日本人類遺伝学会第 28 回大会シンポジウム講演要旨

Abstracts of the Symposium, the 28th Annual Meeting of the Japan Society of Human Genetics

Symposium: **Biotechnology in Human Getetics —From Molecules to Whole Body—**

Introductory Remarks by the Chairman

Hiraku TAKEBE
(Department of Experimental Radiology, Faculty of Medicine, Kyoto Univerisity, Kyoto 606)

Recent development in biotechnology might have caused too much expectation in its application to human genetics. This symposium intended to present and discuss current status of the biotechnology based on the experimental evidences using mainly human and animal materials. The development of the basic technique in biotechnology depended mainly on the works at molecular levels in microorganisms and the successful application in human genetics has been limited to a few attempts, some of which were presented in this symposium. The speakers were selected from those working on the structure and function of the genes of interest in human genetics such as globins, transforming genes, EGF, insulin *etc.* General introduction was given by Dr. Y. Takagi, who is the chairman of the committee on recombinant DNA joint research project of the Ministry of Education, Science and Culture (Monbusho). Drs. Y. Fukumaki, K. Tanaka and N. Shimizu presented their own research reports, the abstracts appearing in this issue. Dr. Z. Ogita, the president-elect for the annual meeting 1984, concluded the symposium by discussing the application to the whole body by his sophisticated micro-technique using mouse embryos.

S1. 人類遺伝学の研究におけるバイオテクノロジー：高木康敬(九大・医・生化). Biotechnology in the Studies of Human Genetics: Yasuyuki TAKAGI (Dept. Biochem., Kyushu Univ. Med. Sch., Fukuoka)

1953 年に Watson, Crick が DNA の二重ラセン構造を提唱して分子遺伝学または遺伝子生化学の基礎を確立してから今年で 30 年になる．この間にまず微生物系で研究は長足の進歩を遂げ，それによって 1973～74 年に新しい強力な手段である組み換え DNA 実験技術がもたらされ，今日，高等・

下等を問わずすべての生物について遺伝情報の本格的な研究が展開されている.

その結果，組み換え DNA 実験が始まってようやく 10 年を経過したにすぎないが，この間にすでにヒトの遺伝子が 100 近く単離され，次々とその構造が解析された．そして真核細胞生物も原核細胞生物の場合と同じような転写開始機構を持つが，その遺伝子は染色体 DNA のごく一部を構成しているにすぎず，1) 遺伝子内にイントロンとエキソンが存在し，2) イントロンに相当する mRNA 部分は形質発現過程において除去，すなわちスプライシングを受け，3) 反復配列，転移性遺伝要素，擬似遺伝子が存在し，また 4) IgG に見られるように個体分化の間に遺伝子は再編成され，5) 進化の過程において遺伝子は重複される，などの新しい知見が得られている.

そして医学の研究においては，現在，ヒト遺伝子の構造研究をさらに広げ，またその知見をもとに形質発現およびその調節機構の解明へとすすみ，遺伝病の本態を遺伝子レベルで解析し，外因性疾患をも含めたすべての疾患における遺伝学的背景を理解するとともに，分化・発生・増殖・老化・免疫など生物の持つ基本的現象の究明を通じて癌をはじめとする代謝性疾患の本質を明らかにしようとしており，すでに DNA による診断が実用化されている.

今後はさらに有機化学的なポリヌクレオチド鎖の合成，遺伝子の真核細胞への導入，細胞融合など多くの新しいバイオテクノロジーの進歩に助けられて，従来の遺伝学とは異なり，遺伝子を分離して構造を決定したうえで，その特定の部位に一定の変異を人工的に導入して表現型が知られていない遺伝子についても，積極的に遺伝子の機能を明らかにしようとする reverse genetics または *in vitro* genetics が推進されるであろう.

S2. DNA 組み換え技術を用いた遺伝病の解析；ヒトグロビン遺伝子の異常について：服巻保幸（九大・医・生化）. **Molecular Analyses of Hereditary Diseases Using DNA Manupulation Techniques; Abnormal Human Globin Genes:** Y. FUKUMAKI (Dept. Biochem., Kyushu Univ. Sch. Med., Fukuoka)

