3-4 Genome-wide Analysis of Gene Functions

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Cellular events are regulated by a number of genes contained in the genome. Understanding the mechanisms of such genetic regulations can lead to a breakthrough in the communications technology. Toward this end, we have carried out genome-wide analysis of about 4,500 genes and their protein products in the fission yeast genome. Here we introduce a DNA microarray for monitoring expression profiles of the genes, and a library of fluorescent proteins for imaging four-dimensional localization of the gene products.

Keywords
Genome, Gene expression, Genetic network, DNA microarray

1 Preface

Genetic information of a single human cell corresponds to computer memory of about 1 Gbyte. It is a difficult question to regard this memory size as too big or too small. Considering that a whole human body is developed from a single fertilized egg with a memory of 1 Gbyte and that it engages in highly intellectual activities, however, most people might feel it as too small. In a human adult, on the contrary, there seems to exist approximately 1 trillion cells. If each cell is equivalent to computer memory of 1 Gbyte, it is clear that a human adult has an inconsiderable amount of memory in total. Furthermore, it is quite a wonder that a human adult builds a network of 1 trillion cells and engages in coordinated activities. Living organisms use a simple chemical substance, DNA, as their memory for genetic information, and establish a flexible information processing system based on it. It is one of the projects of our Cell Biology Group to study and understand control mechanisms, or algorithms, for information processing in living organisms. In this report, we will introduce two research projects which have been conducted by Cell Biology Group for genome-wide analysis of gene functions. One is a research to monitor the ON/OFF state of all genes in living cells in order to analyze how cells respond to different environmental conditions. Another is a research to create a four-dimensional (three-dimensional space plus time) map for intracellular localization in order to determine where and how the produced proteins move within a cell. Through extension of these researches, it is expected that the genetic information processing mechanisms in living organisms are understood as an integrated system that can lead to the development of a new information processing principle [1].

2 Genome-wide analysis of gene functions

For comprehensive genome-wide analysis of gene functions, it is important to choose an appropriate living organism. We use human cells for live cell imaging researches. In considering an organism for research on genome-wide research, however, it is suitable to choose an organism that has a small genome size and shares cellular activities common to humans. As such an organism, we chose fission yeast. This organism was chosen because it is easy to handle, and its genome size is one hundredth the human genome, and yet has a life cycle of sexual reproduction (meiosis), in addition to a usual cell division cycle (mitosis), common to higher organisms like humans. Since the complete nucleotide sequences of its genome was published and the number of its genes are about 4,500 [2]. We will introduce a comprehensive analysis research on expressions of the entire set of genes using DNA microarray and a research on creating image database to present intracellular localization of proteins produced from the genes.

2.1 Gene expression analysis using DNA microarray

We produced DNA microarray as a method to detect the ON/OFF of gene expression. Here follows its principle. DNA microarray is produced by spotting the DNAs derived form each gene in an array of columns and rows on a glass slide. During gene expression, mRNAs are produced from DNA templates. The mRNAs produced from a specific gene DNA bind to these DNAs since their nucleotide sequences are complementary. However, the mRNAs do not bind to DNA of different genes. The mRNAs prepared from cells are labeled with fluorescent dyes, and reacted to the DNA microarray. By reading the profile of binding to a specific gene, the ON/OFF results of gene expression can be detected. In Fig. 1, red and green spots represent the ON and OFF states of the gene, respectively. Figure 2 shows an apparatus which spots DNAs on a glass slide. Since this apparatus is capable of arranging a spot of 150 μm in diameter at a distance of 200 μm, it is possible to arrange about 4,500 genes of fission yeast within a single microarray for simultaneous analysis. In such an experiment, it is important to adhere the DNAs stably on a glass slide. In our DNA microarrays, the single-stranded DNAs are covalently bound to the glass slide through amino groups, and data with high accuracy and reproducibility can be obtained. Using these methods, we are now researching on the gene expression profile in response to environmental conditions. By studying and understanding the alteration of gene expression in genome-wide and their regulatory mechanisms, it is possible to understand the information processing algorithm of living cells.

2.2 Four-dimensional mapping of intracellular localization of proteins

By using a DNA microarray, it becomes possible to measure expression levels of the genes. Next, an important question is whether proteins are actually produced from RNAs and where these proteins are working in the cell. As one of the means for visualizing proteins in the cell, fluorescent proteins can be generated by fusing the jellyfish green fluorescent protein (GFP) to a specific protein at the gene level. By observing such fluorescently-tagged proteins on the fluorescence microscope systems developed by our Cell Biology Group, it is possible to observe the intracellular localization of the proteins within living cells. By the use of genomic DNA fragments of fission yeast randomly fused with the GFP gene, we have succeeded in mapping the intracellular localization of about 250 gene products of fission yeast [3]. Figure 3 shows selected examples of such results. Through a series of these researches, many useful information on cell structure and its dynamics were obtained and the understanding of the mechanisms of genetic information processing were significantly progressed [4]-[8]. For the detailed academic achievements, refer to our Web page of Cell Biology Group [9]. Since this library of fluorescent proteins is limited to 250 gene products (proteins) among the 4,500 genes of fission yeast, however, we are now trying to extend this study to a larger number of proteins. As for now, we have constructed about

