Takeda Award 2002 Achievement Facts Sheet

Techno-Entrepreneurial Achievements for Individual/Humanity Well-Being

Technical Achievement: The Development and Promotion of DNA Microarrays

Awardees:

Patrick O. Brown (Stanford University and Howard Hughes Medical Institute) for the development of DNA microarrays with pre-synthesized DNA probes and the promotion of the technology by releasing the production methods on the Internet

Stephen P. A. Fodor (Affymetrix, Inc.) for the development of high-density DNA microarrays using photolithographic fabrication, and the promotion of the technology by commercialization of the GeneChip® array.

The prize is awarded jointly to Patrick O. Brown and Stephen P. A. Fodor.

(Awardees are listed in alphabetical order.)

Executive Summary

The achievements by Patrick O. Brown and Stephen P.A. Fodor provide methods that enable the simultaneous analysis of sequences and functions of large numbers of genes. Life processes are controlled with expression and suppression of genes in interacted fashions and without DNA microarrays it was almost impossible to analyze dynamic expression and interactions of genes due to changes in environment surrounding cells. DNA microarrays enable researchers to follow time courses of expression of multiple genes under different conditions in quantitative and simultaneous fashions. Their methods represent a significant technical jump from previous technologies. The main application fields of DNA microarrays are gene expression analysis and genetic analysis. In gene expression analysis, the gene expression "scripts" that specify the distinctive properties and behavior of each different cell in our bodies, and the changes in gene expression during development, in physiological responses, and in disease, can be followed with DNA microarrays by monitoring the messenger RNAs that specify which proteins are made in each cell, and in what amounts. Changes in gene expression due to pathological processes such as cancer and viral diseases, and due to cellular responses to medication, can be visualized by DNA microarrays as changes in the fluorescent pattern. The

application of DNA microarrays to drug discovery, diagnosis and therapy has already begun.

DNA microarrays are also used to detect small differences in genes (single nucleotide polymorphism, SNP) between individuals, which exist in one to hundreds or thousands in genes and correspond to expression differences in individuals. SNP research is very important, because it can lead to tailor-made medications, in which an individual can be diagnosed and treated according to his or her own genetic information.

DNA microarrays are very small glass or silicon supports with hundreds of thousands of different oligonucletides or cDNAs on their surface. Target DNA or RNA from a sample mixture binds to certain oligonucleotides or cDNAs on the surface, and these are identified by detecting emission from a fluorophore attached to the target. The methods utilize hybridization, base pair formation in which complementary strands of DNA bind together. Target DNAs or RNAs are identified by their positions on the surface, and the quantity of each target is measured by the strength of its emission.

In 1991, Stephen P. A. Fodor reported the fabrication of DNA microarrays by combining the photolithographic method, used in semiconductor fabrication, and combinatorial synthesis of oligonucleotides on the surface of glass chips, while working for Affymax, an early combinatorial chemistry venture. In 1993, he co-founded Affymetrix, a spin-off of Affymax, in order to develop oligonucleotide arrays with hundreds of thousands of oligonucleotides on the surface of the supports. In 1994, Affymetrix commenced manufacturing and selling the first DNA microarray, GeneChip®, thus realizing the DNA microarray market.

Patrick O. Brown developed an idea for mechanically arraying cDNA on the surface of a glass support so that researchers could prepare DNA microarrays mounting their choice of array content. He led his research group to develop a method for fabricating DNA microarrays utilizing a robot to spot numerous cDNAs on the surface of easily available slide glass. In 1996, he disclosed the know-how, tools, and designs for the fabrication of DNA microarrays on the Internet so that scientists could make their own DNA microarrays in their own laboratories. This disclosure of information for the self-fabrication of DNA microarrays has prompted the wide-spread use of DNA microarrays with pre-synthesized probes.

The uses of DNA microarrays developed by Brown and Fodor have different features and complement each other. Commercialization of the GeneChip® array and promotion of self-made DNA microarrays by disclosing information on the Internet have synergistically prompted the wide-spread use of DNA microarrays. DNA microarrays have been used not only in the field of medicine, but also in the field of agriculture for the purposes of effective breeding and increasing tolerance to the environment. Their achievements deserve the Takeda Award.

