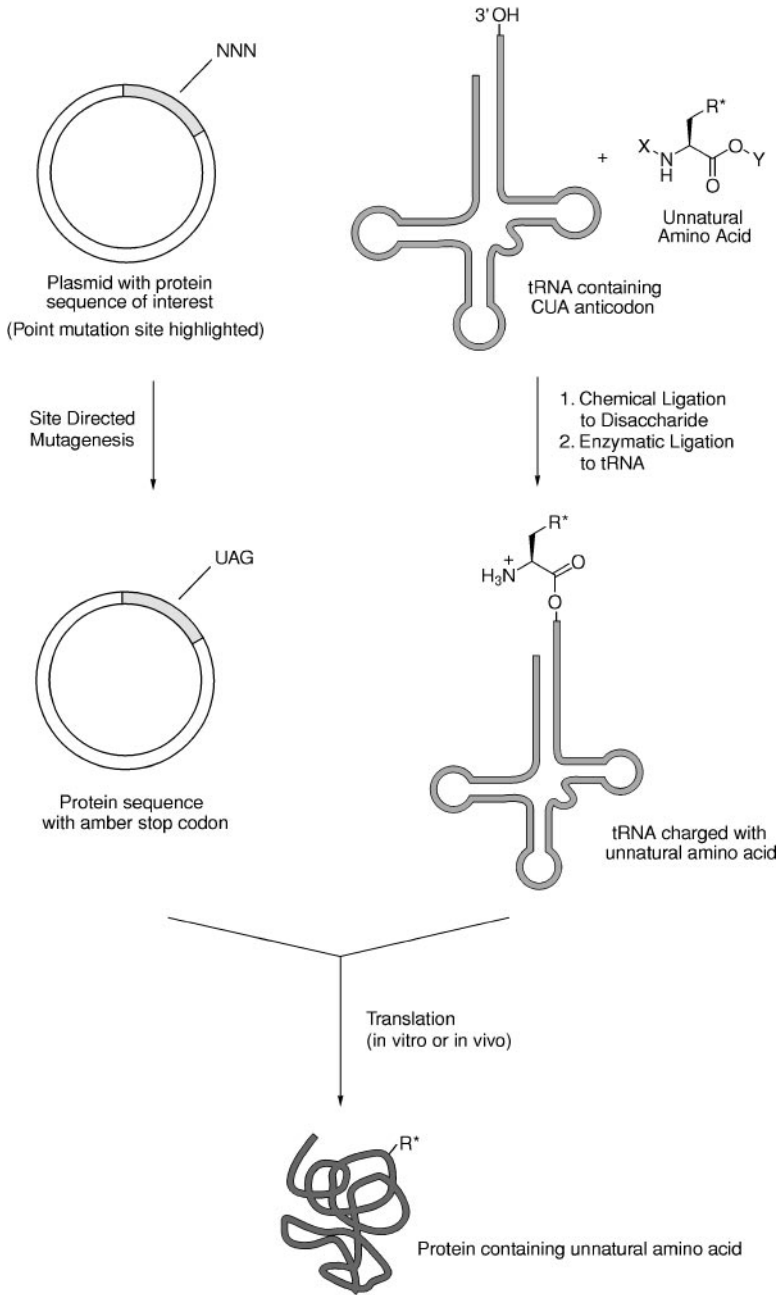


Figure 8 Schematic of nonsense suppression mutagenesis. Use of site directed mutagenesis and the generation of unnatural tRNA molecules provides a route to proteins containing an unnatural amino acid.

nonsense suppression mutagenesis *in vivo*, efforts are underway to eliminate the necessity of externally generating and delivering chemically modified tRNAs. Initial progress has been made in developing a native translational system capable of catalytically and specifically generating unnatural amino-acetylated tRNAs, and continued progress in this area is expected (Liu et al. 1997, Wang et al. 2001).

Oligonucleotides

Oligonucleotides are synthetically tractable biopolymers, readily accessible through solid phase synthesis, and their availability for molecular biology is taken for granted. Similarly, the ability to chemically modify and incorporate unnatural nucleotides synthetically and enzymatically has proved crucial in a diverse range of applications, including sequencing, hybridization, antisense, and *in vitro* evolution. Recent work has focused attention on other areas where unnatural nucleotides and oligonucleotides are useful. The work of Verdine and co-workers highlights the potential for using unnatural nucleotides to probe protein-DNA interactions (Huang et al. 1998). HIV reverse transcriptase (RT) is a viral protein essential for HIV proliferation, thus inhibiting its activity is a major target of drug treatment. Crystal structures have been solved for the RT holoenzyme, RT complexed with double-stranded DNA, and RT bound to non-nucleoside inhibitors (Das et al. 1996, Ding et al. 1995, Hsiou et al. 1996, Jacobomolina et al. 1993, Jager et al. 1994, Kohlstaedt et al. 1992, Ren et al. 1995, Rodgers et al. 1995). Still, structural characterization of the catalytic complex between RT, a template:primer, and a nucleotide triphosphate could provide new insights into the catalytic mechanism of HIV RT and provide a better picture of how mutations in RT lead to drug resistance. Attempts to crystallographically characterize the catalytic complex bound to dideoxynucleotides failed because of poorly diffracting crystals (Huang et al. 2000). To stabilize this complex, Verdine and co-workers turned to a combined chemical-genetic approach. Oligonucleotides containing a thiol-bearing guanosine were synthesized (Figure 9A). From a model based on the crystal structure of the RT-duplex DNA complex, a residue on one face of an α -helix was mutated to a cysteine to form a disulfide cross-link between the protein and the DNA. Studies revealed that stable cross-linking was possible, and a structure of this cross-linked complex was solved (Huang et al. 1998). Interestingly, in this structure the disulfide tether between protein and DNA is not visible and suggests that complex formation is not the result of rigid linkage, but instead because of an increase in effective concentration of protein and DNA. Thus the unnatural nucleotide provided a means for observing binding interactions not easily amenable to structural study. Unnatural nucleotides have also been employed to address specific mechanistic questions involving the enzymic processing of oligonucleotides (Bruner et al. 2000), and the rich chemistry of unnatural oligonucleotides suggests the potential to probe a whole host of other interesting DNA-protein interactions.

Another area of study is the development of nucleotide analogs containing nucleic acids that do not associate by standard hydrogen bonding. An interesting example of such a system is the incorporation of pyrene nucleotides (Figure 9B) (Matray & Kool 1999). The pyrene group is a large hydrophobic aromatic ring system that occupies approximately the same amount of space as a hydrogen-bonded pair of nucleic acids. Kool and co-workers found that this nucleotide could be incorporated by a polymerase; however, incorporation was only possible opposite abasic sites. Using this technique, they were able to sequence DNA stretches and determine the positions of abasic sites: Abasic sites occur regularly in damaged DNA and are difficult to distinguish from other types of DNA damage (Maulik et al. 1999). Attempts have also been made to develop unnatural nucleotides that can associate without standard hydrogen bonding and show relatively little affinity for natural nucleotides (Benner et al. 1998, Guckian et al. 2000, Wu et al. 2000). Some success has been achieved. However, neither these, nor the pyrene nucleotides, can be extended by natural polymerases following initial incorporation. Efforts to engineer polymerase to accept and extend these nucleotides are underway (Lutz et al. 1999).

