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Solution structure of epidermal growth factor. It is based on a model by Iain Campbell of Oxford University, produced on a SiliconGraphics workstation using Polygen software, and shows the electrostatic potential at the surface.

EMERGING

by William J. Netzer

The most productive discovery technologies are likely to be those that provide information relevant to the decisions of a pharmaceutical company to undertake further development of lead compounds; i.e., to traverse the costly R&D pipeline leading to pharmaceutical markets. So believes Alejandro Zaffaroni, a founder of Palo Alto (CA) companies Alza, Syntex, and DNAX (a unit of Schering-Plough).

Yet while the judicious choice of drug candidate compounds influences research productivity, the intellectual groundwork underlying these choices traditionally has taken many years to evolve. Now, new discovery technologies and constellations of existing technologies, combined with information management techniques, are likely to accelerate and improve that process. The U.S. arm of Zaffaroni's latest venture, Affymax Research Institute (also in Palo Alto), is a drug discovery company that very much holds to this philosophy. Its researchers expect to accelerate lead compound discovery and to hasten development of a critical information base—and thus better predict both the likelihood of developing successful compounds and their probable course of development.

The process of developing finished drugs from leads is generally long, convoluted, and costly; further, a plethora of promising lead compounds is likely to spur competition among discovery companies for the development resources large firms can provide. Thus, in allocating resources to innovative drug discovery technologies, developing companies such as Affymax must also consider the R&D practices and goals of large manufacturers. Biotechnical suitors may have more to offer than just promising molecules: information linking molecular structure to activity and selectivity, elucidation of biomolecular targets, and preliminary toxicological data.

Fallout Along the R&D Pipeline

Usually, lead compounds spawn numerous chemical derivatives that are evaluated through batteries of assays to assess their value as potential drugs. Successive stages of escalating commitment and resource allocation characterize the process, and compounds will fall out at all stages along the route to commercialization.

For example, at Pfizer (New York), according to the company's information specialist Bob Hollis, for every 15 compounds that are nominated for clinical trials (i.e. compounds that begin animal toxicology), on the average six drop out during animal testing because of toxicity or because production cannot be scaled up to sustain human trials. The remainder enter Phase I human trials, where another three will drop out because of inability to address salient clinical parameters. Those remaining go on to Phase II, where four more will drop out due to lack of (or nominal) efficacy.

That means only two out of every 15 compounds enter Phase III, where one or both still may fall by the wayside due to a variety of causes—e.g. lack of efficacy, the emergence of rare side effects, results of long-term animal toxicology, or a competing compound in parallel development showing greater promise. The bulk of the pharmaceutical industry's R&D spending (about $7–9 billion per year, or about 73 percent) goes toward evaluating these drug candidates. And industry estimates for producing a successful drug range from $100–125 million. Weeding out useless compounds before substantial resources are allocated for development—and picking more fruitful leads—therefore have been long-standing industry goals.

Starting with Screening

Receptor screens have become fairly common in the pharmaceutical industry. Merck (Rahway, NJ), perhaps the most prolific screener, uses about 60 different receptors to screen approximately 40,000 chemical entities each year. The vast majority are natural products (derived mainly from microbial broths) and result in a total

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Receptor binding is one of neurobiotechnology's tools for novel drug discovery. This computer image depicts a ligand/receptor binding interaction. Pink spheres represent atoms comprising the binding domain of the receptor. The ligand is depicted by the red to yellow color.

TOOLS FOR DISCOVERING DRUGS

of about 500,000 assays, according to Alan Oliff, director of research at Merck's West Point (PA) facility. The company's CCK antagonist, asperlicin, now in clinical trials for irritable bowel syndrome, is a recent fruit borne of this screening process, as was the cholesterol-lowering drug, Mevacor, a decade ago.

It would be a mistake, however, to view the goals of pharmaceutical R&D solely in terms of achieving a single drug resulting from one lead compound. Drug development is a creative process that attempts to link chemical structure with biological effect. It often results in growth and modification of an initial therapeutic concept. The payoff, therefore, may go well beyond one marketable drug.