Achievement and Creativity

1. What are DNA microarrays?

A gene consists of double stranded DNA molecules that form a helical structure. The double stranded DNA molecules unzip into two complementary strands when heated. Upon cooling, each of these can recombine or bind to a new DNA molecule that contains the same sequence as the complementary strand. This process of two complementary strands binding together is called hybridization and this reaction is used to detect DNA or RNAs of interest (targets) on the surface of the DNA microarray. Certain oligonucleotides (short chains of nucleic acids) or cDNAs (complementary DNAs synthesized from mRNA) are attached or synthesized on the surface of the DNA microarray to bind their complementary strands. These oligonucleotides and cDNA are called probes, and the targeted DNAs or RNAs can be detected by hybridization reactions with probes on the surface of the DNA microarray. A DNA microarray contains tens of thousands of oligonucletides or cDNAs attached on the surface; thus tens of thousands of genes or RNAs can be simultaneously detected on a single array.

A use of hybridization reactions to identify the location of certain nucleic acids sequences were reported by several researchers. Gillespie discussed the idea of using support bound DNA to hybridize to complementary RNA[1)]. Pardue and Gall[2)], and Jones and Robertson[3)] reported in situ hybridization, in which the positions of specific sequences can be located in the nucleus or chromosomes by hybridization reactions on cells fixed to microscopic slides. Later, the multicolor fluorescent labeling technique was developed for the analysis of multiple probes for in situ hybridation[4, 5)]. In the late 1970s, Kafatos et al., introduced dot blots, a technique for analyzing multiple hybridization targets in parallel by applying them to a filter in a defined pattern[6)]. In the dot blots, multiple targets are arrayed on the support and the probe, normally a single sequence, is labeled and applied under hybridization conditions to the membrane. Saiki et al. introduced a variant of dot blots, reverse dot blots, in which multiple probes are attached as an array to the membrane support and the target to be analyzed is labeled[7)].

The emergence of the Human Genome Project prompted various technological developments. One is the idea of using high-density oligonucleotide arrays for sequence analysis. Southern and Maskos reported in 1992 the synthesis of oligonucleotide arrays, which were a low-density prototype array[8,9)]. About one hundred different oligonucleotides up to 19 bases in length were synthesized in situ on a glass microscopic slide using parallel lines of silicon rubber tubing[8)]. These oligonucleotide arrays were tested to examine the feasibility of using them for sequence analysis. Many of the basic ideas of DNA arrays such as arraying probes on solid supports and in situ preparation of probes, were already established by Maskos and Southern, and other researchers[10)], but how to prepare high-density microarrays was a

completely different matter, requiring novel engineering intellect and knowledge, the photolithographic fabrication of the high-density oligonucleotide microarrays, and the robot spotting fabrication of DNA microarrays with pre-synthesized probes.

2. Photolithographic fabrication of high-density DNA microarrays - GeneChip® arrays

In the late 1980s, combinatorial chemistry was developed to perform fast and effective drug discovery research using the parallel synthesis of compounds and high-throughput screening systems. Some of the early features of combinatorial chemistry include building up versatile combinatorial compound libraries using solid-phase synthesis of linear compounds such as peptides, and high-throughput random screening for bioactive compounds using highly sensitive biological systems such as antigen-antibody reactions. In 1988, Fodor joined Affymax, an early combinatorial chemistry venture, and started working to fabricate high-density arrays of biomolecules. His background in biochemistry and biophysics, and his experience in combinatorial chemistry led Fodor to combine the technologies of different fields, such as electronics, biology, and combinatorial chemistry, to produce high-density oligonucleotide arrays. The technologies included the photolithographic fabrication method used in semi-conductor fabrication, the parallel synthesis strategy and solid-phase synthesis of nucleic acids from combinatorial chemistry[11-13]. Although there were some technical issues to be resolved, the photolithographic fabrication of high-density oligonucleotide arrays became more and more promising and the potential commercial possibilities became more and more clear. Bearing these in his mind, in 1993 Fodor co-founded Affymetrix, a spin-off of Affymax, to develop the technology for high-density oligonucleotide arrays[14]. In 1994, Affymetrix commenced manufacturing and selling the first DNA microarray, GeneChip® arrays, which were loaded with hundreds of thousands oligonucleotides on the supports. Their technology is one of the rare cases of happy marriage between technologies used in the information and electronic fields, and those in the life science field[15].

Figure 1 shows the process for the photolithographic fabrication of high-density oligonucleotide arrays. In this process, bases attached to the surface are protected by photo-labile protecting groups. Before initiating the coupling reaction, the protecting groups can be photo-chemically eliminated by light in a predefined region, and the coupling reaction commences when bases are added. By continuing this process, hundreds of thousands of different oligonucleotides can be synthesized on a very small area of glass support[16]. Since the yield of coupling reaction on the support is less 100%, the length of oligonucleotide is limited.

The GeneChip® array was the first commercial DNA microarray, and swiftly spread into laboratories because of its ready availability as off-the-shelf products, notwithstanding the inflexibility of custom production.

