Functional genomics: from genes to new therapies

Mark R. Dyer, Dalia Cohen and Paul L. Herrling

The application of genomics in pharmaceutical R&D is presently one of the central issues in the industry. The evolution of functional genomics approaches and their integration into a technology platform for therapeutic discovery is a challenging and complex process. In this review, the authors describe how functional genomics will offer significant opportunities in the search for causal and disease-modifying therapies for better treatment of society's most outstanding medical needs.

The Human Genome Project is an international research effort to generate detailed genetic and physical maps of the human genome and to sequence the DNA of the estimated 70,000–100,000 genes[1–3]. This initiative is having a profound impact on academic and biomedical research. Currently there is an accelerated development of scientific approaches, technologies and informatics tools, which are the fundamental components needed to harness raw genomics data, to elucidate normal gene function in healthy individuals and understand gene dysfunction in disease states. The Human Genome Project and genomics research have shown that many diseases, such as Alzheimer's disease[4,5], Parkinson's disease[6,7], diabetes[8], asthma[9] and rheumatoid arthritis[10] to name just a few, have an important genetic component that interacts with environmental factors to manifest disease syndromes. Often, it will be more practical to address the genetic basis of diseases rather than to modify environmental and behavioural factors. In many cases the contribution of the latter to disease onset and progression remains unknown.

As a better insight is gained into the genetic and molecular basis of diseases of major significance, pharmaceutical discovery efforts will, increasingly, yield causal and disease-modifying therapeutics to address major unsatisfied medical needs. The correlation and linkage of genetic information concerning disease states and its translation into disease-relevant therapeutic targets for focused biomedical research is a central issue in the pharmaceutical and biotechnology industries. In the 1970s, relatively few well-characterized enzymes and receptors were available to pharmaceutical researchers. This changed in the 1980s with the advances in molecular biology and recombinant DNA technology and has resulted in the exponential increase in the number of genes and proteins available for pharmaceutical research. The current need is for approaches that enable the industry to select the disease-relevant molecular targets for therapeutic discovery activities.

Functional genomics can be described as the combined scientific, technological and informatics approaches that are being applied to bridge successfully genomics research with the process of discovery of disease-relevant therapeutic targets (Fig. 1). As even the largest companies do not have the resources to address all potential disease targets in their research programmes, those that can rapidly identify and assign function for key genes and proteins in disease states and, accordingly, focus their activities, will gain a key advantage over their competitors. Building a competitive functional genomics technology platform and integrating it with the downstream process of therapeutic discovery is a complex challenge and Novartis' recent major investment in this field highlights many of the important questions and issues being addressed by research management and scientific associates in the industry.

Mark R. Dyer and **Paul L. Herrling***, Novartis Pharma, CH-4002, Basel, Switzerland. **Dalia Cohen**, Novartis Pharmaceuticals, 556 Morris Avenue, Summit, NJ 07901, USA. *tel: +41 61 324 6284, fax: + 41 61 324 2424, e-mail: paul.herrling@pharma.novartis.com

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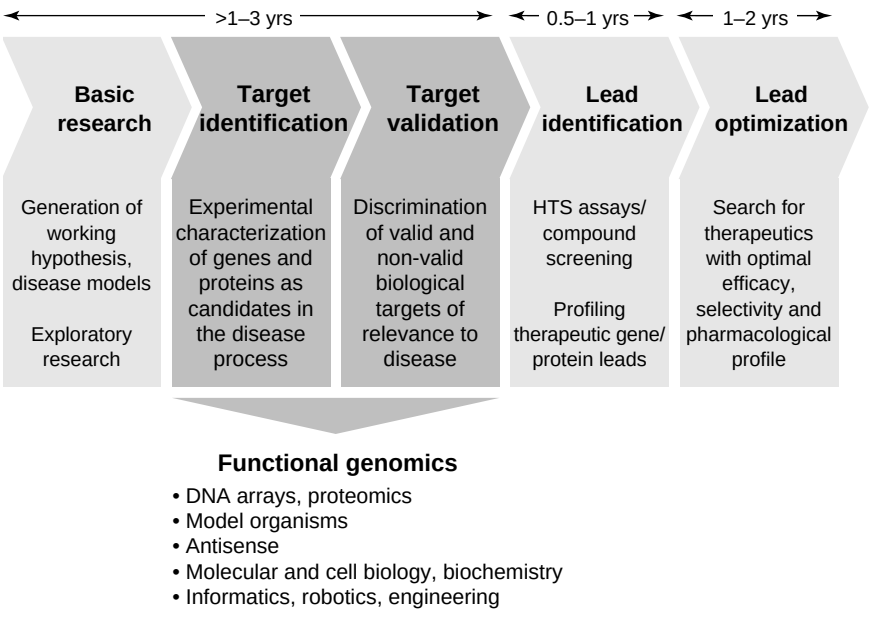