Take Knoll Pharmaceuticals' (Ludwigshafen, F.R.G.) Verapamil, a calcium channel antagonist used to treat cardiovascular disease and hypertension. It was discovered in 1962 after testing more than 500 derivatives of the lead compound, papaverine. At least 300 additional compounds were then synthesized. Some were used to define the actual calcium channel and to develop the pharmacology of this system to its present state. And company spokespersons note that the chemistry upon which these calcium antagonists are based continues to yield new compounds, with new patents still being filed.

Similar examples of evolving families of drugs are the beta adrenoreceptor blockers (e.g. Inderal, Tenormin) and agonists (e.g. Ventolin), whose discoveries have entailed both development of new therapeutic targets and growing knowledge of receptor subtypes.

Receptors and Primary Screening

In primary pharmaceutical screening, the specific binding of a ligand to a receptor acts as a selective device, or predictor of biological effect. This may take the form of a binding assay that measures specificity and affinity of binding, or a functional assay signaling the immediate effect or consequence of binding—i.e. whether a ligand acts as an agonist or an antagonist.

Although a drug's effects are generally the result of numerous molecular interactions, receptor assays provide an approximation of activity and a high-throughput way to screen leads. Additionally, they provide a means for deriving structure activity relationships (SARs), which are correlations of biological activity with molecular structure. In this context, screening can provide data for rational drug design.

At Nova Pharmaceutical (Baltimore, MD), receptor binding assays are used extensively for screening its own compounds, as well as those of corporate partners. Once a promising lead has been identified, chemists analyze its structure and produce variants that are then put back into the original binding assays to assess how changes in structure affect affinity for a receptor. Large pharmaceutical companies have for years used similar approaches involving whole animal systems and tissue assays. Binding assays, according to Nova researcher Paul Sweetnam, are able to provide a fairly good indication of potency by measuring the affinity of a ligand for a receptor.

The importance of affinity in defining the value of a lead compound has been fundamental to pharmacological screening, says Joshua Boger, president of Vertex Pharmaceuticals (Cambridge, MA) and former director of basic chemistry at Merck. Boger has noted that many screening programs have failed to generate useful leads—even though tens of thousands of compounds were tested.

Essentially, a useful lead exhibits extremely high affinity for a receptor. The stringency of this criterion is motivated by a desire for potency—to minimize dosages and side effects. Boger notes that while screens often resolve many weak (low-affinity) leads, pharmaceutical companies discard them because they have not been willing to attempt development where potency would have to be raised by perhaps several orders of magnitude.

Boger believes that screening can generate numerous low-affinity leads, and that they can provide data for computational techniques that link chemical structure to biological activity. He also notes that one of the demonstrated strong-points of computer-assisted drug design is the abili-

U.S. FIRMS WITH STRUCTURE-BASED DRUG DESIGN PROGRAMS

Firms Dedicated to Rational Drug Design
(location, year founded)

Agouron Pharmaceuticals (La Jolla, CA, 1984). The oldest and most visible company founded to pursue rational drug design (RDD). Its primary target is to develop inhibitors to the TS (thymidylate synthase) enzyme to combat cancer, especially solid tumors. It has designed six families of molecules, and expects to begin human clinical testing in 1990 or 1991. Also anti-viral drug development, including four inhibitors of HIV (human immunodeficiency virus) — reverse transcriptase, protease, integrase, p24. Another TS inhibitor program is aimed at psoriasis; others are exploring receptors for cyclophilin, human insulin, herpes, glucocorticoid (anti-inflammatory drugs), and the oncogene *ras* protein.

BioCryst (Birmingham, AL, 1986). Lead project on purine nucleoside phosphorylase inhibitors, for cancer and some auto-immune diseases, is at preclinical stage. Other projects include inhibitors of aldose reductase (diabetes, cataracts), collagenase (connective tissue disorders), and factor D (ischemic insult, immune complex disorders).

