*Proc. Natl. Acad. Sci. USA*
Vol. 81, pp. 4534–4538, July 1984
Medical Sciences

# Aberrant expression of an amplified c-*myb* oncogene in two cell lines from a colon carcinoma

(marker chromosomes/myeloblastosis virus/tumorigenesis/cellular oncogene)

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*Contributed by J. Michael Bishop, March 23, 1984*

ABSTRACT **Two cell lines (COLO 201 and COLO 205) derived independently from a single adenocarcinoma of the human colon each harbored an approximately 10-fold amplification of the cellular oncogene c-*myb* and a proportional abundance of the 4-kilobase mRNA derived from c-*myb*. By contrast, expression of c-*myb* could not be detected in cells from a variety of other solid tumors, including other colon carcinomas. Analysis of the amplified DNA with restriction endonucleases failed to reveal any topographical abnormalities within c-*myb*. Neither COLO 201 nor COLO 205 carry the double minute chromosomes and homogeneously staining regions of chromosomes that frequently serve as karyotypic signatures of amplified DNA. Instead, amplified c-*myb* is carried on what appear to be disomic or trisomic copies of the same anomalous marker chromosome that is characteristic of both COLO 201 and COLO 205. The karyological origin of this abnormal chromosome is not presently apparent. Our findings show c-*myb* expression by cells outside of the hemopoietic lineage, raise the possibility that amplification and/or ectopic expression of c-*myb* may have contributed to the genesis of the tumor from which the cells of COLO 201 and COLO 205 arose, and suggest that amplification of cellular oncogenes may be a more common factor in tumorigenesis than might have been suspected from available karyological data.**

Avian myeloblastosis virus (AMV) is a chicken retrovirus that causes myeloblastic leukemia in birds and transforms myelomonocytic hemopoietic cells in culture (1, 2). The tumorigenicity of avian myeloblastosis virus has been attributed to the viral oncogene v-*myb* (3), which arose by transduction of the cellular oncogene c-*myb* (4). Expression of c-*myb* in normal cells and tissues has to date been observed only in hemopoietic lineages (5, 6) and has been implicated by circumstantial evidence in the genesis only of hemopoietic neoplasms (6).

We now describe *ca.* 10-fold amplification and abundant expression of c-*myb* in two cell lines (COLO 201 and COLO 205; COLO is the designation of tumor cell lines established in Colorado), derived independently from a single adenocarcinoma of the human colon (7). Our findings (*i*) add to the growing list of human tumors whose origins may be traced in part to the amplification and enhanced expression of cellular oncogenes (8–12), (*ii*) dramatize the likelihood that amplification of DNA in tumor cells occurs more frequently than would be suspected from the frequency of double minute chromosomes and homogeneously staining regions of chromosomes in tumor cells, (*iii*) provide an example of c-*myb* expression by cells not in a hemopoietic lineage, and (*iv*) raise the possibility that amplification and/or ectopic expression of c-*myb* may have helped to engender the carcinoma from which the cells of COLO 201 and COLO 205 originally arose.

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## MATERIALS AND METHODS

Tumor cells were obtained from American Type Culture Collection. The recombinant plasmid clones containing chicken viral oncogene inserts have been described (13–16). Other oncogene DNA fragments were prepared from recombinant plasmids supplied as follows: v-*myb* (K.-H. Klempnauer); human c-*myb* (pF8; ref. 17); c-Ha-*ras*, c-Ki-*ras*, and N-*ras* (C. Tabin). Radioactive DNAs were prepared with reverse transcriptase and either [$^{32}$P]dCTP or [$^{3}$H]dCTP to achieve specific activities of 2–5 × $10^8$ cpm/$\mu$g and 1–2 × $10^7$ cpm/$\mu$g of DNA template, respectively. Procedures for transfer of RNA and DNA to nitrocellulose, hybridizations, and washings were those reported earlier (11). RNA dot blots were spotted on nitrocellulose from RNA dissolved in 14.8% formaldehyde containing 12× NaCl/Cit (1× NaCl/Cit is 0.15 M NaCl/0.015 M sodium citrate, pH 7).

Chromosomal spreads and trypsin/Giemsa staining (G-banding) followed published procedures (18). *In situ* hybridizations were performed as described by Harper and Saunders (19).

