Journal of Cellular Biochemistry 33:267–288 (1987)
Cellular and Molecular Biology of Tumors and
Potential Clinical Applications 107–128

Oncogene Amplification and Chromosomal Abnormalities in Small Cell Lung Cancer

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Twelve cell lines isolated from patients with small cell lung cancer have been studied for amplification of the three characterised members of the myc proto-oncogene family (c-myc, N-myc, and L-myc) and for abnormalities of chromosome 3. Ten of these lines were being studied for the first time. Ten of the 12 small cell lung cancer cell lines had amplification of one member of the myc proto-oncogene family. Amplification of c-myc was observed in only one small cell lung line—a "morphological variant." One "classic" small cell lung cancer line expressed c-myc but had no obvious amplification of the gene. N-myc and L-myc were more commonly amplified than c-myc. Chromosomal abnormalities (mainly deletions) in chromosome 3 were observed in all small cell lung carcinoma cell lines examined. When the small cell lung carcinoma lines were grouped according to "classic" or "variant" characteristics, it was found that the "classics" had deletions of the short arm of chromosome 3, whereas the "biochemical variants" had deletions of the long arm of chromosome 3. The extent of the deletions varied between cell lines. For the deletion in the short arm of chromosome 3 the minimum common region of overlap was assigned to bands 3p23–3p24.

Key words: c-myc, N-myc, L-myc, chromosome 3, oncogene amplification, chromosomal abnormalities, small cell lung cancer

In a significant number of tumour types, the activation of a proto-oncogene is associated with a chromosomal abnormality—a translocation, inversion, or deletion [for review, see ref 1]. Examples of consistent chromosomal abnormalities are the translocations in Burkitt's lymphoma involving the c-myc gene on chromosome 8 [2] and the Philadelphia chromosome in acute myeloid leukaemia involving the c-abl gene on chromosome 9 [3]. The proto-oncogene mapping to the chromosomal abnormality may not be the only activated oncogene in the tumour, however: in in vitro transformation experiments, oncogenes have been shown to be required to act synergistically to bring about transformation [for recent review, see ref 4]. Wilm's tumour

Received August 11, 1986; revised and accepted October 29, 1986.

and retinoblastoma provide likely in vivo examples of more than one gene being involved in the transformed phenotype [5,6]. The two genes may be activated by different mechanisms—mutations in the coding or regulatory regions of the gene or amplification of the gene are two possible mechanisms other than chromosomal rearrangement.

Some cell lines isolated from patients with small cell lung cancer (SCLC) exhibit amplified genes from the myc gene family [7–12] and some show chromosomal abnormalities—most notably a deletion in the short arm of chromosome 3 ($3p^-$) [13–18]. The purpose of this present study was to characterise the same set of cell lines (mainly isolated from patients in Cambridge, UK) with respect to both amplification and $3p^-$ status. These 2 abnormalities may be independent or synergistic factors in the transformed phenotype of SCLC cell lines.

Dr. J. Minna and his coworkers have developed a classification of SCLC cell lines based on certain in vitro morphological and biochemical criteria [19]. The biomarkers include the expression of the lung APUD (amine precursor uptake and decarboxylation) enzyme L-3,4-dihydroxyphenylalanine decarboxylase (DDC), neurone-specific enolase (NSE), the brain isoenzyme of creatine kinase (CK-BB), and bombesin-like immunoreactivity (BLI, a gastrin-releasing peptide hormone). All SCLC cell lines exhibit elevated levels of CK-BB and NSE. Two subtypes can be distinguished, however—"classic" and "variant." "Classic" cell lines, accounting for 70% of SCLC cell lines, grow as tightly packed floating aggregates, have high levels of all four biomarkers, and possess neuroendocrine tissue-related dense-core vesicles. The "variant" cell lines (30% of all SCLC cell lines) do not express DDC or BLI but can be further subdivided into "biochemical" and "morphological" subclasses on a morphological basis [11]. The "biochemical variants" have "classic" morphology, while the "morphological variants" show less differentiated morphology than "classic" lines (they resemble undifferentiated large cell lung carcinoma [LCLC], having more cytoplasm and one or more prominent nucleoli, and grow as loose-floating aggregates).

The "morphological variant" SCLC cell lines have increased tumorigenicity in nude mice and are more resistant to X-rays compared with "classic" cell lines [20–22]. It seems likely that these cell lines are the in vitro correlate of the mixed SCLC/LCLC histogenic subtype [9]. Patients with such tumours have poorer prognosis than patients with histologically pure SCLC [23,24]. "Morphological variant" SCLC cell lines either express their phenotype at the onset of culture or acquire it in vitro with the loss of APUD marker expression and the development of c-myc amplification [9]. The "morphological variants" may therefore represent a form of tumour progression, perhaps in association with amplification of the c-myc gene [7,11,25]. In neuroblastoma, N-myc amplification and/or overexpression correlates with progression toward more malignant stages [25–28]. This study of myc family amplification is therefore interesting in the light of the SCLC subclass phenotypes; gene amplification may have important implications for diagnosis and treatment of patients with SCLC.

MATERIALS AND METHODS

Cell Lines: Origin, Establishment, and Culture

Controls for molecular studies. The following cell lines were used as controls: JI (Burkitt's lymphoma cell line (t2;8) expressing c-myc RNA) [29], COLO

320 HSR (colon carcinoma cell line with amplified copies of the c-myc gene associated with homogeneously staining regions) [30], Kelly (neuroblastoma line with amplification and expression of the N-myc gene) [31].

SCLC cell lines (classified as detailed above). The SCLC cell lines used were as follows: from the USA—NCI N417 ("morphological variant," established from a nude mouse heterotransplant of an untreated tumour sample) [19]; NCI H69 ("classic," established from a patient receiving prior therapy) [19]; from Sutton, UK—MAR, POC, FRE ("classics," cultured directly from a surgically treated patient, from a xenografted tumour from a chemotherapy-treated patient, and directly from a chemotherapy-treated patient, respectively), MOR (adenocarcinoma). The following lines were established in Cambridge, UK, by the authors (see Table I): NCI H69-LX4 (a multi-drug-resistant variant of NCI H69 [32]); COR-L42, -L47, -L51, -L88 ("classics"); COR-L27, -L24, -L103 ("biochemical variants," COR-L24 and -L103 coming from the same patient before and after chemotherapy, respectively). All COR lines were established directly in culture from pleural effusion, lymph node, or marrow aspirate samples from untreated patients (except COR-L103) in Cambridge [33].

All cell lines were routinely passaged in RPMI 1640 medium plus 10% foetal calf serum and the antibiotics penicillin and streptomycin. NCI H69-LX4 had 0.04 μg ml^{-1} adriamycin added to the growth medium. Kelly, COR-L88, and COR-L23 were passaged using trypsin treatment. All remaining lines grew as floating aggregates and were subcultured by being allowed to settle out, all but a small volume of medium being removed and replaced with fresh medium. Aggregates were mechanically dispersed on subculturing.

TABLE I. Properties of Cell Lines Established in Cambridge (UK) [32]

Line no.	Cytology[a]	L-dopa decarboxylase[b]	Ck-BB[c]	NSE[d]	Months in culture[e]
COR-L23	LCLC	−0.16	2.8; 0.0	9.7	3
COR-L42	SCLC	−0.09; +0.04; +0.05; 0.76	>543; >366	18.5	24
COR-L51	SCLC	+1.73; +3.04	500; >714	16.0	6
COR-L88	SCLC	+2.92; +5.66; +1.27	>312; >565	NA	9
COR-L47	SCLC	+2.14; +0.97; +1.18	52.4; >680	21.5	9
COR-L24	SCLC	+0.06; +0.15; 0.09	280; 253	18.1	18
COR-L27	SCLC	−0.17; +0.04	>700	16.3	12

[a]As kindly classified by Dr. A. Gazdar: LCLC, large cell lung carcinoma; SCLC, small cell lung cancer.
[b]All sample and control values are expressed as cpm mg^{-1} protein. The human SCLC cell line NCI H69 and the mouse tumour line EMT6 served as positive and negative controls, respectively. Values given (V) were calculated as follows: V = (Z−Y/X−Y), where X = cpm mg^{-1} NCI H69 control; Y = cpm mg^{-1} protein EMT6 control; Z = cpm mg^{-1} protein test cells. (A negative value indicates a lower activity for the test cells than for EMT6.)
[c]Values given are ng mg^{-1} protein.
[d]Values are % specific binding of NSE by the monoclonal antibody B12/A6, calculated as (cpm bound by McAb − cpm bound by P3NSO spent medium/input cpm × 100). P3NSO is a nonsecreting mouse myeloma. Values represent the mean of triplicate determinations, from within a single experiment, which varied by less than 5%. Binding of B12/A6 to nonpulmonary cell lines is low, eg, T lymphoblastoid (target cell Molt 4) = 1.5% specific binding. (Data reproduced with kind permission of Dr. J. Reeve [49].) NA, not available.
[e]Estimated times for non-Cambridge lines (NCI H69, MAR, POC, FRE) = >18 months.

Isolation of DNA

Logarithmically growing stock cultures of cells in suspension or in trypsinised monolayers were pelleted, resuspended in 10 mM Tris, 5 mM ethylenediamine tetraacetic acid (EDTA), pH 8, and lysed by the addition of 1% lithium dodecyl sulphate. The mixture was then subjected to four phenol-chloroform extractions and DNA precipitated from the final aqueous phase with 0.2 M sodium acetate and ethanol. The DNA was dissolved and stored in sterile distilled water at −20°C.