CrysChem, subsidiary of Synbiotics (Riverside, CA, 1987). The company will crystallize Fab fragments of antibodies, then use molecular modeling to design ligands to bind antibody and receptor. As well, it will synthesize decapeptides corresponding to the hypervariable loops of anti-receptor antibodies.

Protos, subsidiary of Chiron (Emeryville, CA, 1988). Chiron supplies company with genetic engineering expertise and products; e.g. purified receptor proteins for growth factors such as EGF. Partnership with J&J (wound healing) and Warner Lambert (Morris Plains, NJ) (cancer, central nervous system disorders).

Receptech, subsidiary of Immunex (Seattle, WA, 1989). Will develop soluble receptors as treatments for auto-immune disorders and anti-inflammatory diseases, as well as conduct initial development of Immunex's other long-term projects (see, e.g., Sterling Drug, below).

Vertex Pharmaceuticals (Cambridge, MA, 1989). Initial projects focus on organ transplant rejection (cyclophilin), autoimmune disease (various targets), antivirals (e.g. HIV protease), and emphysema (leukocyte elastase).

Pharmaceutical/ Biopharmaceutical Firms

Abbott Laboratories (Abbott Park, IL). Primary emphases on acquired immunodeficiency syndrome (AIDS) therapeutics and renin inhibitors (hypertension). Early-stage programs with structural targets related to neurological and immune disorders.

Bristol-Myers Squibb (New York). Recently announced intent to establish an RDD unit.

Burroughs Wellcome (Research Triangle Park, NC). Main focus on non-peptide inhibitors of HIV reverse transcriptase. Also continuing work on dihydrofolate reductase (DHFR) inhibitors to combat certain cancers.

DuPont (Wilmington, DE). Developing phospholipase A2 inhibitors as anti-inflammatories.

Genentech (So. San Francisco, CA). Modeling the anti-HIV activity of potential AIDS therapeutics; studying growth hormone receptor interactions. Also studying lymphocyte attachment mechanisms to attempt to block cell adhesion to tissues (tumor metastases, autoimmune diseases).

Hoffmann-La Roche (Nutley, NJ). Focus on non-peptide inhibitors of DHFR (cancer), beta-lactamase (bacterial infections), and HIV protease. Collaboration with Genentech on argatroban. Joint venture with Genetics Institute (Cambridge, MA) to develop inhibitors of HIV reverse transcriptase.

Johnson & Johnson (New Brunswick, NJ). Subsidiary Ortho Pharmaceutical engaged in R&D agreement with Scripps (La Jolla, CA).

Eli Lilly (Indianapolis, IN). Joint venture with Agouron. While targets have not been disclosed, they are suspected to include AIDS-associated proteins.

Merck (Rahway, NJ). Projects include non-peptide inhibitors of HIV protease, carbonic anhydrase (diuretics), and leukocyte elastase (emphysema).

Pfizer (New York). Independent programs, along with SmithKline Beecham (SB) and Upjohn, toward development of non-peptide inhibitors of HIV protease. SB and Upjohn have first-generation inhibitors (peptides) in hand.

Schering-Plough (Kenilworth, NJ). Pursuing strucure of gamma interferon (cancer, immune disorders).

SmithKline Beecham (Philadelphia, PA). See Pfizer.

Sterling Drug (New York). Well-advanced program on anti-rhinovirus 14 compounds (common cold). Lead compound in clinical trials. Also pursuing synthetic mimics of interleukin-2 in collaboration with Immunex.

Upjohn (Kalamazoo, MI). See Pfizer.

Some of the information in this table was provided courtesy of Hambrecht & Quist (San Francisco, CA).

ty to design more potent molecules from less potent leads. Vertex is seeking corporate relationships to test this approach, which would require sets of competing ligands (ligands that may occupy the same receptor site).