## RESULTS

**Amplification of c-*myb* in the COLO 201 and COLO 205 Cell Lines.** During the course of a survey for amplification of cellular oncogenes in cells derived from human tumors (10, 11, 20), we discovered evidence for amplification of c-*myb* in COLO 201 and COLO 205 (Fig. 1). DNAs from various sources were cleaved with the restriction endonuclease *Eco*RI, fractionated by electrophoresis in an agarose gel, immobilized on nitrocellulose, and analyzed by hybridization with a radioactive molecular clone of v-*myb* (Fig. 1). The results revealed 2.8-kilobase-pair (kbp) and 2.0-kbp fragments of DNA that were more abundant in the DNA from COLO 201 and COLO 205 cells than in DNAs from other sources (Fig. 1). Both fragments are characteristic of human c-*myb* (17, 21). Quantitative analyses revealed that the two fragments of c-*myb* DNA are amplified *ca.* 10-fold over normal in the cells of COLO 201 and COLO 205 (see Fig. 1). We found no evidence for amplification of several other cellular oncogenes, including c-*myc*, c-Ha-*ras*, c-Ki-*ras*, and N-*ras* (data not shown).

We and others have found that amplification of cellular oncogenes is sometimes accompanied by rearrangement of DNA within the amplified domain (10, 12). Therefore, we used restriction endonucleases to map the topography of the amplified c-*myb* in COLO 201 and 205 cells (Fig. 2). No evi-

Abbreviations: G-banding, trypsin/Giemsa staining; kbp, kilobase pairs.

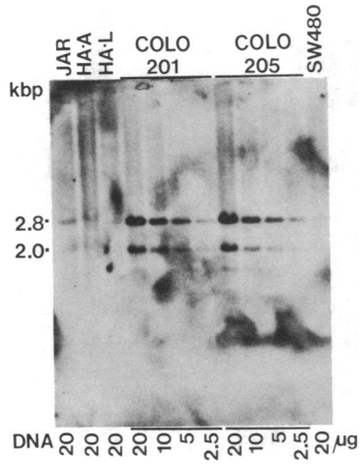


FIG. 1. Analysis of c-*myb* DNA in tumor cells. DNA from COLO 201 and 205 cells and other tumor cells was digested with *Eco*RI, and the indicated amounts of DNA were run in a 0.8% agarose gel, blotted to nitrocellulose, and probed with $^{32}$P-labeled v-*myb* *Kpn* I fragment (4). It can be seen from serial dilution of the samples that the c-*myb*-specific signals at 2.8 kbp and 2.0 kbp are very intense in COLO 201/205 samples, with about 2.5 μg of DNA giving the same signal intensity as given by 20 μg of DNA from other tumors. Thus, c-*myb* is approximately 8-fold amplified in COLO 201 and slightly more in COLO 205. Other tumor cells were as follows: JAR, a choriocarcinoma; HA-A and HA-L, melanomas; SW480, colon carcinoma.

dence of rearrangement emerged. We conclude that c-*myb* (and presumably a larger domain of surrounding DNA) is amplified but otherwise undisturbed in the DNA of COLO 201 and COLO 205.

**Expression of c-*myb* in COLO 201 and COLO 205.** Amplification of DNA is generally accompanied by enhanced

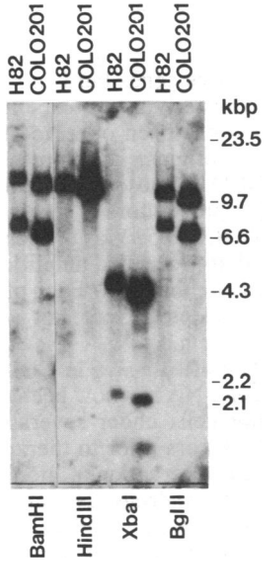


FIG. 2. Mapping of the c-*myb* locus by restriction endonuclease cleavage. Fifty micrograms of DNA from H82 lung carcinoma cells and 10 micrograms of DNA from COLO 201 cells were cleaved with the restriction endonucleases indicated, run in an 0.8% agarose gel, blotted to nitrocellulose, and probed with the nick-translated pF8 c-*myb* plasmid. The sizes of *Hin*dIII-cleaved DNA markers are given in kbp. Small differences in the mobilities of the fragments result from loading different amounts of DNA to the gel. Normal-sized fragments (see refs. 17 and 21) also were generated by cleavage with other restriction enzymes of COLO 205 DNA.