3. Robot spotting fabrication of DNA microarrays with pre-synthesized probes (Stanford type microarray)

As the Human Genome Project continued toward its goal of characterizing the genomes of human and selected model organisms, and more and more information about genome sequence was accumulating, it became clear that new and much larger scale analytical methods were necessary to analyze gene differences between species and the functions of newly identified genes. High-density DNA microarrays were known for large scale DNA analysis[11, 12], but the demand for inexpensive custom microarrays was mounting from researchers who were studying genes in different fields. Brown seized this opportunity and led his research group to develop the technology for fabricating inexpensive DNA microarrays suitable for custom preparation. In 1995, he succeeded in fabricating cDNA microarrays using a robot spotter[17] and reported the first application of cDNA microarray to the gene expression studies[18].

The novelties in their technology for the fabrication of DNA microarrays are the uses of high precision, high-speed robot spotter and cDNA or pre-synthesized oligonucleotides as probes. The use of the high precision, high-speed robot spotter allows researchers to prepare high-density microarrays on a very small area of solid support with good reproducibility. The use of cDNA or pre-synthesized oligonucleotides as probes gives researchers flexibility to prepare their own microarrays with their choice of probe content. Brown and his group developed a robotic spotting machine for arraying, and, in 1996, released information as to know-how, tools, and the design for the fabrication of DNA microarrays on the Internet[19,20]. This disclosure of information for the self-fabrication of DNA microarrays has prompted the wide-spread use of DNA microarrays with pre-synthesized probes. It also paved ways for many companies to develop their own DNA microarrays with pre-synthesized probes based on their own technologies such as ink jet and spotting methods, and let them to enter to DNA microarray market.

Figure 2 shows a diagram for the fabrication of DNA microarrays with pre-synthesized probes using a robot spotter. Microarrays are fabricated on poly-L-lysine-coated microscope slides with a custom-built spotting machine fitted with printing tips. The tips are loaded with probes from 96-well microtiter plates and deposit small amounts of probe on the surface of the slides. After hybridization, the microarrays are scanned with a laser fluorescent scanner.

Since DNA microarrays can identify numerous genes at the same time, they are mostly used in the fields of gene expression and genetic analyses instead of being used as sequencers. Brown and his co-workers reported a quantitative analytical method for gene expression by conducting co-hybridization on a DNA microarray[18, 21]. Figure 3 shows a process diagram of gene expression analysis on DNA microarrays. Messenger RNA extracted from target tissue is

labeled with green fluorescent dye and mRNA from reference tissue is labeled with red fluorescent dye. They are co-hybridized on a DNA microarray with cDNA as probes. The DNA microarray is scanned with a laser and the fluorescent patterns are memorized in a computer. The patterns are superimposed on the computer screen and analyzed. If the quantities of both mRNA are the same, the overlapping part turns yellow. If mRNA in the target tissue is more abundant in the reference tissue, the overlapping part turns green, and will turn red in the opposite situation. This way, researchers can tell which genes are activated or suppressed in target tissue. This method enables researchers to conduct quantitative gene expression studies and has become an indispensable method in genetic and pathological studies.

4. Comparison of some of the features of DNA microarrays

Some of the features of DNA microarrays are summarized at Table 1. The photolithographic fabrication method yields very high-density DNA microarrays (the GeneChip®), but the length of the probes is limited. The densities of the DNA microarrays fabricated by robot spotting (Stanford type) are about one-tenth those of GeneChip® but they can load very long oligonucleotides or cDNAs. The Stanford type needs self-construction of arrayers, but is flexible for custom preparation. The GeneChip® arrays are suitable for SNP studies (as described later), because DNA microarrays with a single base variation are easily fabricated by the photolithographic method. DNA microarrays with pre-synthesized probes are suitable for gene expression studies because they can load cDNA as probes. These are some of the features of both types of DNA microarrays and thus both types of DNA microarrays complemented each other.