In fact, Affymax has developed technologies for rapidly generating ordered sets of ligands without applying conventional synthetic chemistry. These novel systems—collectively called the Affinity Matrix™—yield enormous chemical diversity and a concomitant capacity to evaluate compounds rapidly for biological activity. Data-basing the information and merging computer-aided drug design techniques with expert systems hopefully will provide a basis for more practical rational drug design approaches.

Another rational approach to molecular design is to derive structural information about a receptor, which usually involves calculating a structure based on crystallographic data. But obtaining useful crystals is often an awesome undertaking. Some suggest that, because of the difficulty of crystallizing and modeling receptors, to mass-screen a million compounds for a lead is likely, in many cases, to be easier and more productive.

Vertex researchers, by contrast, are using molecular biology to modify proteins so that they can be crystallized more readily, hopefully without significantly altering their active sites. Recombinant DNA technology has made it possible to modify proteins either by removing, substituting, or adding peptides or individual residues. It may also be possible to devise recombinant proteins that are more readily crystallized by screening variants (empirically) or by more rational approaches. Numerous laboratories have produced soluble forms of cell-

AI MAY MAKE THINGS MORE RATIONAL

One of the dangers of using a classical medicinal chemistry approach," warns Chris Floyd, a medicinal chemist with British Bio-technology Ltd (BBL, Oxford), is that you are always working with a particular group of compounds and derivatives." This is one reason BBL is participating in the Castlemaine project, a $1.2 million collaborative venture to use artificial intelligence (AI) techniques to gain a better understanding of the drug development process and, eventually, to produce software to assist drug design. The discovery of a lead compound can limit a drug development program, Floyd explains, because it focuses the investigator's efforts on a particular family of chemical derivatives.

Participating in the project are BBL, Cambridge software companies Logica and CamAxys, and the University of Edinburgh's department of artificial intelligence, the U.K.'s foremost academic group in the application of AI to design. The collaboration's multidisciplinary approach already has helped shed new light on the drug development process by forcing drug designers to stand back and analyze what it is they are actually doing. Based on discussions with Floyd and his colleagues at BBL, Logica's Gareth Lloyd has defined four basic tasks performed by the medicinal chemist: selecting a group of compounds, organizing information about those compounds, modeling the pharmacophore, and designing something novel. Nothing startling there, but by trying to represent the knowledge that medicinal chemists use in a variety of ways without knowing the significance of the information itself, the AI specialists found that they were starting to mimic aspects of rational drug design. Instead of relating chemical entities by structure, Lloyd points out, they can be related by chemical group or by similar properties at the functional level. In this way, the effort may clarify relationships between compounds of differing chemical structures—but similar stereochemical arrangements of functions.

This is precisely the approach that led to the development of Hoffmann-La Roche's (Basel, Switzerland) angiotensin-converting enzyme inhibitor. According to Floyd, by extensive computer modeling of existing Merck and Squibb (New York) products, Roche chemists "defined areas of space where interactions were supposed to occur," and then designed molecules to fit. Although Floyd is skeptical about the extent to which drug design is at this point rational ("the rationale is usually more obvious *post hoc*," he says), he is impressed by the way the AI specialists have been able to analyze the information hierarchy used by drug designers.

When the AI system reaches the prototype stage it will be tested out on BBL's development programs for PAF (platelet activating factor) antagonists where, according to Floyd, "there is quite a bit of knowledge, several series of compounds, and many blind alleys." It will be interesting to see if the computer makes the same choices as the experts.

John Pardon, who heads up BBL's efforts in drug design and computational chemistry, already sees benefits from the project. By June 1992, when Castlemaine finishes, he "would like to get some [software] that would work. But the project is already making us sit down and rationalize our strategies."

—JH

surface receptors by expressing only the extracellular, hydrophilic domains (a form that can be crystallized). As well, Vertex is using Space Shuttle missions to attempt to grow more ordered crystals in microgravity for crystallographic analysis and structural mapping.