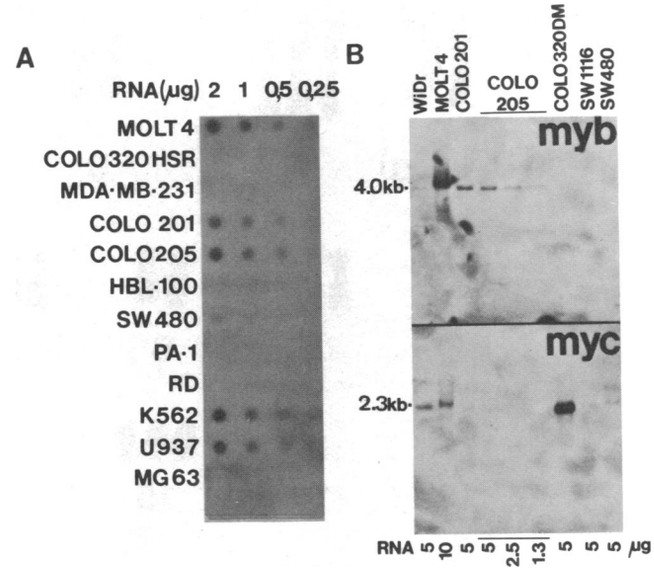


FIG. 3. (*A*) Analysis of c-*myb* RNA in tumor cells. Two micrograms of polyadenylylated RNA from the tumor cells were denatured for 5 min at 50°C in 14.8% formaldehyde containing 12× NaCl/Cit, diluted serially at a 1:2 (vol/vol) ratio, applied to nitrocellulose, and probed with a 2.0-kbp *Eco*RI fragment of human c-*myb*. The cells analyzed were as follows: MOLT4, T-cell leukemia; COLO 320 HSR; SW480, colon carcinoma; MDA-MB, breast carcinoma; RD, rhabdomyosarcoma; K562, erythroleukemia; U937, monocytic leukemia; MG-63, osteosarcoma. (*B*) Blot hybridization analysis of c-*myb* RNA in tumor cells. Indicated amounts of polyadenylylated RNA were run in a denaturing 0.8% agarose gel. The nitrocellulose filter replica was probed with radioactive v-*myb* and autoradiographed (*Upper*). The hybrids were then melted and washed by boiling, and the filter was reprobed with v-*myc* (*Lower*) (*Pst* I fragment; see ref. 22). The cell lines analyzed were: MOLT4, T-cell leukemia; and colon carcinomas WiDr, COLO 320 DM, SW1116, and SW480. kb, Kilobases.

expression of genes within the amplified domain (8–12). We analyzed the expression of c-*myb* in the COLO and other cell lines by hybridization with RNAs immobilized on nitrocellulose (Fig. 3*A*). Expression of c-*myb* in roughly equivalent amounts was readily detectable in cells from various leukemias, as expected from previous reports (5, 6), and in both COLO 201 and COLO 205. By contrast, cells from other tumors (most notably, the SW480 line from an adenocarcinoma of the human colon) contained little or no c-*myb* RNA.

The size of c-*myb* RNA from various sources was examined by electrophoresis in agarose gels and molecular hybridization (Fig. 3*B*). As before, c-*myb* RNA was found only in COLO 201, COLO 205, and leukemia cells (exemplified by the T-cell leukemia MOLT4 in Fig. 3*B*), and its size (approximately 4 kilobases) was as expected for normal c-*myb* (17, 21). We conclude that amplification of c-myb in COLO 201 and COLO 205 has been accompanied by production of abundant c-*myb* mRNA. The apparently normal size of the RNA is in accord with our previous conclusion that the topography of c-*myb* has not been disturbed by amplification.

**The Cytogenetics of Amplified c-*myb* in COLO 201 and COLO 205.** Previous studies have found amplified cellular oncogenes in either double minute chromosomes or homogeneously staining regions of marker chromosomes (10, 11). Neither of these karyotypic abnormalities were apparent, however, when the chromosomes of COLO 201 and COLO 205 were analyzed by G-banding (Fig. 4). Instead, the cells contained several abnormal "marker" chromosomes, one of which (here denoted mar 1; M2 in ref. 7) was either disomic or trisomic in all of the metaphase spreads examined. The same marker chromosomes were apparent in early karyo-