Figure 1. *Snapshot of the pharmaceutical discovery process and integration of functional genomics technologies. External collaborations with the biotechnology and academic sectors contribute at this and other stages of the pharmaceutical R&D process – they are an important source of new technologies, disease-relevant targets, therapeutic candidates and clinical expertise.*

Strategic considerations in building a competitive functional genomics platform

The race to discover, develop and market new therapeutics is a fiercely competitive one, requiring significant investment in human creativity, money and time. Currently, marketed drugs interact with ~400 genes or gene products and estimated numbers of important genes for disease predisposition, onset and progression range from 3000–10,000 out of the total of ~100,000 genes in the human genome. Hence, many opportunities remain to identify novel genes and proteins as proprietary targets for pharmaceutical research. Novartis' decision to build up in-house functional genomics (Box 1) took into account several strategic factors:

Delivery of validated targets. The success of functional genomics in pharmaceutical research will be measured ultimately in terms of productivity in contributing validated, disease-relevant targets, which can then be exploited for therapeutic discovery by disease-oriented research groups. The availability of in-house functional genomics expertise is considered essential as a 'provider' to meet the complex genomics-related needs of research programmes in the company's core disease areas. Similarly, a fully integrated in-house functional genomics group can benefit from uninhibited exchange of disease know-how and models available from their in-house partners. A parallel initiative and major investment in agricultural genomics further augments the possibilities for the sharing of expertise and technologies in intramural collaborations within the company.

Focusing existing activities. Within Novartis Research, several programmes existed with established expertise that could be conveniently refocused into the new functional genomics group. As an example, the company's strengths in antisense research and a leading position in nucleotide chemistry built up since the early 1990s, made this programme ideally suited for redirection to address the need for the profiling of gene function.

Achieving critical mass. It was also clear that the many in-house activities in genomics research needed a focus to allow critical mass allocation to central problems in the field, such as high-throughput functional profiling methodology.

Need for strong in-house expertise. Although functional genomics approaches are the focus of dedicated efforts in the biotechnology sector, the majority of these smaller companies excel in one or a small subset of the necessary technologies or informatics tools. This firmly underscored the requirement for a strong in-house group to build a comprehensive platform that integrates approaches and technologies generated in-house with those acquired via external collaborations. Clearly, it is important to have a high-calibre team of scientists who can assess the scientific value of the myriad approaches and tools offered by biotechnology and academia, and prioritize their importance for in-house use. In cases where external alliances are initiated, it is necessary to organize the collaboration formally, such that a company gains scientific expertise and technology transfer[11]. Integration of these externally acquired assets will take place in Novartis' functional genomics centres of excellence.

Major out-sourcing. Alternative models for the implementation of functional genomics, whereby a major part of the

programme is accessed via an external research alliance, probably limits the optimization of the interface with in-house discovery efforts owing to divergent partner goals, proprietary conflicts and potentially non-aligned corporate cultures. Such collaborations will need creative, highly dedicated management and continuous inter-company dialogue to achieve their aims.

Scientific and technology challenges in functional genomics

Presently, a large and rapidly growing amount of gene-sequence information is available from the different genome projects in the public domain, biotechnology sector and from pharmaceutical gene-discovery efforts. The challenge in establishing a competitive functional genomics programme is the ability to handle the enormous amount of information resulting from the analysis of differential gene-expression studies. For example, differential gene expression in normal versus diseased tissues, samples under selective stress or genetic pressure, or in cells and tissues treated with different therapeutic agents. Genes and their products identified from differential display studies need to be functionally evaluated to ascertain their relevance and importance in disease onset and progression, prior to their incorporation into screening assays for new therapeutic leads. Currently, a key limiting factor in functional genomics that slows its applications is the lack of fully automated, high-throughput functional profiling technologies to process the increasingly large amount of raw genomics and differential gene and protein display data.