Oligosaccharides

Another area that highlights the potential of chemical approaches to generate natural and unnatural biomolecules is in the synthesis of complex sugars. Oligosaccharides, glycoproteins, and glycolipids, play a role in cell recognition, cell adherence, and protein structure. However, a key difficulty in studying the role of these sugar-containing molecules in vitro and in vivo has been their synthesis. Like proteins and nucleic acids, complex sugars are oligomers of monomeric units. Unlike proteins and nucleic acids, oligosaccharides are branched biopolymers, where branching can occur at one of a number of chemically similar sites. Glycosidic bonds also can be formed in either of two orientations at the anomeric position, increasing the potential number of isomers. Furthermore, no simple genetic encoding dictates the pattern of connectivity and, in fact, many glycoproteins exist in a range of different glycoforms.

Although direct chemical synthesis has given access to a number of sugars, the difficulties of this approach have forced exploration of other avenues. Simpler chemical (Seeberger & Haase 2000), enzymatic (Koeller & Wong 2001), and biosynthetic (Yamase et al. 2000) methods to generate complex sugars have been pursued with some success, and recent progress in solid-phase oligosaccharide synthesis (Plante et al. 2001) points to a future where rapid access to any desired complex sugar will be synthetically accessible.

As an alternative to the direct synthesis of full sugars, Bertozzi and co-workers have developed a semisynthetic method for derivatizing surface glycoproteins (Mahal et al. 1997, Saxon & Bertozzi 2000). In their approach, cells are treated with either *N*-levulinoylmannosamine (ManLev; Figure 10A) or *N*-azidoacetylmannosamine, ketone- or azide-containing analogs of *N*-acetylmannosamine,

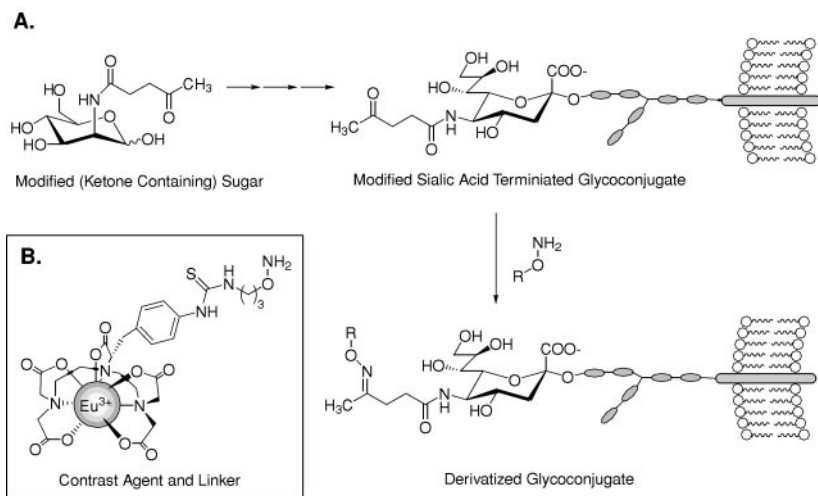


Figure 10 (A) Scheme for the derivatization of cell surface oligosaccharides. Introduction of a modified mannose sugar results in its conversion to a modified sialic acid and ultimately to its incorporation into glycoconjugates as the terminal sugar. This modified glycoconjugate can be selectively chemically derivatized with a range of possible chemical groups. (B) The chemical structure of a studied derivatization agent. This compound consists of both a MRI contrast agent and a linker that provides reactivity with the modified glycoconjugates.

respectively. These sugars are ultimately converted biosynthetically to ketone- or azide-bearing sialic acid sugar analogs. Sialic acid is a common terminal component of mammalian glycoconjugates, thus this approach delivers a chemically reactive handle, a ketone or azide, to the surface of a cell. Specific derivatization of this group provides a way to further elaborate the sugar and offers a general means of modifying the cell surface. As an application of this approach, Bertozzi and co-workers showed that it was possible to specifically deliver a modified magnetic resonance imaging contrast reagent (Figure 10B) to Jurkat cells fed the ManLev sugar (Lemieux et al. 1999). As cancer cells express heightened levels of sialic acid on their cell surface, this approach might be amenable to improved cancer imaging. Further use of cell surface remodeling offers the potential to modify and study cell-surface interactions. A potential future goal is to increase the specificity of cell surface handle incorporation so that derivatization of specific cell glycoproteins would be possible. Altogether, oligosaccharide synthesis and modification continues to be an active and exciting area of chemical research, and we expect significant progress in the coming years. A more in depth discussion of this field can be found in this volume (Saxon & Bertozzi 2001).

Second Messengers

A final area that demonstrates the utility of chemical approaches is the use of unnatural small biomolecules. Changes in the concentration of small biomolecules, such as nucleoside triphosphates, metal ions, and phospholipids, play an important role in propagating and regulating signaling pathways, metabolic activity, and transcription. One application of unnatural small biomolecules is to probe the temporal and spatial aspect of these fluctuations *in vivo*. While methods such as direct perfusion, microinjection, and using cells that have been permeabilized provide a means of addressing some of these changes, the use of chemical analogs offers the potential for greater control with a larger range of biomolecules. Recent generation and use of photolytically cleavable caged second messengers highlight this potential.

It is well established that changes in Ca^{2+} concentration initiate a range of biochemical events and that these changes can occur as Ca^{2+} oscillations. However, less clear is what role these oscillations play *in vivo* and whether oscillating levels of inositol 1,4,5-triphosphate (IP_3) initiate these changes in calcium concentration (Berridge 1993, Fewtrell 1993, Jacob 1990, Tsien & Tsien 1990). To address these issues, Tsien and co-workers synthesized a cell-permeable caged form of IP_3 (Figure 11) (Li et al. 1998). This molecule was assembled from *myo*-inositol and included a photocleavable protecting group at the 6-hydroxyl, an important interaction site with the IP_3 receptor, and protection of the phosphates with an esterase labile group. They demonstrated that this molecule was internalized in astrocytoma cells as an inactive form at relatively high (μM) concentrations. Moreover, active compound was released upon ultraviolet irradiation. Using the caged IP_3 analog, they modulated the amplitude, frequency, and number of IP_3 oscillations to determine their relative contributions. By releasing the same amount of analog over a range of time intervals (a single large pulse, short pulses at intervals from 0.5 to >2 min, and a slow continuous release) they found maximal expression of a downstream reporter gene was effected by a spike of IP_3 at a 1-min interval. This period corresponds roughly to physiological Ca^{2+} ion oscillations and suggests a potential role for Ca^{2+} ion and IP_3 oscillations.