A variety of new companies utilizing crystallography and computational chemistry have recently been founded, each approaching drug discovery and rational design in different ways (see table). Protos (Emeryville, CA, a wholly owned subsidiary of Chiron) is obtaining cloned (soluble) receptor proteins and peptide growth factors from its corporate parent. Through crystallography and modeling the company aims to discover non-peptide analogs of various growth factors—a goal shared by many drug companies. Research director Steve Rosenberg believes ultimately that crystal structures of receptors, as opposed to structures of sets of ligands, will be most valuable in rational design. He bases that belief on the notion that even sets of competing ligands may represent only portions of a receptor cavity's available binding modes: they may fill overlapping but different parts of the cavity, and even structures occupying overlapping volumes may be bound to the receptor through differing interactions (e.g., quantities of hydrogen bonds, van der Waals forces, etc.). Hence, a pharmacophore calculated solely from ligands may represent only a portion of the actual receptor site. (Also, a receptor site that binds known ligands may represent only one of several targets for achieving modulation of receptor function.)

CrysChem (San Diego, CA), a subsidiary of Synbiotics, takes another approach to crystallography and rational drug design. During the past few years, Synbiotics has developed a technology involving antibodies that act either as surrogates of the binding sites of other receptors or as surrogates of ligands. Initially, the company focused on veterinary products and envisioned surrogate receptors as high-throughput pharmacological screens. Earlier this year, however, Synbiotics created another subsidiary, ImmunoPharmaceutics, headed by Manfred Wolff, to target human therapeutics. Wolff was formerly vice president of pharmaceutical discovery at Allergan (Irvine, CA). Rather than go the screening route, ImmunoPharmaceutics will employ a variety of techniques to extract information directly from the antigen binding sites of its surrogate receptors (antibodies), based on the assumption that those reagents (and their anti-idiotypes) contain information pertaining to the three-dimensional and electronic characteristics of a pharmacophore. Antibody-based receptor surrogates are not replicas of natural receptors, but may embody SARs for classes of potential ligands, and so may represent alternative systems for drug discovery and design.

ChrysChem is crystallizing antibodies (Fab' fragments) rather than other receptors which would have to be approached on a case-by-case basis. It will then do subsequent molecular modeling to design ligands that would bind both surrogate receptor (antibody) and actual receptor. Addi-

tionally, the two Synbiotics' subsidiaries will synthesize decapeptides corresponding to the hypervariable loops of anti-receptor antibodies (surrogate ligands). According to Wolff, some of these peptides bind to the actual receptor as competitive ligands. These peptides may then be used as bridges to design non-peptide analogs (peptide-mimetics)—the actual drug leads.

Receptor Selectivity

Profile™ is a set of receptor binding assays now used at Nova to screen compounds against an assortment of different receptor types and subtypes. Its purpose is to determine whether a ligand binds to more than one kind of receptor. A ligand might, for example, bind to both beta 1 and beta 2 adrenoreceptors. The significance of this cross-reactivity would depend on the affinity of the ligand for each receptor subtype and on what kind of drug was being sought.

If a selective beta 1 antagonist was sought, a ligand that bound to both subtypes with high affinity might be problematic. But a problem might not exist if the ligand's affinity for beta 1 receptors was several orders of magnitude greater than its affinity for beta 2 receptors. (A selective beta 1 ligand will have specificity for heart beta adrenoreceptors, as opposed to beta 2 receptors, which occur more frequently in the peripheral vasculature and bronchi.)

The binding of a ligand to several receptor types might result in its abandonment or in chemical modification to achieve greater selectivity. In other instances cross-reactivity might be welcomed when it resulted in a new therapeutic target, based on binding of a ligand to a previously unsuspected receptor.

A drug's selectivity is also influenced by its bioavailability, however, which in turn is influenced by its pharmacokinetics and by its metabolism, as well as by the variability of the target itself.

Bioavailability is generally not addressed by receptor screens, unless, for example, the receptor is found within a cell. Ligand Pharmaceuticals (San Diego, CA), for one, uses cells expressing intracellular receptors, such as those that bind to steroid hormones. In these functional assays, a positive not only signals specificity for the receptor, but also the ability to cross the cell membrane.