Box 1. Investing in functional genomics

Novartis was the first large company to make a major investment in functional genomics for pharmaceutical and agricultural applications. In the healthcare setting, the company has established an in-house functional genomics capability for therapeutic discovery that will involve ~100 scientists and technical staff. This capability is complemented by the foundation of the Novartis Institute for Functional Genomics (NIFG) in La Jolla, CA, USA. The new institute will be staffed by a further 100 scientists when it is fully operational towards the end of 1999. Success of the functional genomics initiative will depend on full cooperation and interaction of these research groups and their optimal fit with pharmacogenomics efforts in the company's development functions (Fig. I). Presence in the field is further strengthened by functional genomics approaches being pursued by our colleagues in the agribusiness sector.

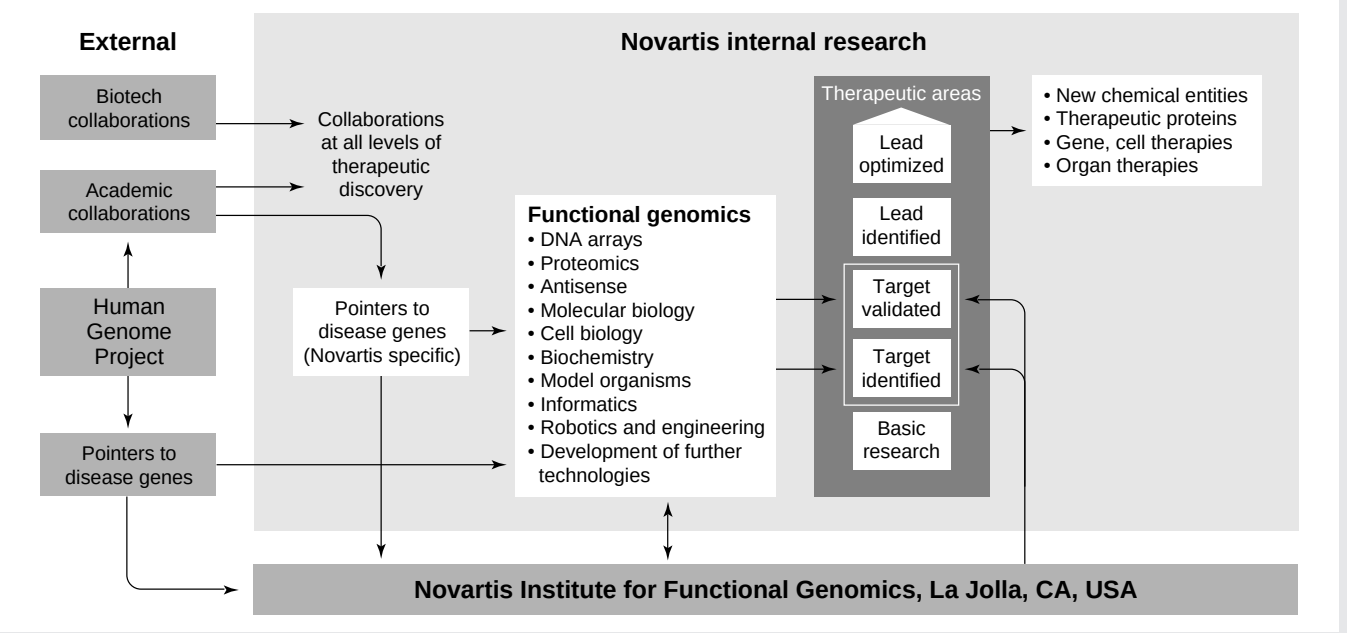

Figure I. *A strong functional genomics platform is necessary to integrate approaches and technologies generated in-house with those acquired via external collaborations. Such integration will take place in Novartis' functional genomics centres of excellence – Basel, Switzerland; Summit, NJ, USA and the NIFG, which is a subsidiary of the independent Novartis Foundation.*

Functional genomics technologies and informatics tools need to be used in an interactive manner in order to assign gene function successfully, place individual genes into biological pathways, predict which pathways initiate the disease process and use this information to screen and optimize therapeutic leads and candidates. It will also be necessary to model accurately the dynamic interactions of cell signalling pathways to improve the prediction of effects resulting from their experimental or therapeutic manipulation. Complementary approaches for candidate disease gene and protein discovery, high-throughput functional profiling tools, strategies involving use of diverse model organisms, and high-powered informatics will all contribute to identification of new therapeutic targets.