Other photocleavably protected biomolecules have been studied, including the neurotransmitter glutamate (Furuta et al. 1999), chelated Ca^{2+} (Adams et al. 1997), and nitric oxides (Namiki et al. 1999). Not only have these molecules been useful for probing temporal questions, but they also are beginning to be more useful for probing questions of spatial control. Recently, Tsien and co-workers have developed a new photocleavable caging group, brominated 7-hydroxycoumarin-4-ylmethanol (Bhc) (Furuta et al. 1999), that is more susceptible to cleavage by two-photon excitations, a method that provides both long-wavelength excitation as well as very high-resolution three-dimensional control (Denk et al. 1990). Protection of the neurotransmitter glutamate with Bhc allowed them to perform the first three-dimensionally resolved map of the glutamate sensitivity of neurons in intact brain slices (Namiki et al. 1999). Continued progress and use of the temporal and spatial control of small biomolecules is anticipated.

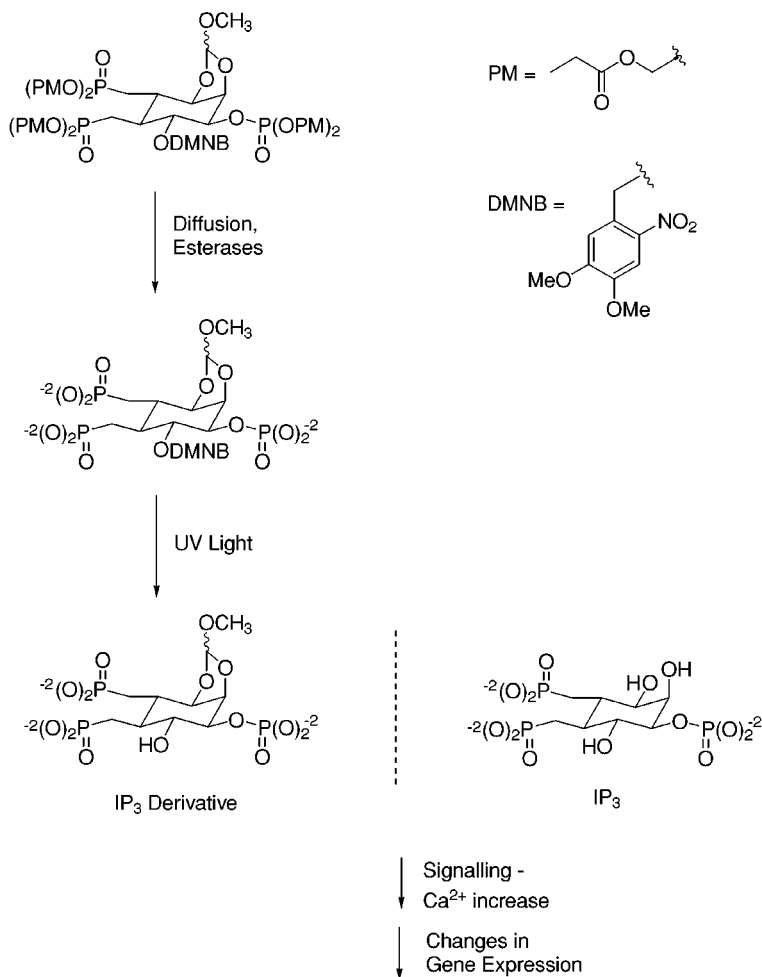


Figure 11 Chemical scheme depicting the results of introducing and activating a caged IP₃ derivative. Chemical structures are shown for the caged, cell-permeant IP₃ derivative, the caged IP₃ derivative, and the uncaged IP₃ derivative (*left*). For comparison, the structure of the natural IP₃ molecule is depicted on the *bottom right*.

CONCLUSIONS AND FUTURE DIRECTIONS

Biological systems are composed of complex assemblies and networks of diverse interacting components (e.g., proteins, oligonucleotides, oligosaccharides, lipids, and metabolites). Moreover, these molecules are themselves structurally complicated. To understand biological systems, it is necessary to generate these components. However, many of these biomolecules are not directly genetically encoded,

and, thus, alternative strategies for obtaining them are necessary. Chemical methods have provided access to both natural and unnatural biomolecules, offering new insights into their structure and function. Additionally, some of these molecules have been used to probe cellular complexes and networks to better understand them. The current challenge is to develop new methods sufficiently simple to be of general use that provide access to a more complete set of biomolecules, and that place these biomolecules in a cellular context.

As our understanding of biological systems evolves, the spatial and temporal nature of complex processes must be deciphered. Genetics only provides a glimpse into the rapid dynamics and graded responses of many biological phenomena because no single genetic method provides a means to perturb protein activity or protein-protein interactions in a rapid, tunable, and conditional manner. The use of small, cell-permeable molecules with high specificity for their targets has begun to provide a clearer picture of the dynamic and graded nature of biological processes. However, new chemical approaches for discovering these tools will need to be developed. Methods that enable genetic-like specificity with chemical control will be particularly valuable in these efforts.

ACKNOWLEDGMENTS

We are grateful to B.C. Freeman and members of the Shokat lab for helpful discussions and critical comments on this manuscript. M.S.K. thanks the National Institutes of Health (AI10611-01) and P.J.A. thanks the Susan G. Komen Breast Cancer Foundation (PDF 2000 78) and the American Cancer Society (PF-01-095-01-TBE) for postdoctoral fellowships. K.M.S. thanks the National Institutes of Health (CA70331-05 and AI44009-03), the GlaxoSmithKline Fund, and the Sandler Family Supporting Foundation for generous financial support.

Visit the Annual Reviews home page at www.AnnualReviews.org

LITERATURE CITED

- Adams SR, Lev-Ram V, Tsien RY. 1997. A new caged Ca^{2+} , azid-1, is far more photosensitive than nitrobenzyl-based chelators. *Chem. Biol.* 4:867–78
- Benner SA, Battersby TR, Eschgfaller B, Hutter D, Kodra JT, et al. 1998. Redesigning nucleic acids. *Pure Appl. Chem.* 70:263–66
- Berridge MJ. 1993. Inositol trisphosphate and calcium signalling. *Nature* 361:315–25
- Bishop AC, Kung CY, Shah K, Witucki L, Shokat KM, Liu Y. 1999. Generation of monospecific nanomolar tyrosine kinase inhibitors via a chemical genetic approach. *J. Am. Chem. Soc.* 121:627–31
- Bishop AC, Shah K, Liu Y, Witucki L, Kung C, Shokat KM. 1998. Design of allele-specific inhibitors to probe protein kinase signaling. *Curr. Biol.* 8:257–66
- Bishop AC, Shokat KM. 1999. Acquisition of inhibitor-sensitive protein kinases through protein design. *Pharmacol. Ther.* 82:337–46
- Bishop AC, Ubersax JA, Petsch DT, Matheos DP, Gray NS, et al. 2000. A chemical switch for inhibitor-sensitive alleles of any protein kinase. *Nature* 407:395–401

