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Amplification and Expression of Protooncogenes in Human Small Cell Lung Cancer Cell Lines

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ABSTRACT

Amplification and expression of 16 protooncogenes were examined in 12 established small cell lung cancer (SCLC) cell lines. Seven of 12 cell lines showed a 20- to 35-fold amplification of the c-*myc* oncogene, 3 cell lines showed an 80- to 130-fold amplification of N-*myc* oncogene, and one cell line had a simultaneous amplification of the c-*myb* and N-*myc* oncogene. In this cell line both oncogenes were transcriptionally highly active at the same time. A variant subpopulation of SCLC expressed an 8.5-kilobase v-*fms* homologous transcript at high levels but without amplification of the c-*fms* gene. All cell lines examined had similar RNA levels of the N-*ras*, Ki-*ras*, Ha-*ras*, and c-*raf*1 oncogenes. DNA amplification, however, was undetectable. The protooncogenes c-*fes*, c-*fos*, and c-*erb*B were expressed very weakly and the transcripts of the oncogenes c-*mos*, c-*sis*, c-*erb*A, c-*src*, and c-*abl* were not observed in any of the 12 SCLC-cell lines. From these data we conclude that beyond the oncogenes *myc* and *myb*, oncogenes whose gene products are GTP binding proteins and phosphokinases may also be necessary to develop and keep the malignant state of SCLC. The v-*fms* homologous transcript found may be involved in the transition of the classic cell type to the variant cell type of SCLC.

INTRODUCTION

Lung cancer has been classified clinically into SCLC[1] and non-SCLC, based on differences among tumor spread at the time of diagnosis, response to applied treatment modalities, and overall survival. SCLC accounts for about 20–25% of all new cases of human lung cancer, and 2-year survival rates are still within a 10–20% range, despite intensive clinical research. Based on the expression of DDC, a key enzyme of the diffuse neuroendocrine system (1), established SCLC cell lines can be subclassified *in vitro* into a classic cell type with measurable activity of DDC and a variant cell type without enzyme activity. Beyond this feature other parameters differ between both subclasses of SCLC cell lines: the classic cell type expresses bombesin-like immunoreactivity, has higher levels of neuron-specific enolase, a longer population doubling time in suspension culture, a lower cloning efficiency in soft agarose, and a longer latent phase of nude mouse heterotransplants than the variant cell type (2, 3).

As a rule, variant cell lines have a higher degree of DNA amplification and a higher level of RNA expression of the c-*myc* oncogene than do classic cell lines (4). This suggests a possible function of the c-*myc* oncogene and probably other cellular oncogenes in the transition of the classic to the variant cell type and the malignant behavior of variant cell lines (4–6).

Cellular oncogenes, originally identified by their structural similarity to oncogene sequences in the genome of acutely transforming retroviruses and/or by their capability to transform NIH-3T3 cells in transfection assays, may play an essential role in controlling normal cell growth and development (7–12). In activated forms, which one can find in many human and animal tumor cells, cellular oncogenes may be involved in neoplasmic transformation of cells and tumor establishment. Point mutations, deletions, translocations, and gene amplification could be mechanisms that give quiescent protooncogenes a transforming potency (13–22).

Beyond c-*myc* amplification, L-*myc* and N-*myc* amplifications were found in SCLC cell lines with increased RNA expression almost proportional to the corresponding gene. Amplifications of L-*myc* and N-*myc* oncogenes have no constant association with both cell types of SCLC. In all SCLC cell lines examined, if at all, only one gene out of three *myc*-related oncogenes was amplified (23, 24).

With regard to these findings and the results of Slamon *et al.* (25), who found a number of transcriptionally active protooncogenes in 20 fresh human cancer specimens of different histological types including lung cancer, we performed similar analyses of 16 oncogenes in 12 permanent SCLC cell lines.

MATERIALS AND METHODS

Cell Lines. The SCLC cell lines used were established in our laboratory (SCLC-16H, SCLC-21H, SCLC-22H, SCLC-24H, SCLC-86M) or were donated by Drs. Carney and Gazdar, NCI, Bethesda, MD (NCI-H69, NCI-H82, NCI-H146, NCI-N417, NCI-H526, NCI-N592) and Drs. Pettengill and Sorenson, Dartmouth Medical School, Hanover, NH (DMS-79). All 12 cell lines grew as floating cell aggregates in liquid culture, formed colonies in soft agarose, were tumorigenic in athymic nude mice, were free of *Mycoplasma* contamination, and expressed human isoenzymes distinct from HeLa cells. Their morphological, biochemical, and chromosomal features have been described in detail (2, 3, 26–28). Cell lines SCLC-22H, SCLC-24H, SCLC-86M, NCI-H69, NCI-H146, and NCI-N592 expressed DDC activity and thus belonged to the classic cell type of SCLC; all other cell lines (SCLC-16H, SCLC-21H, NCI-H82, NCI-N417, NCI-H526, DMS-79) had undetectable DDC activity and were subclassified as variant SCLC cell lines (2, 3). The RNA expression and DNA amplification of c-*myc* and N-*myc* were previously analyzed by Little *et al.* (4) and Nau *et al.* (23) for the NCI cell lines H69, H82, H146, N417, H526, and N592. Griffin and Baylin (5) could demonstrate c-*myb* transcripts in the cell lines H69 and H82 but not in N417. These cell lines were included as positive controls for these protooncogenes. All cell lines were kept continuously growing in RPMI 1640 medium supplemented with 10% v/v fetal bovine serum, designated R10 medium (both purchased from GIBCO, Paisley, United Kingdom) in a well humidified atmosphere of 5% CO_2/95% air at 37°C.

Purification of DNA. High-molecular weight cellular DNA was prepared from 1–2 × 10^8 cells obtained from logarithmically growing culture. The cells were washed with phosphate-buffered saline and resuspended in 10 ml lysis buffer (0.5% SDS, 0.1 M NaCl, 0.02 M Tris-HCl, pH 7.4), 0.001 M EDTA, and proteinase K, 100 μg/ml). This solution was incubated overnight at 37°C and extracted once with phenol and twice with chloroform:isoamylalcohol (24:1). DNA was precipitated with 0.15 M sodium acetate (pH 5.2) and 2 volumes of cold 100% ethanol pooled off and dried under vacuum. DNA was dissolved and stored in water at 4°C.

