Expanding the HTS paradigm

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▼ The efficiency with which compounds in need of biological evaluation can be identified using HTS has placed increasing pressure on both pharmaceutical and biotech lead-development programs. At the same time, increased screening capacity has created more demand for novel validated disease targets. These developments have led to new opportunities for the use of HT technologies at numerous stages of the drug discovery process.

High-throughput screening is the rapid analysis of chemical libraries containing from hundreds-of-thousands to millions of compounds for those exhibiting a desired biological activity. This is achieved using automated, robust miniaturized assays. However, just a small fraction of primary 'actives' flowing from this process can easily overwhelm traditional follow-up testing, creating a new rate-limiting step in the lead-development pipeline. Finding a solution to the 'shifting bottlenecks' in drug discovery can only be developed through collaborations between biological and physical sciences, engineering, and informatics, and will enable HT technologies to be applied to medicinal chemistry, structural biology, pharmacology, ADME studies and toxicology. For example, voltage-gated ion channels represent an important target area in neuroscience, yet reliance on single-channel current recordings limits the rate at which leads from HTS and medicinal chemistry can be characterized. High-throughout electrophysiology technologies, particularly automated patch-clamp assays, are being developed by several bioengineering companies and have the potential to improve throughput by one to two orders-of-magnitude, relative to manual methods [1].

High-throughput meets high-resolution

Rapid analog synthesis and combinatorial chemistry have contributed to substantial increases in compound archives and, in turn, to the number of lead structures requiring prioritization. Thus, information-rich assays, such as those based on fluorescence microscopy, can add significant value to each datapoint. By providing a window into the cell, microscopy reveals the distribution and morphology of proteins and organelles. Subcellular responses to pharmacological agents, such as the trafficking of G-protein-coupled receptors, or the nuclear translocation of transcription factors, can be monitored using a combination of molecular sensors, including those based on variants of green fluorescent protein, and automated high-speed scanning microscopes. The latest instruments, capable of kinetic multiparameter outputs, operate at rates comparable to those of microtiter-plate readers and use a combination of sophisticated autofocusing techniques, optical designs and image-processing software. Screens based on changes in cellular phenotype represent an improvement in the cell-based assay and yield information about the mechanism-of-action of a compound beyond that obtained with current reporter gene formats. Phenotypic-based assays have broad applications in HTS, pharmacological profiling and target validation.

On a slightly more distant horizon, *in vivo* imaging – a powerful non-invasive technology previously limited to clinical diagnostics – is now accessible to pharmacologists and biologists. The anatomical and physiological data provided by positron emission tomography (PET), magnetic resonance imaging (MRI), and optical projection tomography (OPT), for example, will bring unprecedented insight into drug action early in development [2,3]. Recent advances in this technology include compact scanners for use with small animals, improved access to PET tracers, and the synthesis of novel MRI contrasting agents that can report on biological processes. Further developments in this technology will make HT micro-PET of transgenic mice possible, which would move receptor-occupancy studies forward in the lead optimization process, where crucial questions of brain penetration and dose-response relationships can supplement current parameters of compound-series structure–activity relationships. In addition, HT-MRI or -OPT performed on rodents, for example, could provide 3D images of the effects of a compound on tumor metastasis or neuronal apoptosis, thereby circumventing the need for tissue histo- or immuno-pathological endpoints.

Between thought and expression

Crucial to the success of any drug is its metabolic and pharmacokinetic properties, such as its bioavailability, biological half-life and the nature of its metabolic by-products. Quantitation of such parameters is often dependent on analyte identification by chromatograpy and mass spectrometry (e.g. from plasma samples). Integration of automated sample preparation with more-efficient separation techniques and sensitive detection methods is a bottleneck currently being addressed [4]. ADME studies performed *in vitro*, such as

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the use of isolated hepatocytes or liver microsomes to study drug metabolism, are being adapted to assay formats similar in design to those currently running on HTS platforms with the aid of fluorogenic cytochrome-P450 substrates, and are readily configured as HT systems.

