Focus on Phosphat

High-throughput screening improves the odds by using kinases as targets for drug discovery.

BY MARK S. LESNEY

DEVELOPING EFFECTIVE DRUGS TARGETED to specific physiological processes is the ultimate goal of medicinal chemistry. Currently, the G-protein coupled receptors (GPCRs) take pride of place as specific metabolic receptors for the majority of target-directed drug discovery. But coming in at a close second are the kinases.

According to Serenex's Steven Hall and Triad Therapeutics' Hakim Djaballah, chairs of the Protein Phosphorylation Drug Discovery World Summit held in San Diego, March 2003, "Kinase research now accounts for nearly 25% of the programs of the majority of pharmaceutical and biotech companies." To be financially competitive and, more importantly, to discover the most promising drug candidates in the quickest possible fashion, the development of the right high-throughput method has become the core of much of this research.

Like the GPCRs, kinases are involved in the all-critical function of signal transduction. Part of the regulatory and response mechanism of individual cells and organs, kinases help maintain health, promote growth and development, and resist disease. In fact, as detailed previously, kinases provide the means through which the "sensory" GPCRs pass their message to the individual cells via the phosphorylation of specific proteins (*1*).

Unfortunately, the message is not always the correct one. In conditions such as cancer or autoimmune disease, the wrong signal or a badly timed one leads to damaging responses (e.g., tumor growth, inappropriate angiogenesis, erroneous apoptosis). Drugs that inhibit the aberrant expression of a kinase in a patient can stop the signaling cascade in its tracks and mean the difference between life and death. The best example of this, of course, is the much-touted drug Glivec—the first drug designed to inhibit a specific kinase responsible for the development of a unique form of leukemia (see box, "Gleanings from Glivec"). Because of this success, and because biochemists have speculated for years about the potential value of kinases as drug targets, these phosphate-transferring enzymes have become today's hot topic of drug research (*2*). To a lesser extent, their sister enzymes, the phosphatases, have also become a thriving field for study (see box, "Not forgetting phosphatases").

Kinase kinds

Although all kinases perform a similar biochemical function—moving a phosphate molecule from a nucleoside triphosphate donor to a protein or lipid acceptor—their substrate or cofactor specificity varies greatly. But, of the hundreds of available human kinases, a few overarching classes do stand out that are gener-

