

## Loss of Heterozygosity on Chromosome 1p in Thyroid Adenoma and Medullary Carcinoma, but not in Papillary Carcinoma

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We analyzed 53 loci on 21 chromosomes other than chromosome 4 to detect possible loss of heterozygosity in 31 thyroid tumors using polymorphic DNA markers that detect allelic deletions at specific chromosomal loci. Loss of heterozygosity on chromosomes 1, 7 and 12 was detected in one follicular thyroid adenoma, and on chromosome 1 in two medullary thyroid carcinomas. However, no loss of heterozygosity was detected at any of the loci examined in papillary thyroid carcinomas. These results suggest that chromosomal loss detected in thyroid adenoma is one of the signals for risk of premalignant transformation, and that inactivation of unknown genes on chromosome 1p contributes to tumorigenesis of medullary thyroid carcinoma. Some genetic changes other than chromosomal losses may participate in the tumorigenesis of papillary thyroid carcinoma.

Key words: Thyroid tumor — Loss of heterozygosity — Chromosome 1

Clinically, apparent nodules of the thyroid are found in 4 to 7% of the adult population and are more common in women than in men.<sup>1)</sup> Various tumors such as benign adenomas, well differentiated carcinomas and anaplastic malignant carcinomas originate from follicular epithelial cells, and one-third of all medullary thyroid carcinomas originating from thyroid C cells are hereditary. Therefore, thyroid tumors provide an attractive model for study of the mechanism of tumorigenesis.

The growth of thyroid cells is under regulatory control by TSH and many growth factors,<sup>2)</sup> but the factors that disrupt this regulation are still not known. Exposure to radiation and iodine-deficiency may play roles in the pathogenesis of thyroid tumors. There are reports that the incidence of thyroid lymphoma is increased in patients with Hashimoto's thyroiditis,<sup>3)</sup> and that both follicular and papillary thyroid carcinoma may progress to undifferentiated carcinoma.<sup>4)</sup> However, little is known about the molecular mechanisms involved in tumorigenesis in the thyroid gland.

Recently, two important molecular events in tumorigenesis have been identified; activations of oncogenes and inactivation of tumor suppressor genes.<sup>5)</sup> Activations of oncogenes due to point mutations, gene amplifications, and rearrangements of protooncogenes have been detected in many malignant tumors.<sup>6)</sup> By the study of DNA sequence polymorphisms, it is now possible to detect mitotic recombination and chromosomal deletion

localized in a small region of the chromosome as loss of heterozygosity even in a solid tumor.<sup>7)</sup> This loss of heterozygosity on a specific chromosome is thought to be important in tumorigenesis, by causing a defect of tumor suppressor genes. To date, there has been no report describing allele loss in thyroid adenoma or papillary thyroid carcinoma. Therefore, to determine whether such changes occur in benign and malignant tumors of the thyroid, we studied the constitutional and tumor genotypes of these tumors using polymorphic DNA probes to detect possible loss of heterozygosity on a specific chromosome.

### MATERIALS AND METHODS

**Human tissue samples** Thirty-five primary thyroid tumors, 27 samples of normal thyroid tissue and 7 samples of peripheral leukocytes were obtained from 34 patients at surgery. The tumors included 8 follicular adenomas, 19 papillary carcinomas and 8 medullary thyroid carcinomas (1 sporadic, 1 MTCWP, 2 MEN 2A and 4 MEN 2B). Since papillary thyroid carcinomas often contain much stroma, the samples were examined microscopically to assess how much nontumorous tissue they contained, and only 15 of the 19 samples of papillary carcinoma, in which more than 70% of the tissue consisted of tumor cells, were analyzed for loss of heterozygosity.

**Isolation of DNA** High-molecular-weight DNA was prepared from the tumor tissues and peripheral leukocytes by the method of Blin and Stafford.<sup>8)</sup>

Abbreviations: MEN, multiple endocrine neoplasia; RFLP, restriction fragment length polymorphism; TSH, thyrotropin; MTC, medullary thyroid carcinoma.