High-throughput methods

High-throughput measurement of the relative abundance of particular mRNAs within the pool of cellular messages can be achieved using differential display approaches based on differential display, reverse transcriptase polymerase chain reaction and DNA array technologies. Although the differential display methods can be performed with limiting amounts of biological samples (as is often the case with clinically obtained materials), they are difficult to perform, semi-quantitative and cumbersome. A more direct and straightforward approach involves measuring the amount of labelled RNA or cDNA hybridized to a known capture sequence. Several different technologies have been developed using hybridization to allow for concurrent screening of the relative expression of hundreds to thousands of genes. These approaches include array-based technologies that can identify specific expressed gene products on high-density formats, including filters, microscope slides, or microchips, and solution-based technologies. However, relatively large amounts of test sample are required and low abundance mRNAs might not be detected. In spite of the latter, the relative simplicity of array technologies provides an opportunity to identify rapidly genes that are differentially expressed and they will provide an important tool once the technology matures[12–14].

Proteomics

The proteomics approach enables the identification of differentially expressed proteins and study of their post-translational modifications. This approach is an important companion to gene-expression studies because there is often an insufficient correlation between the level of expression of different genes and the relative abundance of the corresponding proteins. Also, as a protein and its post-translational modifications are not directly encoded for by the same gene, the complete structure of an individual protein cannot be determined by reference to its gene sequence alone. Current proteomics technology is still being developed for the needs of pharmaceutical research, and as yet does not allow in-depth analysis of protein profiles. In addition, it requires large amounts of test samples, which are not always available from clinical sources[15].

Functional profiling

The available tools for functional profiling of genes range from: transfection of cDNA expression libraries constructed in a variety of vectors (plasmids, retroviral vectors); antisense oligonucleotide libraries; ribozymes; *in situ* hybridization methodology; and antisense sequences for the validation of specific genes. Model organisms, such as yeast, *Caenorhabditis elegans*, *Drosophila* and the mouse represent some of the most important experimental systems available to understand gene function and are being used for *in vivo* gene profiling[16]. Furthermore, the opportunity to manipulate homologous genes in these organisms will provide important contributions for the identification of gene function and give valuable clues as to their potential role as a disease mediator in humans. The yeast and the *C. elegans* genomes have been fully sequenced and, in the near future, the *Drosophila* genome will also be completed. A complementary effort is being made to sequence the mouse genome. This cross-species genomics information represents an important additional resource in understanding gene function.

Integrating the new technologies

One of the advantages of applying genomics in large pharmaceutical companies is the extensive knowledge and the availability of in-house model systems to study disease pathophysiology. For many years pharmaceutical researchers in their efforts to understand disease processes and identify new therapeutics have established comprehensive *in vitro* and *in vivo* assays for modelling diseases. Integration of functional genomics technologies with more established scientific disciplines (e.g. protein chemistry, biochemistry, pharmacology and physiology) offers a pharmaceutical company the opportunity to build a unique technology platform. Those companies that get the integration process right and can rapidly identify principle disease-relevant targets will have a competitive advantage in filling their R&D pipelines.

Compared with the development of biology-oriented technologies, less effort has been addressed to the computational biology underlying data analysis and interpretation; the establishment of so-called dry labs. This key area needs

to be strengthened if the enormous amounts of genomics data are to be handled in a meaningful manner[17,18].

In building a functional genomics platform it is important to maintain a flexible position with respect to the use of component technologies, and exploit new technological and computing advances as soon as they mature to meet the requirements of pharmaceutical research. Clearly no single functional genomics approach or technology will meet all pharmaceutical discovery needs, and many new technologies and informatics tools will need to be developed in-house or acquired via external collaborations with leading players in the biotechnology and academic sectors (Box 1). A further means of achieving critical mass in functional genomics technologies has resulted in the evolution of industry consortia, whereby pharmaceutical companies can jointly minimize the risks and costs of new technology development, whilst sharing the rewards of the consortia endeavours.

The decision on new technology development in-house versus technology acquisition via external collaboration is an important one and relies on multiple factors:

- Strategic requirements;
- Existing in-house expertise and capacity;
- Availability for partnering of leading external programmes;
- Willingness of the external partner to transfer technologies and tools into the in-house group;
- Availability of a partner who is committed to mutual goals of the collaboration, and contributes to the optimal blend of 'personal chemistry' needed to forward the joint programme;
- Price tag on externally acquired approaches versus financial and time-line associated costs of developing similar technologies in-house;
- Proprietary and patenting issues.