Preparation of Total Cellular RNA

Cells in log phase were pelleted and resuspended in a drop of medium before adding 6 M guanidine hydrochloride, 0.2 M sodium acetate, pH 5.5 (20 ml per 5×10^7 cells), and vortexing. The DNA was sheared with a VirTis homogenizer. RNA was precipitated by addition of a half volume of ethanol and placed at −20° overnight. The RNA was pelleted by centrifugation and resuspended by vigorous homogenisation in 7 M urea, 0.35 M NaCl, 50 mM Tris pH 7.5, 1 mM EDTA, 0.2% sodium dodecyl sulfate (SDS). After a single phenol-chloroform extraction, RNA was recovered by ethanol precipitation.

Preparation of Cytoplasmic RNA

Pelleted log phase cells were resuspended in 0.9% saline, lysed with a 0.5% NP40 buffer (0.14 M NaCl, 1.5 mM $MgCl_2$, 10 mM Tris, pH 8.6), then layered onto a sucrose cushion (0.14 M NaCl, 1.5 mM $MgCl_2$ 10 mM Tris, pH 8.6, 1% NP40, 24% sucrose). After centrifugation at 3,000 rpm, 4°C, for 25 min, the upper layer was removed, and an equal volume of PK buffer (0.2 M Tris pH 7.5, 0.3 M NaCl, 0.025 M EDTA, 2% SDS, 400 μg/ml Proteinase K) was added and incubated at 37°C for 30 min, followed by incubation at 65°C for 3 min. After a single phenol-chloroform extraction, RNA was recovered by ethanol precipitation.

Filter Hybridisation

DNA. Fifteen micrograms of genomic DNA was completely digested with EcoRI at 37°C and size fractionated in 0.8% agarose gels, using as size markers λDNA digested with HindIII and end labeled with [α^{32}P]-dCTP and Klenow polymerase. The DNA was denatured and transferred to nylon (Hybond^{TM}-N, Amersham) according to Southern [34]. Filters were treated with ultraviolet light for 2–5 min.

RNA. Ten-microgram samples of total cellular RNA were glyoxylated [35] and size fractionated in 1.4% agarose gels. λDNA digested with HindIII and end labeled with [α^{32}P]-dCTP and Klenow polymerase was glyoxylated and run as size markers. RNAs were transferred to nylon (Hybond^{TM}-N, Amersham) by Northern blotting, and filters were baked for 2 hr at 80°C.

Filters with RNA or DNA were treated identically. After 1 hr at 65°C in hybridisation solution (6 × SSC, 0.2% polyvinylpyrrolidone, Ficoll 400, and bovine serum albumin, 0.1% sodium dodecyl sulphate, 50 μg/ml sonicated denatured salmon sperm DNA, 5% dextran sulphate), denatured nick-translated probe [36] (specfic activity at least 10^8 cpm/μg) was added to a final concentration of 10^6 cpm/ml. The probes were restriction enzyme fragments purified from agarose gels. Hybridisation was overnight at 65°C. Filters were washed free of unhybridised probe at 65°C using 0.1 × SSC, 0.1% SDS, prior to autoradiography.

Probe removal prior to rehybridising with a second probe was achieved by incubating DNA filters at 45°C for 130 min in 0.4 M NaOH followed by a 30-min incubation in 0.1 × SSC, 0.1% (w/v) SDS, 0.2 M Tris-HCl, pH 7.5. RNA filters were washed for 2 hr at 65°C in 0.005 M Tris-HCl, pH 8.0, 0.002 M Na_2 EDTA, 0.002% w/v polyvinylpyrrolidone, Ficoll 400, and bovine serum albumin. Filters were autoradiographed overnight to assess the effectiveness of probe removal.

G-Banding

Cells growing in log phase were treated with 0.05 μg/ml of colcemid 1½ and 3 hr before harvesting by centrifugation. The pelleted cells were swollen in 0.075 M KCl for 5 min, fixed twice in 3:1 methanol-acetic acid; then stored for up to 18 hr at −20°C. G-banding was performed by the trypsin method [37].

RESULTS

myc Gene Amplification in SCLC Cell Lines

Southern filter hybridisation analysis was used to evaluate the copy number of each of the characterised members of the myc gene family (c-myc, N-myc, and L-myc) in the SCLC cell lines, using placenta DNA as a single-copy control. Filters were hybridised first with a myc gene probe and second with an immunoglobulin k-chain constant region gene probe [38], which acted as an internal control for the amounts of DNA transferred to the filter. The results are summarised in Table II. A very high proportion of the SCLC cell lines studied—ten out of 12—showed amplifi-

TABLE II. myc Gene Amplification and Chromosome 3 Abnormalities

Cell line	myc amplification	Chromosome 3 abnormalities
Classic SCLC		
NCI H69[a]	N-myc × 80	del in 3p, del 3pq
NCI H69-LX4[b]	N-myc × 80	del 3p
FRE[c]	N-myc × 50	del 3p
MAR[c]	N-myc × 90	del 3p, del 3pq
POC[c]	N-myc × 100	del 3p
COR-L42[b]	?	del 3p
COR-L51[b]	—	t(3;11)(p14;p15.2) iso 3q
COR-L88[b]	L-myc × 15	del 3p
COR-L47[b]	L-myc × 25	del 3p
Variant SCLC[d]		
COR-L24[b] (BV)	L-myc × 10	Normal 3p, del 3q
COR-L103[b] (BV)	L-myc × 10	Normal 3p, del 3q
COR-L27[b] (BV)	L-myc × 10	Normal 3p, del 3q or iso 3q
NCI N417[a] (MV)	c-myc × 50	Normal 3p, $3q^+$
Large cell lung carcinoma		
COR-L23[b]	c-myc × 30	Complex rearrangements
Adenocarcinoma		
MOR[c]	c-myc × 2	N.D.

[a]From U.S.A.
[b]From Cambridge, UK.
[c]From Sutton, UK.
[d]BV, "biochemical variant"; MV, "morphological variant."

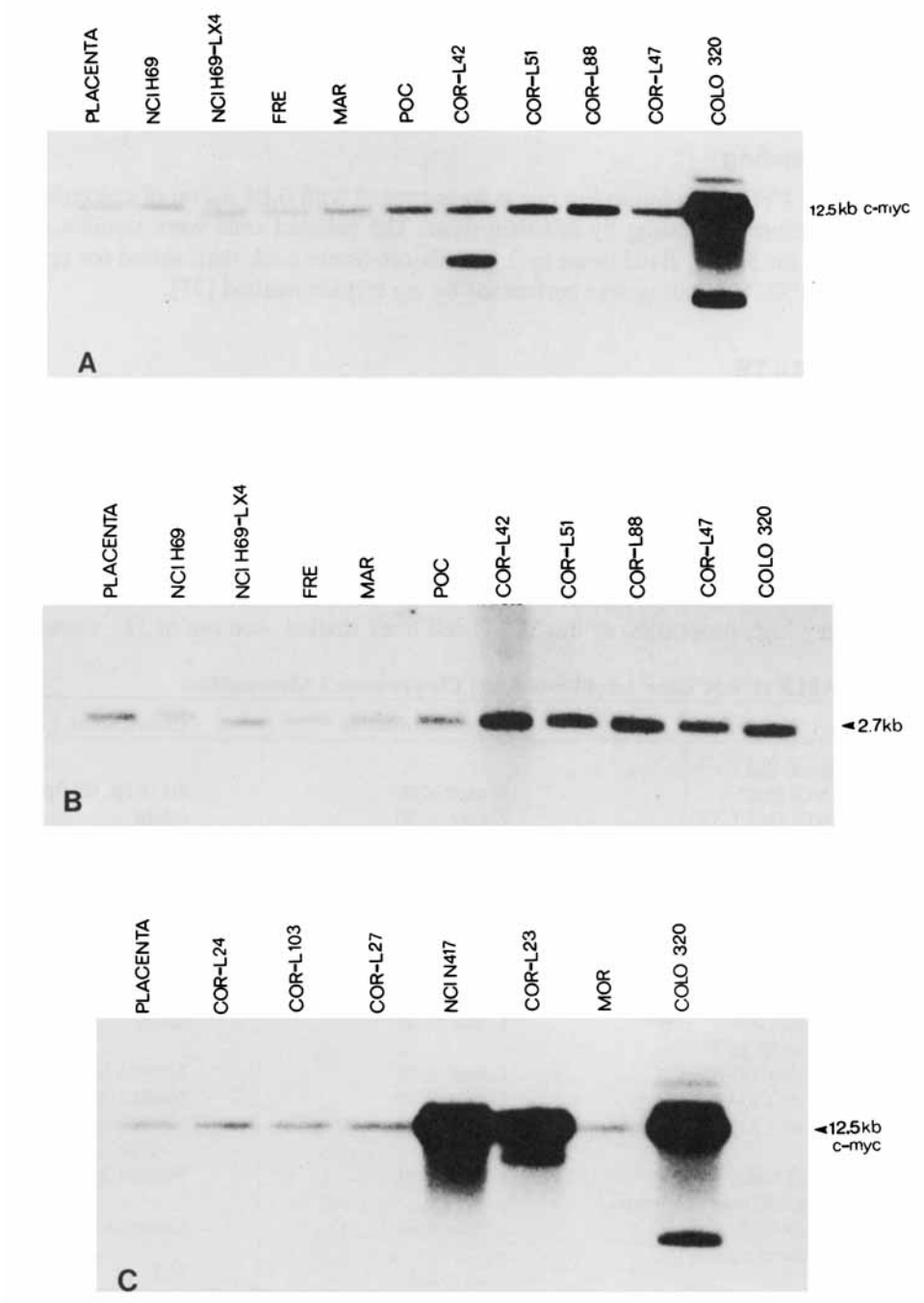


Figure 1 (Continued on following page)

