PRESENTATIONS FROM KEIO

Human Gene Mapping and Chromosome Sorting by Laser Technology

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Abstract. More than 30 human genes have been mapped by a combination of the fluorescent *in situ* hybridization (FISH) and spot blot hybridization in this laboratory for the past five years. Furthermore, the Keio strategy has been established for the molecular analysis of the human genome using flow-sorted human chromosomes. (Keio J Med 42 (4): 212–216, December 1993)

Key words: fluorescent *in situ* hybridization, spot blot hybridization, flow cytometry, gene mapping and human genome

Introduction

Recently, mapping and sequencing of the entire human genome have become a major interest in medical genetics and human molecular biology.¹ The human genome DNA consists of 6×10^9 nucleotides which are distributed into 46 chromosomes; 22 pairs of autosomes (numbered 1–22) and a pair of sex chromosomes (X and Y). These chromosomes can be identified individually by conventional chromosome banding techniques. However, progress in the analysis of the complex human genome depends on rapid and accurate mapping methods of cDNA clones and physical isolation of individual chromosomes. In this paper, we describe a progress report of our research using the advanced methods for gene mapping by *in situ* hybridization (FISH) and for chromosome sorting by flow cytometry both of which use computer-assisted laser technology.

Results and Discussion

Human gene mapping by fluorescence in situ hybridization

The most advanced method for identifying a small DNA segment on a particular chromosomal region is fluorescence *in situ* hybridization. We have modified

the FISH method in a way that signal detection and chromosome banding can be performed simultaneously.² Using a confocal-laser scanning microscopy with computerized image analysis, we were able to map several new cDNA clones and/or genomic DNA clones on the particular human chromosomes (Table 1). One of such example is the mapping of epidermal growth factor receptor (EGFR) gene on the p12 band of human chromosome 7 (Fig. 1). This EGFR gene was assigned to chromosome 7 as the first gene of a membrane receptor for hormone-like proteins in 1980³ and since then amplification of the EGFR gene and its association with certain cancer has been discovered.⁴ However, it is only recent that the precise localization of this gene became possible. Another example is the mapping of myelin P0 protein gene (MPZ) on the q22–q23 region of human chromosome 1.⁵ Interestingly, the locus of the MPZ gene coincides with the genetically determined locus for Charcot-Marie-Tooth (CMT) disease type 1B and therefore it was postulated that the human MPZ gene may be a candidate gene for CMT1B in contrast to the peripheral myelin protein 22 (PMP22) for CMT1A.⁵ More recently, a mutation in the MPZ gene was in fact found in the patients with CMT1B.⁶ Similar to these genes, human hepatocyte growth factor (HGF) gene was mapped on the 7q21.1.² L-gulonolactone oxidase is a key enzyme to produce vitamin C but is deficient in human. This

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Table 1 Mapping of Human Genes

Marker Name	Gene Symbol	Location ^a	Method [*]	Reference
Myeloperoxidase	MPO	17	a,c	12
Interleukin 4	IL4	5	a	13
Phosphoribosyl pyrophosphate synthetase 1	PRPS1	Xq21–qter	c	14
Phosphoribosyl pyrophosphate synthetase 2	PRPS1	Xper–q21	c	14
PRPS1-like 1	PRPS1L1	7p22–qter	c	14
PRPS1-like 2	PRPS1L2	9	c	14
ADP/ATP translocator	ANT	4	a,c	15
Calpain, large-polypeptide L1	CAPN1	11	c	16
Calpain, large-polypeptide L2	CAPN2	1	c	16
Calpain, large-polypeptide L3	CAPN3	15	c	16
Calpain, small-polypeptide	CAPN4	19	c	16
Protein 80K-H (substrate for protein kinase C)	G19P1	19	a	17
Protein 80K-L (substrate for protein kinase C)	MACS	6q15–qter	a	7
80K-L like 1	MACSL1	21	a	7
ATP synthase, H ⁺ transporting β	ATPSB	12p13–qter	a	8
ATPSB-like 1	ATPSBL1	2	a	8
ATPSB-like 2	ATPSBL2	17	a	8
Calpastatin	CAST	5	b	18
Bullous pemphigoid antigen 1 (230/240 kD)	BPAG1	6pter–q15	a	9
Hepatocyte growth factor	HGF	7q21.1	a,b	2
L-gulonolactone oxidase pseudogene	GULOP	8p21.1	a,b	submitted
Cytochrome c-1	CYC1	8q24	b	19
Protein 14–3–3	YWHA1	22	a	20
Complement related protein SP–40, 40	CLI	8	a	21
Glial fibrillary acidic protein	GFAP	17	a	22
Fatty-acid-Coenzyme A ligase, long-chain-2	FACL2	4	a	23
2',3'-cyclic nucleotide 3'phosphohydrolase	CNP	17	a	24
Xanthine dehydrogenase (oxidase)	XDH	2	a	25
Myelin protein zero	MPZ	1q22–q23	a,b	5
Peripheral myelin protein 2	PMP2	8q21.3–q22.1	a,b	26
Epidermal growth factor receptor	EGFR	7p12	a,b,c	3,27,submitted