Type	GeneChip® arrays	Stanford type microarray
Manufacturing method	Photolithographic fabrication	Robot spotting of pre-synthesized probes
Availability	Readily available (off-the-shelf products)	Need construction or purchase of arrayers
Custom preparation	Not flexible	Flexible
Probes	Oligonucleotides (limited length)	cDNA or oligonucleotides (no limited length)
Density (cm^{-2})	up to 500,000 [22]	up to 50,000 [18]

5. Repercussion effects

Although deciphering the draft sequence of the human genome revealed that the human genome contains about 30 thousand genes[23], the functions of most genes remain un-clarified. Before the

emergence of DNA microarrays, it was not possible to compare various genes at the same time. DNA microarrays enable researchers to simultaneously detect genes expressed under various disease conditions, such as cancer, and clarify the mechanisms of disease development. In 1999 Lander et al. reported that a use DNA microarrays enabled to classify cancer types more accurately than traditional histological methods[24]. S.H. Friend et al. used DNA micro arrays to analyze primary breast tumors of 117 patients and found that certain patterns of gene expression profiles strongly suggest clinical metastasis at later stage, indicating a use of DNA microarray gene expression profiling as a diagnostic tool for later stage metastasis[25]. DNA microarrays are also used to detect small differences in genes (single nucleotide polymorphism, SNP) between individuals, which correspond to expressed differences in individuals[26,27]. SNP research is very important, because it can lead to tailor-made medicine, in which an individual can be diagnosed and treated according to his or her own genetic information. DNA microarrays have also been used in the field of agriculture for the purposes of effective breeding and increasing tolerance to the environment as information of plant genomes and cDNAs have been accumulating. The ability to use DNA microarrays to collect global gene expression data quantitatively and systematically has opened up an entirely new field of computational biology.

Commercialization of the GeneChip array and the promotion of self-made DNA microarrays by disclosing information on the Internet have synergistically prompted the wide-spread use of DNA microarrays. According to market analysis, the market for DNA microarrays was $400 -550 million dollars in 2000[28,29]. The share of commercial arrays is 41% and that of the self-made arrays is 39% of the total market. Twenty percent of end-users use both types[28]. The main player in the commercial microarray market is Affymetrix. As academic institutions and pharmaceutical companies move to more aggressively adopt microarrays and prices start to come down, the market is expected to grow to $1-2.2 billion dollars in 2005[28,29].

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The Takeda Award 2002
Individual/Humanity Well-Being
Profiles

Patrick O. Brown

1976 B.A. in Chemistry from University of Chicago
1980 Ph.D. in Biochemistry from the University of Chicago
1982 M.D. from the University of Chicago
1988 Assistant Professor of Pediatrics and of Biochemistry at Stanford University, and Assistant Investigator at the Howard Hughes Medical Institute
1995 Associate Professor of Biochemistry at Stanford University
1997 Associate Investigator at the Howard Hughes Medical Institute
2000-present Professor of Biochemistry at Stanford University
2002 Investigator at the Howard Hughes Medical Institute

Honors:

1998 Jacob Heskel Gabbay Award in Biotechnology and Medicine for the development of microarray methods for the analysis of gene expression on a genome-wide scale (with Stephen P.A. Fodor)
2000 National Academy of Sciences Award in Molecular Biology for his intellectual leadership in functional genomics, most notably the development of a reliable and accessible DNA microarray system to measure genome-wide gene expression
2001 Millennium Award for Genomics Research in Clinical Immunology
2002 Discover Magazine Award for Innovation
He is honored with many other awards.

Stephen P. A. Fodor

1978 B.S. in Biology from Washington State University
1982 M.S. in Biochemistry from Washington State University
1985 Ph.D. in chemistry from Princeton University
1989 Joined Affymax Research Institute
1993 Distinguished Inventor's Award by the Intellectual Property Owner's Association
1993 Founded Affymetrix Inc.
1995-1999 President and CEO of Affymetrix Inc.
1999-present Chairman and CEO of Affymetrix Inc.
2000 Founded Perlegen Sciences, Inc.

Honors:

1983 Association of Princeton Graduate Alumni Teaching Honor

1992 AAAS Newcomb-Cleveland Award for Outstanding Publication in *Science*

1993 Distinguished Inventor's Award by the Intellectual Property Owner's Association

1998 Jacob Heskel Gabbay Award in Biotechnology and Medicine for the development of microarray methods for the analysis of gene expression on a genome-wide scale (with Patrick O. Brown)

2000 Rising Star Award for Silicon Valley

2000 Foundation Fighting Blindness Visionary Award

He is honored with many other awards.