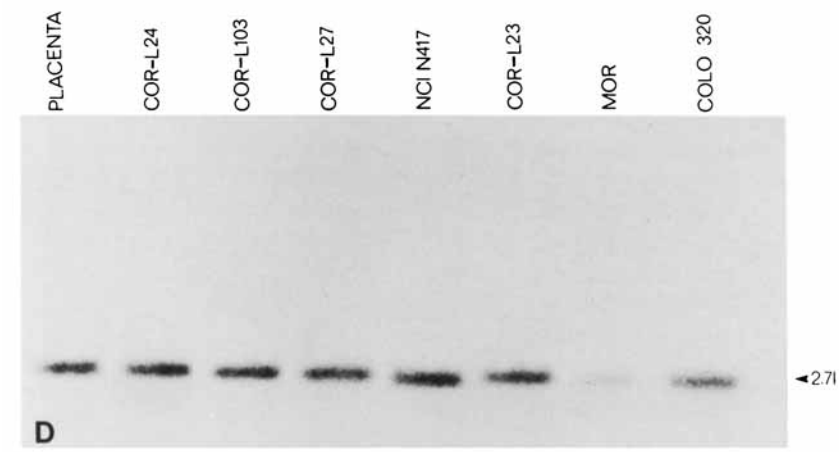


Fig. 1. Amplification of c-myc gene. Ten micrograms of DNA from the cell lines shown in the figure was digested with EcoRI and fractionated in 0.8% agarose gels. After transfer to nylon, the filters were hybridised first with a c-myc exon 1 probe (see text) (**A,C**) and, following autoradiography and removal of the signal, reprobed with an immunoglobulin K-chain constant region gene probe (**B,D**) (A and B therefore represent the same filter as do C and D). The c-myc probe hybridises to a 12.5-kb EcoRI fragment, the immunoglobulin probe to a 2.7-kb EcoRI fragment. Fragment sizes were estimated by coelectrophoresis of bacteriophage lambda DNA digested with HindIII.

cation of c-myc, N-myc, or L-myc. No cell line had amplification of more than one member of the myc gene family (Figs. 1–3) as reported by Dr. J. Minna's laboratory [7–9,12].

From analyses of metaphase spreads it was found that all the SCLC cell lines showing myc gene amplification, with the single exception of COR-L47, had chromosomal markers of gene amplification—double minutes (DM) or homogeneously staining regions (HSR)—previously shown to be the location of amplified c-myc [30] and N-myc [31] genes. NCI H69 and NCI N417 had an HSR on chromosomes 12 and 1, respectively. Chromosome 1p+ markers in COR-L88, -L24/-L103, and -L27 were probable HSRs. DMs were observed in NCI H69-LX4, MAR, FRE, POC, and the LCLC cell line COR-L23. The lines COR-L42 and COR-L51, with no apparent myc gene amplification, had no visible HSR or DM.

Our c-myc probe (Sp65 myc HS1 #31) was a 3-kilobase-pair (kb) HindIII-SacI fragment of exon 1/intron 1 that did not cross-hybridise with N- or L-myc (the three characterised myc genes do not have exon 1 sequence homology [39,8,40]). In all DNAs studied the c-myc probe detected a 12.5-kb EcoRI fragment (Fig. 1A,C) which represents the germline c-myc gene [41,42]. Amplification of c-myc was observed only in the "morphological variant" SCLC cell line NCI N417, in the LCLC cell line COR-L23, possibly in the adenocarcinoma MOR (Fig. 1C,D shows that although MOR appears to have single-copy c-myc, the filter has less MOR DNA, and other filters show 2–3 copies of c-myc in this cell line), and in our positive control COLO 320 HSR. COR-L23 and COLO 320 HSR also showed unique amplified EcoRI fragments with the c-myc probe (Fig. 1C) in several DNA preparations. Such fragments are consistent with possible c-myc rearrangements that may have occurred prior to amplification. This phenomenon is not manifest at the RNA level (see below and Fig. 4C). The novel fragments may alternatively represent polymorphisms of the

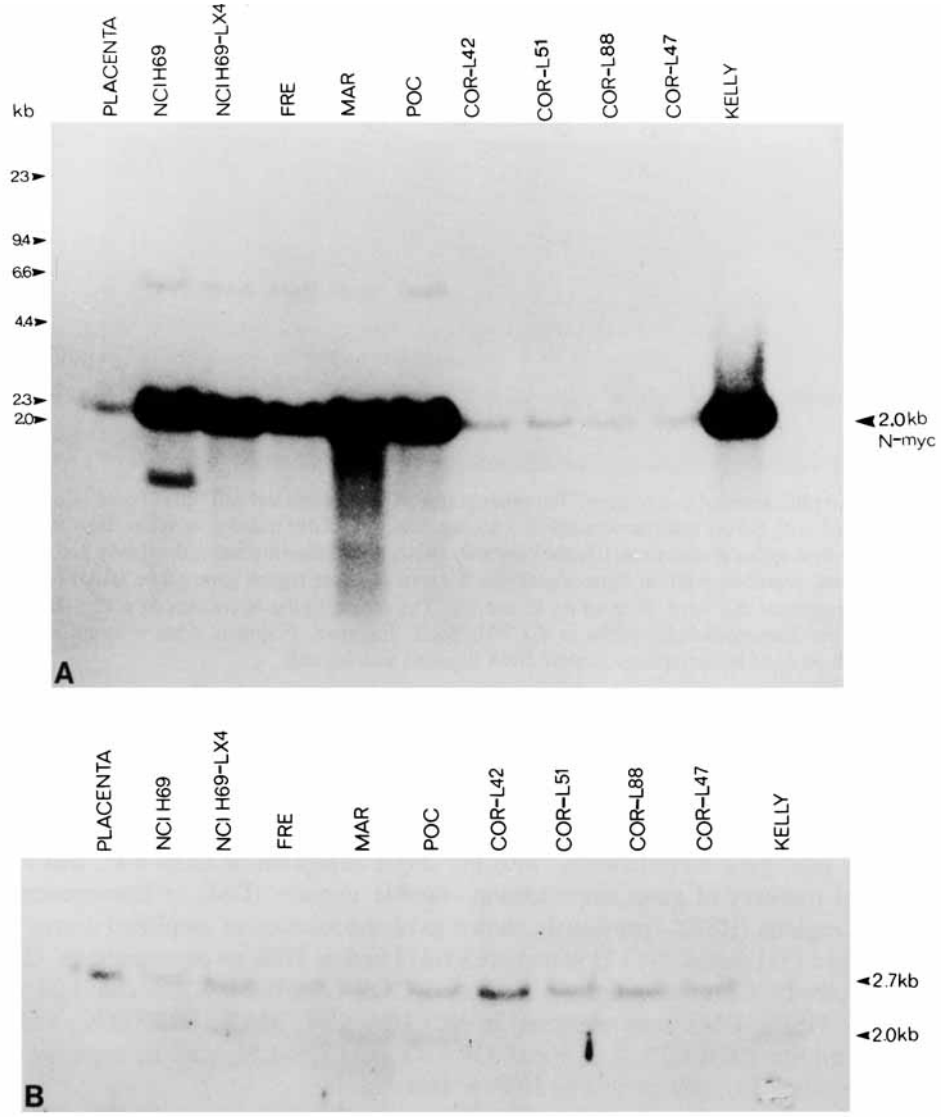
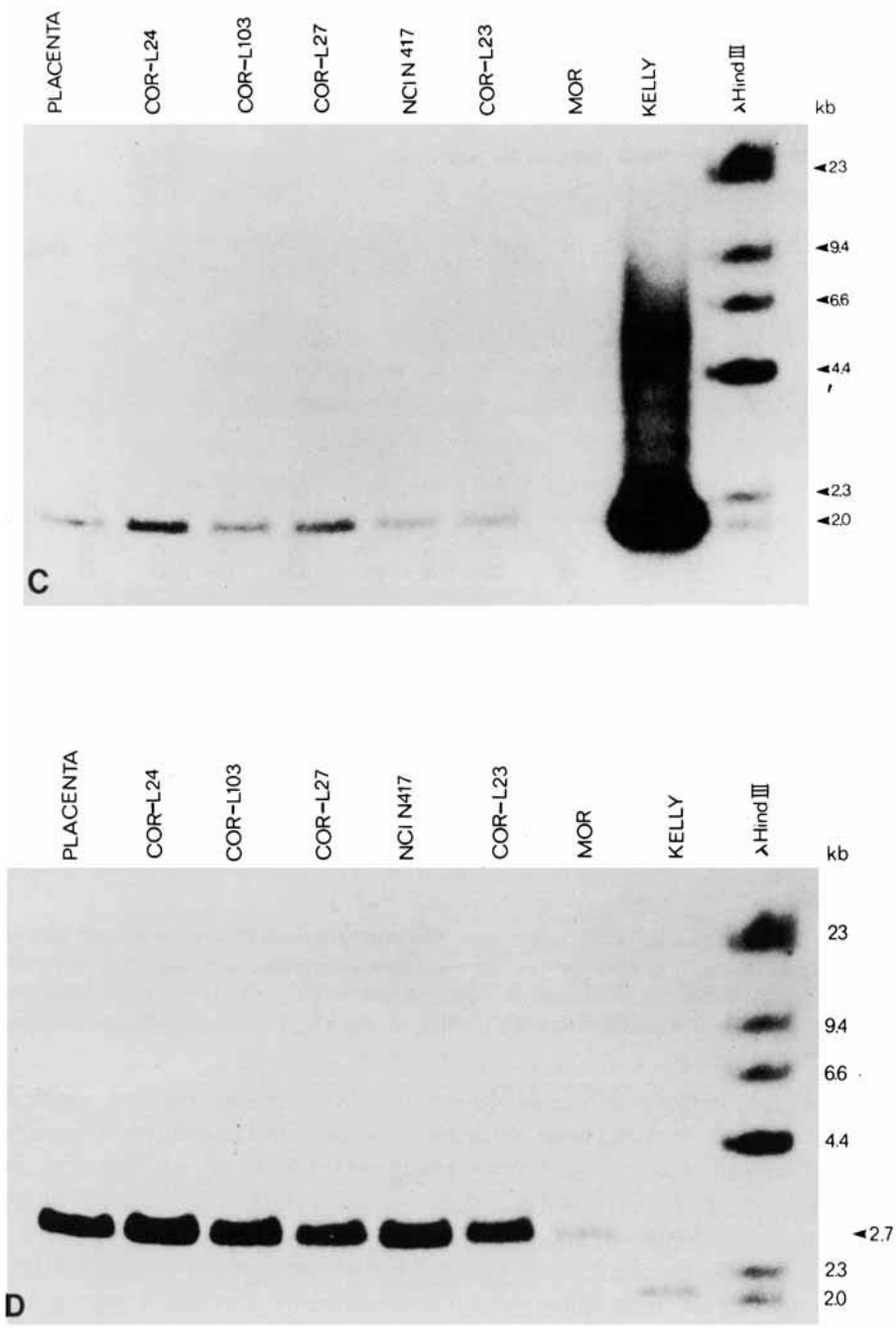