遺伝病の病因を分子レベルで解析するさいのモデルとして，グロビン鎖の産生障害をきたす遺伝病（サラセミア）をとりあげ，DNA 組み換え技術との関連から，その解析例を提示した．1) サザーン・ブロッティング法による解析例；δ 鎖および β 鎖を欠くホモ接合型 $\delta\beta$-サラセミア患者の白血球から DNA を調製し，γ, δ, β グロビン遺伝子および γ-δ グロビン遺伝子間領域をプローブにしてサザーン・ブロッティングを行った．その結果，δ, β グロビン遺伝子を含み，$^{A}\gamma$ グロビン遺伝子の近傍におよぶ 2 万塩基対以上にわたる欠失が確認された．この欠失は従来から γ グロビン遺伝子の発現に抑制的に働くと推測されている領域を含んでおり，本症例は新しい型の $\delta\beta$-サラセミアと考えられる．2) 塩基配列決定法による解析例；β 鎖を全く産生しないホモ接合型 β°-サラセミア患者の β グロビン遺伝子をクローン化し，その塩基配列を決定した．その結果，41, 42 番目のコドンに 4 塩基の欠失が認められた．このため 59 番目が終止コドンとなり，β 鎖の産生が全くみられない．3) *in vivo* 遺伝子発現系を用いた解析例；正常な β 鎖をわずかながら産生する β^{+}-サラセミアの遺伝子の塩基配列が決定され，第 1 介在塩基配列内 110 番目の塩基グアニンのアデニンへの変異が認められた．そこでこの β グロビン遺伝子を SV40 の後期遺伝子領域に組み換えたウイルスを作製した．これをサル線維芽細胞に感染後 polyA^{+} RNA を抽出し，第 2 エキソン内の一部をプライマーにして cDNA を作製し（プライマー伸長法）解析を行った．その結果，正常の β グロビン遺伝子を用いた場合得られる mRNA のほかに，これより 19 塩基長い mRNA の存在を確認し，この 19 塩基が第 1 介在塩基配列に由来す

ることを塩基レベルで明らかにした．また患者の網状赤血球の RNA の中にも，プライマー伸展法・S1 マッピング法により同様の異常な mRNA を確認した．以上の結果から，この β^{+}-サラセミアでは，介在塩基配列内の変異によりスプライシングの受容部位が形成され，これを用いた異常なスプライシングがおき，正常なスプライシングが障害をうけるものと結論された．以上のように，遺伝病の解析は構造解析（サザーン・プロッティング法，塩基配列決定法による）で病因が明らかになる場合，またこれに機能解析（*in vivo*, *in vitro* 遺伝子発現系による）を加えることによりはじめてその病因が明らかになる場合があり，今後は組織特異性や発生に伴う発現の調節などが反映できる遺伝子発現系の開発が望まれる．

S3. Genomic Rearrangements of the SV40 Sequences in the Chinese Hamster Embryo Fibroblasts (CHEF/18) Cells Transfected by the SV40 DNA with Flanking Mouse Sequences: **Kiyoji TANAKA** (Dept. Med. Geriatr., Osaka Univ., Osaka) **and Ruth SAGER** (Dana Farber Cancer Inst., Boston).

The 8.8 kb EcoRI fragment containing SV40 early sequences and their flanking mouse sequences which were cloned into lambda gt WES were transfected into CHEF/18 cells. The foci which arose were picked-up and cloned. The five parental clones (205SV33-4E, 205SV33-26E, 205SV21-21E, 205SV21-24E and 205SV53-2E) which were found to have only one copy of SV40 DNA were chosen. To examine the stability of the integrated SV40 DNA in the parental clones, the integration patterns of SV40 DNA in the subclones from each parental clone was compared. The arrangements of SV40 sequences were examined by blot hybridization with ^{32}P-labeled nick translated SV40 DNA following BamHI or EcoRI digestion. The patterns of subclones from 205SV33-4E were different from one clone to another, while all the subclones from 33-26E maintained the same restriction patterns as those of the parental clone. In 205SV21-24E, almost all the subclones gained the extra fragments which were not seen in parental 205SV21-24E. About half of the subclones from 205SV21-21E showed the different patterns from those of the parental clone. In the subclones from 205SV53-2E, three out of 12 subclones contained extra new fragments. These results indicate that there are significant differences in the stability of the integrated SV40 sequences among different parental clones; 205SV33-4E is a very unstable clone while 205SV33-26E is very stable. 205SV53-2E is also a stable clone. Both 205SV21-24E and 21-21E are intermediate.

Properties of the parental clones were examined. All the clones made tumors in nude mice, although the appearance of the tumors was delayed in 205SV33-26E. All the clones also expressed normal sizes of large T (94K daltons), non-viral middle T (53K daltons) and small T (17K daltons) antigens. However, the amount of large T antigen in 205SV33-26E was less than that of other clones.

To examine whether there are any differences between stable and unstable clones in the

genomic sequences containing SV40 DNA and mouse flanking sequences, the restriction enzyme mappings were carried out with 18 enzymes using SV40 DNA as probe. 205SV33-4E retained whole 8.8 kb EcoRI fragment which was transferred, while in 205SV33-26E and 53-2E, almost all the 5′ flanking mouse sequences and all the 3′ flanking mouse sequences were missing. In 205SV33-26E, the BamHI (2469) and SfaNI (2255) SV40 sites were missing but HpaI site (2604) was retained, indicating that 205SV33-26E has a deletion in the 3′ end region of the transcription template for early mRNA, although the whole translation template for large T antigen was retained. In 205SV53-2E, both transcription and translation templates were intact. In 205SV21-24E and 21-21E, about one-third of the 5′ flanking mouse sequences were missing and part of the 3′ flanking mouse sequences were also missing. SV40 sequences were intact.