Preparation of RNA. Logarithmically growing cells (10^8) were lysed in 5 ml cold lysis buffer, briefly vortexed, and cytoplasmic RNA was

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[1] The abbreviations used are: SCLC, small cell lung cancer; DDC, dihydroxyphenylalanine-decarboxylase; kb, kilobase(s); SSC, saline-sodium citrate (1× SSC is 0.15 M sodium chloride:0.015 M sodium citrate); SDS, sodium dodecyl sulfate; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; NCI, National Cancer Institute.

separated from nuclei by a sucrose cushion. Cellular proteins were hydrolyzed by proteinase K digestion (200 μg/ml) for 30 min at 37°C. The solution was extracted once with phenol:chloroform: isoamylalcohol (25:24:1), and the RNA was precipitated with 2.5 volumes ethanol and tested for integrity in a 1% glyoxal-agarose gel. For preparation of polyadenylated RNA, cytoplasmic RNA was purified by chromatography on oligodeoxythymidylate cellulose as described by Aviv and Leder (29).

Southern Hybridization. Restriction endonuclease-digested DNA was electrophoresed in 1% agarose gel, transferred to nitrocellulose by the method of Smith and Summers (30), prehybridized [30–50% formamide (dependent on homology between probe and DNA)-5× SSC-5× Denhardt's solution-50 mM sodium phosphate buffer, pH 6.5-250 μg/ml denatured salmon DNA] and hybridized (30–50% formamide-5× SSC-1× Denhardt's solution-20 mM sodium phosphate buffer, pH 6.5-10% dextran sulfate-100 μg/ml denatured salmon DNA) to nick-translated probes (Table 1). After hybridization the filters were washed initially 4 times for 5 min in 2× SSC-0.1% SDS, at room temperature, then 3 times for 20 min in 0.1× SSC-0.1% SDS at 50°C and exposed to Kodak XAR-5 film for various periods.

Northern Hybridization. For Northern blots, cytoplasmic or polyadenylated RNA was electrophoresed in glyoxal gel, transferred with 20× SSC to nitrocellulose, and hybridized in the presence of 10% dextran sulfate and 50% formamide. Filters were washed essentially as described for Southern hybridization. Twenty μg of cytoplasmic or 5 μg polyadenylated RNA were loaded in each lane (31).

RNA Dot Blot Analysis. Cytoplasmic RNA was denatured in 6× SSC-0.22 M formaldehyde for 15 min at 50°C, diluted serially, and spotted on nitrocellulose equilibrated with 20× SSC. The blots were prehybridized and hybridized as described for Southern hybridization (32).

RESULTS

Protooncogene Expression in SCLC Cell Lines. Cytoplasmic RNA was isolated from 12 established SCLC cell lines, the same RNA batches were analyzed by the dot and Northern blot techniques with ^{32}P-labeled oncogene probes listed in Table 1 using a β-actin probe as internal control probe, and the dot blot autoradiographs were quantified by densitometer tracing (DU 6 Beckmann photometer). To simplify the evaluation of data we estimated the intensity of hybridization corresponding to a scale of hybridization signal intensities (Fig. 1*A*). The scale ranges from 0, *i.e.*, no expression detected, to ++++ for high level expression. A summary on the expression of 9 oncogenes is given in Table 2. The weak hybridization signals of v-*fes*, v-*fos*, and v-*erb*B were negative corresponding to the scale of intensity given in Fig. 1*A*. We could not detect transcripts homologous to *abl*, *mos*, *erb*A, *sis*, and *src* probes. For the protooncogenes N-*ras*, Ki-*ras*, Ha-*ras*, and c-*raf*1 we found similar levels of this transcripts in all SCLC cell lines examined. RNA expression related to the cellular oncogenes c-*fms*, c-*myc*, c-*myb*, and N-*myc*, however, differed considerably among the SCLC cell lines.

Table 1 *Oncogene probes*

Plasmid	Fragment used as probe	kb pair	Ref.
c-*myc*	*Cla*I-*Eco*RI	1.3	4
p-*myc*	*Pst*I-*Pst*I	1.5	43
p-v-*fos*	*Pst*I-*Pst*I	1.1	44
p-*erb*/t	v-*erb*A: *Pvu*II-*Sst*I	0.8	
	v-*erb*B: *Hin*dII-*Pvu*II	0.9	45
p-N-*ras*	*Eco*RI-*Eco*RI	1.1	46
p-Ki-*ras*	*Eco*RI-*Eco*RI	1.0	47
p-HaSV-BS9	*Eco*RI-*Eco*RI	0.4	48
abl-pK2	*Eco*RI-*Eco*RI	1.6	49
c-*mos* (pHM2A)	*Eco*RI-*Eco*RI	2.7	50
N-*myc* (pNB1)	*Eco*RI-*Bam*HI	1.0	51
v-*fes*	*Pst*I-*Pst*I	0.5	52
v-*fms*	*Pst*I-*Pst*I	1.5	53
v-*src*	*Pvu*II-*Pvu*II	0.8	54
v-*sis*	*Pst*I-*Pst*I	1.2	55
c-*raf*1 (pHE1)	*Eco*RI-*Eco*RI	1.8	
p-*myb*	*Bam*HI-*Bam*HI	1.0	56
β-actin	*Bgl*II-*Eco*RI	0.5	