Table I. Probes Used in This Study and Results of Loss of Heterozygosity

Probe	Local-ization	Enzyme	No. of tumor	Informa-tive cases	Loss in tumor	Probe	Local-ization	Enzyme	No. of tumor	Informa-tive cases	Loss in tumor
D1S57	1pter	<i>TaqI</i>	24	16	1	PLAU	10q24	<i>BamHI</i>	10	10	0
D1Z2	1p36	<i>TaqI</i>	10	10	0	WT	11p13	<i>TaqI</i>	18	13	0
GLUT1	1p35	<i>BglII</i>	14	6	0	D11S12	11p15	<i>MspI</i>	26	26	0
D1S7	1p35	<i>HinfI</i>	28	18	0	APOA1	11q23	<i>TaqI</i>	24	1	0
MYCL	1p32	<i>EcoRI</i>	30	17	3	INT2	11q23	<i>TaqI</i>	27	17	0
D1S64	1p	<i>MspI</i>	15	12	0	KRAS2	12p12	<i>TaqI</i>	29	4	1
D1S60	1p	<i>BamHI</i>	31	7	0	D12S4	12cen	<i>MspI</i>	4	4	0
D1S73	1p	<i>TaqI</i>	29	15	0	RB	13q14	<i>BamHI</i>	20	17	0
AMY	1q21	<i>PstI</i>	22	15	0	D13S2	13q23	<i>MspI</i>	21	5	0
AT3	1q23	<i>PstI</i>	27	24	1	D14S16	14	<i>PstI</i>	24	2	0
D1S8	1q42	<i>AluI</i>	28	22	0	D15S18	15q11	<i>BglII</i>	31	20	0
NMYC	2p14	<i>PvuII</i>	31	22	0	D16S79	16p13	<i>TaqI</i>	27	18	0
TGFA	2p13	<i>TaqI</i>	26	25	0	TP53	17p13	<i>BglII</i>	31	11	0
EARBA2	3pter	<i>EcoRI</i>	18	0	—	D17S30	17p	<i>MspI</i>	27	27	0
D3S3	3p14	<i>MspI</i>	26	25	0	D17S4	17q23	<i>PvuII</i>	14	3	0
D5S2	5pter	<i>MspI</i>	27	10	0	PALB	18q11	<i>MspI</i>	28	19	0
D5S4	5p	<i>MspI</i>	29	4	0	DCC	18q	<i>MspI</i>	31	6	0
MYB	6q	<i>EcoRI</i>	25	16	0	D19S7	19cen	<i>MspI</i>	23	19	0
EGFR	7q31	<i>HindIII</i>	27	26	0	C3	19q13	<i>TaqI</i>	14	0	—
MET	7q31	<i>TaqI</i>	19	10	1	D19S9	19q12	<i>EcoRI</i>	10	0	—
MOS	8q11	<i>MspI</i>	31	0	—	D20S5	20p15	<i>MspI</i>	26	26	0
PAS	9q11	<i>HindIII</i>	10	10	0	D20S4	20p13	<i>MspI</i>	26	10	0
D10S24	10p13	<i>MspI</i>	26	8	0	D20S6	20p	<i>TaqI</i>	14	14	0
IRBP	10p11	<i>MspI</i>	30	10	0	D21S11	21q11	<i>EcoRI</i>	31	16	0
D10S5	10q21	<i>TaqI</i>	26	1	0	D22S1	22p	<i>TaqI</i>	14	14	0
D10S1	10q22	<i>BglII</i>	31	9	0	D22S9	22q11	<i>MspI</i>	23	7	0
D10S4	10q22	<i>TaqI</i>	24	7	0						

**DNA probes** The polymorphic DNA probes and restriction endonucleases used in this study are listed in Table I. These probes, which detected restriction fragment polymorphism (RFLP) at 53 loci on 21 autosomal chromosomes other than chromosome 4, were radiolabeled by the random hexanucleotide primer method.<sup>9)</sup>

**Southern blot analysis** DNAs (5 µg) were digested with appropriate restriction endonucleases for 12 h under the conditions recommended by the supplier (Toyobo, Japan). The resulting DNA fragments were separated by electrophoresis in 0.7% agarose gel and transferred to a nylon membrane (Hybond-N, Amersham) by the method of Southern.<sup>10)</sup> DNAs on the filter were hybridized at 42°C for 16 h in a mixture of 50% formamide, 6× standard saline citrate (SSC), 5× Denhard's solution, 10 mM EDTA, 0.5% sodium dodecyl sulfate (SDS), 100 µg/ml of denatured salmon sperm DNA, <sup>32</sup>P-labeled denatured probe DNA and 100 mg/ml dextran sulfate. The filter was then washed twice in 2×SSC for 10 min, once in 1×SSC with 0.1% SDS for 30 min, and once in

0.1×SSC with 0.1% SDS for 10 min at 65°C and subjected to autoradiography using Kodak X-ray film (XRP-5 or XAR-5) with a Dupont Cronex intensifying screen at -70°C. When necessary, the membranes were reprobbed after removing the first probe.