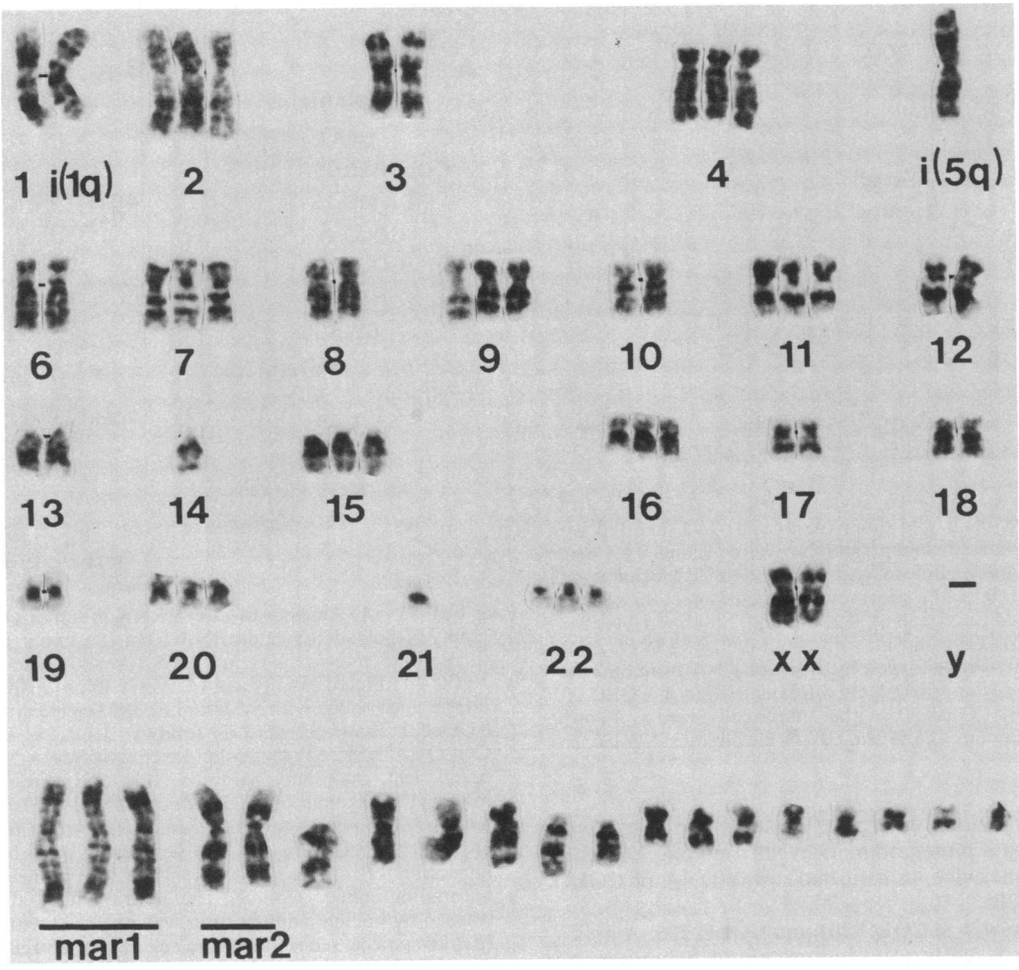


FIG. 4. A typical G-banded karyotype of COLO 205. Marker chromosomes are grouped in the lower part of the figure. Three copies of the longest marker, mar 1 (M2 in ref. 7) are present. COLO 201 metaphases also contained two to three copies of the mar 1 chromosome (not shown).

types of cell lines, even though our present analysis suggests that the modal number of chromosomes has been reduced in the interim, from bimodal 75/72 to 69 in COLO 201 and from 75 to 73 in COLO 205 (7).

The chromosomal location of amplified c-*myb* in COLO 201 and COLO 205 was sought with hybridization *in situ* (Fig. 5). Silver grains in the autoradiograms clustered over the proximal portion of the long arm of the abnormal chromosome denoted mar 1: 56% of all grains (total 57) in 10 metaphase spreads of COLO 201 and 50.6% of 348 grains in 20 metaphase spreads of COLO 205 (data not shown). In less than 5% of the cells, grains occasionally were observed also over a characteristic chromosome of group D, but these were a negligible portion of the total and have been ignored in our interpretation of the data. The normal chromosomal position of human c-*myb* is 6q22-24 (23). Because we have yet to identify the karyotypic origin of the mar 1 chromosome in COLO 201 and COLO 205, we cannot determine whether amplification of c-*myb* has been accompanied by translocation of the gene. But it is clear that the amplified DNA must be divided into as many as three units because the mar 1 chromosome in COLO 201 and COLO 205 is di- or even trisomic, and each copy of the chromosome bears amplified c-*myb* detectable by hybridization *in situ* (Fig. 5 and data not shown).