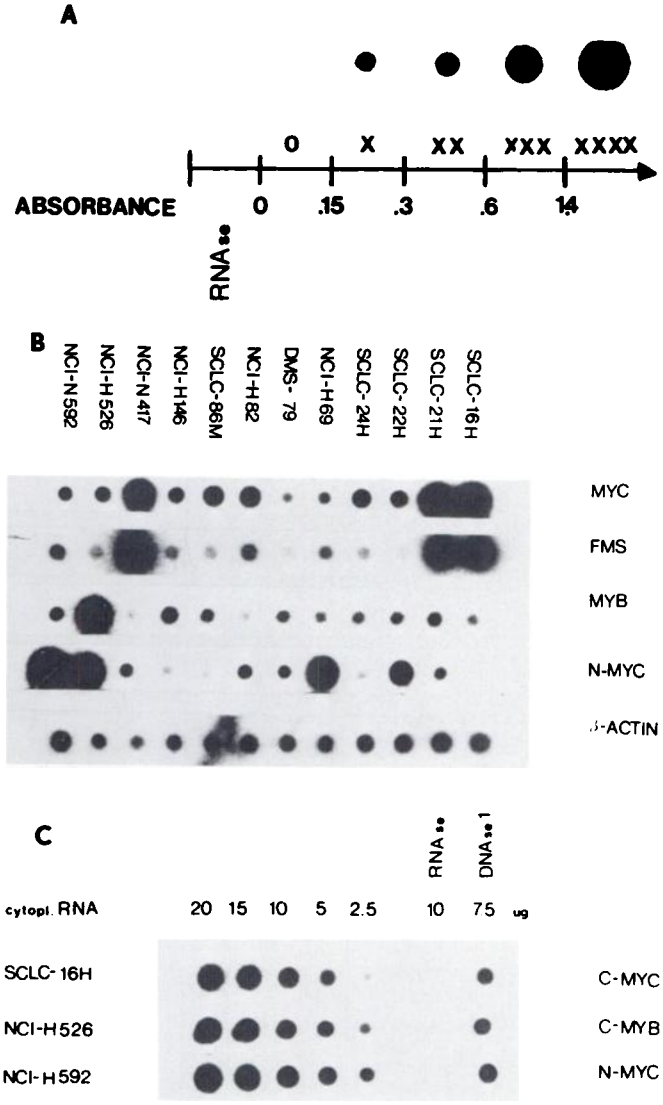

Fig. 1. *A*, scale of intensity of hybridization. The relative intensity was determined by making densitometer tracings (DU 6 spectrophotometer). *B*, dot blot hybridization of the *myc*, *myb*, N-*myc*, *fms*, and β-actin probe as internal control. The first dilution step (20 μg) is given. *C*, reduction of the spot intensity to undetectable levels in cytoplasmic (*cytopl.*) RNA preparations preincubated with RNase A and T1. Integrity of the spot intensity after preincubation with DNase 1 demonstrates that the signal is due to hybridization of the oncogene probes to RNA.

In order to exclude false-positive hybridization signals due to soiling of RNA preparations with genomic DNA, we blotted RNase T1 and RNase A digested probes from all cell lines tested as negative controls. A representative example is shown in Fig. 1*C*. We also digested RNA probes with DNase 1 to demonstrate that the intensity of the signal does not change thereby.

The sizes of transcripts homologous to protooncogenes c-*fms*, c-*myc*, N-*myc*, and c-*myb* were determined by the Northern blot technique (Fig. 2). We found one c-*myc* homologous transcript of 2.3 kb with the human c-*myc* *Cla*I/*Eco*RI probe and one c-*myb* related transcript of 3.5 kb (Fig. 2, *A* and *B*). The transcript sizes correspond to previously reported data from SCLC cell lines (4, 5). The N-*myc* probe hybridized to a 3.5-kb

Table 2 *Summary of results on the expression of protooncogenes in SCLC cell lines*

Cell line	Cell type	c-*myc*	*fms*	*myb*	N-*myc*	N-*ras*	Ki-*ras*	Ha-*ras*	*raf*1	*fes*
SCLC-16H	Variant	xxxx[a]	xxx	x	x	xxx	xxx	xx	xx	0
SCLC-21H	Variant	xxxx	xxx	xx	x	xxx	xxx	xx	xx	x
SCLC-22H	Classic	xxx	0	xx	xxx	xxx	xxx	xx	xx	0
SCLC-24H	Classic	xxx	0	x	x	xxx	xxx	xx	xx	x
NCI-H69	Classic	xx	x	x	xxxx	xx	xxx	x	xx	x
DMS-79	Variant	xx	0	x	xx	xxx	xxx	x	xx	0
NCI-H82	Variant	xxx	xx	0	xx	xxx	xxx	x	xx	x
SCLC-86M	Classic	xxx	x	x	0	xxx	xxx	xx	xx	0
NCI-H146	Classic	xx	x	xx	0	xxx	xxx	xx	xx	0
NCI-N417	Variant	xxx	xxxx	0	xx	xx	xxx	xx	xx	0
NCI-H526	Variant	x	x	xxxx	xxxx	xxx	xxx	xxx	xxx	x
NCI-N592	Classic	x	xx	xx	xxxx	xxx	xxx	xx	xx	x

x–xxxx, level of expression; 0, no expression detected.