- Braisted AC, Judice JK, Wells JA. 1997. Synthesis of proteins by subtiligase. *Methods Enzymol.* 289:298–313
- Bruner SD, Norman DPG, Verdine GL. 2000. Structural basis for recognition and repair of the endogenous mutagen 8-oxoguanine in DNA. *Nature* 403:859–66
- Chang YT, Gray NS, Rosania GR, Sutherlin DP, Kwon S, et al. 1999. Synthesis and application of functionally diverse 2,6,9-trisubstituted purine libraries as CDK inhibitors. *Chem. Biol.* 6:361–75
- Cochran AG. 2000. Antagonists of protein-protein interactions. *Chem. Biol.* 7:R85–94
- Cotton GJ, Muir TW. 1999. Peptide ligation and its application to protein engineering. *Chem. Biol.* 6:R247–56
- Cotton GJ, Muir TW. 2000. Generation of a dual-labeled fluorescence biosensor for Crk-II phosphorylation using solid-phase expressed protein ligation. *Chem. Biol.* 7:253–61
- Coward P, Wada HG, Falk MS, Chan SDH, Meng F, et al. 1998. Controlling signaling with a specifically designed G(i)-coupled receptor. *Proc. Natl. Acad. Sci. USA* 95:352–57
- Das K, Ding JP, Hsiou Y, Clark AD, Moreels H, et al. 1996. Crystal structures of 8-Cl and 9-Cl TIBO complexed with wild-type HIV-1 RT and 8-Cl TIBO complexed with the Tyr181Cys HIV-1 RT drug-resistant mutant. *J. Mol. Biol.* 264:1085–100
- Davies SP, Reddy H, Caivano M, Cohen P. 2000. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem. J.* 351:95–105
- Davis ST, Benson BG, Bramson HN, Chapman DE, Dickerson SH, et al. 2001. Prevention of chemotherapy-induced alopecia in rats by CDK inhibitors. *Science* 291:134–37
- Dawson PE, Muir TW, Clark-Lewis I, Kent SBH. 1994. Synthesis of proteins by native chemical ligation. *Science* 266:776–79
- Degtarev A, Lugovskoy A, Cardone M, Mulley B, Wagner G, et al. 2001. Identification of small-molecule inhibitors of interaction between the BH3 domain and Bcl-x_L. *Nat. Cell Biol.* 3:173–82
- Denk W, Strickler JH, Webb WW. 1990. 2-Photon laser scanning fluorescence microscopy. *Science* 248:73–76
- Ding J, Das K, Tantillo C, Zhang W, Clark AD, et al. 1995. Structure of HIV-1 reverse transcriptase in a complex with the non-nucleoside inhibitor alpha-apa-R-95845 at 2.8 angstrom resolution. *Structure* 3:365–79
- Diver ST, Schreiber SL. 1997. Single-step synthesis of cell-permeable protein dimerizers that activate signal transduction and gene expression. *J. Am. Chem. Soc.* 119:5106–9
- Dougherty DA. 2000. Unnatural amino acids as probes of protein structure and function. *Curr. Opin. Chem. Biol.* 4:645–52
- Drees BL. 1999. Progress and variations in two-hybrid and three-hybrid technologies. *Curr. Opin. Chem. Biol.* 3:64–70
- Ellman JA. 1996. Design, synthesis, and evaluation of small-molecule libraries. *Acc. Chem. Res.* 29:132–43
- Farrar MA, Alberolaila J, Perlmutter RM. 1996. Activation of the Raf-1 kinase cascade by coumermycin-induced dimerization. *Nature* 383:178–81
- Fewtrell C. 1993. Ca²⁺ oscillations in non-excitable cells. *Annu. Rev. Physiol.* 55:427–54
- Furuta T, Wang SSH, Dantzer JL, Dore TM, Bybee WJ, et al. 1999. Brominated 7-hydroxycoumarin-4-ylmethyls: photolabile protecting groups with biologically useful cross-sections for two photon photolysis. *Proc. Natl. Acad. Sci. USA* 96:1193–200
- Gray NS, Wodicka L, Thunnissen AM, Norman TC, Kwon S, et al. 1998. Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors. *Science* 281:533–38
- Guckian KM, Krugh TR, Kool ET. 2000. Solution structure of a nonpolar, non-hydrogen-bonded base pair surrogate in DNA. *J. Am. Chem. Soc.* 122:6841–47
- Guo Z, Zhou D, Schultz PG. 2000. Designing small-molecule switches for protein-protein interactions. *Science* 288:2042–45
- Habelhah H, Shah K, Huang L, Ostareck-Lederer A, Burlingame AL, et al. 2001.

- ERK phosphorylation drives cytoplasmic accumulation of hnRNP-K and inhibition of mRNA translation. *Nat. Cell Biol.* 3:325–30
- Hanke JH, Gardner JP, Dow RL, Changelian PS, Brissette WH, et al. 1996. Discovery of a novel, potent, and Src family-selective tyrosine kinase inhibitor: study of Lck- and FynT-dependent T cell activation. *J. Biol. Chem.* 271:695–701
- Heinrich SU, Mothes W, Brunner J, Rapoport TA. 2000. The Sec61p complex mediates the integration of a membrane protein by allowing lipid partitioning of the transmembrane domain. *Cell* 102:233–44
- Ho SN, Biggar SR, Spencer DM, Schreiber SL, Crabtree GR. 1996. Dimeric ligands define a role for transcriptional activation domains in reinitiation. *Nature* 382:822–26
- Holsinger LJ, Spencer DM, Austin DJ, Schreiber SL, Crabtree GR. 1995. Signal transduction in T lymphocytes using a conditional allele of Sos. *Proc. Natl. Acad. Sci. USA* 92:9810–14
- Hsiou Y, Ding J, Das K, Clark AD, Hughes SH, Arnold E. 1996. Structure of unliganded HIV-1 reverse transcriptase at 2.7 angstrom resolution—implications of conformational changes for polymerization and inhibition mechanisms. *Structure* 4:853–60
- Huang HF, Chopra R, Verdine GL, Harrison SC. 1998. Structure of a covalently trapped catalytic complex of HIV-1 reverse transcriptase: implications for drug resistance. *Science* 282:1669–75
- Huang HF, Harrison SC, Verdine GL. 2000. Trapping of a catalytic HIV reverse transcriptase-template: primer complex through a disulfide bond. *Chem. Biol.* 7:355–64
- Hunter T. 2000. Signaling—2000 and beyond. *Cell* 100:113–27
- Huse M, Chen YG, Massague J, Kuriyan J. 1999. Crystal structure of the cytoplasmic domain of the type I TGF beta receptor in complex with FKBP12. *Cell* 96:425–36
- Huse M, Holford MN, Kuriyan J, Muir TW. 2000. Semisynthesis of hyperphosphorylated type I TGF beta receptor: addressing the mechanism of kinase activation. *J. Am. Chem. Soc.* 122:8337–38
- Ihle JN. 2000. The challenges of translating knockout phenotypes into gene function. *Cell* 102:131–34
- Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, et al. 2001. Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* 409:1060–63
- Itoh N, Yonehara S, Ishii A, Yonehara M, Mizushima S, et al. 1991. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. *Cell* 66:233–43
- Jacob R. 1990. Calcium oscillations in electrically non-excitable cells. *Biochem. Biophys. Acta* 1052:427–38
- Jacobomolina A, Ding JP, Nanni RG, Clark AD, Lu XD, et al. 1993. Crystal structure of human immunodeficiency virus type-1 reverse transcriptase complexed with double-stranded DNA at 3.0 angstrom resolution shows bent DNA. *Proc. Natl. Acad. Sci. USA* 90:6320–24
- Jager J, Smerdon SJ, Wang JM, Boisvert DC, Steitz TA. 1994. Comparison of three different crystal forms shows HIV-1 reverse transcriptase displays an internal swivel motion. *Structure* 2:869–76
- Klas C, Debatin KM, Jonker RR, Krammer PH. 1993. Activation interferes with the apoptosis pathway in mature human T-cells. *Int. Immunol.* 5:625–30
- Knockaert M, Gray N, Damiens E, Chang YT, Grellier P, et al. 2000. Intracellular targets of cyclin-dependent kinase inhibitors: identification by affinity chromatography using immobilized inhibitors. *Chem. Biol.* 7:411–22
- Kodadek T. 2001. Protein microarrays: prospects and problems. *Chem. Biol.* 8:105–15
- Koeller KM, Wong C-H. 2001. Enzymes for chemical synthesis. *Nature* 409:232–40
- Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. 1992. Crystal structure at 3.5 angstrom resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science* 256:1783–90
- Lemieux GA, Yarema KJ, Jacobs CL, Bertozzi