RESULTS

**Loss of heterozygosity at RFLP loci on chromosomes 1, 7 and 12 in follicular adenoma** DNA samples from all the patients showed constitutional homozygosity on ERBA2 (3pter), MOS (8q11), C3 (19q13) and D19S9 (19q12), indicating that they were not informative at these loci (Table I). As summarized in Table II, 38 informative loci were obtained in eight follicular adenomas on 19 chromosomes other than chromosomes 4, 8 and 9. Loss of heterozygosity was observed in one adenoma, but no common loss of any allele was detected. In this tumor, loss of heterozygosity was observed at MYCL (1p32), AT3 (1q23), MET (7q32) and KRAS2 (12p12)

Table II. Loss of Heterozygosity at Chromosomal Loci in Human Follicular Thyroid Adenoma

Case No.	Chromosome retaining heterozygosity	Chromosome showing loss of heterozygosity
1	1, 3, 7, 10, 11, 13, 17, 20, 22	ND
2	1, 2, 3, 6, 7, 10, 11, 12, 15, 16, 17, 20, 22	ND
3	2, 3, 6, 10, 11, 15, 17, 20, 22	1, 7, 12
4	1, 2, 3, 7, 10, 12, 17, 20, 21, 22	ND
5	1, 2, 6, 7, 10, 11, 14, 15, 16, 17, 20, 21, 22	ND
6	1, 2, 5, 6, 7, 10, 17, 19, 20, 22	ND
7	1, 2, 3, 6, 7, 11, 14, 15, 16, 18, 19, 20, 21	ND
8	5, 10, 11, 15, 16, 21, 22	ND

ND: not detected.

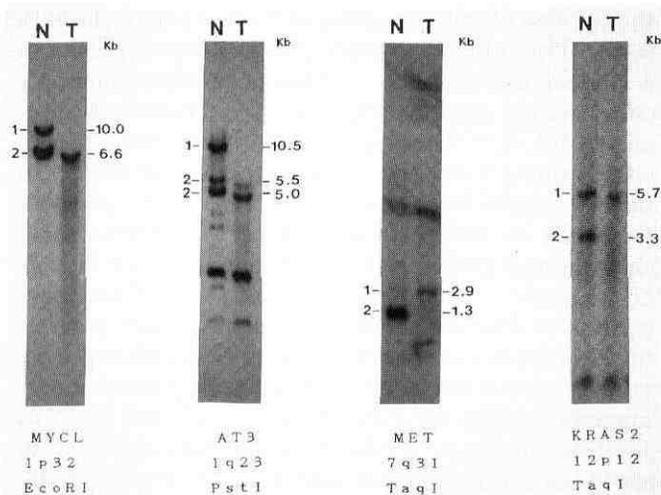


Fig. 1. Loss of constitutional heterozygosity at loci on chromosomes 1, 7 and 12 in a follicular thyroid adenoma. DNAs of tumor and peripheral leukocytes from the patient were digested with appropriate restriction enzymes, separated by electrophoresis in 0.7% agarose gel and transferred to a nylon membrane. The membrane was hybridized to <sup>32</sup>P-labeled DNA probes for loci on chromosomes 1, 7 and 12. Numbers 1 and 2 on the left indicate the observed alleles. Lane N contains the digest of DNA from peripheral leukocytes. Lane T contains the digest of DNA from the tumor. kb; kilobase.

(Fig. 1). In this case, loss of heterozygosity should extend from the short arm to the long arm of chromosome 1.

**No loss of heterozygosity at RFLP loci on any chromosome in papillary thyroid carcinoma** As summarized in Table III, in 15 specimens of papillary thyroid carcinomas, 47 informative loci were obtained on 19 chromosomes other than chromosomes 4, 8 and 14. No loss of heterozygosity was observed at any RFLP loci on any chromosome.