## DISCUSSION

**Is Expression of c-*myb* in COLO 201 and COLO 205 an Ectopic Function?** Amplification of DNA enhances the expression of previously active genes but is not known to activate the expression of otherwise quiescent genes. Because expression of c-*myb* has been observed previously only in cells of hemopoietic origins, its expression in the cells of COLO 201 and COLO 205 poses an enigma. Might c-*myb* be active in the normal progenitors of the tumor that gave rise to COLO 201 and COLO 205? Was the gene abnormally activated by an event that preceeded amplification? Or did amplification itself activate the gene? Each of these possibilities represents an unprecedented event, but we cannot presently choose from among them.

**The Origins of Amplified c-*myb* in COLO 201 and COLO 205.** Amplification of DNA has now been observed in a variety of human tumor cells under several different circumstances: in primary tumors prior to therapy (refs. 20 and 24 and our unpublished observations), after selection of cells for resistance to cytotoxic drugs (24), and in tumor cells that have been propagated for extended periods in culture (24). Any of these circumstances could apply to COLO 201 and COLO 205. First of all, it is possible that amplification of c-*myb* was present at the time the tumor was resected and explanted into culture. We used cells derived from the passage 8 and 32 without variation in results. Moreover, present karyotypic evidence indicates that COLO 201 and COLO 205 are independently established lines, yet both display roughly the same amplification of DNA and abundance of c-*myb* RNA—as if these abnormalities preceeded establishment of the cell lines. Second, the patient from whom the tumor cells were obtained had been treated with 5-fluoroura-