- CR. 1999. Exploiting differences in sialo-side expression for selective targeting of MRI contrast reagents. *J. Am. Chem. Soc.* 121:4278–79
- Li WH, Llopis J, Whitney M, Zlokarnik G, Tsien RY. 1998. Cell-permeant caged InsP(3) ester shows that Ca^{2+} spike frequency can optimize gene expression. *Nature* 392:936–41
- Lin H, Cornish W. 2001. In vitro protein-protein interaction assays: beyond proteins. *Agnew. Chem. Int. Ed.* 40:871–75
- Liu DR, Magliery TJ, Pasternak M, Schultz PG. 1997. Engineering a tRNA and aminoacyl-tRNA synthetase for the site-specific incorporation of unnatural amino acids into proteins in vivo. *Proc. Natl. Acad. Sci. USA* 94:10092–97
- Liuzzi M, Deziel R, Moss N, Beaulieu P, Bonneau AM, et al. 1994. A potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity in vivo. *Nature* 372:695–98
- Lutz S, Burgstaller P, Benner SA. 1999. An in vitro screening technique for DNA polymerases that can incorporate modified nucleotides. Pseudothymidine as a substrate for thermostable polymerases. *Nucl. Acids Res.* 27:2792–98
- Mahal LK, Yarema KJ, Bertozzi CR. 1997. Engineering chemical reactivity on cell surfaces through oligosaccharide biosynthesis. *Science* 276:1125–28
- Maly DJ, Choong IC, Ellman JA. 2000. Combinatorial target-guided ligand assembly: identification of potent subtype-selective c-Src inhibitors. *Proc. Natl. Acad. Sci. USA* 97:2419–24
- Matray TJ, Kool ET. 1999. A specific partner for abasic damage in DNA. *Nature* 399:704–8
- Maulik G, Botchway S, Chakrabarti S, Tetradis S, Price B, Makrigiorgos GM. 1999. Novel non-isotopic detection of MutY enzyme-recognized mismatches in DNA via ultrasensitive detection of aldehydes. *Nucl. Acids Res.* 27:1316–22
- Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ. 1999. Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* 286:971–74
- McMillan K, Adler M, Auld DS, Baldwin JJ, Blasko E, et al. 2000. Allosteric inhibitors of inducible nitric oxide synthase dimerization discovered via combinatorial chemistry. *Proc. Natl. Acad. Sci. USA* 97:1506–11
- Namiki S, Kaneda F, Ikegami M, Arai T, Fujimori K, et al. 1999. Bis-*N*-nitroso-caged nitric oxides: photochemistry and biological performance test by rat aorta vasorelaxation. *Bioorg. Med. Chem.* 7:1695–702
- Noren CJ, Anthony-Cahill SJ, Griffith MC, Schultz PG. 1989. A general method for site-specific incorporation of unnatural amino acids into proteins. *Science* 244:182–88
- Ogasawara J, Suda T, Nagata S. 1995. Selective apoptosis of Cd4(+)Cd8(+) thymocytes by the anti-Fas antibody. *J. Exp. Med.* 181:485–91
- Paulus H. 1998. The chemical basis of protein splicing. *Chem. Soc. Rev.* 27:375–86
- Peterson RT, Link BA, Dowling JE, Schreiber SL. 2000. Small molecule developmental screens reveal the logic and timing of vertebrate development. *Proc. Natl. Acad. Sci. USA* 97:12965–69
- Peterson SA, Klabunde T, Lashuel HA, Purkey H, Sacchettini JC, Kelly JW. 1998. Inhibiting transthyretin conformational changes that lead to amyloid fibril formation. *Proc. Natl. Acad. Sci. USA* 95:12956–60
- Pfeifer BA, Admiraal SJ, Gramajo H, Cane DE, Khosla C. 2001. Biosynthesis of complex polyketides in a metabolically engineered strain of *E. coli*. *Science* 291:1790–92
- Plante OJ, Palmacci ER, Seeberger PH. 2001. Automated solid-phase synthesis of oligosaccharides. *Science* 291:1523–27
- Qureshi SA, Kim RM, Konteatis Z, Biazzo DE, Motamedi H, et al. 1999. Mimicry of erythropoietin by a nonpeptide molecule. *Proc. Natl. Acad. Sci. USA* 96:12156–61
- Redfern CH, Coward P, Degtyarev MY, Lee EK, Kwa AT, et al. 1999. Conditional expression and signaling of a specifically