Toxicogenomics, an emerging branch of genomics, uses gene chips containing tens-of-thousands of oligonucleotides or cDNAs per cm^2 to link compound-induced changes in gene expression to toxicity. These expression profiles can reveal, for example, how a given chemical substance might affect levels of xenobiotic-metabolizing enzymes that could lead to potentially dangerous drug–drug interactions, or activate gene clusters known to be induced by DNA-damaging agents or endocrine disruptors [5]. Toxicological data obtained in this way can provide a genome-wide view of the effect of a compound on cellular function, thus providing an early warning of potential adverse effects. Approaches to automation depend, in part, on both optimization of RNA preparation from tissue samples, and integration of databases and informatics software required for the identification and interpretation of the numerous drug-signature correlations revealed by these studies.

Gene hunting

Targets entering HTS should be of therapeutic significance; that is, the role of the protein in human physiology and disease should be clearly understood before costly drug development resources are committed. For example, in a recently reported case that is symptomatic of the industry, a clinical candidate showing positive results in rats consumed $71 million in R&D before it was terminated because of a lack of efficacy during limited clinical trials [6].

Looking for targets in the genomes of infectious pathogens and humans is a major goal of the post-genomic era. Today's pharmaceuticals are directed towards <2% of the proteins encoded by the human genome [7], leaving ample opportunity for discovery of novel disease-intervention points. Of the ~30,000 human genes unearthed by genomic sequencing, those capable of modulating disease remain largely unknown.

Identifying proteins involved in disease, without the insight gained from years of intensive biomedical research that accompanies many of today's validated targets, is a daunting task. Only with HT technologies, which enable parallel acquisition of many thousands of datapoints, will it be possible to elucidate many uncharted protein–protein interactions, differential-expression patterns, and/or protein modifications that underlie various conditions. Bioanalytical methods based on two-hybrid systems, protein microarrays, and multidimensional electrophoretic or chromatographic separations coupled with mass spectroscopy, will help script a 'Who's who and with whom' of protein identities and associations. Identification of members of signal transduction or metabolic pathways, ligands for orphan receptors, or proteins post-translationally modified in disease, will be aided with structural and comparative studies in non-human species. Large-scale mapping of interacting proteins in yeast has already been achieved using two-hybrid and protein array approaches [8,9], and has permitted players of previously unknown function to be recognized as members of known metabolic pathways.

For tomorrow's medicines

HT-enabled technologies will be fundamental to more-efficient clinical trials that are based on well-characterized surrogate disease-markers in genotype-selected populations. For example, in contrast to traditional trials, which enrole thousands of patients from a relatively broad enrollment criteria, Genentech (http://genentech.com) gained FDA approval for trastuzumab – a treatment for metastatic breast cancer – using data from two Phase III clinical trials with <700 patients selectively overexpressing tumor-associated epidermal growth factor receptor 2. Future trial designs will incorporate dozens of prognostic reporter genes or proteins accessible through microarray technology, eventually paving the way to 'personalized medicines' – drugs prescribed or tailor-made according to the genetic makeup of an individual.

The immediate promise of HT technologies extends beyond HTS and lies in compressing the preclinical development timeline. Through acquisition of comprehensive efficacy and safety profiles, facilitated by parallel evaluation of pharmacological, metabolic, and toxicological properties of a lead-compound series, the probability of a successful clinical outcome will improve. Upstream of HTS, relevant target-identification will depend on integrating the developing fields of functional and structural proteomics with maturing genetic protocols for isolating disease-dependent genes. Innovative solutions to shorten time-consuming procedures, such as the application of RNA-interference technology to create the equivalent of gene-knockouts in mammalian cell lines, will be crucial to rapid progress. Given that HT technologies often involve a fusion of biology, automation and complex data-analysis, their success is contingent on assembly of a multidisciplinary team of scientists, engineers and bioinformatics experts. High-throughput screening has revolutionized compound screening: now is the time to expand the paradigm.

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