Fig. 2. Amplification of N-myc gene. Ten micrograms of DNA from the cell line shown in the figure was digested with EcoRI and filters prepared as for Figure 1. The two filters were probed first with the Nb-1 probe (see text) (**A,C**) and second with the immunoglobulin gene probe (**B,D**). The Nb-1 probe hybridises to a 2.0-kb EcoRI fragment. Note that in B and D the Nb-1 signal has not been completely removed from those lines with amplification of the N-myc gene. (Continued on following page)

c-myc gene, which are as yet unreported. The novel fragment of ~8.5 kb in COR-L42 (Fig. 1A) is interesting; no RNA species of this line hybridised to the c-myc probe (Fig. 4A), nor were N- or L-myc found to be amplified or expressed in it (Figs. 2A, 3A, 5A, 6A). As both N-myc and L-myc were originally identified by their limited sequence homology to exons 2 and 3 of c-myc, COR-L42 may have amplification of some other myc-related gene.

The N-myc probe Nb-1 (ATCC No: 41011, a 1-kb EcoRI-BamHI fragment), which represents 318 base pairs of intron 1 and 679 base pairs of exon 2 of the N-



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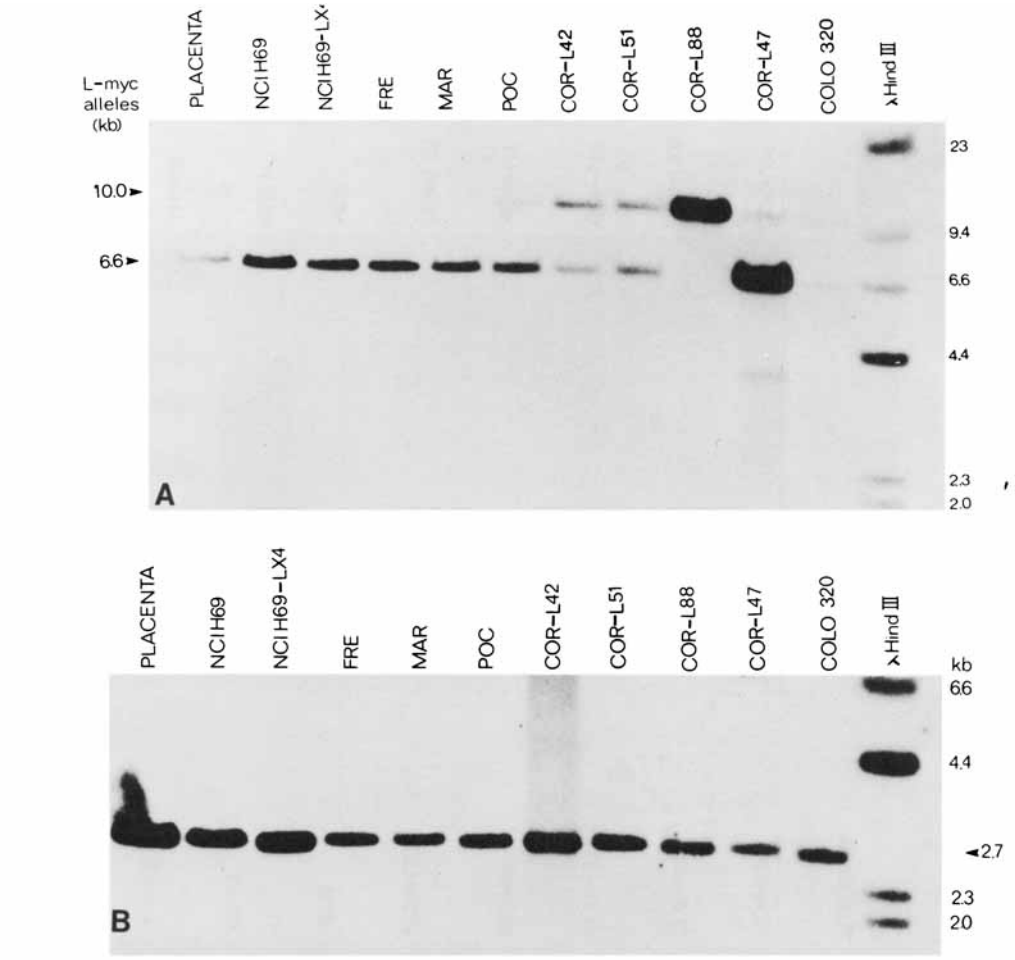
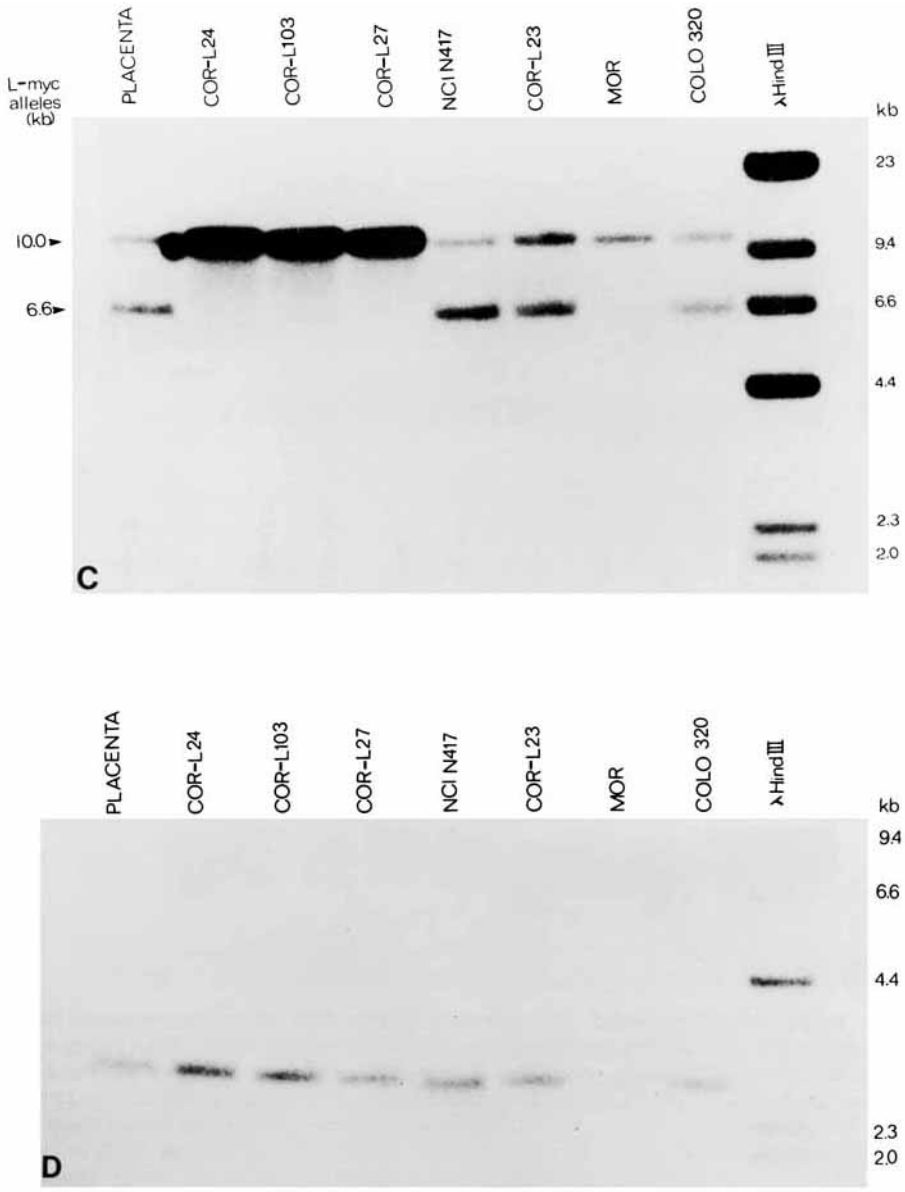