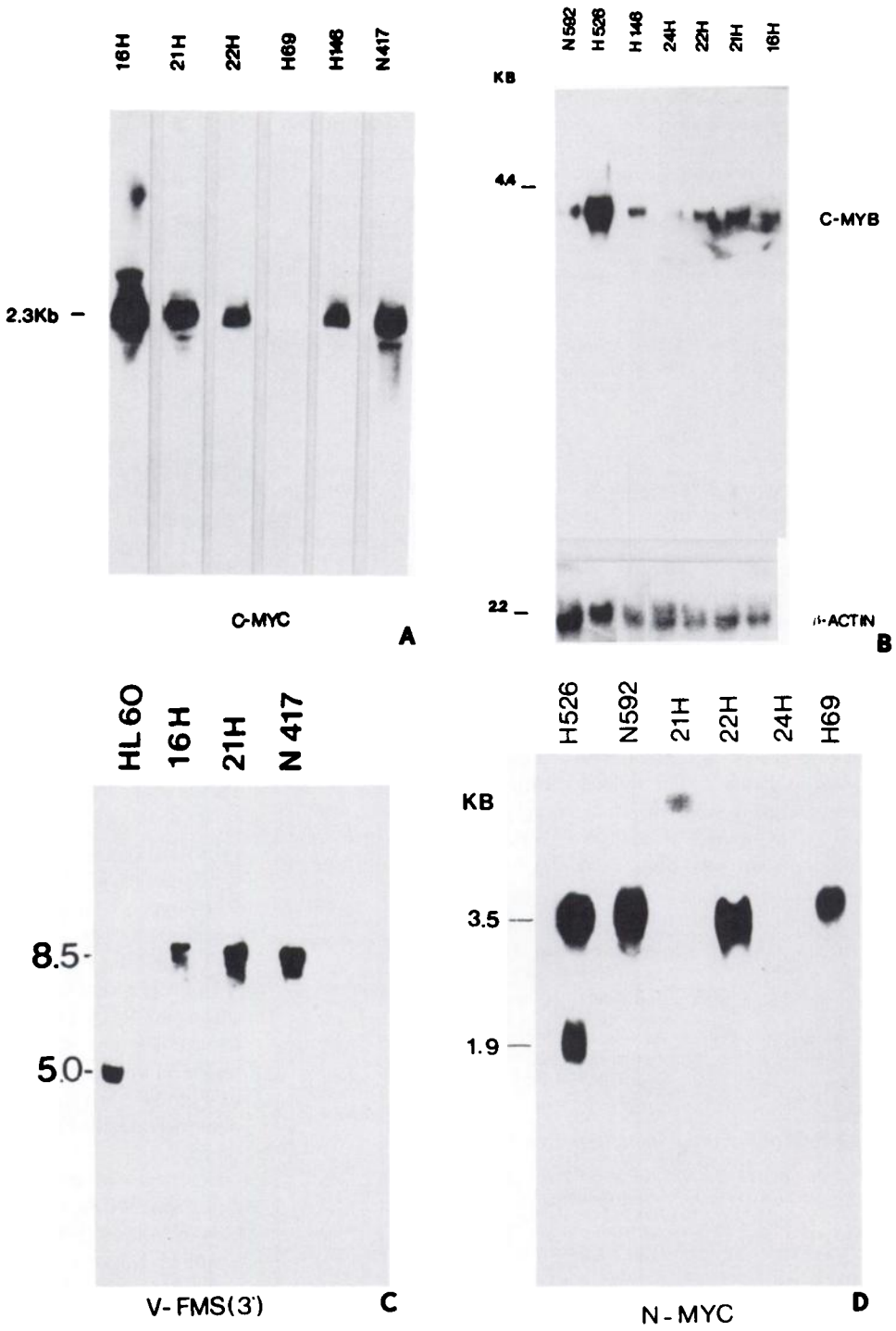

Fig. 2. *A*, *B*, *C*, Northern blot analysis showing the sizes of mRNA transcripts detected in SCLC related to c-*myc*, N-*myc*, and c-*myb*. The c-*myb* filter was boiled for 10 min in twice-distilled water to remove the c-*myb* probe and subsequently rehybridized to a β-actin probe. The analysis was performed on 20 μg cytoplasmic RNA. *D*, Northern blot analysis of an 8.5-kb v-*fms*-related transcript in cell lines with high level of *fms* expression. Five μg poly(A+)RNA were loaded in each *lane* for SCLC cell lines and 30 μg total cellular RNA were used for TPA-induced differentiation HL60 (3.3×10^{-8} M for 30 h).

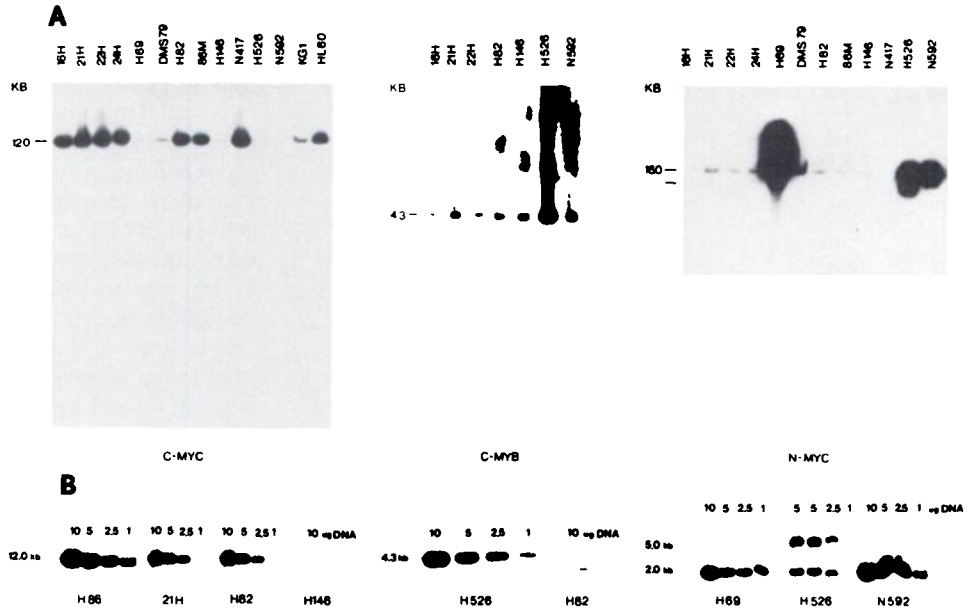

Fig. 3. *A*, Southern blot analysis of DNA from SCLC cell lines with ^{32}P-labeled v-*myc*, v-*myb*, and pNB1 oncogene probes (Table 1). A 12.0-kb *Eco*RI fragment hybridized in all SCLC cell lines with the v-*myc* probe, a 4.3-kb *Bam*HI fragment with the v-*myb* probe, and 16.0-kb *Hind*III fragment with the pNB1 probe. In addition to the 16.0-kb fragment, a 14.0-kb *Hind*III fragment of NCI-H526 also hybridized with the pNB1 probe. *B*, different amounts of DNA were digested with *Eco*RI for Southern blot analysis probed with v-*myc* and pNB1 probes and digested with *Bam*H1 for Southern blot analysis with the v-*myb* probe. The appropriate ^{32}P-labeled bands were cut out and liquid scintillation was counted.

RNA from cell lines which highly expressed this oncogene (SCLC-22H, NCI-H526, NCI-H69, NCI-N592) and to an additional transcript of 1.9 kb in the variant cell line NCI-H526 as recently described by Nau *et al.* (23) (Fig. 2*C*).

A v-*fms* related transcript of 8.5 kb was found in polyadenylic acid RNA of the variant cell lines NCI-N417, SCLC-16H, and SCLC-21H. The TPA-induced 5.0-kb v-*fms* homologous transcript of the promyelocytic cell line HL 60 was detected by the same v-*fms* probe (33) (Fig. 2*D*).

We also hybridized the RNA from TPA-induced HL60 cells and the RNA from the SCLC cell line NCI-N417 to an *Eco*RI/*Eco*RI fragment of p-*cfms* 104 (5′ end probe) obtained from A. Ulrich which represents the extracellular domain of the c-*fms* product (34). With this probe we could demonstrate the 5.0-kb c-*fms* RNA from HL60 cells but not the 8.5-kb transcript from SCLC cells (data not shown).