ILLUSTRATION: MICK WIGGINS

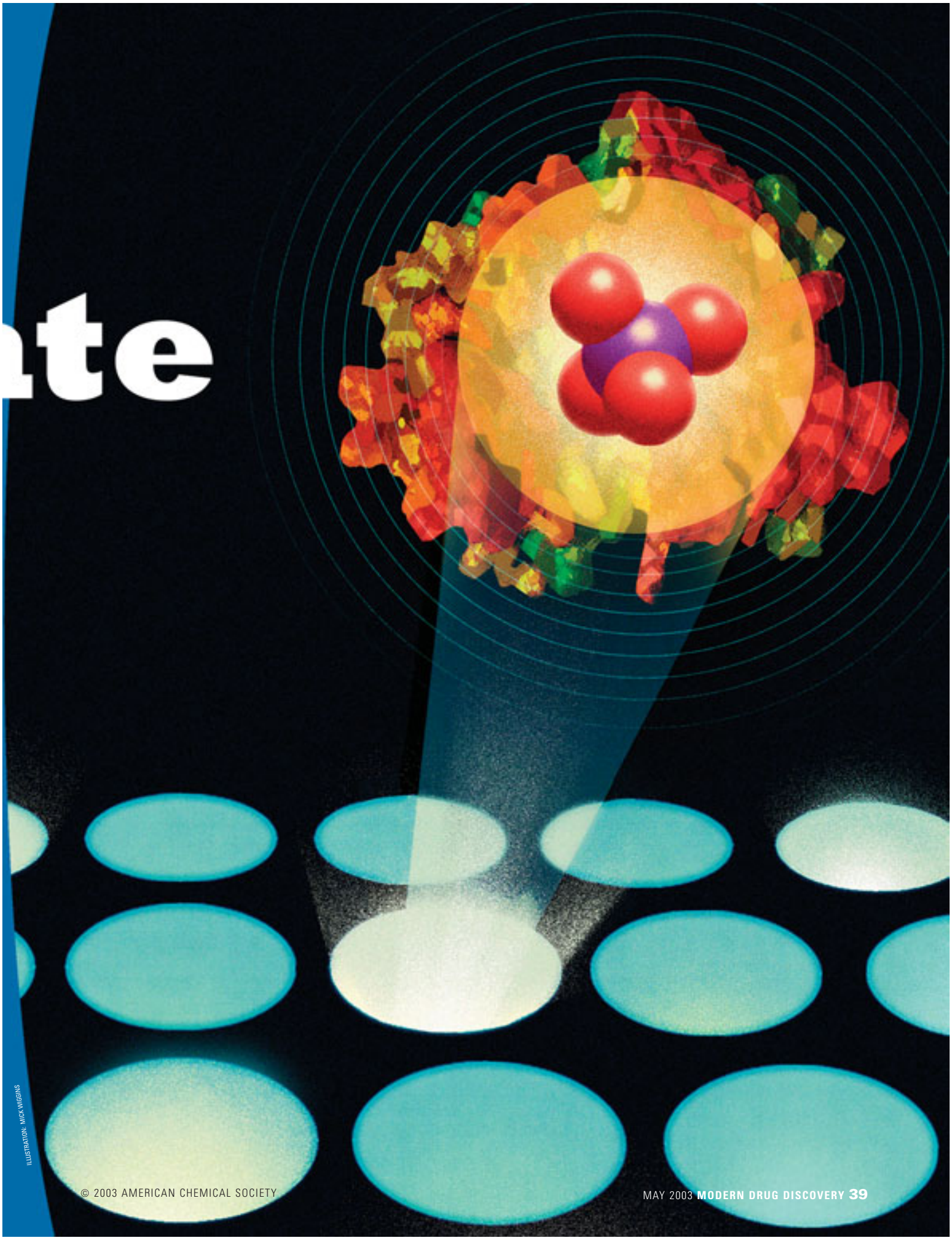

ILLUSTRATION: MICK WIGGINS

ally based on the nature of the different protein or lipid substrate involved or the activating molecules that act as cofactors. For example, only certain amino acids in a protein are amenable to phosphorylation. Certain kinases have thus evolved to be selectively capable of phosphorylating only serine or threonine residues; others can only phosphorylate tyrosines. This separation of substrate residue is often used as part of the classification scheme. And, although no taxonomy can strictly match kinase kind with physiological effect, there is a degree of correlation. Among the methods used to group kinases, the most classic taxonomy is that developed by Steven K. Hanks for human kinase classification. It contains five broad groups separated on the basis of sequence similarity of the catalytic domains (*http://pkr.sdsc.edu/html/pk_classification/pk_catalytic/pk_hanks_class.html*).

The AGC group includes the cyclic nucleotide-regulated protein kinase A and the diacylglycerol-activated/phoshpholipid-dependent protein kinase C families. *The CaMK group* comprises those kinases regulated by Ca^{2+}-calmodulin (CaM). Many of these are important in the process of neural transmission.

The CMGC group contains the cyclin-dependent kinases (CDKs) as well as the mitogen-activated protein kinases (MAPKs). These enzymes are key to a number of signal transduction pathways and in the cascade responses of cells to external stimuli. The p38 MAPK, for example, regulates the production of various cytokines, including tumor necrosis factor alpha and interleukins that are involved in inflammatory diseases. Vertex is investigating a number of p38 MAPK inhibitors in clinical trials to examine their anti-inflammatory effects on rheumatoid arthritis and other inflammatory diseases (*www.vrtx.com/AutoimmuneInflammation.html*).

The PTK group contains both the membrane- and non-membrane-spanning protein tyrosine kinases, including a wide variety of growth factor receptors. Finally, there is the *OPK* or *other protein kinases* category. These include kinases with unique activities, including kinases with leucine zipper domains and those involved in transcriptional control. Many of these families have been reorganized in the new classification scheme of the Human Kinome Project.

Promega offers a good overview of the role of a lot of the well-known kinases in signal transduction and a list of some of the many "minor" kinases (*www.promega.com/guides/sigtrans_guide/Default.htm*).

It should be no surprise that, faced with such a complicated taxonomy and such profound importance to cellular regulation, researchers have avidly attacked the so-called kinome—the sum total of kinase genes expressed in a species—for all the same reasons that hold in exploration of genomes and proteomes. The Human Kinome Project is a collaboration between the Salk Institute and the biotechnology company Sugen (*3*). Using data mining techniques, the researchers determined that 518 distinct kinases were encoded by the human genome, 100 of which had not been previously studied. They added four groups to the Hanks and Hunter classification and greatly extended the families and subfamilies within them. In the future, cross comparisons among other species, especially in manipulable model systems, will provide detailed information about the role of these various kinases in human physiology.

On a practical note, many companies have developed microar-

Gleanings from Glivec

Much of the excitement in the field of kinase research is due to the recent success of Glivec (imatinib; Gleevec in Europe). A lead compound identified in a screen for protein kinase C (PKC) inhibitors, the drug was specifically modified to be a selective inhibitor of the unique BCR/ABL-fusion tyrosine kinase produced from a reciprocal translocation event between chromosomes 9 and 22 that results in human chronic myelogenous leukemia (CML). By selective modification, the PKC inhibitory effect was eliminated while tyrosine kinase activity was enhanced. The addition of a polar side chain to the drug made it orally available. X-ray crystallography and docking studies showed that Glivec bound specifically to the ATP-binding site of an inactive form of the kinase. The drug induces remission in many cases of CML, but it is not curative and leukemia cells can become resistant to it. However, the drug is synergistic with a variety of other anticancer drugs. Researchers are also testing Glivec for effectiveness against several other cancers that involve other unique kinases that are also bound by the compound (*2*).