Fig. 3. Amplification of the L-myc gene. Ten micrograms of DNA from the cell lines shown in the figure was digested as for Figure 1. The two filters were probed first with the L-myc probe (see text) (**A,C**) and second with the immunoglobulin gene probe (**B,D**). The L-myc probe hybridises to the two L-myc EcoRI polymorphic fragments of 10.0 and 6.6 kb. D is a very short exposure. (Continued on following page)

myc gene, detected a 2-kb EcoRI germline N-myc gene fragment, amplified in four of the classic lines and none of the four "variant" SCLC cell lines (Fig. 2A,C). Only NCI H69 showed a novel slightly amplified fragment of ~1.3 kb (Fig. 2A), which may represent a rearrangement of the N-myc gene (although there is no evidence of such at the RNA level, Fig. 5A).

The L-myc probe, a 1.8-kb SmaI-EcoRI fragment [8] detected a 10.0-kb and/or a 6.6-kb EcoRI fragment(s), which represented the germline L-myc gene in its two EcoRI restriction site polymorphic forms (Fig. 3A,C). In heterozygous cells, only one of the two alleles is amplified; COR-L47 is an example (Fig. 3A). Amplification of L-myc occurred in both "classic" and "variant" SCLC subtypes. The L-myc gene in COR-L47 may be amplified as a relatively short unit, of which there were no visible manifestations in metaphase spreads. COR-L47 did, however, show a rear-



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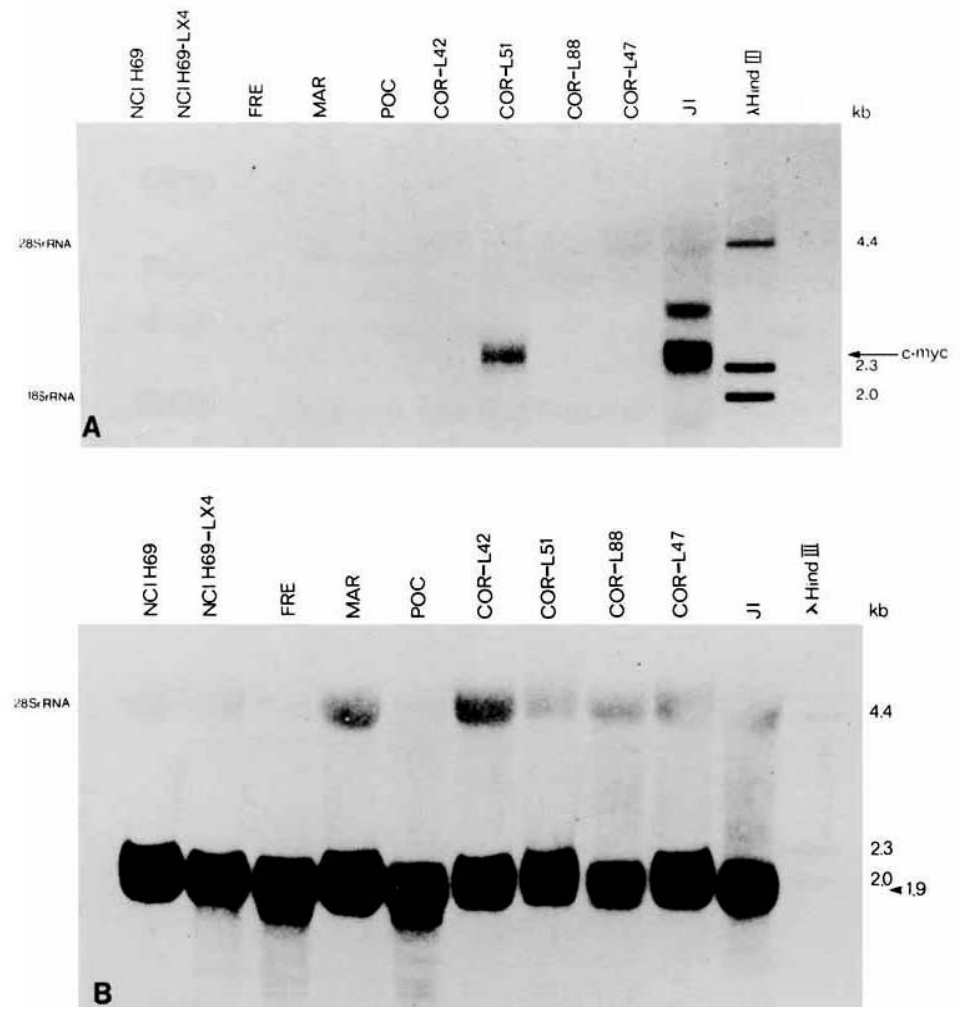
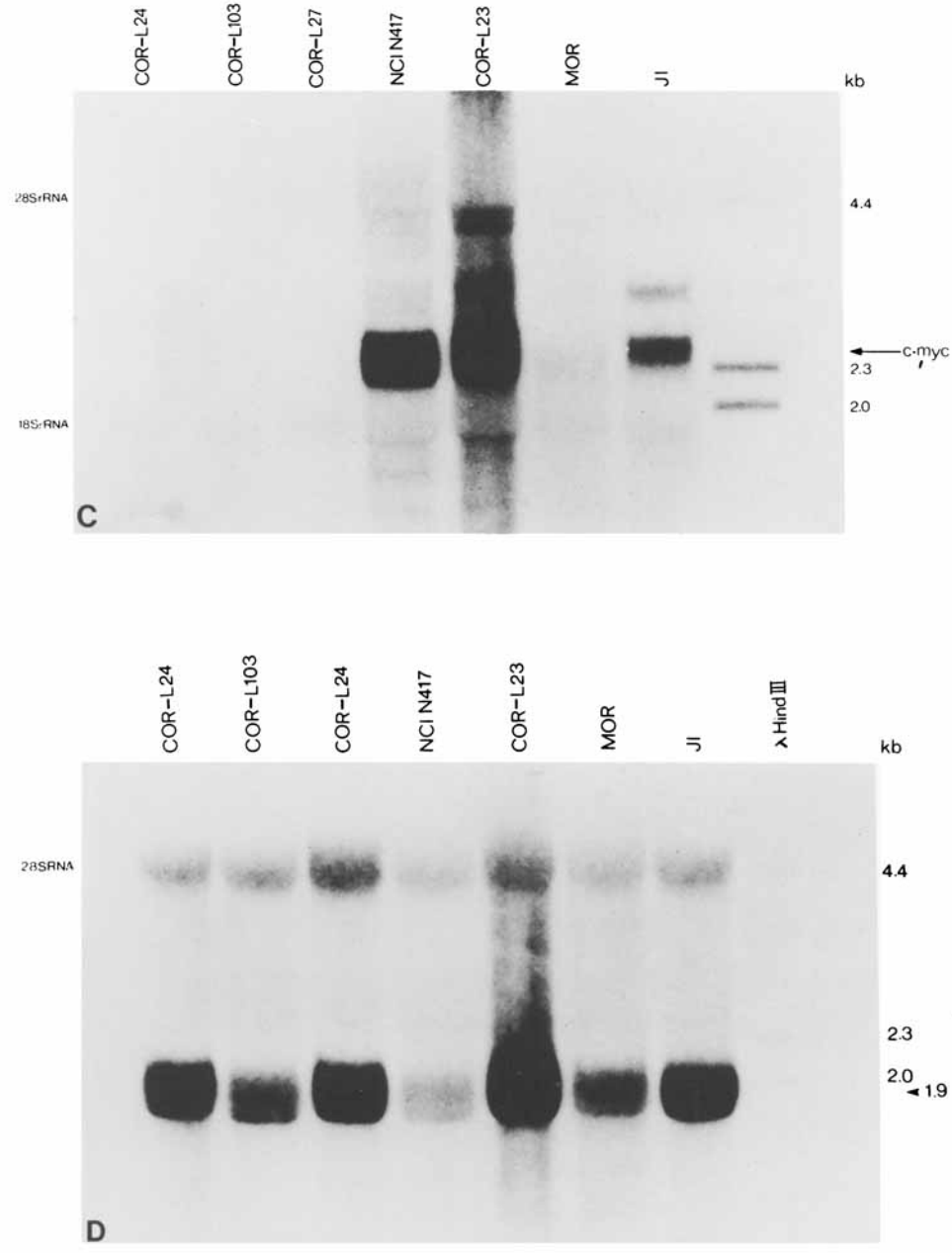