Amplification of Protooncogenes in SCLC Cell Lines. We analyzed the copy number of cellular oncogenes which were transcribed at elevated levels in SCLC cell lines by Southern blot technique. Genomic DNA was extracted, digested with restriction endonucleases, electrophoresed in 1% agarose gel, blotted on nitrocellulose filters, and hybridized with the radiolabeled oncogene probes listed in Table 1. DNA from cell lines SCLC-16H, SCLC-21H, SCLC-22H, SCLC-24H, SCLC-86M, NCI-H82, and NCI-N417 showed a more intense c-*myc* signal in the autoradiographs than the remaining cell lines (Fig. 3). With the c-*myb* probe, the variant cell line NCI-H526 had a multiple stronger hybridization signal than in the other cell lines. As described above, this cell line expressed the c-*myb* transcript at high levels. Cell lines NCI-H69, NCI-H526, and NCI-N592 showed an intense characteristic 16.0-kb *Hind*III digested N-*myc* fragment. Other oncogene probes revealed a uniform labeling of all cell lines. These results suggest amplification of the c-*myc* protooncogene in the cell lines SCLC-16H, SCLC-21H, SCLC-22H, SCLC-24H, SCLC-86M, NCI-H82, and NCI-N417, an amplification of the N-*myc* gene in cell lines NCI-H69, NCI-H526, NCI-N592, and a c-*myb* amplification in cell line NCI-H526. After *Eco*RI and *Hind*III digestion, we found an accessory amplified N-*myc* hybridization band in NCI-H526, as described by Nau *et al.* (23). NCI-H69 also had an unamplified 4.0-kb *Eco*RI fragment (Fig. 4). With other oncogene probes we detected only the characteristic restriction endonuclease fragments.

In order to estimate the degree of amplification, DNA was cleaved by restriction endonucleases, diluted serially, electrophoresed, blotted, and hybridized as described. Hybridization signals were compared with those from cell lines with a known degree of amplification (Fig. 3*B*). A 25- and 47-fold DNA amplification for c-*myc* of the cell lines NCI-H82 and NCI-N417 and a N-*myc* amplification of the cell lines NCI-H69, NCI-H526, and NCI-N592 has been recently reported. The degrees of N-*myc* amplification were specified at 85-fold in NCI-H69 and at 115- (5.0-kb band) and 135- (2.0 kb) fold, respectively, in NCI-H526 (4, 23).

The ^{32}P-labeled hybridization bands were cut out and measured in a scintillation counter. The cell lines SCLC-16H and SCLC-86M had a 30- to 35-fold and the cell lines SCLC-21H, SCLC-22H, and SCLC-24H a 20- to 25-fold amplification of the c-*myc* oncogene. Intensity of signals was compared with NCI-H82 and NCI-H146 which is not amplified for c-*myc* (4). The c-*myb* oncogene of NCI-H526 was amplified 20- to 25-fold as compared to the unamplified cell line NCI-H82. In order to estimate the degree of N-*myc* amplification in NCI-N592, we used NCI-H69, which is supposed to have 85-fold amplification (23). Our analyses showed 130- to 140-fold amplification of N-*myc* oncogene in NCI-N592.

DISCUSSION

We could demonstrate a number of transcriptionally active oncogenes in SCLC cell lines for which 4 patterns of expression were observed: (*a*) five protooncogenes, c-*myc*, c-*raf*1, N-*ras*, Ha-*ras*, and Ki-*ras* were expressed in all cell lines examined. C-*myc* expression differed considerably in level from cell line to cell line. The variant cell lines, excluding NCI-H526 and DMS-79, expressed the c-*myc* oncogene at higher levels than did the classic cell lines. Some classic SCLC cell lines (all established in Marburg; SCLC-22H, SCLC-24H, and SCLC-86M) also had a high degree of c-*myc* amplification. A uniform expression pattern of Ki-*ras*, Ha-*ras*, N-*ras*, and c-*raf*1 was found in all cell lines; (*b*) the oncogenes c-*myb* and N-*myc* were expressed at different levels in some cell lines without correlation between the pattern of expression and the phenotype of SCLC; (*c*) 3 of

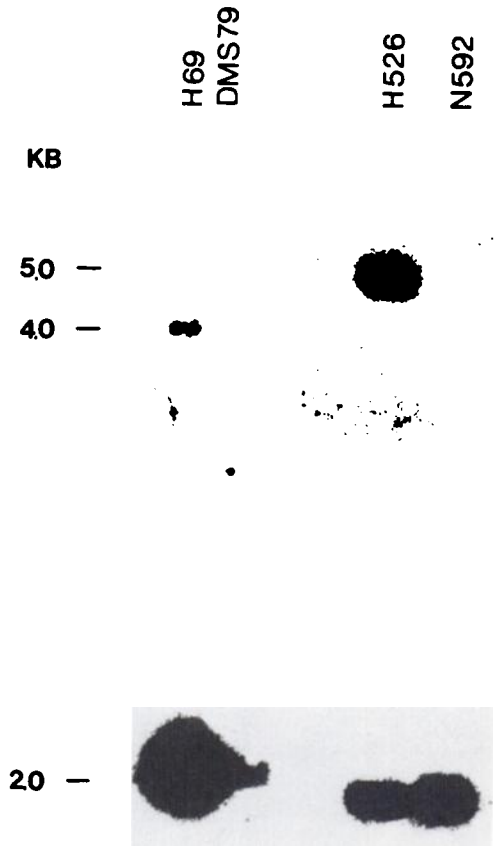

Fig. 4. Southern blot analysis of DNA from NCI-H69, DMS-79, NCI-H526, and NCI-N592 hybridized with ^{32}P-labeled pNB1 DNA. In addition to the amplified 2.0-kb *Eco*R1 fragment an unamplified 4.0-kb fragment of NCI-H69 and an amplified 5.0-kb fragment of NCI-H526 also hybridized with the N-*myc* specific probe pNB1.

6 variant SCLC cell lines expressed a strong 8.5-kb v-*fms* homologous transcript; (*d*) for the oncogenes c-*erb*A, c-*mos*, c-*sis*, c-*ab*1, and c-*src*, we did not observe transcripts in SCLC. The expressions of c-*fos*, c-*fes*, and c-*erb*B were weak because of insufficient contrast with the background and thus were valued as negative.