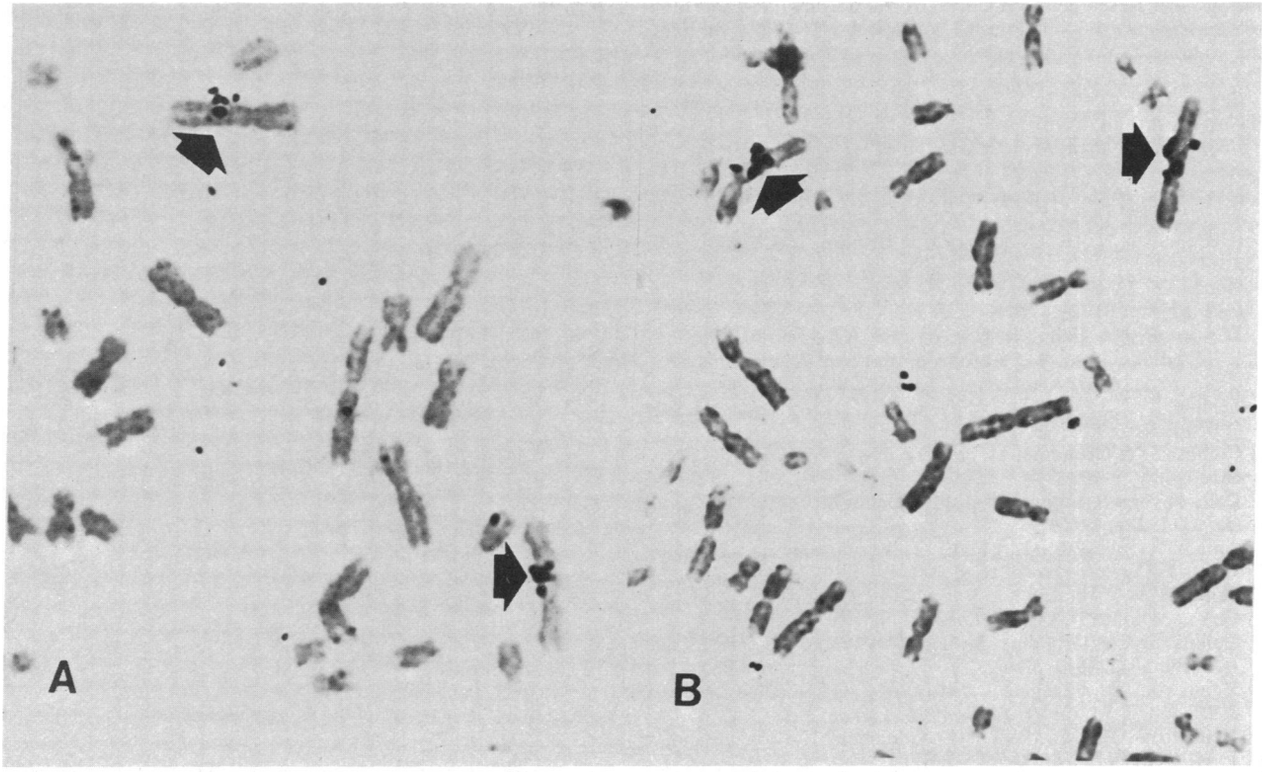


FIG. 5. Localization of c-*myb* in COLO 201/205 metaphases by *in situ* hybridization. (*A* and *B*) Metaphases of COLO 201 and 205 hybridized *in situ* with the 2.0-kbp c-*myb* *Eco*RI fragment (see Fig. 3), respectively, followed by G-banding in *B*. Marker 1 is distinguished by its size and centromere index. Autoradiographic grains are situated in the proximal part of the long arm of mar 1 chromosome (arrows).

cil prior to harvesting the ascites fluid that was used to establish the COLO 201 and COLO 205 lines (7). Although we have no evidence that the amplification of DNA now apparent in the cells mirrors the emergence of resistance to 5-fluorouracil, the cytotoxic agent may have itself elicited adventitious DNA replication that led to amplification of c-*myb* (25, 26). Even if induced in this manner, however, the amplification would probably not have survived subsequent propagation of the cells unless it conferred a selective advantage on the tumor cells. Third, we consider it unlikely that the amplification has arisen during the course of (or as a consequence of) propagation of the established cell lines because of our suspicion that the tumor cells probably contained amplified c-*myb* at the time of the explantation (see above).

**Might Amplification of c-*myb* Contribute to Tumorigenesis?** Amplified DNA generally survives in cells only if the accompanying expression of amplified genes confers a selective advantage on the cells (24, 27). Therefore, we assume that the amplified DNA in COLO 201 and COLO 205 is now essential to cell growth or survival. We cannot be certain, however, that the advantage arises from the enhanced expression of c-*myb*: the domain of amplified DNA is presumably large enough to harbor a number of genes (24, 28), any of which might be responsible for the maintenance of amplification.

**What Is the Likelihood that c-*myb* Itself Might Contribute to the Growth of the Tumor Cells of COLO 201 and COLO 205?** The c-*myb* gene and its progeny viral oncogene (v-*myb*) have been implicated previously only in hemopoietic neoplasms. The viral oncogene induces myelomonocytic leukemias (1, 2), and anomalous expression of c-*myb* consequent to genetic rearrangement has been described in lymphosarcomas originally induced by Abelson leukemia virus (29). On the other hand, no consistent pattern has emerged as yet to describe the relationship between amplification of cellular oncogenes and the types of tumors in which it is found. For example, amplification and enhanced expression of c-*myc* have been described in leukemias (8, 9), in a neuroendocrine tumor of colon (10), and in cells from small-cell carcinomas of the lung (30); none of these correspond with any fidelity to the tumorigenicity of v-*myc*—the viral derivative of c-*myc* (1, 2). Amplification of c-*myb* is not a common feature in colon carcinoma cell lines—we have seen only one additional example (unpublished data) among 20 cell lines examined.

Multiple events contribute to the genesis of human tumors; therefore, it is likely that amplification of c-*myb* in COLO 201 and COLO 205 is but one of several genetic abnormalities that now sustain the growth and malignant phenotype of the tumor cells. The possibilities are best illustrated by the fact that v-*myb* can serve as one of two essential functions (the other being a mutant version of c-Ha-*ras*) required to achieve tumorigenic transformation of rat embryo fibroblasts in culture (refs. 31–33; L. Parada, personal communication). The possible role of other oncogenes in the phenotype of COLO 201 and COLO 205 must be sought by means other than those used in the present study.

The amplified copies of c-*myb* in COLO 201 and COLO 205 apparently are divided into as many as three units located on redundant copies of the same abnormal chromosome mar 1. The amplified DNA is not apparent as either of the typical karyotypic abnormalities—double minute chromosomes and homogeneously staining chromosomal regions. These findings dramatize the fact that amplification of DNA need not be manifest in cytogenetic analyses and raise the possibility that amplification of cellular oncogenes may be a more common factor in tumorigenesis than might have been suspected from available karyological data.

We thank Kalle Saksela for discussions and help, Kirsi Pylkkänen for expert technical help, and Anne Brisk and Janine Marinos for typing. These studies were supported by The Finnish Academy, The

Finnish Cancer Research Foundation, Nordisk Insulinfond and Research and Science Foundation of Lääkefarmos Oy (Finland), and by the National Cancer Institute.

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