Table III. No Loss of Heterozygosity at Chromosomal Loci in Papillary Thyroid Carcinoma

Case No.	Chromosomes retaining heterozygosity
1	1, 2, 3, 5, 7, 9, 10, 11, 15, 17, 18, 19, 20, 21, 22
2	1, 2, 3, 5, 6, 7, 9, 10, 12, 15, 17, 18, 20, 21, 22
3	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 15, 17, 18, 20, 21, 22
4	1, 2, 3, 5, 7, 9, 10, 11, 13, 15, 17, 19, 20, 21, 22
5	1, 2, 5, 7, 9, 10, 11, 13, 15, 16, 17, 18, 20, 22
6	1, 2, 3, 6, 7, 9, 10, 11, 15, 17, 19, 20, 22
7	1, 2, 3, 5, 7, 9, 10, 11, 15, 16, 17, 19, 20, 22
8	1, 2, 3, 5, 6, 7, 9, 10, 11, 15, 17, 19, 20, 22
9	1, 2, 3, 5, 6, 7, 9, 10, 11, 12, 13, 17, 18, 20, 21, 22
10	1, 2, 3, 5, 7, 10, 11, 15, 17, 19, 20, 21, 22
11	1, 3, 6, 7, 11, 16, 17, 18, 19, 20
12	1, 2, 3, 6, 7, 11, 16, 17, 18, 20
13	1, 2, 3, 10, 11, 15, 16, 17, 18, 20
14	1, 2, 3, 6, 7, 11, 16, 17, 18, 19
15	1, 2, 3, 5, 6, 7, 10, 11, 15, 16, 17, 18, 19, 20

No loss of heterozygosity was detected in any chromosome.

**Loss of heterozygosity at RFLP loci on chromosome 1p in thyroid medullary carcinoma** In 8 medullary thyroid carcinomas (MTCs), 32 informative loci were obtained on 16 chromosomes other than chromosomes 4, 8, 12, 13 and 14. In one MTCWP case, loss of heterozygosity was observed at MYCL (1p31) and D1S57 (1pter) (Fig. 2A). This tumor did not give the 1.6 kb *TaqI* fragment of D1S57 or the 6.6 kb *EcoRI* fragment of MYCL. In addition, in one MEN 2B case, loss of heterozygosity was observed at MYCL (1p32) (Fig. 2B). This tumor also did not give the 6.6 kb *EcoRI* fragment of MYCL. These tumors did not show loss of heterozygosity on the other

chromosomes examined, suggesting that these chromosomal losses were not random events.

**Allelic retention at MEN 2A and 2B loci on chromosome 10 in medullary thyroid carcinoma** Recently, the loci of MEN 2A, 2B and MTCWP (medullary thyroid carcinoma without pheochromocytoma) have been assigned to chromosome 10q<sup>11-14</sup>) by genetic linkage studies. As shown in Table I, we used IRBP, D10S24, D10S5, D10S1, D10S4 and PLAU as markers of chromosome 10,

but observed no loss of heterozygosity at these loci in any MTCs or other thyroid tumors (Table IV).

DISCUSSION

Recently, losses of heterozygosity at specific loci on some chromosomes have been observed in several tumors,<sup>7,15)</sup> indicating inactivation of a specific gene with tumor-suppressing activity. There have been few reports about loss of heterozygosity in thyroid tumors other than MTCs. In this study, we analyzed the DNAs from 31 thyroid tumors of benign and malignant type using polymorphic probes to detect possible loss of heterozygosity on chromosomes.

One of 8 follicular thyroid adenomas examined showed loss of heterozygosity at four loci on 3 chromosomes. This is the first report of loss of heterozygosity in a thyroid adenoma. In cytogenetic analyses, multiple chromosomal abnormalities have been detected on chromosomes 1, 4, 7, 9 and 12,<sup>16,17)</sup> and the abnormalities on chromosomes 1 and 7 are consistent with our results. In fact, microfollicular adenomas are considered to progress frequently to follicular carcinomas.<sup>18)</sup> Therefore, these chromosomal losses may be one of the signals for the risk of malignant transformation. In fact, loss of heterozygosity was observed in colon adenomas,<sup>19)</sup> and progression to carcinoma was proposed to be facilitated by a series of genetic alterations such as loss of putative tumor suppressor genes on chromosomes 5, 17 and 18.<sup>20)</sup> In addition, alteration of the *ras* gene may constitute an early step in thyroid tumorigenesis,<sup>21)</sup> but no point mutation of the *ras* gene was detected in the thyroid adenomas we examined (unpublished data).