Potency also influences selectivity. And although it relates to the affinity of a ligand for a receptor, potency may ultimately depend on the physiological events that result from receptor binding.

Rationalizing the Source

Although screening often connotes a reliance on serendipity, choosing a source of chemical diversity often is based on rationales designed to enhance the probability of discovery. There are good reasons for screening natural products, for example. Many drugs including aspirin, digitalis, morphine, and numerous antibiotics were derived from natural sources.

Additionally, substances produced by organisms that function in communication (e.g. growth regulators, toxins, and pheromones) often mimic or inhibit the actions of hormones, transmitters, and other biological modulators; and may therefore bind to receptors that recognize these molecules. This is especially significant since similar receptor specificities occur across species lines and even among different kingdoms.

Mass-screening of microbial broths is common, but now plants are being considered more and more as potential sources. Shaman Pharmaceuticals (San Carlos, CA) hopes to increase the likelihood of finding useful compounds in plants through an ethnobotanical approach. Essentially, the company sends interdisciplinary teams of investigators to tropical areas in South America, Africa, and Southeast Asia to identify medicinal plants used by local shamans. Similarly, Stamford, CT-based start-up VimRx has arrangements with South American and East European research institutes to supply plant compounds for mass screening.

In Vitro Toxicology

In recent years, researchers have been developing *in vitro* assays that assess toxicity to complement or substitute for animal models. While industry and regulators have yet to resolve questions of data interpretation from *in vitro* tests, as well as their relation to standard *in vivo* models, *in vitro* assays have another, to date less explored, use; namely, in the assessment of lead compound toxicity.

Although a lead's toxicity may bear little relationship to that of a final drug, the purpose of a preliminary toxicology screen would be to develop SARs pertaining to aspects of toxicity, such as acute cytotoxicity, which can be resolved by fairly simple systems.

For example, the neutral red (NR) assay developed by Ellen Borenfreund at Rockefeller University (New York) is a quantitative *in vitro* test that measures general acute cytotoxicity. It is based on the accumulation of a weakly cationic red dye by the lysosomes of viable cells. In Borenfreund's versions of the assay the toxic endpoint is cell death or injury indicated by inhibition of NR uptake (expressed generally as NR_{50}).

Borenfreund has used a wide variety of target cells and claims that the choice of target cell is of secondary importance except where cell metabolism is an important factor for modifying toxicity. Her critics contend, however, that the assays may not be able to distinguish organ-specific toxicants or subtle lesions (short of cell death or compromised membrane integrity).

In fact, the generality of NR assays might make these systems useful as preliminary tox screens. Borenfreund has demonstrated broad agreement between NR data and *in vivo* systems, and has shown that, although differences in the magnitude of toxicity exist for different cell types and cells derived from different species, there is often agreement in the rank order of toxicities for a wide assortment of toxicants.

More significantly, Borenfreund has used the NR assay to describe SARs within series of compounds, such as substituted alcohols, toluenes, phenolics, and various metals, and again showed agreement with *in vivo* systems.

One might envision a preliminary tox screen consisting of several cell types to reflect both differentiated targets and metabolic competence. Clonetics (San Diego, CA) sells human keratinocytes and its version of the NR assay (which employs inhibition of cell proliferation as an endpoint). The company claims that this system is particularly relevant to human toxicity because the cells are of human origin, are differentiated, and are metabolically competent; in other words, it expresses the cytochrome monooxygenase (P450) system (at approximately 10 percent of the activity found in liver) which can result in the detoxification of some compounds or in activation of toxicants, a process generally mediated by the liver.

A variety of other *in vitro* assays exist that reflect either finer distinctions regarding targets or cellular injury. These more articulated assays might be assembled in screens based on the specific goals of drug discovery programs. Of course, like the NR assays, most of these systems would not directly address aspects of toxicology such as teratogenicity, chronic toxicity, and pharmacokinetics.

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