- designed G(i)-coupled receptor in transgenic mice. *Nat. Biotechnol.* 17:165–69
- Ren JS, Esnouf R, Garman E, Somers D, Ross C, et al. 1995. High resolution structures of HIV-1 RT from four RT-inhibitor complexes. *Nat. Struct. Biol.* 2:293–302
- Rivera VM, Wang XR, Wardwell S, Courage NL, Volchuk A, et al. 2000. Regulation of protein secretion through controlled aggregation in the endoplasmic reticulum. *Science* 287:826–30
- Rodgers DW, Gamblin SJ, Harris BA, Ray S, Culp JS, et al. 1995. The structure of unliganded reverse transcriptase from the human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* 92:1222–26
- Saxon E, Bertozzi CR. 2000. Cell surface engineering by a modified Staudinger reaction. *Science* 287:2007–10
- Saxon E, Bertozzi CR. 2001. Chemical and biological strategies for engineering cell surface glycosylation. *Annu. Rev. Cell Dev. Biol.* 17:1–23
- Sche PP, McKenzie KM, White JD, Austin DJ. 1999. Display cloning: functional identification of natural product receptors using cDNA-phage display. *Chem. Biol.* 6:707–16
- Sche PP, McKenzie KM, White JD, Austin DJ. 2001. Corrigendum to “Display cloning: function identification of natural product receptors using cDNA-phage display.” *Chem. Biol.* 8:399–400
- Schreiber SL. 2000. Target-oriented and diversity-oriented organic synthesis in drug discovery. *Science* 287:1964–69
- Schwikowski B, Uetz P, Fields S. 2000. A network of protein-protein interactions in yeast. *Nat. Biotechnol.* 18:1257–61
- Seeberger PH, Haase WC. 2000. Solid-phase oligosaccharide synthesis and combinatorial carbohydrate libraries. *Chem. Rev.* 100:4349–93
- Shah K, Liu Y, Deirmengian C, Shokat KM. 1997. Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. *Proc. Natl. Acad. Sci. USA* 94:3565–70
- Shultz MD, Bowman MJ, Ham YW, Zhao XM, Tora G, Chmielewski J. 2000. Small-molecule inhibitors of HIV-1 protease dimerization derived from cross-linked interfacial peptides. *Angew. Chem. Int. Ed.* 39:2710–13
- Sicheri F, Moarefi I, Kuriyan J. 1997. Crystal structure of the Src family tyrosine kinase Hck. *Nature* 385:602–9
- Sieg DJ, Ilic D, Jones KC, Damsky CH, Hunter T, Schlaepfer DD. 1998. Pyk2 and Src-family protein-tyrosine kinases compensate for the loss of FAK in fibronectin-stimulated signaling events but Pyk2 does not fully function to enhance FAK(–) cell migration. *EMBO J.* 17:5933–47
- Spencer DM, Belshaw PJ, Chen L, Ho SN, Randazzo F, et al. 1996. Functional analysis of Fas signaling in vivo using synthetic inducers of dimerization. *Curr. Biol.* 6:839–47
- Spencer DM, Graef I, Austin DJ, Schreiber SL, Crabtree GR. 1995. A general strategy for producing conditional alleles of Src-like tyrosine kinases. *Proc. Natl. Acad. Sci. USA* 92:9805–9
- Spencer DM, Wandless TJ, Schreiber SL, Crabtree GR. 1993. Controlling signal transduction with synthetic ligands. *Science* 262:1019–24
- Stockwell BR, Schreiber SL. 1998. Probing the role of homomeric and heteromeric receptor interactions in TGF-beta signaling using small molecule dimerizers. *Curr. Biol.* 8:761–70
- Tanaka M, Suda T, Takahashi T, Nagata S. 1995. Expression of the functional soluble form of human Fas ligand in activated lymphocytes. *EMBO J.* 14:1129–35
- Taunton J, Hassig CA, Schreiber SL. 1996. A mammalian histone deacetylase related to the yeast transcriptional regulator Rpd3p. *Science* 272:408–11
- Thompson LA, Ellman JA. 1996. Synthesis and applications of small molecule libraries. *Chem. Rev.* 96:555–600
- Tian SS, Lamb P, King AG, Miller SG, Kessler L, et al. 1998. A small, nonpeptidyl mimic of granulocyte-colony-stimulating factor. *Science* 281:257–59

- Tolbert TJ, Wong C-H. 2000. Integrin-mediated synthesis of proteins containing carbohydrates and other molecular probes. *J. Am. Chem. Soc.* 122:5421–28
- Tsien RW, Tsien RY. 1990. Calcium channels, stores, and oscillations. *Annu. Rev. Cell Biol.* 6:715–60
- Uetz P, Giot L, Cagney G, Mansfield TA, Judson RS, et al. 2000. A comprehensive analysis of protein-protein interactions in *Saccharomyces cerevisiae*. *Nature* 403:623–27
- Wallace CJA. 1995. Peptide ligation and semi-synthesis. *Curr. Opin. Biotechnol.* 6:403–10
- Wang L, Brock A, Herberich B, Schultz PG. 2001. Expanding the genetic code of *Escherichia coli*. *Science* 292:498–500
- Weiss EL, Bishop AC, Shokat KM, Drubin DG. 2000. Chemical genetic analysis of the budding-yeast p21-activated kinase Cla4p. *Nat. Cell Biol.* 2:677–85
- Wieser R, Wrana JL, Massague J. 1995. GS domain mutations that constitutively activate T beta R-I, the downstream signaling component in the TGF-beta receptor complex. *EMBO J.* 14:2199–208
- Wu YQ, Ogawa AK, Berger M, McMinn DL, Schultz PG, Romesberg FE. 2000. Efforts toward expansion of the genetic alphabet: optimization of interbase hydrophobic interactions. *J. Am. Chem. Soc.* 122:7621–32
- Xu R, Ayers B, Cowburn D, Muir TW. 1999. Chemical ligation of folded recombinant proteins: segmental isotopic labeling of domains for NMR studies. *Proc. Natl. Acad. Sci. USA* 96:388–93
- Yamase H, Zhao LS, Liu HW. 2000. Engineering a hybrid sugar biosynthetic pathway: Production of L-rhamnose and its implication on dihydrostreptose biosynthesis. *J. Am. Chem. Soc.* 122:12397–98
- Young K, Lin S, Sun L, Lee E, Modi M, et al. 1998. Identification of a calcium channel modulator using a high throughput yeast two-hybrid screen. *Nat. Biotechnol.* 16:946–50
- Zacharias DA, Baird GS, Tsien RY. 2000. Recent advances in technology for measuring and manipulating cell signals. *Curr. Opin. Neurobiol.* 10:416–21
- Zhong WG, Gallivan JP, Zhang YN, Li LT, Lester HA, Dougherty DA. 1998. From ab initio quantum mechanics to molecular neurobiology: a cation-pi binding site in the nicotinic receptor. *Proc. Natl. Acad. Sci. USA* 95:12088–93
- Zhu H, Klemic JF, Chang S, Bertone P, Casamayor A, et al. 2000. Analysis of yeast protein kinases using protein chips. *Nat. Genet.* 26:283–89
- Zutshi R, Brickner M, Chmielewski J. 1998. Inhibiting the assembly of protein protein interfaces. *Curr. Opin. Chem. Biol.* 2:62–66