Olah *et al.*<sup>22)</sup> detected deletion of chromosome 11q in all three papillary thyroid carcinomas they examined by cytogenetic analysis. However, we did not detect loss of chromosome 11q in any papillary thyroid carcinoma in our series. In cytogenetic analysis, chromosomal rearrangement and translocation might be interpreted as

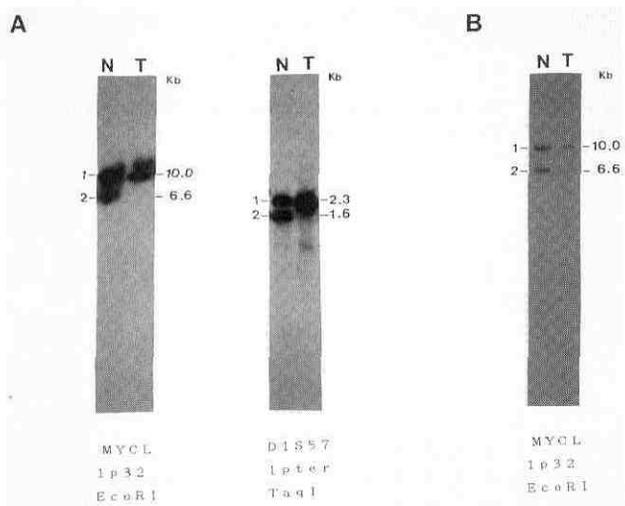


Fig. 2. Loss of constitutional heterozygosity at loci on chromosome 1 in medullary thyroid carcinoma from MTCWP (A) and MEN 2B (B) cases. DNAs of tumor and normal thyroid tissue from patients were digested with appropriate restriction enzymes, separated by electrophoresis in 0.7% agarose gel, and transferred to a nylon membrane. The membrane was hybridized to <sup>32</sup>P-labeled DNA probes for MYCL (2A), DIS57 (2A), and MYCL (2B) loci on chromosome 1. Numbers 1 and 2 on the left indicate the observed alleles. Lane N contains a digest of DNA from normal thyroid tissue. Lane T contains the digest of DNA from the tumor. kb; kilobase.

Table IV. Loss of Heterozygosity at Chromosomal Loci in Medullary Thyroid Carcinoma

Case No.	Chromosomes retaining heterozygosity	Chromosome showing loss of heterozygosity
1 (sporadic)	1, 2, 3, 6, 7, 11, 15, 16, 18, 21	ND
2 (MTCWP)	2, 10, 11, 15, 16, 18, 21	1
3 (MEN2A)	1, 2, 3, 6, 7, 10, 11, 16, 17, 20, 22	ND
4 (MEN2A)	1, 2, 3, 6, 7, 10, 11, 16, 17, 18, 19, 20, 21	ND
5 (MEN2B)	1, 2, 3, 5, 7, 10, 11, 16, 17, 18, 19, 20, 21	ND
6 (MEN2B)	1, 2, 7, 11, 15, 16, 17, 18, 20, 21	ND
7 (MEN2B)	2, 3, 7, 11, 15, 16, 17, 18, 20, 21	1
8 (MEN2B)	1, 2, 10, 11, 15, 16, 18, 21, 22	ND

ND: not detected.

deletion, or artificial chromosomal changes that occurred during the incubation period might be detected.

The p53 protooncogene, mapped to 17p13,<sup>23)</sup> is known to act as a suppressor gene,<sup>24)</sup> but in our series no loss of heterozygosity was detected at the loci of p53 and D17S30 (Table I). Therefore, deletion of the p53 gene may not participate in thyroid tumorigenesis. Similarly, the WT gene, RB gene and DCC gene, mapped to 11p13,<sup>25)</sup> 13q14<sup>26)</sup> and 18q,<sup>27)</sup> respectively, are also thought to be critical suppressor genes, but we detected no loss of heterozygosity at these loci in thyroid tumors (Table I), so these genes may also not participate in tumorigenesis in the thyroid.