In an environment where scientific and technological methodologies are evolving rapidly (and in some cases being quickly superseded), success depends on identifying and integrating the right cutting-edge technologies and informatics tools. The same criteria used for the evaluation of new scientific approaches and technologies need to be used for the evaluation of the multitude of informatics packages available in the public domain and private sector.

The human factor and functional genomics

Creating and maintaining a leading scientific team in this fast-moving environment will remain a key factor for managers of functional genomics programmes. Attracting the best scientists and informatics personnel is a priority process and Novartis is pursuing a proactive approach to staffing its groups. Visits by Novartis research managers and scientists to premier academic institutions and presentation of the company's mission and goals has been one of the best mechanisms to attract high-calibre scientists to the in-house team. A further source of talent has been tapped by recruiting from the best in-house post-doctoral scientists available from a pool of currently around 200 individuals.

Understandably, the challenge of associating gene sequence to biological function is one of the central issues in present-day biology. This, combined with a unique multidisciplinary research environment and availability of up-to-date infrastructure and equipment offered by the pharmaceutical industry, as well as the chance to apply scientific knowledge in the pursuit of superior therapies for important diseases, is attracting many leading academic researchers to industry. In return, the challenge of the pharmaceutical industry is to encourage and nurture scientists to direct their expertise and energies into therapeutic discovery activities. To maintain and coordinate a high-calibre scientific team, a new breed of research managers is evolving. These individuals should be excellent scientists in their own right, have the entrepreneurial and leadership talents needed to manage activities in this fast moving field, and possess a firm grasp of the principles of pharmaceutical R&D.

Outlook and conclusion

Functional genomics approaches and technologies will have an impact on other areas of pharmaceutical R&D beyond discovery research. Pharmacogenomics activities to profile the efficacy and side effects of new and existing therapies in subsets of patients within a given disease category also hold the promise of addressing 'personalized' medical needs. In the molecular diagnostics field, the availability of a meaningful number of precisely located single-nucleotide polymorphic (SNP) sites spanning the genome holds promise for the association of particular genetic loci with major disease states[19]. This information, together with the high-throughput gene-chip technologies, will offer new opportunities for molecular diagnostics and disease predisposition monitoring in large sections of the population. It will also allow much earlier preventive treatment in many slowly evolving diseases.

In conclusion, the genomics revolution is now entering its second phase, whereby the pioneering efforts to map and sequence the human genome, and the enormous

wealth of data they have generated, are now being converted into precise information on gene and protein function in normal and disease states. The progress of functional genomics will focus pharmaceutical research towards disease-relevant targets and provide a starting point for the discovery of causal and disease-modifying therapies to address the most outstanding medical needs of society.

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In short…

Genomics research delivers new insights into the role of the serotonin receptor 5-HT_3. Researchers at The Institute for Genomics Research (TIGR, Rockville, MD, USA), the George Washington University Medical Center (GWUMC, Washington, DC, USA) and the University of Dundee (Dundee, UK), have recently identified a gene that provides the missing component of the 5-HT_3 receptor. This receptor is an important target for anti-nausea drugs and has been implicated in the control of anxiety and psychosis.

The first subunit of the 5-HT_3-receptor complex to be identified, named 5-HT_{3A}, was by molecular cloning techniques in 1991. Cloned receptors that contained only 5-HT_{3A} were found to behave very differently from their natural 5-HT_3 receptor counterparts. For example, the flow of current across the activated receptor is unusually small and the passage of calcium ions is higher than through neuronal 5-HT_3 receptors.

In January this year, Ewen Kirkness at TIGR using database mining discovered a gene for a second subunit (5-HT_{3B}), that has similarity to the 5-HT_{3A} gene. Kirkness and Mike Hanna found the gene to be highly expressed in the amygdala, an area of the brain implicated with emotional behaviour. Tim Hales and Paul Davies at GWUMC then performed functional studies in a human cell line into which the gene had been transferred. They discovered that 5-HT_{3B} combines with the known 5-HT_{3A} subunit to yield receptors with similar functionality to the neuronal 5-HT_3 receptors, that is, enhanced electrical conductance and reduced calcium ion permeability. The combination receptors also show different reaction to drugs, suggesting that it may prove useful to identify new compounds that regulate the 5-HT_3 receptor. Workers at Dundee University using a frog-egg expression system to examine the properties of 5-HT_{3B} have confirmed these findings.

According to Kirkness and Hales, 'This research demonstrates how rapidly accumulating data from the Human Genome Project can be used to obtain new insights to biological processes, and to uncover new targets for the design of more selective pharmaceutical agents.'