Fig. 4. Expression of c-myc RNA. Ten micrograms of RNA from the cell lines shown in the figure was denatured and separated in 1.4% agarose gels. The RNA was total cellular RNA except for FRE, POC, COR-L88, and COR-L103, which were cytoplasmic preparations. The RNA was transferred to nylon, and the filter was hybridised to the c-myc exon 1 probe as described in "Materials and Methods" (**A,C**). The c-myc mRNA runs at ~2.4 kb using coelectrophoresed glyoxylated bacteriophage lambda DNA digested with HindIII as size markers. This c-myc probe hybridises nonspecifically to ribosomal RNA (A,C). Variations in loading and transfer were normalised by reprobing the washed filters with an actin probe pRT3, as shown in **B** and **D**. Actin RNA is 1.9 kb and the pRT3 probe also detects 285 ribosomal RNA. (Continued on following page)

rangement of an L-myc or L-myc-related allele. When digested with EcoRI, HindIII, XbaI, SacI, or KpnI, COR-L47 DNA showed novel hybridising species in comparison with other L-myc amplifed or single-copy cell lines (Fig. 3A and data not shown). The novel fragments were of single-copy intensity and may be an unamplified L-myc allele, perhaps present on a third copy of chromosome 1 (cells of COR-L47 are diploid or tetra/quatraploid). Alternatively, the novel fragments may represent a myc-related gene, which, when amplified, is detectable by some homology with the L-



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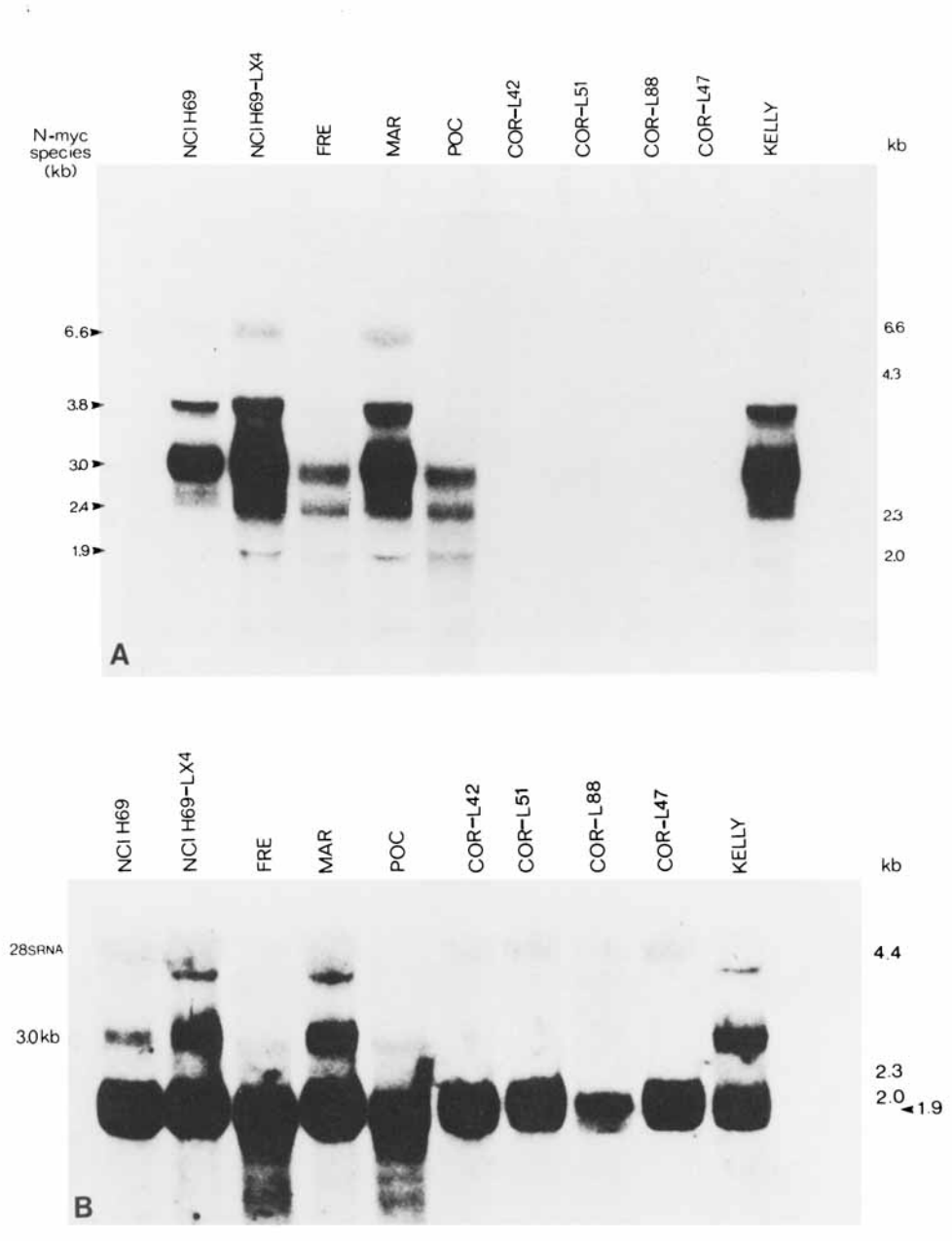
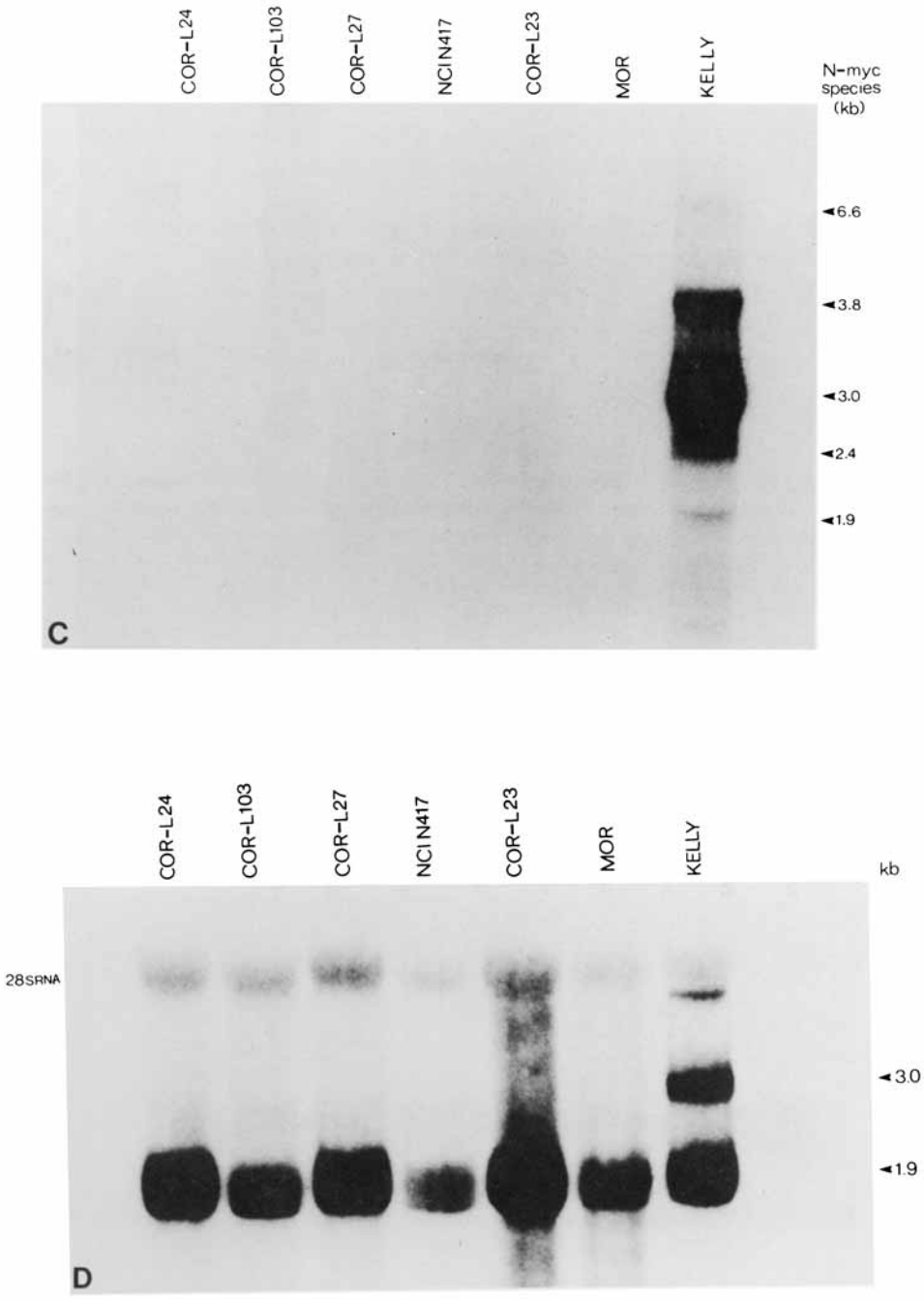