We also found a simultaneous amplification of 2 oncogenes, c-*myb* and N-*myc*, in a single SCLC cell line (NCI-H526), both of which were transcriptionally highly active. Nine of 12 cell lines revealed amplification of one of the 2 *myc*-related oncogenes, N-*myc* and c-*myc*. L-*myc* was not amplified in these cell lines.[2]

Our data on N-*myc* and c-*myc* agree with those of Nau *et al.* (23) who described amplification and elevated expression of either N-*myc* or c-*myc* in most SCLC cell lines. We could not find an exception to the rule expressed by these authors that only one of the 3 *myc*-related oncogenes (N-*myc*, c-*myc*, L-*myc*) is amplified in SCLC cell lines (24).

Amplification and elevated expression of the c-*myc* oncogene may correlate with aggressive growth qualities of the variant cell type of SCLC. Our results suggest that in particular the c-*myc* expression may be associated with the subclassification of SCLC into variant and classic cell types rather than DNA amplification. All cell lines established in Marburg, including classic lines, had a high degree of c-*myc* amplification. The expression, however, was approximately 4 times higher in variant cell lines than in classic cell lines. The high level of c-*myc* expression in variant cell lines cannot therefore be determined only by the degree of amplification. Other factors, possibly oncogene products and growth factors may control the c-*myc* expression in classic cell lines so that the amplification does not proportionally effect the c-*myc* expression (5, 10, 11, 35). N-*myc* amplification did not show any association with the SCLC subclassification.

One variant cell line (NCI-H526) was amplified simultaneously for 2 oncogenes, c-*myb* and N-*myc*. Griffin and Baylin (5) found v-*myb* homologous transcripts in 7 of 8 SCLC cell lines examined (5). We detected c-*myb* transcription in 10 of 12 cell lines. The quantity of c-*myb* transcription, however, was obviously lower than the c-*myc* transcription. The only exception was NCI-H526, which was amplified for the c-*myb* gene. The c-*myb* function and mechanisms of c-*myb* amplification in SCLC still remain unclear because of little knowledge of normal, cellular functions of c-*myb* and of the mechanisms involved in its amplification.

In addition to *myc*-related genes and the c-*myb* oncogene, a number of protooncogenes were transcriptionally active in SCLC cell lines. C-*raf*1, Ki-, N-, and Ha-*ras* oncogenes were expressed at high levels in all cell lines examined but not amplified. Recent findings underline transforming potency of *ras* oncogenes. C-Ha-*ras* from urinary carcinoma cell line T24 can transform rat embryo cells into cells which are capable of forming tumors with high metastatic potential in nude mice (36). The high expression levels of *ras* genes and the c-*raf*1 gene in SCLC suggest that these genes may play an important role in tumor establishment (35, 37).

Three of 6 variant cell lines expressed high levels of a v-*fms* homologous transcript. The putative gene product of c-*fms* is the CSF-1 receptor (38). The Northern blot analysis showed an 8.5-kb transcript in polyadenylic acid RNA from SCLC-16H, SCLC-21H, and SCLC-N417. This v-*fms*-related transcript of SCLC was thus considerably larger than the 5.0-kb v-*fms* homologous transcript of the TPA-induced promyelocytic cell line HL 60 or the 4.3-kb transcript found in human placenta (34). Walker *et al.* (40) found in 3 of 5 cell lines derived from tumors produced by neoplastic transformation of rat epithelial cells high levels of a 9.5-kb v-*fms*-related transcript. Also, the murine myeloid leukemia cell line WEHI-3B expressed 2 v-*fms*-related transcripts, a smaller one of 4.1 kb which may correspond to the 3.7-kb RNA found in human placental tissue (34) and a larger 8.4-kb transcript which probably corresponds to the v-*fms*-related RNA seen in variant SCLC cells and neoplastically transformed rat tracheal cells.

With regard to the high sequence homology between v-*fms* and c-*fms* in the cytoplasmic region and the missing homology of the SCLC RNA to the 5′ c-*fms* probe, we suggest that the gene expressed by SCLC cells is a member of an *fms*-related

[2] B. Johnson, personal communication.

gene family but is not identical to the CSF-1 receptor encoding gene.

The mechanism of the v-*fms*-related gene activation in a subpopulation of variant cell lines remains obscure. Neither the Southern blot analyses of the 3 cell lines showed an amplification nor could we detect a rearrangement with the restriction endonucleases used. Rearrangements that could possibly be picked up by other restriction endonucleases cannot be excluded. Of interest is that only those variant cell lines expressed this v-*fms* homologous transcript which at an earlier point had been classic cell lines. This holds true for SCLC-16H which altered its phenotype after prolonged *in vitro* cultivation (28), for SCLC-21H which supposedly altered its phenotype *in vivo* (40), and for NCI-N417 which altered its phenotype after nude mouse xenotransplantation (3, 40).

As the first step, the coherence between v-*fms*-related expression and the phenotypic transition of SCLC *in vivo* and *in vitro* needs to be proved through other studies. In the next step the gene product of the v-*fms* homologous transcript of SCLC cell lines should be characterized, and experiments with *in vitro* inductions of such a transition should be performed.