Fig.1 Photolithographic fabrication of high density DNA array

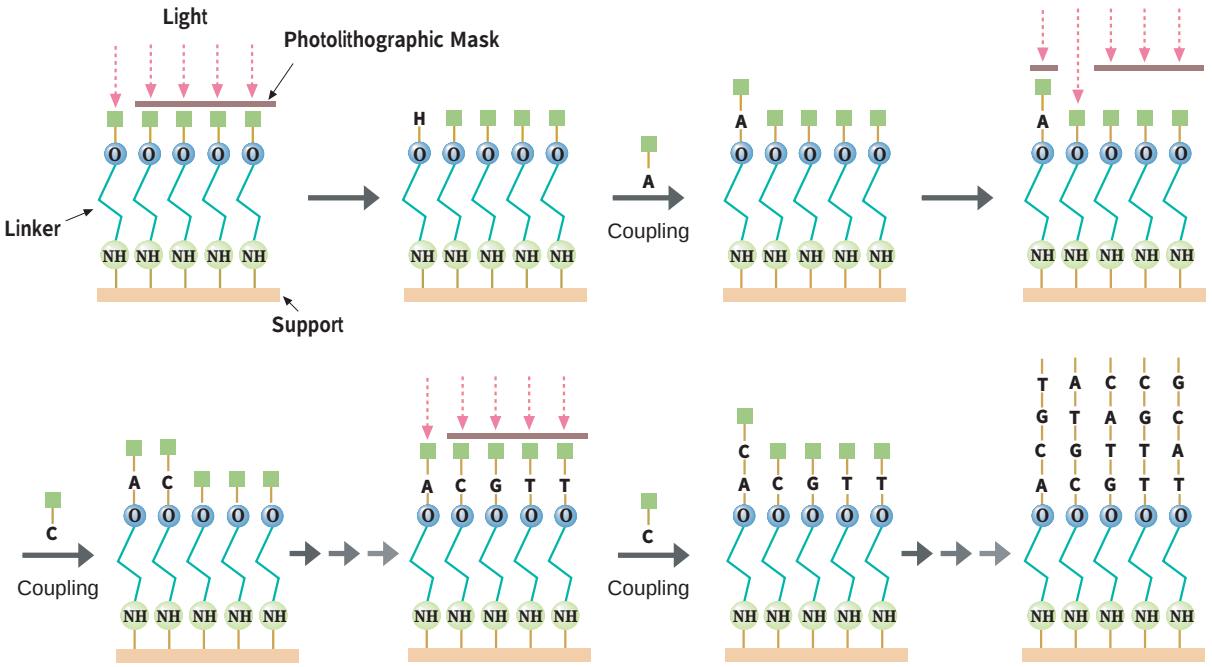

Fig.2 Fabrication of DNA microarray with pre-synthesized probes (cDNA) using a robot plotter

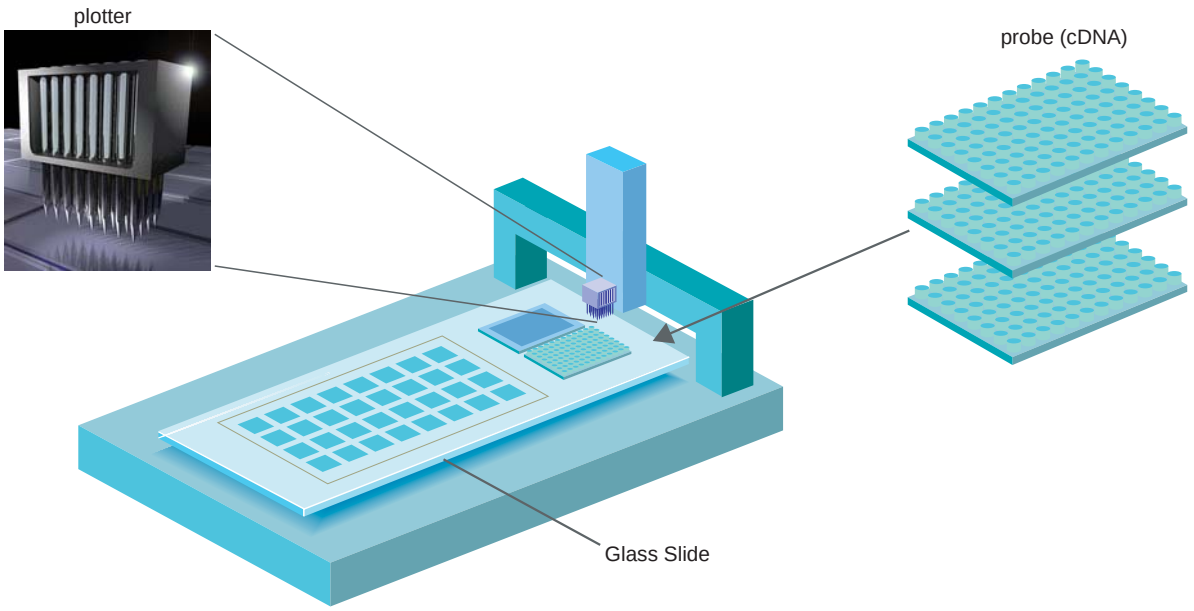

Fig.3 Process diagram of gene expression analysis with DNA microarray