Fig. 5. Expression of N-myc mRNA. Ten micrograms of RNA (samples were total or cytoplasmic just as in Fig. 4) from the cell lines shown in the figure was treated as for Figure 4. After probing the two filters with the Nb-1 probe and detection of a major 3-kb N-myc transcript (**A,C**), they were washed and reprobed with the actin probe (**B,D**). Note that the Nb-1 signal has not been fully removed prior to probing for actin mRNA. (Continued on following page)



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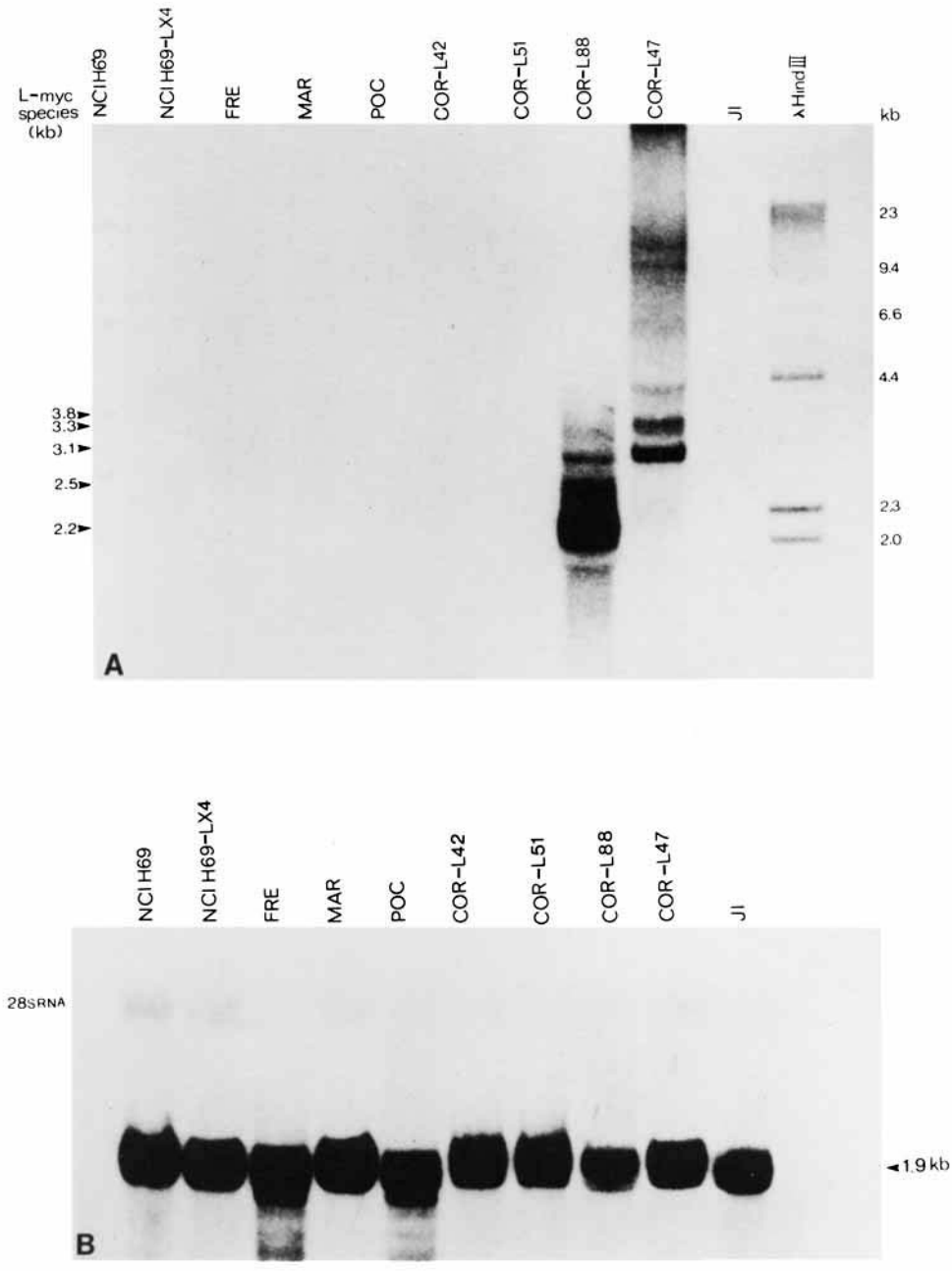

Fig. 6. Expression of L-myc mRNA. Ten micrograms of RNA (samples total or cytoplasmic as in Fig. 4) from the cell lines shown in the figure was treated as for Figure 4. After probing with the L-myc transcript (**A,C**), the figures were reprobed with the actin probe (**B,D**). The major L-myc transcript is 2.2 kb; sizes of other hybridising species are shown. (Continued on following page)

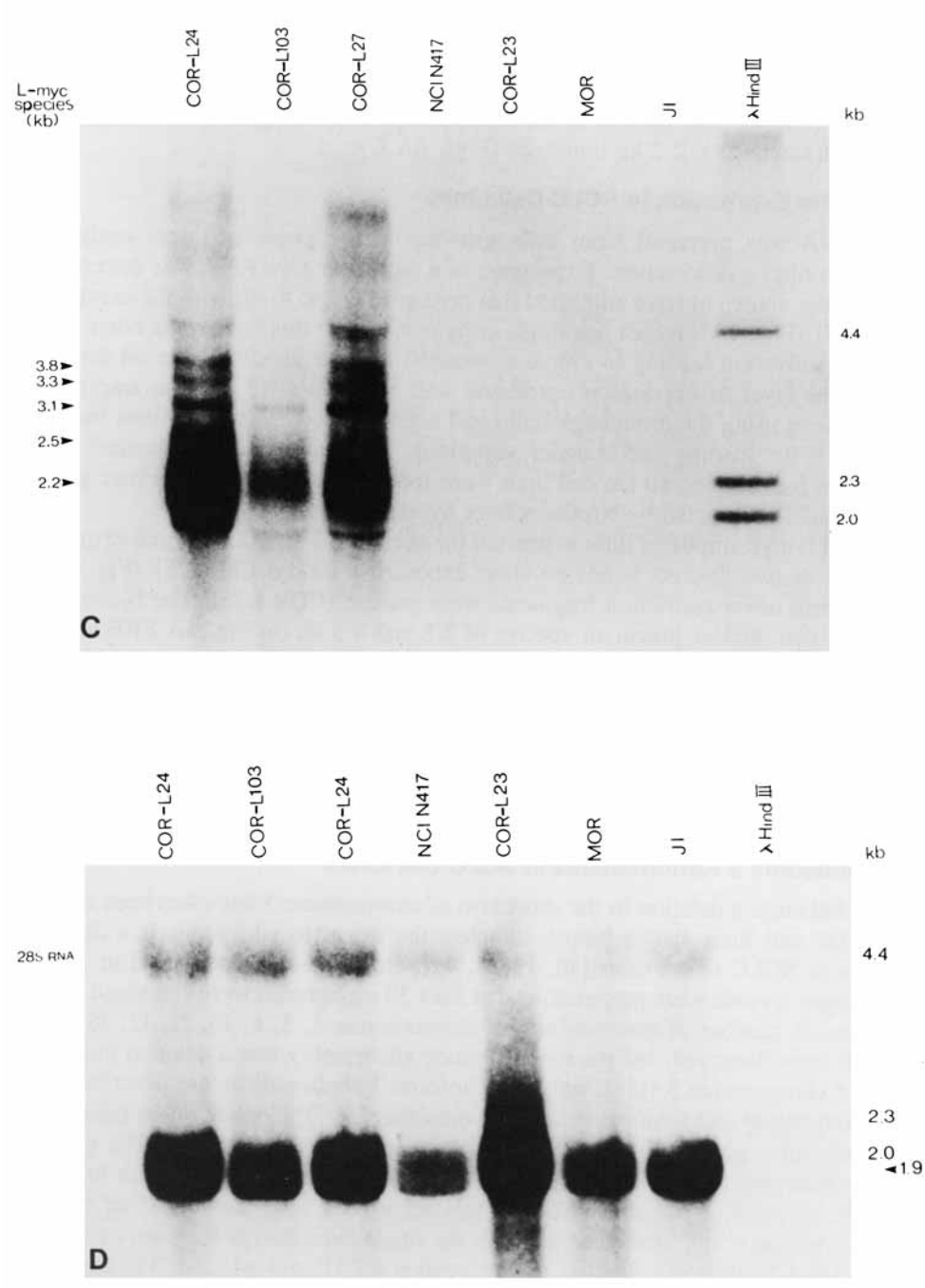

Figure 6 Continued

myc probe. Although there are no reports of more than one member of the characterised myc gene family being amplified in a single SCLC cell line, this apparent exclusion may not be universal and may not extend to more distantly related genes. Interestingly, in COR-L47 the major RNA species hybridising to the L-myc probe was a 3.1-kb species, present in the other L-myc expressing lines at a lower molar ratio than the normal 2.2-kb transcript (Figs. 6A,C).

myc Gene Expression in SCLC Cell Lines

RNA was prepared from cells growing in log phase and was analysed by Northern filter hybridisation. Expression of a particular myc RNA was detected only in cell lines shown to have amplified that myc gene (Figs. 4–6), with the exception of COR-L51 (Fig. 4A), which has single-copy c-myc. For this line, some other mechanism of activation leading to c-myc expression may be involved. For all three myc genes, the level of expression correlated well with the level of gene amplification (determined using the immunoglobulin and actin gene controls in sections band D of Figs. 1–6 for loading and transfer variation). As myc gene amplification seemed exclusive (see above), so the cell lines were found to express only one myc gene (as far as could be detected by Northern filter hybridisation).