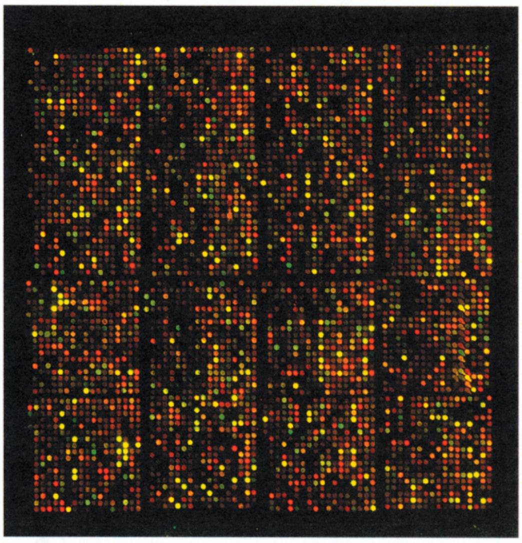
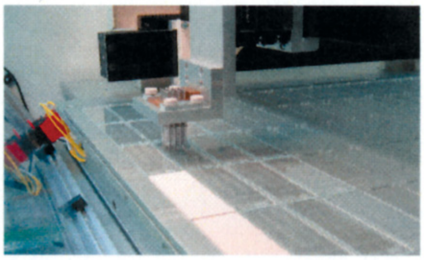


Fig.1 DNA microarray showing ON (red) / OFF (green) of all genes of fission yeast



DNA solution is captured by 16 pin

Spotting DNA solution on the glass slide

Fig.2 DNA microarray spotter

1000 cell strains producing fluorescent proteins. With those cell strains, we are now creating the four-dimensional intracellular localization map of protein localization (Fig. 4). These information is accessible at our Web page of Cell Biology Group.

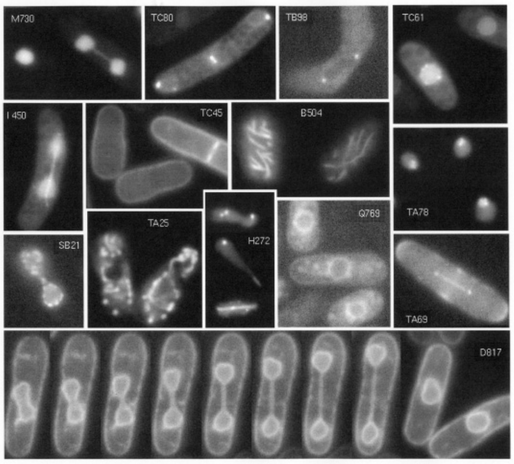


Fig.3 Intracellular localization analysis of proteins

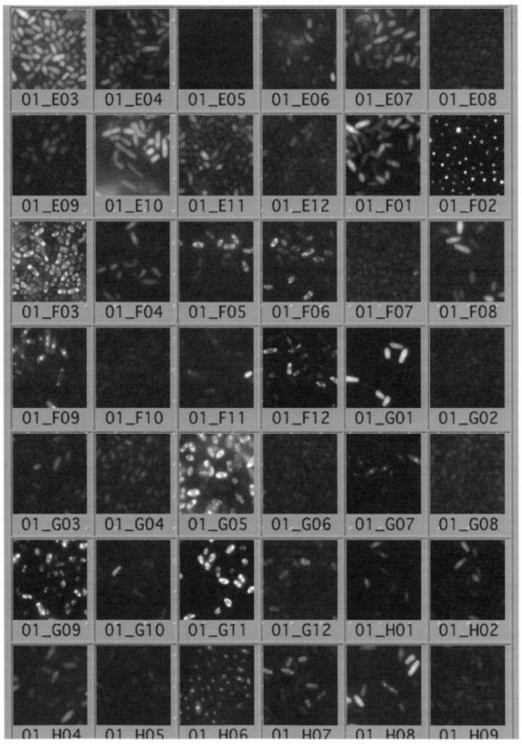


Fig.4 Image database for protein intracellular localization

3 Conclusion

Living organisms survive through unexpected environmental changes, or dangers, using limited information to its maximum extent. Learning the flexible and shrewd information processing strategy of living organisms can provide a unique opportunity to create a human-friendly and flexible communication system. Thus, it is important to study and understand the communication systems based on DNAs as an essence of life. To this end, we have developed tools to analyze those genetic mechanisms in living organisms.

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