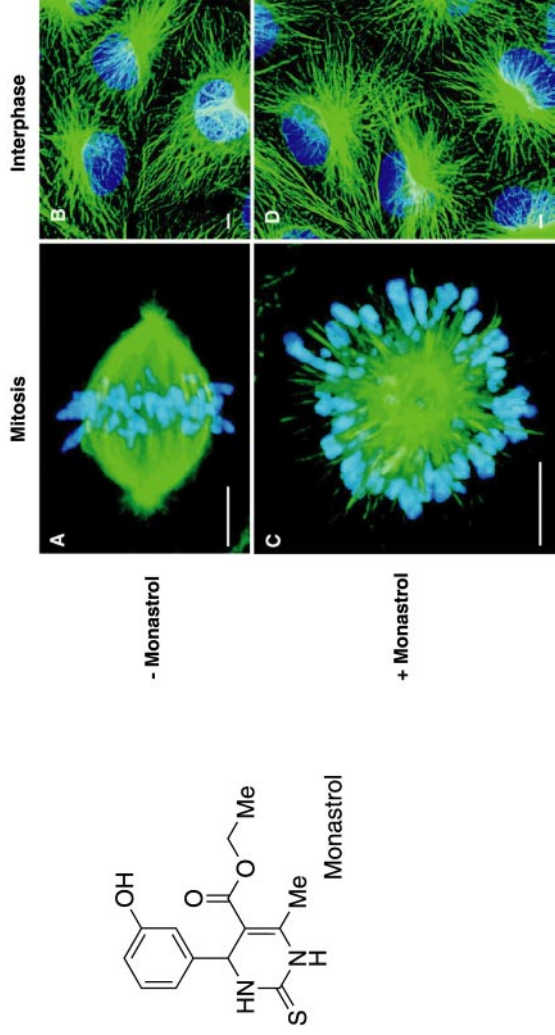


Figure 2 Monastrol causes the formation of monoastrol spindles in mitotic cells. Immunofluorescence staining [α -tubulin (green), chromatin (blue)] of BS-C-1 cells treated for 4 h with 0.4% DMSO (control, A and B) or 68 μ M monastrol (C and D). No difference in distribution of microtubules and chromatin in interphase cells was observed (B and D). Monastrol treatment of mitotic cells replaces the normal bipolar spindle (A) with a rosette-like microtubule array surrounded by chromosomes (C). Scale bars, 5 μ m.

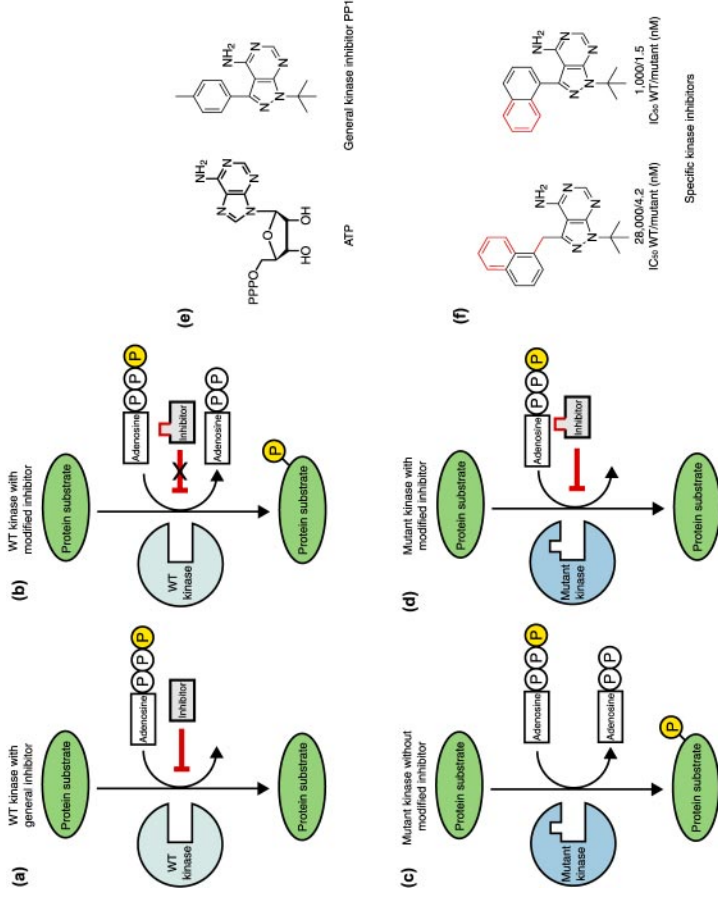


Figure 4 Kinase-specific inhibition can be achieved by coupling chemistry and genetics. (A) Wild-type kinases are inhibited by a non-specific inhibitor. (B) Wild-type kinases are not inhibited by non-specific inhibitor analogs containing a sterically bulky functional group. (C) Engineered kinases show normal kinase activity. (D) Engineered kinases are inhibited by inhibitor analogs that contain a sterically bulky functional group. (E) Chemical structures of ATP and the general kinase inhibitor PP1. (F) Chemical structures of specific kinase inhibitors, IC₅₀ wt/mutant (nM).

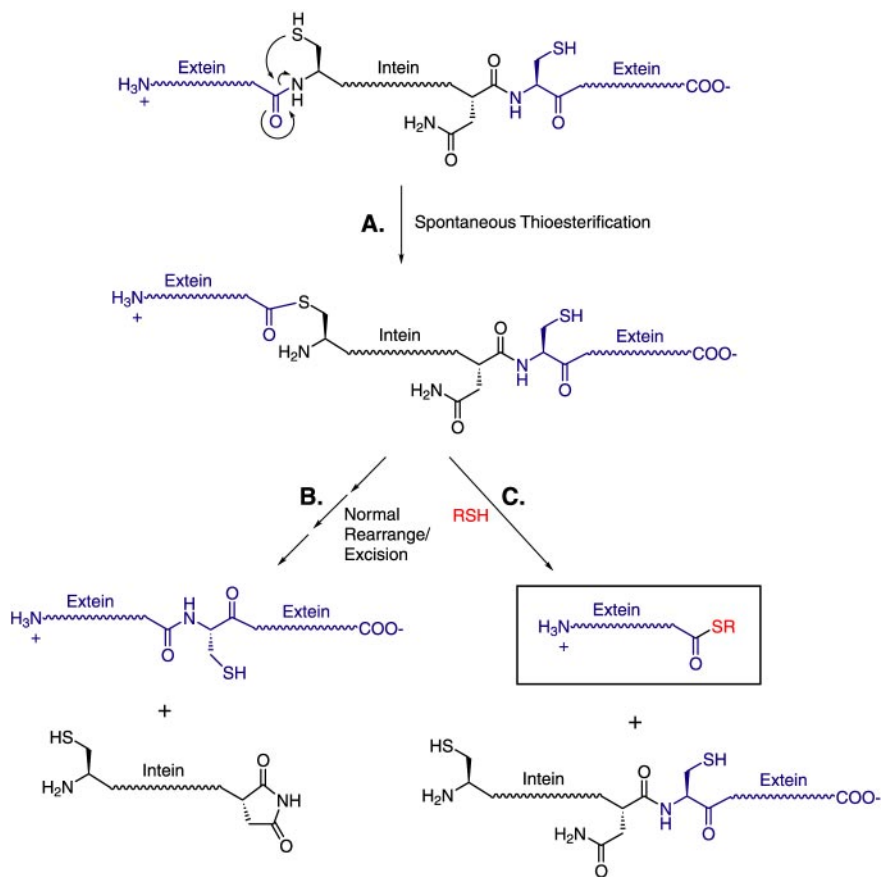


Figure 7 Inteин-mediated rearrangement of proteins to generate either extein-removed protein products or activated carboxy-terminal thioester protein products. (A) Spontaneous backbone rearrangement of an intein-containing protein to generate the activated thioester intermediate. (B) Normal rearrangement of the activated thioester intermediate to generate a final protein product with the intein domain removed. (C) Thiol-mediated interception of the activated thioester intermediate to generate an activated carboxy-terminal thioester protein product.