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REFERENCES

1. Pearse, A. G. E. The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept. J. Histochem. Cytochem., *17:* 303–313, 1969.
2. Carney, D. N., Gazdar, A. F., Bepler, G., Guccion, J. G., Marangos, P. J., Moody, T. W., Zweig, M. H., and Minna, J. D. Establishment and identification of small cell lung cancer cell lines having classic and variant features. Cancer Res., *45:* 2913–2923, 1985.
3. Gazdar, A. F., Carney, D. N., Nau, M. M., and Minna, J. D. Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinctive biochemical, morphological and growth properties. Cancer Res., *45:* 2924–2930, 1985.
4. Little, C. D., Nau, M. M., Carney, D. N., Gazdar, A. F., and Minna, J. D. Amplification and expression of the c-*myc* oncogene in human lung cancer cell lines. Nature (Lond.), *306:* 194–196, 1983.
5. Griffin, C. A., and Baylin, S. B. Expression of the c-*myb* oncogene in human small cell lung cancer. Recent Results Cancer Res., *99:* 237–245, 1985.
6. Mark, G. E., and Rapp, U. R. Primary stucture of v-*raf*: relatedness to the src family of oncogenes. Science (Wash. DC), *224:* 285–289, 1984.
7. Duesberg, P. H. Activated proto-oncogenes: sufficient or necessary for cancer? Science (Wash. DC), *228:* 669–677, 1985
8. Bishop, J. M. Cellular oncogenes and retroviruses. Annu. Rev. Biochem., *52:* 301–354, 1983.
9. Greenberg, M. E., and Ziff, E. B. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (Lond.), *311:* 433–438, "984.
10. Müller, R., Bravo, R., and Buckhardt, J. Induction of c-*fos* gene and protein by growth factors precedes activation of c-*myc*. Nature (Lond.), *312:* 716–720, 1984.
11. Kelly, K., Cochran, B. H., Stiles, C. D., and Leder, P. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet derived growth factor. Cell, *35:* 603–610, 1983.
12. Armelin, H. A., Armelin, M. C. S., Kelly, K., Stewart, T., Leder, P., Cochran, B. H., and Stiles, C. D. Functional role for c-*myc* in mitogenic response to platelet-derived growth factor. Nature (Lond.), *310:* 655–660, 1984.
13. Heisterkamp, N., Stephenson, J. R., Groffen, J., Hansen, P. F., de Klein, A., Bartram, C. R., and Grosveld, G. Localization of the c-*ab*1 oncogene adjacent to a translation break point in chronic myelocytic leukaemia. Nature (Lond.), *306:* 239–242, 1983.
14. Collins, S. J., and Groudine, M. T. Rearrangement and amplification of c-*ab*1 sequences in the human chronic myelogenous leukemia cell line K-562. Proc. Natl. Acad. Sci. USA, *80:* 4813–4817, 1983.
15. Alitalo, K., Schwab, M., Lin, C. C., Varmus, H. E., and Bishop, J. M. Homogeneously staining chromosomal regions contain amplied copies of an abundantly expressed cellular oncogene (c-*myc*) in malignant neuroendocrine cells from a human colon carcinoma. Proc. Natl. Acad. Sci. USA, *80:* 1707–1711, 1983.
16. Kozbor, P., and Croce, C. M. Amplification of the c-*myc* oncogene in one of five human breast carcinoma cell lines. Cancer Res., *44:* 438–441, 1984.
17. Alitalo, K., Winqvist, R., Lin, C. C., de la Chapelle, A., Schwab, M., and Bishop, J. M. Aberrant expression of an amplified c-*myb* oncogene in two cell lines from a colon carcinoma. Proc. Natl. Acad. Sci. USA, *81:* 4534–4538, 1984.
18. Pelicci, P. G., Lanfrancone, L., Brathwaite, M. D., Wolman, S. R., and Dalla-Favera, R. Amplification of the c-*myb* oncogene in a case of human acute myelogenous leukemia. Science (Wash. DC), *224:* 1117–1121, 1984.
19. Dalla-Favera, R., Wong-Staal, F., and Gallo, R. C. Oncogene amplification in promyelocytic leukemia cell line HL-60 and primary leukemic cells of the same patient. Nature (Lond.), *299:* 61–63, 1982.
20. Schwab, M., Alitalo, K., Klempmauer, K. H., Varmus, H. E., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumor. Nature (Lond.), *305:* 245–248, 1983.
21. Santos, E., Martin-Zanca, D., Reddy, E. P., Pierotti, M. A., Della Porta, G., and Barbacid, M. Malignant activation of a Ki-*ras* oncogene in lung carcinoma but not in normal tissue of the same patient. Science (Wash. DC), *223:* 661–664, 1984.
22. Orkin, S. H., Goldman, D. S., and Sallan, S. E. Development of homozygosity for chromosome 11p markers in Wilms' tumour. Nature (Lond.), *309:* 172–174, 1984.
23. Nau, M. M., Brooks, B. J., Carney, D. N., Gazdar, A. F., Battey, J. F., Sausville, E. A., and Minna, J. D. Human small cell lung cancers with amplification and expression of the N-*myc* gene. Proc. Natl. Acad. Sci. USA, *83:* 1092–1096, 1986.
24. Nau, M. M., Burke, J., Brooks, J., Battey, J., Sausville, E., Gazdar, A. F., Kirsch, J. R., McBride, D. W., Bertness, K., Hollis, G. F., and Minna, J. D. L-*myc* a new *myc* related gene amplied and expressed in human small cell lung cancer. Nature (Lond.), *318:* 69–73, 1985.
25. Slamon, D. J., Kernion, J. B., Verma, J. M., and Cline, M. J. Expression of cellular oncogenes in human malignancies. Science (Wash. DC), *224:* 256–262, 1984.
26. Pettengill, O. S., Sorenson, G. D., Wurster-Hill, D. H., Curphey, T. J., Noll, W. W., Cate, C. C., and Maurer, L. H. Isolation and growth characteristics of continuous cell lines from small-cell carcinoma of the lung. Cancer (Phila.), *45:* 906–918, 1980.
27. Bepler, G., Jaques, G., Neumann, K., Aumueller, G., Gropp, C., and Havemann, K. Establishment, growth properties, and morphological characteristics of permanent human small cell lung cancer cell lines. J. Cancer Res. Clin. Oncol., *113:*34–40, 1986.
28. Bepler, G., Jaques, G., Koehler, A., Gropp, C., and Havemann, K. Neuroendocrine markers, classical tumor markers, and chromosomal characteristics in human small cell lung cancer cell lines. J. Cancer Res. Clin. Oncol., *113:*253–259, 1987.
29. Aviv, H., and Leder, P. Purification of biologically active globin messenger RNA by chromatography on oligo thymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA, *69:* 1408–1412, 1972.
30. Smith, G. E., and Summers, M. D. The bidirectional transfer of DNA and RNA to nitrocellulose or diazobenzyloxymethyl-paper. Anal. Biochem., *109:* 123–129, 1980.
31. Thomas, P. S. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA, *77:* 5201–5205, 1980.
32. White, B. A., and Bancroft, F. C. Cytoplasmic dot hybridization. J. Biol. Chem., *257:* 8569–8572, 1982.
33. Sariban, E., Mitchell, T., and Kufe, O. Expression of the c-*fms* proto-oncogene during human monocytic differentiation. Nature (Lond.), *316:* 64–66, 1985.
34. Coussen, S. L., Beveren, C. V., Smith, D., Chen, E., Mitchell, R. L., Isacke, C. M., Verma, J. M., and Ulrich, A. Structural alteration of viral homologue of receptor proto-oncogene *fms* at carboxyl terminus. Nature (Lond.), *320:* 277–280, 1986.
35. Rapp, U. R., Bonner, T. I., Moelling, K., Jansen, H. W., Bister, K., and Ihle, J. Genes and gene products involved in growth regulation of tumor cells. Recent Results Cancer Res., *99:* 221–236.
36. Pozzatti, R., Mushel, R., Williams, J., Padmanabhan, R., Howard, B., Liotta, L., and Khoury, G. Primary rat embryo cells transformed by one or two oncogenes show different metastatic potentials. Science (Wash. DC), *232:* 223–227, 1986.
37. Land, H., Parado, L. F., and Weinberg, R. A. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (Lond.), *304:* 596–602, 1983.
38. Sherr, C. J., Rettenmier, C. W., Sacca, R., Roussel, M. F., Look, A. T., and Stanley, E. R. The c-*fms* proto-oncogene product is related to the receptor for the mononuclear phagocyte growth factor, CSF-1. Cell, *41:* 665–676, 1985.
39. Saksela, K., Bergh, J., Lehto, V. P., Nilsson, K., and Alitalo, K. Amplification of the c-*myc* oncogene in a subpopulation of human small cell lung cancer. Cancer Res., *45:* 1823–1827, 1985.
40. Walker, C., Nettesheim, P., Barrett, J. C., and Gilmer, T. M. Expression of