All c-myc amplified lines expressed the normal 2.2- and 2.4-kb transcripts (only resolved as two discrete bands on short exposures), as did COR-L51 (Fig. 4A,C), even where novel restriction fragments were present (COR-L23). The N-myc probe detected two nuclear precursor species of 3.8 and 6.6 kb (in Fig. 5A FRE and POC are cytoplasmic RNA preparations) and the major 3-kb translated transcript [40] (Fig. 5A,C). The nature of the remaining hybridising species is being further investigated. The L-myc probe detected a major 2.2-kb transcript and several larger species (Fig. 6A,C), one of which is that (3.1 kb) in higher molar ratio in COR-L47 (see above), and two of which are nuclear (3.3 and 3.8 kb; COR-L103 in Fig. 6C was a cytoplasmic preparation).

Chromosome 3 Abnormalities in SCLC Cell Lines

Although a deletion in the short arm of chromosome 3 has often been observed in SCLC cell lines and in biopsy samples, the extent to which this is a diagnostic feature of SCLC is not clear [10, 13–18, 43]. Using the cell lines listed in Table II, metaphase spreads were prepared, and at least 20 metaphases were examined for each cell line. A number of abnormalities of chromosomes 1, 5, 6, 10, 11, 12, 16, 17, 19, and 20 were observed, but the most common abnormality was a deletion in the short arm of chromosome 3 ([44]; only chromosome 3 abnormalities are described here). Both interstitial and terminal deletions were observed. The extent of the deletion was constant for a given cell line but varied between cell lines (Fig. 7). The minimum common region of overlap mapped to the 3p23–3p24 band border, with breakpoint clusters at 3p12, 3p14, and 3p24. In this set of cell lines the SCLC of "classic" phenotype had a $3p^-$ chromosome, with the single exception of the c-myc expressing line COR-L51 (this cell line has a translocation t(3;11)(p14;p15.2)). The three "biochemical variant" SCLC cell lines had a $3q^-$ chromosome, with no minimal region of overlap, while the "morphological variant" NCI N417 had no $3p^-$ or $3q^-$ but showed extra material on 3q.

Although the cells of every cell line examined in this study had an abnormal chromosome 3, they possessed at least one normal chromosome 3. In triploid cells,

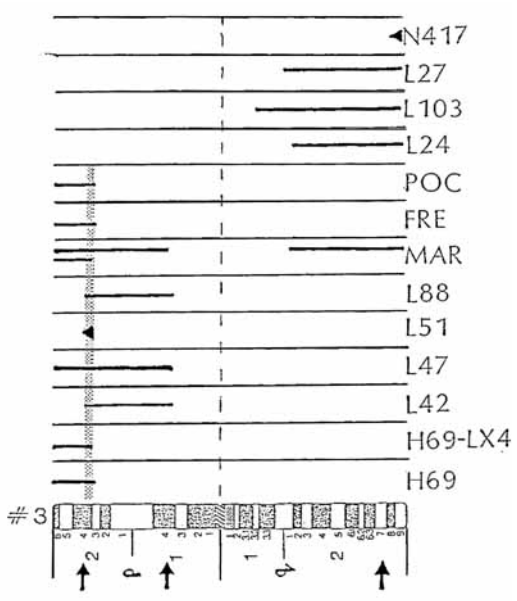

Fig. 7. Abnormalities of chromosome 3 seen in the majority of cells examined from each cell line. Abnormalities of each line are shown in relation to the idiogram of a G-banded chromosome 3. Solid bar, deleted regions of chromosome 3; triangle, translocations; arrow, known constitutive fragile sites on chromosome 3 (3p24.2; 3p14.2; 3q27); shaded area, common region of deletion overlap.

there were always two normal and one abnormal chromosome 3s rather than two abnormal 3s. Tetraploid cells had two normal and two abnormal chromosome 3s.

DISCUSSION

Oncogene Amplification

In order to assess the significance of the myc gene family in SCLC we have studied a set of SCLC cell lines established in Britain for copy number and expression of these genes. Ten of 12 SCLC cell lines showed myc gene amplification and expression, including 5 of 7 lines established from untreated patients. In comparison, Dr. J. Minna has reported at this meeting a finding of c-, N-, or L-myc amplification in 23 of 38 SCLC cell lines. Gene amplification is an adaptive mechanism allowing increased expression of genes conferring a selective advantage on cells [44,45]. Growing the SCLC cells in culture is likely to allow for such selection to occur. Eight of our 12 SCLC cell lines have been in culture for over a year. If, as the high occurrence of myc gene amplification in SCLC cell lines strongly suggests, myc amplification confers some selective advantage on SCLC cells, the time in culture may explain the higher proportion of lines with myc gene amplification. Until something is known of the function of the myc gene products, we cannot explain this selective advantage nor the apparent exclusion of the myc gene family; as found by other workers [8,9,12], no cell line showed amplification or expression of more than one of the three myc genes.

Amplification would certainly appear to be the most common mechanism of myc gene activation in SCLC. All except one of our amplified lines had cytogenetic

markers of gene amplification, and hence translocation of the amplified genes may also have been involved in most cases. Rearrangement of the myc genes was uncommon, perhaps owing to flanking DNA protecting the myc genes from aberrations of translocation. Of the two unamplified lines, COR-L42 may have amplification of a myc-related gene detected by the c-myc exon 1 probe. The other line, COR-L51, expressed c-myc from a single-copy gene. As c-myc is expressed in many normal tissues [46–48], it is perhaps unnecessary to invoke aberrations of the c-myc gene as having activated its expression.

Several reports suggest an association of c-myc amplification with the "morphological variant" subclass of amplified cell lines [7,9,10], believed to represent a more aggressive form of SCLC in patients [9]. These reports document c-myc amplification in a higher proportion of "morphological variant" cell lines than of "biochemical variant" or "classic" cell lines. The present study is in agreement with such observations, c-myc expression being detected in only one "classic" SCLC cell line and in none of the "biochemical variant" cell lines. With only one "morphological variant" line we cannot comment on the importance of c-myc amplification in this phenotype. We note, however, that while it is more common for c-myc to be amplified in this subclass, other myc genes can be amplified and expressed while maintaining the "morphological variant" phenotype [12]. We also found c-myc amplification and expression in a LCLC and an adenocarcinoma cell line; c-myc amplification and expression is thus not exclusive to SCLC among lung cancers. Until more is known about the nature of the contribution of myc gene expression to the cellular phenotype we cannot interpret the apparently more restricted expression of c-myc, as opposed to N- and L-myc among SCLC subclasses.

Chromosome 3

This study included eight "classic" and four "variant" SCLC cell lines, mainly from untreated patients and from different sites. The "classic" cell lines all showed abnormalities in 3p (7 deletions and 1 translocation), whereas the "variant" cell lines had normal 3ps but deletions in 3q. More SCLC cell lines will need to be studied to determine if this distribution of 3p and 3q abnormalities among SCLC subtypes is significant. Published data on 3p deletions in SCLC often do not involve a distinction between "classic" and "variant" lines [16,43,52]. However, Whang-Peng et al [13] reported 3p deletions in both SCLC subtypes. Saksela et al [10] found 3q abnormalities in two "variant" SCLC cell lines, while de Leij et al [17] found 3p deletions in three "variant" lines. Hence, although our "classic" and "variant" lines were distinct in terms of 3p/3q abnormalities, not all pubished data would agree with this.

Chromosome 3 abnormalities are not confined to SCLC; Zech et al [15] reported 3p deletions in a squamous, a LCLC, and an adenocarcinoma cell line. In fact, abnormalities of chromosome 3 are common in both solid and haematopoietic tumours [50], and 3p is the single most consistent site of chromosome change in rhabdomyosarcoma cells [51]. The presence of three constitutive fragile sites on chromosome 3 (Fig. 7) may predispose to such abnormalities [52]. The 3p deletion is therefore not an abnormality specific to lung cancer, but as a consistent feature of at least "classic" SCLC the possible significance of the DNA of this region 3p14–3p24 should be considered. The presence of three copies of chromosome 3 in the majority of the "classic" SCLC cell lines, one copy of which has the $3p^{-}$, raises the possibility that the deletion may contribute to tumorigenesis or tumour progression by uncovering a

recessive mutant allele, analogous to the retinoblastoma system [6]. It will be of interest to determine which genes map to the 3p deletion region. As the c-raf proto-oncogene maps to 3p25 [53], it may be affected by some 3p deletions (Fig. 7). Molecular probes will also allow more accurate deletion mapping than conventional cytogenetics has been able to achieve.

ACKNOWLEDGMENTS

We thank F. Gilbert for the cell line Kelly, Dr. J. Minna for the line NCI N417 and the L-myc probe, Drs. D. Carney and A. Gazdar for the line NCI H69, Dr. M. Ellison for the lines MAR, FRE, POC, and MOR, Dr. G. Lenoir for the line JI, Dr. J.M. Bishop for the line COLO 320 HSR, and DR. J. Rogers for the actin clone pRT3.

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