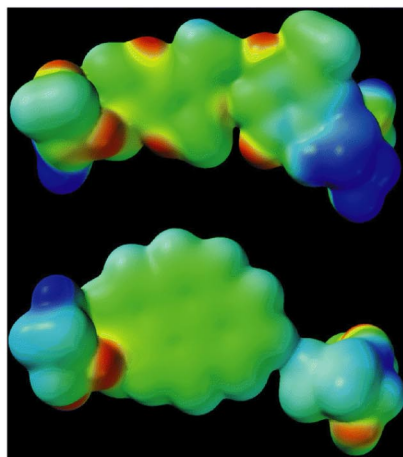
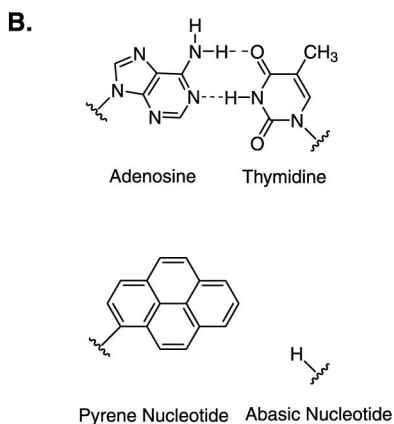
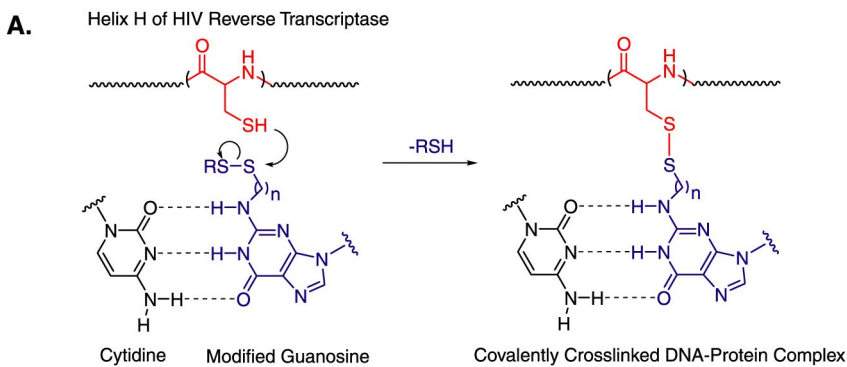


Figure 9 (A) Use of a modified guanosine nucleotide to covalently trap the protein-DNA interaction between HIV reverse transcriptase and its DNA substrate. (B) Comparison of standard base pair interactions (adenosine and thymidine) to a novel pyrene-containing and abasic nucleotide pair. The chemical structures of the pairs are depicted on the left. The space filling models are shown on the right.



CONTENTS

CHEMICAL AND BIOLOGICAL STRATEGIES FOR ENGINEERING CELL SURFACE GLYCOSYLATION, <i>Eliana Saxon and Carolyn Bertozzi</i>	1
THROMBOSPONDINS: MULTIFUNCTIONAL REGULATORS OF CELL INTERACTIONS, <i>Josephine C. Adams</i>	25
SALMONELLA INTERACTIONS WITH HOST CELLS: TYPE III SECRETION AT WORK, <i>Jorge E. Galán</i>	53
PATTERNING MECHANISMS CONTROLLING VERTEBRATE LIMB DEVELOPMENT, <i>Javier Capdevila and Juan Carlos Izpisua Belmonte</i>	87
IDENTIFICATION OF SELF THROUGH TWO-DIMENSIONAL CHEMISTRY AND SYNAPSES, <i>Michael L. Dustin, Shannon K Bromley, Mark M. Davis, and Cheng Zhu</i>	133
POLARIZED CELL GROWTH IN HIGHER PLANTS, <i>Peter Hepler, L. Vidali, and A. Cheung</i>	159
BOUNDARIES IN DEVELOPMENT: FORMATION AND FUNCTION, <i>Kenneth D. Irvine and Cordelia Rauskolb</i>	189
MOLECULAR BASES OF CIRCADIAN RHYTHMS, <i>Stacey L. Harmer, Satchidananda Panda, and Steve A. Kay</i>	215
EARLY EYE DEVELOPMENT IN VERTEBRATES, <i>Robert L. Chow and Richard A. Lang</i>	255
RNP LOCALIZATION AND TRANSPORT IN YEAST, <i>Pascal Chartrand, Robert H. Singer, and Roy M. Long</i>	297
VERTEBRATE SOMITOGENESIS, <i>Olivier Pourquié</i>	311
ANIMAL CELL CYTOKINESIS, <i>Michael Glotzer</i>	351
STEM AND PROGENITOR CELLS: ORIGINS, PHENOTYPES, LINEAGE COMMITMENTS, AND TRANSDIFFERENTIATIONS, <i>Irving L. Weissman, David J. Anderson, and Fred Gage</i>	387
RECENT ADVANCES IN CHEMICAL APPROACHES TO THE STUDY OF BIOLOGICAL SYSTEMS, <i>Michael A. Shogren-Knaak, Peter J. Alaimo, and Kevan M. Shokat</i>	405
EMBRYO-DERIVED STEM CELLS: OF MICE AND MEN, <i>Austin G. Smith</i>	435

HOW MATRIX METALLOPROTEINASES REGULATE CELL BEHAVIOR, <i>Mark D. Sternlicht and Zena Werb</i>	463
BIOLOGICAL BASKET WEAVING: FORMATION AND FUNCTION OF CLATHRIN-COATED VESICLES, <i>Frances M. Brodsky, Chih-Ying Chen, Christine Knuehl, Mhairi C. Towler, and Diane E. Wakeham</i>	517
GETTING THE MESSAGE ACROSS: THE INTRACELLULAR LOCALIZATION OF mRNAs IN HIGHER EUKARYOTES, <i>Isabel M. Palacios and Daniel St. Johnston</i>	569
CELLULAR FUNCTION OF PHOSPHOINOSITIDE 3-KINASES: IMPLICATIONS FOR DEVELOPMENT, IMMUNITY, HOMEOSTASIS, AND CANCER, <i>Roy Katso, Klaus Okkenhaug, Khatereh Ahmadi, Sarah White, John Timms, and Michael D. Waterfield</i>	615
CONTROL OF EARLY SEED DEVELOPMENT, <i>Adbul M. Chaudhury, Anna Koltunow, Thomas Payne, Ming Luo, Mathew R. Tucker, E. S. Dennis, and W. J. Peacock</i>	677
PEROXISOME BIOGENESIS, <i>P. Edward Purdue and Paul B. Lazarow</i>	701
THE MOLECULAR BASIS OF SISTER-CHROMATID COHESION, <i>Janice Y. Lee and Terry L. Orr-Weaver</i>	753
LEFT-RIGHT ASYMMETRY DETERMINATION IN VERTEBRATES, <i>Mark Mercola and Michael Levin</i>	779
INDEXES	
Subject Index	807
Cumulative Index of Contributing Authors, Volumes 13–17	841
Cumulative Index of Chapter Titles, Volumes 13–17	844
ERRATA	
An online log of corrections to <i>Annual Review of Cell and Developmental Biology</i> chapters may be found http://cellbio.AnnualReviews.org/errata.shtml	