These results indicate that the flanking mouse sequences could be involved in the rearrangements in the unstable clone and that the deleted 3′ end region of the transcription template for early mRNA in 205SV33-26E may contain the polyadenylation signal for early mRNA and the lack of the signal may contribute to significantly lower expression of T antigen. The decreased level of T antigen may lead decreased level of viral replication and stable arrangements of integrated SV40 sequences. DNA sequencing analysis in the 5′ flanking mouse sequences is in progress. This region may contain either mouse DNA replicating origin, enhancer (promotor) or enzymes involved in DNA recombinations.

S4. レセプター機能疾患の基礎研究と治療に向けてのバイオテクノロジー：ハイブリッドホルモンの応用：清水信義（慶大・医・分子生物）. **Biotechnology for Study and Treatment of Receptor-deficicient Diseases: Use of the Hybrid Hormones: N. SHIMIZU** (Dept. Mol. Biol., Keio Univ. Med. Sch., Tokyo)

細胞の代謝，増殖や分裂は，EGF（上皮増殖因子）やインスリンのようなポリペプチドホルモンによって制御されている．これらのホルモンは，細胞膜の特異的なレセプターに結合してシグナルを誘導する．レセプターに結合した EGF やインスリンは複合体として集合し，エンドサイトーシスによって細胞内に陥入し種々のオルガネラに伝播されるとともにリソゾーム内で分解される．一方，レセプターの燐酸化反応がホルモン依存的にチロシン残基に起こる．ポリペプチドホルモン作用の分子機構や細胞生物学に関するこのような新しい発展につれて，近年，ホルモンレセプター系の機能欠損に起因する疾患が認識されつつある．われわれは，1) ホルモンレセプター系の細胞遺伝学的解析と，2) レセプター機能欠損の修復を目指したバイオテクノロジーの開発を試みている．第 1 の目的のためにはインスリンや EGF にジフテリア毒素やリシンの A 鎖を接合した毒性ハイブリッドホルモンを作製し，ポリペプチドレセプター系の細胞変異株を分離している (*BBRC* **91**: 143, 1979; *FEBS Lett.* **118**: 274, 1980; *PNAS*, **78**: 445, 1981; *J. Supramol. Sti. Cell. Biochem.* **16**: 105, 1981; *Cold Spring Harbor Conf. Cell Prolif.* **9**: 397, 1982). 第 2 の目的のためには，α2-マクログロブリン (α2M) のような独自の膜レセプターに結合する血清タンパクにインスリンや EGF を接合して，α2M のレセプターを介して，ホルモンを細胞内に搬入することを試みている (*FEBS Lett.* **152**: 131, 1983). 本講演で

は，このようなハイブリッドホルモン分子の作製，化学的性状，生物活性などに関する最近の知見をまとめて紹介するとともに，レセプター疾患治療のバイオテクノロジーとしての可能性と問題点について言及した．

S5. A Developmental Engineering Approach to Human Genetics: Zen-ichi OGITA
(Dept. Biochem. Pathol., Res. Inst. Oriental Med., Toyama Med. Pharm. Univ., Toyama)

The elucidation of genetic phenomena by the investigating method a la Mendel based upon mating experiments has a limited scope of analysis and, in the case of man, has a limited application. For man, there can be no marriage with a mating experiments as its object, and yet very few infants are born. Moreover, observers and persons to be observed are, both men with destined lengths of life, and this makes it particular and difficult to deal with the spheres of human genetic investigations. Man is not appropriate as a subject for applying the investigating method a la Mendel with which are observed the traits transmitted from parents to children over each generation.

However, a method of forming hybrid cells was established, which is capable of creating *in vitro* new combinations of genomes and chromosomes using somatic cells, and a gene engineering technique was also established, which creates new combinations of genes by cutting and fusing DNA *in vitro*, whereby establishing conditions for *in vitro* genetics were settled. Thus, new aspects were opened to human genetics as well as genetics a la Mendel which has been obliged to be applied only among the same biological species capable of mating. The preparing method of chimera animals by Tarkowski in 1961 and Mintz *et al.* in 1962 is making its unexpected evolution to those investigations in which the interaction between the cells with more than 2 sorts of different genotypes can be analyzed through its developmental process and yet even within each organ of an individual.

Furtheremore, if a preparing method of chimera animals among different species of animals is developed, human gene expression will be able to be observed on each organ or tissue of an experimental animal by transferring human chromosome or gene into its early embryo. Therefore, it is not a dream that the treatment of a human hereditary disease will be developed through the elucidation of the process of its onset in mice. Especially, great hopes are desired of replacing human productive age by that of mice so as to shorten the time of generation by means of a surprising method, quite beyond conception, of expressing human gene in the mouse individual. Thus, if such developmental engineering investigating technique is available, not only the disadvantage, destined to human genetics, that observers and persons to be observed are, both, men with destined lengths of life, will be able to be compensated, but also an analysis will be able to be made of the mechanism of control in the developmental process of human genes.

Discussions were made on what evolution of human genetics will follow and how it should evolve through respective combination and systematization of these gene engineering, cell engineering and developmental engineering investigating techniques.