Not forgetting phosphatases

The mirror image of the kinases, phosphatases remove the phosphate groups from phosphorylated proteins and can be divided into two major groups: those that remove phosphates from phosphotyrosines and those that alter phosphoserines and phosphothreonines (*3*). Whereas the kinases achieve catalytic specificity by altered domains that recognize specific motifs in their substrates, the phosphatases achieve specificity mainly by changes in their constituent parts that localize the enzyme complex in various parts of the cell where their activity is required. Their catalytic regions, by contrast, are highly conserved. In addition, some phosphatases are regulated in vivo by protein modulators, such as calmodulin, or show metal cation requirements. Specific phosphatases have been found associated with a wide variety of cancers, and many companies are looking into using these enzymes as therapeutic targets. For example, Maxia Pharmaceuticals, a subsidiary of Incyte, is working to develop orally available, small-molecule phosphatase inhibitors of the MKP-1 and the Cdc25 phosphatases. Furthermore, in 1991, cyclosporin, the critically important immunosuppressant drug that made organ transplants routinely feasible, was shown to be a specific inhibitor of calcineurin, a CaM-dependent protein phosphatase (*1*).

rays to target kinases for purposes such as drug development. For example, PerkinElmer developed a Micromax direct-system focused array that contains 196 human kinases and 54 phosphatase genes. As with other microarray technology, the ultimate hope of researchers is to differentiate specific target genes activated across disease and normal physiological states. These could then be used in high-throughput screening to find inhibitors that could act as lead compounds.

The role of kinases, and the potential for kinase inhibitors in immunosuppression and the treatment of various cancers, has been much studied (*1*), but more recently, they have been deemed potentially useful in the treatment of inflammatory diseases, including arthritis, cirrhosis, dermatitis, and arteriosclerosis (*4*). Researchers at Eli Lilly (Indianapolis) and the Imperial College of London recently raised the issue of kinases as therapeutic targets for congestive heart failure, especially because of the apparent role of the protein kinase C family (which comprises at least 12 serine/threonine kinases) in cardiac hypertrophy (*5*). In addition, in a variety of human and rodent cell culture models, the activities of certain MAPK and lipid kinases also appear to be important in heart disease development.

Many companies, including Amersham, Applied Biosystems, Promega, and Upstate, provide a variety of high-throughput screening tools that researchers can use to assay the different forms of kinases and test for specific types of kinase inhibitors. These assays incorporate various colorimetric, luminescent, and fluorescent technologies to provide the level of screening required. Vertex has a patent on mutagenesis techniques to develop hybrid kinases as surrogate targets for drug design and compound screening.

In an even more theoretical approach to the problem of targeting, researchers are also looking to find and model more specific binding domains and sequence motifs that

Some commercial suppliers of high-throughput kinase assays

Albany Molecular Research Inc.
BioSource International, Inc.
Cetek Corp.
Ciphergen Biosystems
GeneFormatics
Invitrogen Corp.
Jerini Array Technologies/Jerini AG
Kinexus Bioinformatics
Li-Cor Biosciences
LifeSpan BioSciences
Luminex Corp.
Nanogen
PanVera Corp.
PPD Discovery
Promega Corp.
ProQinase GmbH
Structural Bioinformatics, Inc.
Upstate, Inc.

could one day provide high-throughput screening tools or even the information required for specific molecular design of potential inhibitors. For example, Michael Yaffe and colleagues at the Massachusetts Institute of Technology (Cambridge) and the Harvard Medical School (Boston) developed a proteomic approach for identifying the binding domains that modulate kinase-dependent signaling pathways (*6*). They used a screening library of partially degenerate phosphopeptides built around a particular protein kinase phosphorylation motif to "pull out" kinases that bound them.

If drugs targeted to kinases are ever to fulfill their overall promise of being a grand suite of effective compounds and not just the blockbuster few like Glivec, far more work must be done in focusing on individual members and unique docking sites on the hundreds of kinases in the human kinome. High-throughput screening for general classes of kinases, although invaluable for discovering appropriate potential targets to select from in the first place, cannot be the end-all and be-all of targeting. Unique sites on specific kinases related to the individual metabolic processes in particular diseases must be located for appropriate drug discovery.

Ultimately, says the National Institutes of Health's Elizabeth Nabel, broad-spectrum inhibitors are not the answer "because each of the targets . . . is comprised of families of related proteins whose members are involved in both beneficial and pathological processes" (*4*). Dealing with this problem will require all of the collective expertise of molecular modelers and medicinal chemists. But this seeming obstacle provides both the challenge and the opportunity for unique disease control (and unique economic niches) for those researchers and companies pursuing the vast drug promise involved when focusing on phosphate. With luck and skill, the current boom in clinical trials of kinase inhibitors (see "Clinical Trials Track", p 59) might only hint at the things to come.

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