a *fms*-related oncogene in carcinogen-induced neoplastic epithelial cells. Proc. Natl. Acad. Sci. USA, *84:* 1804–1808, 1987.
41. Gonda, T. J., and Metcalf, D. Expression of *myb, myc* and *fos* proto-oncogene during the differentiation of a murine myeloid leukaemia. Nature (Lond.), *310:* 249–251, 1984.
42. Bepler, G., Jaques, G., Havemann, K., Koehler, A., Johnson, B. E., and Gazdar, A. F. Characterization of two cell lines with distinct phenotypes established from a patient with small cell lung cancer. Cancer Res., *47:* 1883–1891, 1987.
43. Vennström, B., Moscovici, C., Goodman, H. M., and Bishop, J. M. Molecular cloning of the avian myelocytomatosis virus genome and recovery of infectious virus by transfection of chicken cells. J. Virol., *39:* 625–631, 1981.
44. Curran, T., Peters, G., Van Beveren, C., Teich, N. M., and Verma, I. M. FBJ murine osteosarcoma virus: identification and molecular cloning of biologically active proviral DNA. J. Virol., *44:* 674–682, 1982.
45. Vennström, B., Fanshier, L., Moscovici, C., and Bishop, J. M. Molecular cloning of the avian erythroblastosis virus genome and recovery of oncogenic virus by transfection of chicken cells. J. Virol., *36:* 575–585, 1980.
46. Murray, M. J., Cunningham, J. M., Parada, L. F., Dautry, F., Lebowitz, P., and Weinberg, R. A. The HL-60 transforming sequence: a *ras* oncogene coexisting with altered *myc* genes in hematopoietic tumors. Cell, *33:* 749–757, 1983.
47. Ellis, R. W., DeFeo, D., Shin, T. Y., Gonda, M. A., Young, H. A., Tsuchida, N., Lowy, D. R., and Scolnick, E. M. The p21 *src* genes of Harvey and Kirsten sarcoma viruses originate from divergent members of a family of normal vertebrate genes. Nature (Lond.), *292:* 506–511, 1981.
48. Ellis, R. W., DeFeo, D., Maryak, I. M., Young, H. A., Shih, T. Y., Chang, E. H., Lowy, D. R., and Scolnick, E. M. Dual evolutionary origin for the rat genetic sequences of Harvey murine sarcoma virus. J. Virol., *36:* 408–420, 1980.
49. Srihivasan, A., Reddy, E. P., and Aaronson, S. A. Abelson murine leukemia virus: molecular cloning of infectious integrated proviaral DNA. Proc. Natl. Acad. Sci. USA, *78:* 2077–2081, 1981.
50. Watson, R., Oskarsson, M., and Vande Wonde, G. F. Human DNA sequence homologous to the transforming gene (*mos*) of Moloney murine sarcoma virus. Proc. Natl. Acad. Sci. USA, *79:* 4078–4082, 1982.
51. Schwab, M., Alitalo, K., Klempnauer, K. H., Varmus, H. E., Bishop, J. M., Gilbert, F., Brodeur, G., Goldstein, M., and Trent, J. Amplified DNA with limited homology to *myc* cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. Nature (Lond.), *305:* 245–248, 1983.
52. Sherr, C. J., Fedele, L. A., Oskarsson, M., Maizel, J., and Woude, G. V. Molecular cloning of Shyder-Theilen feline leukemia and sarcoma viruses: comparative studies of feline sarcoma virus with its natural helper virus and with Moloney murine sarcoma virus. J. Virol., *34:* 200–212, 1980.
53. Donner, L., Fedele, L. A., Garon, C. F., Anderson, S. J., and Sherr, C. J. McDonough feline sarcoma virus: characterization of the molecularly cloned provirus and its feline oncogene (v-*fms*). J. Virol., *41:* 489–500, 1982.
54. DeLorbe, W. J., Luciw, P. A., Goodman, H. M., Varmus, H. E., and Bishop, J. M. Molecular cloning and characterization of avian sarcoma virus circular DNA molecules. J. Virol., *36:* 50–61, 1980.
55. Robbins, K. C., Devare, S. G., and Aaronson, S. A. Molecular cloning of integrated simian sarcoma virus: genome organization of infectious DNA clones. Proc. Natl. Acad. Sci. USA, *78:* 2918–2922, 1981.
56. Perbal, E., and Baluda, M. A. Avian myeloblastosis virus transforming gene is related to unique chicken DNA regions separated by at least one intervening sequence. J. Virol., *41:* 250–257, 1982.