SI-3. PRADER-WILLI SYNDROME: CONSIDERATION OF A QUESTION IN ITS CLINICAL, CYTOGENETIC AND MOLECULAR ASPECTS. Norio NIIKAWA (Dept. Hum. Genet., Nagasaki Univ. Sch. Med., Nagasaki)

Prader-Willi syndrome (PWS) is a multiple congenital anomaly/mental retardation syndrome characterized by severe muscle hypotonia in infancy, a peculiar facies, small hands and feet, hypopigmented skin, hypogonadism, poly/hyperphagia and subsequent obesity and diabetes in childhood. Because of these diverse phenotypes, PWS is thought as one of "contiguous gene syndromes." Almost all patients are sporadic, suggesting fresh mutants for autosomal dominant gene(s). Although the basic cause is unknown, 50–100% of patients show chromosome abnormality involving the 15q11.2 region. However, the cytogenetic data so far reported were diverse and controversial, *i.e.*, monosomy, disomy, trisomy, or even tetrasomy for a 15q11.2 band. Several hypotheses have been proposed, but none seems convincing to explain these complex findings. Molecular approaches were done to see a DNA deletion in PWS patients using 15q11.2-specific DNA clones as probes. However, these studies could not detect all-or-nothing results for the deletion either, suggesting that these clones would be out-side of the PWS loci. Besides PWS, it was found that patients with Angelman syndrome (AS) also have a 15q11-12 deletion, leading to another confusion.

In this symposium, the results of previous clinical, cytogenetic and molecular studies including ours on PWS and AS are reviewed. The strategy and the data of our recent approach to clone the PWS gene(s) by the microdissection/microcloning technique are also presented. With this technique, we have cloned chromosomal DNAs directly from the region at 15q11.2. Of 30 clones analyzed, one showed a one-copy density on the Southern blot of two AS patients, and the other two clones showed one-copy signal on the blots of a PWS patient, suggesting candidate clones each for the AS and the PWS genes.

SI-4. MOLECULAR ANALYSIS OF FAMILIAL POLYPOSIS COLI. Yuchio YANAGAWA and Takehiko SASAZUKI (Dept. Gen. Med. Inst. Bioreg., Kyushu Univ., Fukuoka)

An inherited cancer syndrome, familial polyposis coli (FPC) is an autosomal dominant genetic trait with high penetrance of the mutated gene and is characterized by the hundreds of adenomatous polyps with a high risk at development of colorectal carcinoma. Linkage analysis with polymorphic DNA markers demonstrated that the FPC gene is localized on the long arm of chromosome 5. In order to identify the FPC gene and analyze its functions, we have investigated the molecular mechanism of tumorigenesis in FPC.

1) We observed loss of heterozygosity in colorectal tumors from patients with FPC on 15 chromosomes and in nonpolyposis colorectal carcinomas (NPCC) on 7 chromosomes. Frequent loss of heterozygosity in colorectal carcinomas from FPC patients were observed on chromosomes 5 (24%), 14 (20%), 17 (31%), 18 (40%), and 22 (33%), and also on chromosomes 5 (32%), 14 (30%), 17 (27%), 18 (20%), and 22 (19%), in NPCC.

Loss of heterozygosity in colorectal adenoma was less than 7% on 9 chromosomes. These results suggest that tumor suppression genes for colorectal carcinogenesis may locate on the above chromosomes and that the FPC gene on chromosome 5 may be a tumor suppressor gene.

2) To identify the existence and localization of tumor suppressor genes on human

chromosome 5, we introduced a whole or truncated human chromosome 5 into murine hepatoma cell line, 7R1 and constructed microcell hybrids, BG15-6 and BG15-9, containing a whole human chromosome 5 and a truncated human chromosome 5 (5pter-q23), respectively.

We found that 5q31-qter region of human chromosome 5 possessed the ability to suppress the tumor formation in nude mice, and that the proximal region of 5q23, where the FPC gene was located, could dramatically reduce the colony formation in soft agar.

3) The role of the loss of human chromosome 5 in the process of malignant transformation in colorectal carcinomas were investigated by introducing a chromosome 5 into a human colorectal carcinoma cell line, SW620, which possessed a deletion of long arm of chromosome 5. We found that introduced human chromosome 5 suppressed the growth rate of SW620, further suggesting that tumor suppressor gene may locate on chromosome 5.

シンポジウム II. 優性遺伝性疾患への実験的アプローチ

Symposium II. Experimental Approaches to Autosomal Dominant Disorders

Chairpersons: Zenichi OGITA (Toyama) and Ichiro MATSUDA (Kumamoto)

SII-1. APPROACHING GENETIC DISEASES BY "REVERSE GENETICS."

Yusuke NAKAMURA (Dept. Biochem., Cancer Inst., Tokyo)

Development of genetic linkage maps of human chromosomes, with markers based on RFLPs (restriction fragment length polymorphisms), has led to localization of genes responsible for many genetic diseases. The conventional approach to studying genetic mutations causing hereditary diseases is to identify an abnormal protein or metabolic product first and then to isolate the gene in order to characterize the mutations at the DNA level. For most genetic diseases, however, the biochemical abnormalities have not been identified; linkage mapping makes it possible to begin the process of identifying a mutation from the other direction, or "reverse genetics." Genetic diseases such as adenomatous polyposis coli (APC), multiple endocrine neoplasia types I and II (MEN I and II), or von Recklinghausen neurofibromatosis (NF I), for example, have so far defied efforts to identify the abnormal gene products. However, as polymorphic DNA markers are able to distinguish two cytogenetically identical chromosomes which have been inherited by a given individual from each parent, one can examine the pattern of co-segregation between the unknown gene causing the phenotype (*i.e.*, affected or unaffected for genetic diseases) and genotype (alleles for RFLP markers). Because primary maps of markers are now available for almost all chromosomes, this approach can localize an unknown gene to a particular chromosomal region as a first step toward its isolation; when the gene is cloned and its mutation(s) characterized at the molecular level, the function of its product and the biochemical consequences of mutation can be determined.

I have been using reverse genetics to approach the isolation of the genes responsible