* a): spot blot hybridization, b): FISH, c): somatic cell hybrid panel

pseudogene (GULOP) was mapped 8p21.1 (submitted). There are several other genes related to neuronal functions (Table 1).

Human gene mapping by spot blot hybridization

Mapping of human genes and analysis of the human genome can be facilitated if individual chromosomes are obtained with high purity and in large quantities. Flow cytometry provides a fast and accurate method to measure the minute quantity of DNA in each human chromosome and flow-machines with sorting capabilities have provided a method for the fractionation and collection of individual chromosomes. We have established a method to sort human chromosomes at high resolution

Fig 1 FISH mapping of EGF receptor gene. Fluorescent signals after *in situ* hybridization of EGFR cDNA probes are shown on Q-banded metaphase chromosomes of GM130B lymphoblastoid cells. EGFR genes are located on the band p12 of chromosome 7.

Fig 2 Flow karyotypes of human lymphoblastoid GM130B cells. (A) A typical flow karyotype after metaphase chromosomes were stained with propidium iodide. (B) A flow karyotype after staining with Hoechst 33258. Metaphase chromosomes were prepared by the polyamine/digitonin method and analyzed by a FACS-440 cell sorter.

using a conventional cell sorter, equipped with a single argon ion laser after optimizing the flow system and improving the protocol for preparation and staining of chromosomes¹⁾ (Fig 2A and 2B). Under our conditions using two fluorescent reagents, 18 different chromosomes are separated as single peaks. A dual-laser flow system was also used to improve the resolution, but the separation pattern generated by the single laser system was sufficient for many experimental purposes.¹

Collecting some 50,000 chromosomes each as small spots on nitrocellulose membrane filters and performing hybridization with a human gene probe of interest, we developed a convenient gene mapping method (spot blot hybridization) and assigned the chromosome which carries a particular gene (Table 1). These included genes for protein 80K-L (substrate for protein kinase C) (MACS),⁷

ATP synthase β -subunit (ATPSB)⁸ and bullous pemphigoid antigen 1 (BPAG1).⁹ Use of the flow-sorted translocation chromosomes enabled us to regionally localize a particular gene (Table 1). The spot blot hybridization with a panel of flow-sorted human chromosomes was useful for finding pseudogenes and homologous sequences such as MACSL1,⁷ ATPSBL1 and ATPSBL2.⁸

The Keio strategy for human genome analysis

Relatively large quantities ($\sim 1 \times 10^7$) of the sorted chromosomes have been used to produce a chromosome-specific clone library of numerous DNA segments. These clone libraries are being used as marker probes for genetic linkage analysis, physical mapping of the individual chromosomes, and the molecular analysis of genetic diseases. We are particularly engaged in the analysis of Down syndrome and cat eye syndrome which are associated with chromosomes 21 and 22, respectively, using cosmid clone libraries under the Keio Strategy (Fig 3).^{10,11} Chromosome-specific cosmid clones were gridded at high density on nylon filters (HDR filter) and screened with selected YAC (yeast artificial chromosome) clones. These clones are being ordered by contig mapper and/or restriction enzyme analysis. Cosmid clones in a particular contig are being used for cDNA isolation and genomic sequencing.

Thus, the flow-cytometry and scanning microscopy with laser optics have numerous applications to the analysis of human genome and important disease gene hunting.

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Fig 3 A simplified diagram of the Keio strategy II⁺.

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