Our results indicate that the frequency of loss of heterozygosity at loci on the chromosomes is very low in papillary thyroid carcinoma, as in stomach cancer.<sup>28)</sup> The chromosomal lesions in papillary thyroid carcinoma may be too small to be detected by Southern blot analysis with currently available probes. Some structural alterations of protooncogenes leading to oncogene activation was reported on the *ras* gene (point mutation<sup>29)</sup>), *trk* gene (rearrangement<sup>30)</sup>) and PTC gene (rearrangement to *ret* gene<sup>31)</sup>) in papillary thyroid carcinoma. Thus, in papillary thyroid carcinoma, activation of oncogenes rather than inactivation of tumor suppressor genes may be important in tumorigenesis or tumor development.

The gene responsible for MEN 2A was mapped to chromosome 10,<sup>11,12)</sup> but in MEN 2A tumor (MTC and pheochromocytoma) loss of heterozygosity has not been detected frequently on this chromosome.<sup>32)</sup> We also did not observe loss of heterozygosity in any thyroid tumors including MTCs of MEN 2A and 2B at several loci (IRBP3, D10S24, D10S5, D10S1, D10S4, PLAU) on chromosome 10. Therefore, in MTCs associated with MEN 2A and 2B, plural genetic abnormalities may be necessary for tumorigenesis. On the other hand, two cases of MTCs showed loss of heterozygosity; one tumor of MTCWP showed loss of heterozygosity at MYCL (1p32) and D1S57 (1pter), and the other tumor of MEN 2B showed loss of heterozygosity at MYCL (1p32). Mathew *et al.*<sup>33)</sup> previously reported loss of heterozygosity at D1S7 (1p35) in 3 of 8 MTCs (38%), and Okazaki *et al.*<sup>34)</sup> also reported allele loss in 5 of 34 MTCs (15%) at the same locus (D1S7). In our study, no loss of

heterozygosity was observed at this locus on chromosome 1 (Table I). Moreover, recently, Yang *et al.*<sup>35)</sup> reported that the frequency of loss of heterozygosity in MTCs was 50% at MYCL, which is similar to our finding. These data suggest that one of the critical suppressor genes involved in the tumorigenesis and/or progression of MTC may be located in the region of 1p32. Loss of heterozygosity on 1p has also been observed in tumors originating from neural crest cells, such as neuroblastoma,<sup>36)</sup> melanoma,<sup>37)</sup> MTC<sup>32)</sup> and pheochromocytoma.<sup>33,38)</sup> Therefore, we suppose that genetic alterations on chromosome 1p may be a common mechanism of tumorigenesis in cells of neural crest cell origin, and may also be necessary for tumorigenesis and/or progression of MTC of MEN 2A and 2B in addition to genetic changes on chromosome 10.

In summary, our data suggest that the multiple chromosomal losses detected in thyroid adenoma are one of signals of the premalignant status, and that inactivation of some unknown genes on chromosome 1p is involved in tumorigenesis of MTC. Furthermore, genetic changes other than chromosomal losses may contribute to the tumorigenesis of papillary carcinoma of the thyroid.

#### ACKNOWLEDGMENTS

We thank Drs. T. Sekiya, A. S. Bleaudat, S. C. Bock, J. Breslow, C. D. Bridges, W. Cavenee, T. P. Dryja, R. R. Frant, M. Jansen, N. J. Jeffreys, M. Richard, J-L. Mandel, J. Minna, B. D. Nelkin, C. G. P. Mathew, M. Mueckler, P. Derynek, P. Pearson, D. J. Show, G. F. Sounders, G. D. Stewart, R. A. Weinberg, B. N. White, R. White, J. Harlay, H. McDermid, G. I. Lieu, M. McCoy, X. O'Breakefiels, L. Crawford, G. Fey, J. Yokota, D. A. Haber, and B. Vogelstein for providing polymorphic DNA probes. DNA probes were also provided by the Japanese Cancer Research Resources Bank and the American Type Culture Collection. We also thank Dr. H. Morizumi for pathological diagnoses. This work was supported by a Grant-in-Aid for Research on Intractable Disease from the Ministry of Health and Welfare of Japan and a Grant-in-Aid for Scientific Research (No. 02771799) from the Ministry of Education, Science and Culture of Japan.

(Received April 30, 1991/Accepted July 17, 1991)

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